FENNEMA'S FOOD CHEMISTRY

FIFTH EDITION

edited by

Srinivasan Damodaran
Kirk L. Parkin

FENNEMA'S
FOOD CHEMISTRY
FIFTH EDITION

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

FENNEMA'S
FOOD CHEMISTRY
FIFTH EDITION

edited by

Srinivasan Damodaran
Kirk L. Parkin

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

© 2017 by Taylor & Francis Group, LLC
CRC Press is an imprint of Taylor & Francis Group, an Informa business

No claim to original U.S. Government works

Printed on acid-free paper
Version Date: 20170124

International Standard Book Number-13: 978-1-4822-0812-2 (Paperback) 978-1-4822-4361-1 (Hardback)

This book contains information obtained from authentic and highly regarded sources. Reasonable efforts have been made to publish reliable data and information, but the author and publisher cannot assume responsibility for the validity of all materials or the consequences of their use. The authors and publishers have attempted to trace the copyright holders of all material reproduced in this publication and apologize to copyright holders if permission to publish in this form has not been obtained. If any copyright material has not been acknowledged please write and let us know so we may rectify in any future reprint.

Except as permitted under U.S. Copyright Law, no part of this book may be reprinted, reproduced, transmitted, or utilized in any form by any electronic, mechanical, or other means, now known or hereafter invented, including photocopying, microfilming, and recording, or in any information storage or retrieval system, without written permission from the publishers.

For permission to photocopy or use material electronically from this work, please access www.copyright.com (http://www.copyright.com/) or contact the Copyright Clearance Center, Inc. (CCC), 222 Rosewood Drive, Danvers, MA 01923, 978-750-8400. CCC is a not-for-profit organization that provides licenses and registration for a variety of users. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

Trademark Notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation without intent to infringe.

Library of Congress Cataloging-in-Publication Data

Names: Fennema, Owen R. | Damodaran, Srinivasan. | Parkin, Kirk L. (Kirk Lindsay), 1955-
Title: Fennema's food chemistry.
Other titles: Food chemistry
Description: Fifth edition / [edited by] Srinivasan Damodaran & Kirk L. Parkin. | Boca Raton : CRC Press, 2017. | Includes bibliographical references and index.
Identifiers: LCCN 2016027062| ISBN 9781482243611 (hardback : alk. paper) | ISBN 9781482208122 (pbk. : alk. paper) | ISBN a9781482208139 (e-book) | ISBN 9781482243666 (e-book) | ISBN 9781482208146 (e-book)
Subjects: LCSH: Food--Analysis. | Food--Composition.
Classification: LCC TX541 .F65 2017 | DDC 664/.07--dc23
LC record available at https://lccn.loc.gov/2016027062

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com

Contents

Preface vii
Memorial Resolution of the Faculty of the University of Wisconsin–Madison ix
Editors xiii
Contributors xv

Chapter 1 Introduction to Food Chemistry 1
Owen R. Fennema, Srinivasan Damodaran, and Kirk L. Parkin

SECTION I Major Food Components

Chapter 2 Water and Ice Relations in Foods 19
Srinivasan Damodaran

Chapter 3 Carbohydrates 91
Kerry C. Huber and James N. BeMiller

Chapter 4 Lipids 171
David Julian McClements and Eric Andrew Decker

Chapter 5 Amino Acids, Peptides, and Proteins 235
Srinivasan Damodaran

Chapter 6 Enzymes 357
Kirk L. Parkin

Chapter 7 Dispersed Systems: Basic Considerations 467
Ton van Vliet and Pieter Walstra

SECTION II Minor Food Components

Chapter 8 Vitamins 543
Jesse F. Gregory III

Chapter 9 Minerals 627
Dennis D. Miller

Chapter 10 Colorants 681

Steven J. Schwartz, Jessica L. Cooperstone, Morgan J. Cichon, Joachim H. von Elbe, and M. Monica Giusti

Chapter 11 Flavors 753

Robert C. Lindsay

Chapter 12 Food Additives 803

Robert C. Lindsay

Chapter 13 Bioactive Food Components: Nutraceuticals and Toxicants 865

Hang Xiao and Chi-Tang Ho

SECTION III Food Systems

Chapter 14 Characteristics of Milk 907

David S. Horne

Chapter 15 Physiology and Chemistry of Edible Muscle Tissues 955

Gale M. Strasburg and Youling L. Xiong

Chapter 16 Postharvest Physiology of Edible Plant Tissues 1017

Christopher B. Watkins

Index 1087

Preface

Welcome to the fifth edition of *Fennema's Food Chemistry*. The 11-year interval from the fourth edition has prompted transitions in contributors and content evoked by the waxing and waning of careers, and discoveries based on another decade of research and development. New contributors and co-contributors appear for chapters on "Water and Ice", "Colorants", "Bioactive Substances: Nutraceuticals and Toxicants", "Characteristics of Milk" and "Postharvest Physiology of Edible Plant Tissues". The Chapters titled ''Physical and Chemical Interactions of Components in Food Systems" and "Impact of Biotechnology on Food Supply and Quality" have been omitted from the 5th Edition. In contrast, some things never changed. Dr. Robert Lindsay has been the sole author of the "Food Additives" chapter in all five editions (titled "Other desirable constituents of food" in the first edition) and "Food Flavors" in the second through the fifth edition, despite our efforts to find a better contributor. Some acts are just tough to follow. We are greatly appreciative of the authors' efforts in preparing the fifth edition, for the seriousness and dedication they invested in preparing chapter revisions and complete rewrites to bring the content of *Fennema's Food Chemistry* up to date as much as possible.

On a very sad and somber note, many of you are aware that Dr. Owen Fennema made his final transition in life in August 2012. Sir Isaac Newton said, "If I have seen further it is by standing upon the shoulders [*sic*] of giants." For those of us privileged to have had our lives touched by Owen, we have benefited from the perch of insight he offered, enabling us to see further than we could have on our own. He also inspired us by the manner in which he conducted himself as a scientist, professional, and human being. This fifth edition is dedicated to Dr. Owen Fennema, and, in that context, we share with you the following two documents.

Srinivasan Damodaran and Kirk L. Parkin
Madison, Wisconsin, USA

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Memorial Resolution of the Faculty of the University of Wisconsin–Madison

ON THE DEATH OF PROFESSOR EMERITUS OWEN R. FENNEMA

Professor Emeritus Owen Fennema, age 83, of Middleton, passed away due to complications from bladder cancer, surrounded by family on Wednesday, August 1, 2012, at Agrace Hospice Care. Owen was born on January 23, 1929, in Hinsdale, Illinois, the son of Nicolas (a dairy plant owner) and Fern (First) Fennema. He moved to Winfield, Kansas graduating from high school in 1946. He met his beloved wife, Elizabeth (nee Hammer) in high school and they were married on August 22, 1948.

Owen attended Kansas State University, obtained a B.S. degree in Dairy Industry in 1950, and promptly completed an M.S. degree in Dairy Industry at UW-Madison in 1951. Owen served from 1951 to 1953 as 2nd Lieutenant Ordnance in the U.S. Army, stationed in Fort Hood, Texas. He and Elizabeth moved to Minneapolis, MN in 1953 where Owen worked for the Pillsbury Company in the research department. In 1957, they moved to Madison where Owen went to graduate school and received his Ph.D. in Dairy and Food Industries (minor in biochemistry) in 1960.

Owen was hired as an Assistant Professor in food chemistry in 1960, was promoted to Associate (1964) and Full Professor (1969), served as department Chair 1977–1981, and remained a Professor of Food Science at UW-Madison until his retirement in 1996. During that time, he excelled in every facet of his service to the Food Science department, The College of Agricultural and Life Sciences, the UW-Madison campus, the Food Science profession, and international community.

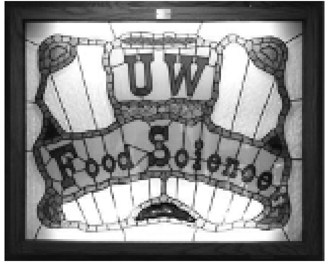
In research, Professor Fennema positioned his group at the leading edge in several areas, the most noteworthy and formative being low temperature biology of foods and model food systems, and edible films. Holistic approaches were taken to define and understand the physical, chemical and biological behaviors of food systems that affect characteristics related to food quality. His fundamental discoveries of the complexities of interactions between phase behavior, (bio)chemical reactivity and solute transport in food systems evolved scientific paradigms in these areas, many of which still guide professionals today. Revealing the nature, influence and control of water and ice in foods was a mainstay of Professor Fennema's research career as reflected by the content of his several hundred scholarly publications and book chapters, along with ~60 theses/dissertations completed by the graduate students he mentored. Among the many honors and awards Owen received for his research activities, the most prestigious were Fellow, and Advancement of Application of Agricultural and Food Chemistry Award, the highest honor from the Agricultural and Food Chemistry Division of American Chemical Society (ACS); Fellow from the Institute of Food Technologists (IFT) and the Nicholas Appert Award (IFT's highest honor); and the Honorary Doctoral degree in Agriculture and Environmental Science from Wageningen Agricultural University, The Netherlands.

In the classroom, Professor Fennema was a gifted communicator and facilitator of student learning. He was legendary in his meticulous organization of course content, and his lectures were crystal clear, like the "water and ice" he frequently studied in research. His focus on explaining principles, coupled with illustrated examples (updated regularly) provided students

with a “real world” feel. Owen’s classroom presence and enthusiasm in the delivery of the material “[brought] the subject matter to life”. Owen had a genuine interest in student learning, would encourage questions, and then take time inside and out of class to help students put it all together. Students came to know Owen as a respectful advocate of theirs, and they found inspiration from his total commitment to their education. In a lifetime of stellar achievements, Owen was recognized world-wide for the publication of a seminal book for food science students and scholars, now titled “Fennema’s Food Chemistry”, published in four editions and multiple languages, and widely used today throughout the world. He considered this one of his greatest achievements as an instructor. He has received many accolades from colleagues and students, including “phenomenal teacher”, a “titan in his field” and a “father of food science”, and he mentored individuals who later became some of the most prestigious leaders in the food science world. To nobody’s surprise, Professor Fennema was awarded the William V. Cruess Award for Excellence in Teaching from IFT, a UW-Madison Distinguished Teaching Award and a Fulbright distinguished lecturer award, Madrid, Spain.

Owen served on numerous professional boards and committees, including the American Chemical Society, the Council for Agriculture Science and Technology and the Institute of Food Technologists (IFT), for which he served in multiple capacities, including treasurer from 1994–1999 and president from 1982 to 1983. Owen was editor-in-chief of IFT’s peer-reviewed journals from 1999 to 2003 when he facilitated a complete reversal of their decline in quality and relevance, ascending to its present stature as an impactful and respected journal among food science scholars. He served on several National Advisory Councils and was recognized by a U.S. FDA Director’s special citation award (2000).

Owen was a citizen of the world, as evidenced by his many contributions to international food science, not the least of which was his service to the International Union of Food Science and Technology (IUFoST). He served in various capacities in IUFoST, gave lectures around the world, and served as major professor to numerous international students. From 1999 to 2001 Owen served as the first President of the International Academy of Food Science and Technology. Owen truly had a global influence, impacting both lives and educational programs of numerous institutions. He was a man without prejudice as illustrated by being one of the first American food scientists to be invited to South Africa, and upon acceptance, insisted that he speak at black institutions in South Africa.



Despite the awards and accolades, Owen remained a humble and caring individual. To his mentors he was always available, had unlimited patience and became a friend for life. Because of the demands on Owen’s time, his students and colleagues would often try to converse with him at every opportunity, sometimes during his frequent walks about campus. Owen’s legendary gait made it difficult for others to keep pace with him, risking the inability to engage in intelligent discourse with him while also gasping for air. Professionally, Owen was often so far out ahead of the rest of us, that we too wondered how we could keep pace.

Owen was also an accomplished poet, wood worker, carpenter and artisan of leaded glass. He was a truly gifted artist, and many of his works are hanging in UW-Madison buildings, IFT headquarters in Chicago, and in private homes of friends and acquaintances. One beautiful piece greets visitors arriving through the main entrance to our beloved Babcock Hall.

Owen touched the lives of many people, including students, colleagues, friends and family. In the last weeks of his life, many people wrote comments and letters to him about what a great teacher and mentor he was and the enormous impact he made on their lives. “As a distinguished scholar, world renowned professor and kind and caring friend, he was an inspiration to us all.”

We are caressed by water as we enter this world, water sustains us as the essence of life, and an overflow of tears accompanies our leaving loved ones behind. Owen studied water his entire professional life. It is easy to picture him now, “playing” with water, looking at us with his habitual wry

grin, knowing something we don't, but eager to share it—ever the teacher. Although we mourn his passing, we will cherish the gift he has left us, the indelible impression of the value of dedication, selflessness, humanity and example.

MEMORIAL COMMITTEE
Srinivasan Damodaran
Daryl B. Lund
Kirk L. Parkin, *Chair*

© University of Wisconsin–Madison, with permission

Among Dr. Owen Fennema's many talents, he had a way with words, including the ability to pose science blended with the art of a storyteller. Here is an excerpt from Dr. Fennema's chapter on "Water and Ice," page 18, from Food Chemistry, Third Edition.

PROLOGUE: WATER—THE DECEPTIVE MATTER OF LIFE AND DEATH

Unnoticed in the darkness of a subterranean cavern, a water droplet trickles slowly down a stalactite, following a path left by countless predecessors, imparting, as did they, a small but almost magical touch of mineral beauty. Pausing at the tip, the droplet grows slowly to full size, then plunges quickly to the cavern floor, as if anxious to perform other tasks or to assume different forms. For water, the possibilities are countless. Some droplets assume roles of quiet beauty - on a child's coat sleeve, where a snowflake of unique design and exquisite perfection lies unnoticed; on a spider's web, where dew drops burst into sudden brilliance at the first touch of the morning sun; in the countryside, where a summer shower brings refreshment; or in the city, where fog gently permeates the night air, subduing harsh sounds with a glaze of tranquility. Others lend themselves to the noise and vigor of a waterfall, to the overwhelming immensity of a glacier, to the ominous nature of an impending storm, or to the persuasiveness of a tear on a woman's cheek. For others the role is less obvious but far more critical. There is life - initiated and sustained by water in a myriad of subtle and poorly understood ways - or death inevitable, catalyzed under special circumstances by a few hostile crystals of ice; or decay at the forest's floor, where water works relentlessly to disassemble the past so life can begin anew. But the form of water most familiar to humans is none of these; rather, it is simple, ordinary, and uninspiring, unworthy of special notice as it flows forth in cool abundance from a household tap. "Humdrum," galunks a frog in concurrence, or so it seems as he views with stony indifference the watery milieu on which his very life depends. Surely, then, water's most remarkable feature is deception, for it is in reality a substance of infinite complexity, of great and unassessable importance, and one that is endowed with a strangeness and beauty sufficient to excite and challenge anyone making its acquaintance.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Editors

Srinivasan Damodaran is a professor of food chemistry at the University of Wisconsin–Madison. He is editor of the book *Food Proteins and Lipids* (Plenum Press) and coeditor of the book *Food Proteins and Their Applications* (with Alain Paraf) (Marcel Dekker, Inc.) and author/coauthor of 12 patents and more than 157 professional papers in his research areas, which include protein chemistry, enzymology, surface and colloidal science, process technologies, and industrial biodegradable polymers. He is a fellow of the Agriculture and Food Chemistry Division of the American Chemical Society. In the fall of 2016, Dr. Damodaran was selected to be the first recipient of the "Owen R. Fennema Professorhip in Food Chemistry" award made possible by private gifts to fund an endowment to honor and preserve the legacy of personal and professional accomplishments achieved by Dr. Fennema. He is on the editorial board of *Food Biophysics* journal. Dr. Damodaran received his BSc (1971) in chemistry from the University of Madras, Madras (now Chennai), India, MSc (1975) in food technology from Mysore University, Mysore, India, and PhD (1981) from Cornell University, Ithaca, New York.

Kirk L. Parkin is professor in the Department of Food Science at the University of Wisconsin–Madison, where he has been on the faculty for more than 31 years. His research and teaching interests revolve around food chemistry and biochemistry, with 3 patents and about 110 refereed journal publications in the areas of marine food biochemistry, postharvest physiology and processing of fruit and vegetable products, fundamental and applied enzymology, and potentially health-promoting bioactive compounds from foods of botanical origin. He has been appointed as the College of Agricultural and Life Sciences Fritz Friday Chair of Vegetable Processing Research for much of the last 19 years, and was elected fellow of the Agricultural and Food Chemistry Division of the American Chemical Society in 2003. Dr. Parkin serves as associate editor for *Journal of Food Science* and on the editorial board of *Food Research International*. Dr. Parkin received his BS (1977) and PhD (2003) in food science from the University of Massachusetts Amherst and MS (1979) in food science from the University of California, Davis.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Contributors

James N. BeMiller
Department of Food Science
Purdue University
West Lafayette, Indiana

Morgan J. Cichon
The Ohio State University
Columbus, Ohio

Jessica L. Cooperstone
The Ohio State University
Columbus, Ohio

Srinivasan Damodaran
Department of Food Science
University of Wisconsin–Madison
Madison, Wisconsin

Eric Andrew Decker
Department of Food Science
University of Massachusetts
Amherst, Massachusetts

Owen R. Fennema

M. Monica Giusti
Department of Food Science
The Ohio State University
Columbus, Ohio

Jesse F. Gregory III
Food Science and Human Nutrition
University of Florida
Gainesville, Florida

Chi-Tang Ho
Department of Food Science
Rutgers University
New Brunswick, New Jersey

David S. Horne
Center for Dairy Research
University of Wisconsin–Madison
Madison, Wisconsin

Kerry C. Huber
Department of Animal and Food Science
Brigham Young University–Idaho
Rexburg, Idaho

Robert C. Lindsay
University of Wisconsin–Madison
Madison, Wisconsin

David Julian McClements
Department of Food Science
University of Massachusetts
Amherst, Massachusetts

Dennis D. Miller
Department of Food Science
Cornell University
Ithaca, New York

Kirk L. Parkin
Department of Food Science
University of Wisconsin–Madison
Madison, Wisconsin

Steven J. Schwartz
The Ohio State University
Columbus, Ohio

Gale M. Strasburg
Department of Food Science and Human Nutrition
Michigan State University
East Lansing, Michigan

Ton van Vliet
Wageningen Agricultural University
Wageningen, the Netherlands

Joachim H. von Elbe
University of Wisconsin–Madison
Madison, Wisconsin

Pieter Walstra
Wageningen Agricultural University
Wageningen, the Netherlands

Christopher B. Watkins
School of Integrative Plant Science
Cornell University
Ithaca, New York

Hang Xiao
Department of Food Science
University of Massachusetts
Amherst, Massachusetts

Youling L. Xiong
Department of Animal and Food Sciences
University of Kentucky
Lexington, Kentucky

1 Introduction to Food Chemistry

Owen R. Fennema, Srinivasan Damodaran, and Kirk L. Parkin

CONTENTS

1.1 What Is Food Chemistry? 1
1.2 History of Food Chemistry 2
1.3 Approach to the Study of Food Chemistry 5
  1.3.1 Analysis of Situations Encountered during the Storage and Processing of Food 8
1.4 Societal Role of Food Chemists 13
  1.4.1 Why Should Food Chemists Become Involved in Societal Issues? 13
  1.4.2 Types of Involvement 14
References 15

1.1 WHAT IS FOOD CHEMISTRY?

Food science deals with the physical, chemical, and biological properties of foods as they relate to stability, cost, quality, processing, safety, nutritive value, wholesomeness, and convenience. Food science is a branch of biological science and an interdisciplinary subject involving primarily microbiology, chemistry, biology, and engineering. Food chemistry, a major aspect of food science, deals with the composition and properties of food and the chemical changes it undergoes during handling, processing, and storage. Food chemistry is intimately related to chemistry, biochemistry, physiological chemistry, botany, zoology, and molecular biology. The food chemist relies heavily on knowledge of the aforementioned sciences to effectively study and control biological substances as sources of human food. Knowledge of the innate properties of biological substances and mastery of the means of manipulating them are common interests of both food chemists and biological scientists. The primary interests of biological scientists include reproduction, growth, and changes that biological substances undergo under environmental conditions that are compatible or marginally compatible with life. To the contrary, food chemists are concerned primarily with biological substances that are dead or dying (postharvest physiology of plants and postmortem physiology of muscle) and changes they undergo when exposed to a wide range of environmental conditions. For example, conditions suitable for sustaining residual life processes are of concern to food chemists during the marketing of fresh fruits and vegetables, whereas conditions incompatible with life processes are of major interest when long-term preservation of food is attempted. In addition, food chemists are concerned with the chemical properties of disrupted food tissues (flour, fruit and vegetable juices, isolated and modified constituents, and manufactured foods), single-cell sources of food (eggs and microorganisms), and one major biological fluid, milk. In summary, food chemists have much in common with biological scientists, yet they also have interests that are distinctly different and are of the utmost importance to humankind.

1.2 HISTORY OF FOOD CHEMISTRY

The origins of food chemistry are obscure, and details of its history have not yet been rigorously studied and recorded. This is not surprising since food chemistry did not acquire a clear identity until the twentieth century, and its history is deeply entangled with that of agricultural chemistry for which historical documentation is not considered exhaustive [1,2]. Thus, the following brief excursion into the history of food chemistry is incomplete and selective. Nonetheless, available information is sufficient to indicate when, where, and why certain key events in food chemistry occurred and to relate some of these events to major changes in the wholesomeness of the food supply since the early 1800s.

Although the origin of food chemistry, in a sense, extends to antiquity, the most significant discoveries, as we judge them today, began in the late 1700s. The best accounts of developments during this period are those of Filby [3] and Browne [1], and these sources have been relied upon for much of the information presented here.

During the period of 1780–1850, a number of famous chemists made important discoveries, many of which related directly or indirectly to food, and these works contain the origins of modern food chemistry. Carl Wilhelm Scheele (1742–1786), a Swedish pharmacist, was one of the greatest chemists of all time. In addition to his more famous discoveries of chlorine, glycerol, and oxygen (3 years before Priestly, but unpublished), he isolated and studied the properties of lactose (1780), prepared mucic acid by oxidation of lactic acid (1780), devised a means of preserving vinegar by the application of heat (1782, well in advance of Appert's "discovery"), isolated citric acid from lemon juice (1784) and gooseberries (1785), isolated malic acid from apples (1785), and tested 20 common fruits for the presence of citric, malic, and tartaric acids (1785). His isolation of various new chemical compounds from plant and animal substances is considered the beginning of accurate analytical research in agricultural and food chemistry.

The French chemist Antoine Laurent Lavoisier (1743–1794) was instrumental in the final rejection of the phlogiston theory and in formulating the principles of modern chemistry. With respect to food chemistry, he established the fundamental principles of combustion organic analysis, he was the first to show that the process of fermentation could be expressed as a balanced equation, he made the first attempt to determine the elemental composition of alcohol (1784), and he presented one of the first papers (1786) on organic acids of various fruits.

Nicolas-Théodore de Saussure (1767–1845), a French chemist, did much to formalize and clarify the principles of agricultural and food chemistry provided by Lavoisier. He also studied CO_2 and O_2 changes during plant respiration (1804) and the mineral contents of plants by ashing and made the first accurate elemental analysis of alcohol (1807).

Joseph Louis Gay-Lussac (1778–1850) and Louis-Jacques Thenard (1777–1857) devised in 1811 the first method to determine percentages of carbon, hydrogen, and nitrogen in dry vegetable substances.

The English chemist Sir Humphrey Davy (1778–1829) in the years 1807 and 1808 isolated the elements K, Na, Ba, Sr, Ca, and Mg. His contributions to agricultural and food chemistry came largely through his books on agricultural chemistry, of which the first (1813) was *Elements of Agriculture Chemistry, in a Course of Lectures for the Board of Agriculture* [4]. His books served to organize and clarify knowledge existing at that time. In the first edition he stated,

> All the different parts of plants are capable of being decomposed into a few elements. Their uses as food, or for the purpose of the arts, depend upon compound arrangements of these elements, which are capable of being produced either from their organized parts, or from the juices they contain; and the examination of the nature of these substances is an essential part of agricultural chemistry.

In the fifth edition he stated that plants are usually composed of only seven or eight elements and that "the most essential vegetable substances consist of hydrogen, carbon, and oxygen in different proportion, generally alone, but in some few cases combined with azote [nitrogen]" (p. 121) [5].

The works of the Swedish chemist Jons Jacob Berzelius (1779–1848) and the Scottish chemist Thomas Thomson (1773–1852) resulted in the beginnings of organic formulas, "without which organic analysis would be a trackless desert and food analysis an endless task" [3]. Berzelius determined the elemental components of about 2000 compounds, thereby verifying the law of definite proportions. He also devised a means of accurately determining the water content of organic substances, a deficiency in the method of Gay-Lussac and Thenard. Moreover, Thomson showed that laws governing the composition of inorganic substances apply equally well to organic substances, a point of immense importance.

In a book entitled *Considérations générales sur l'analyse organique et sur ses applications* [6], Michel Eugene Chevreul (1786–1889), a French chemist, listed the elements known to exist at that time in organic substances (O, Cl, I, N, S, P, C, Si, H, Al, Mg, Ca, Na, K, Mn, and Fe) and cited the processes then available for organic analysis: (1) extraction with a neutral solvent, such as water, alcohol, or aqueous ether; (2) slow distillation or fractional distillation; (3) steam distillation; (4) passing the substance through a tube heated to incandescence; and (5) analysis with oxygen. Chevreul was a pioneer in the analysis of organic substances, and his classic research on the composition of animal fat led to the discovery and naming of stearic and oleic acids.

Dr. William Beaumont (1785–1853), an American Army surgeon stationed at Fort Mackinac, MI, performed classic experiments on gastric digestion that destroyed the concept existing from the time of Hippocrates that food contained a single nutritive component. His experiments were performed during the period 1825–1833 on a Canadian, Alexis St. Martin, whose musket wound afforded direct access to the stomach interior, thereby enabling food to be introduced and subsequently examined for digestive changes [7].

Among his many notable accomplishments, Justus von Liebig (1803–1873) showed in 1837 that acetaldehyde occurs as an intermediate between alcohol and acetic acid during fermentation of vinegar. In 1842, he classified foods as either nitrogenous (vegetable fibrin, albumin, casein, and animal flesh and blood) or nonnitrogenous (fats, carbohydrates, and alcoholic beverages). Although this classification is not correct in several respects, it served to distinguish important differences among various foods. He also perfected methods for the quantitative analysis of organic substances, especially by combustion, and he published in 1847 what is apparently the first book on food chemistry, *Researches on the Chemistry of Food* [8]. Included in this book are accounts of his research on the water-soluble constituents of muscle (creatine, creatinine, sarcosine, inosinic acid, lactic acid, etc.).

It is interesting that the developments just reviewed paralleled the beginning of serious and widespread adulteration of food, and it is no exaggeration to state that the need to detect impurities in food was a major stimulus for the development of analytical chemistry in general and analytical food chemistry in particular. Unfortunately, it is also true that advances in chemistry contributed somewhat to the adulteration of food, since unscrupulous purveyors of food were able to profit from the availability of chemical literature, including formulas for adulterated food, and could replace older, less-effective empirical approaches to food adulteration with more efficient approaches based on scientific principles. Thus, the history of food chemistry and food adulteration are closely interwoven by the threads of several causative relationships, and it is therefore appropriate to consider the matter of food adulteration from a historical perspective [3].

The history of food adulteration in the currently more developed countries of the world falls into three distinct phases. From ancient times to about 1820, food adulteration was not a serious problem, and there was little need for methods of detection. The most obvious explanation for this situation was that food was procured from small businesses or individuals and transactions involved a large measure of interpersonal accountability. The second phase began in the early 1800s, when intentional food adulteration increased greatly in both frequency and seriousness. This development can be attributed primarily to increased centralization of food processing and distribution, with a corresponding decline in interpersonal accountability, and partly to the rise of modern chemistry, as already mentioned. Intentional adulteration of food remained a serious problem until about 1920, which marks the end of phase two and the beginning of phase three. At this point,

regulatory pressures and effective methods of detection reduced the frequency and seriousness of intentional food adulteration to acceptable levels, and the situation has gradually improved up to the present time.

Some would argue that a fourth phase of food adulteration began about 1950, when foods containing legal chemical additives became increasingly prevalent, when the use of highly processed foods increased to a point where they represented a major part of the diet of persons in most of the industrialized countries, and when contamination of some foods with undesirable by-products of industrialization, such as mercury, lead, and pesticides, became of public and regulatory concern. The validity of this contention is hotly debated and disagreement persists to this day. Nevertheless, the course of action in the next few years seems clear. Public concern over the safety and nutritional adequacy of the food supply continues to evoke changes, both voluntary and involuntary, in the manner in which foods are produced, handled, and processed, and more such actions are inevitable as we learn more about proper handling practices for food and as estimates of maximum tolerable intake of undesirable constituents become more accurate.

The early 1800s was a period of especially intense public concern over the quality and safety of the food supply. This concern, or more properly indignation, was aroused in England by Frederick Accum's publication *A Treatise on Adulterations of Food* [9] and by an anonymous publication entitled *Death in the Pot* [10]. Accum claimed that "Indeed, it would be difficult to mention a single article of food which is not to be met with in an adulterated state; and there are some substances which are scarcely ever to be procured genuine" (p. 14). He further remarked, "It is not less lamentable that the extensive application of chemistry to the useful purposes of life, should have been perverted into an auxiliary to this nefarious traffic [adulteration]" (p. 20).

Although Filby [3] asserted that Accum's accusations were somewhat overstated, it was true that the intentional adulteration of several foods and ingredients prevailed in the 1800s, as cited by Accum and Filby, including annatto, black pepper, cayenne pepper, essential oils, vinegar, lemon juice, coffee, tea, milk, beer, wine, sugar, butter, chocolate, bread, and confectionary products.

Once the seriousness of food adulteration in the early 1800s was made evident to the public, remedial forces gradually increased. These took the form of new legislation to make adulteration unlawful and greatly expanded efforts by chemists to learn about the native properties of foods, the chemicals commonly used as adulterants, and the means of detecting them. Thus, during the period 1820–1850, chemistry and food chemistry began to assume importance in Europe. This was possible because of the work of the scientists already cited and was stimulated largely by the establishment of chemical research laboratories for young students in various universities and by the founding of new journals for chemical research [1]. Since then, advances in food chemistry have continued at an accelerated pace, and some of these advances, along with causative factors, are mentioned in the following text.

In 1860, the first publicly supported agriculture experiment station was established in Weede, Germany, and W. Hanneberg and F. Stohmann were appointed director and chemist, respectively. Based largely on the work of earlier chemists, they developed an important procedure for the routine determination of major constituents in food. By dividing a given sample into several portions, they were able to determine moisture content, "crude fat," ash, and nitrogen. Then, by multiplying the nitrogen value by 6.25, they arrived at its protein content. Sequential digestion with dilute acid and dilute alkali yielded a residue termed "crude fiber." The portion remaining after removal of protein, fat, ash, and crude fiber was termed "nitrogen-free extract," and this was believed to represent utilizable carbohydrate. Unfortunately, for many years chemists and physiologists wrongfully assumed that like values obtained by this procedure represented like nutritive value, regardless of the kind of food [11].

In 1871, Jean Baptiste Duman (1800–1884) suggested that a diet consisting of only protein, carbohydrate, and fat was inadequate to support life.

In 1862, the Congress of the United States passed the Land-Grant College Act, authored by Justin Smith Morrill. This act helped establish colleges of agriculture in the United States and

provided considerable impetus for the training of agricultural and food chemists. Also in 1862, the U.S. Department of Agriculture was established, and Isaac Newton was appointed the first commissioner.

In 1863, Harvey Washington Wiley became chief chemist of the U.S. Department of Agriculture, from which office he led the campaign against misbranded and adulterated food, culminating in passage of the first Pure Food and Drug Act in the United States (1906).

In 1887, agriculture experiment stations were established in the United States following enactment of the Hatch Act. Representative William H. Hatch of Missouri, Chairman of the House Committee on Agriculture, was author of the act. As a result, the world's largest national system of agriculture experiment stations came into existence, and this had a great impact on food research in the United States.

During the first half of the twentieth century, most of the essential dietary substances were discovered and characterized, namely, vitamins, minerals, fatty acids, and some amino acids.

The development and extensive use of chemicals to aid in the growth, manufacture, and marketing of foods was an especially noteworthy and contentious event in the middle 1900s.

This historical review, although brief, makes the current food supply seem almost perfect in comparison to that which existed in the 1800s. However, at this writing, several current issues have replaced the historical ones in terms of what the food science community must address in further promoting the wholesomeness and nutritive value of foods, while mitigating the real or perceived threats to the safety of the food supply. These issues include the nature, efficacy, and impact of nonnutrient components in foods, dietary supplements, and botanicals that can promote human health beyond simple nutrition (Chapter 13), molecular engineering of crops (genetically modified organisms [GMOs]) (principally in Chapter 16) and the benefits juxtaposed against the perceived risks to safety and human health, and the comparative nutritive value of crops raised by organic vs. conventional agricultural methods.

1.3 APPROACH TO THE STUDY OF FOOD CHEMISTRY

Food chemists are typically concerned with identifying the molecular determinants of material properties and chemical reactivity of food matrices and how this understanding is effectively applied to improve formulation, processing, and storage stability of foods. An ultimate objective is to determine cause-and-effect and structure–function relationships among different classes of chemical components. The facts derived from the study of one food or model system can be applied to our understanding of other food products. An analytical approach to food chemistry includes four components, namely, (1) determining those properties that are important characteristics of safe, high-quality foods; (2) determining those chemical and biochemical reactions that have important influences on loss of quality and/or wholesomeness of foods; (3) integrating the first two points so that one understands how the key chemical and biochemical reactions influence quality and safety; and (4) applying this understanding to various situations encountered during formulation, processing, and storage of food.

Safety is the first requisite of any food. In a broad sense, this means a food must be free of any harmful chemical or microbial contaminant at the time of its consumption. For operational purposes this definition takes on a more applied form. In the canning industry, "commercial" sterility as applied to low-acid foods means the absence of viable spores of *Clostridium botulinum*. This in turn can be translated into a specific set of heating conditions for a specific product in a specific package. Given these heating requirements, one can then select specific time–temperature conditions that will optimize retention of quality attributes. Similarly, in a product such as peanut butter, operational safety can be regarded primarily as the absence of aflatoxins—carcinogenic substances produced by certain species of molds. Steps taken to prevent growth of the mold in question may or may not interfere with retention of some other quality attribute; nevertheless, conditions producing a safe product must be employed.

A list of quality attributes of food and some alterations they can undergo during processing and storage is given in Table 1.1. The changes that can occur, with the exception of those involving nutritive value and safety, are readily evident to the consumer.

Many chemical and biochemical reactions can alter food quality or safety. Some of the more important classes of these reactions are listed in Table 1.2. Each reaction class can involve different reactants or substrates depending on the specific food and the particular conditions for handling, processing, or storage. They are treated as reaction classes because the general nature of the

TABLE 1.1
Classification of Alterations That Can Occur during Handling, Processing, or Storage

Attribute	Alteration
Texture	Loss of solubility
	Loss of water-holding capacity
	Toughening
	Softening
Flavor	Development of
	Rancidity (hydrolytic or oxidative)
	Cooked or caramel flavors
	Other off-flavors
	Desirable flavors
Color	Darkening
	Bleaching
	Development of desirable colors (e.g., browning of baked goods)
Nutritive value	Loss, degradation, or altered bioavailability of proteins, lipids, vitamins, minerals, and other health-promoting components
Safety	Generation of toxic substances
	Development of substances that are protective to health
	Inactivation of toxic substances

TABLE 1.2
Some Chemical and Biochemical Reactions That Can Lead to Alteration of Food Quality or Safety

Types of Reaction	Examples
Nonenzymic browning	Baked goods, dry and intermediate moisture foods
Enzymic browning	Cut fruits and some vegetables
Oxidation	Lipids (off-flavors), vitamin degradation, pigment decoloration, proteins (loss of nutritive value)
Hydrolysis	Lipids, proteins, vitamins, carbohydrates, pigments
Metal interactions	Complexation (anthocyanins), loss of Mg from chlorophyll, catalysis of oxidation
Lipid isomerization	*cis* → *trans* isomerization, nonconjugated → conjugated
Lipid cyclization	Monocyclic fatty acids
Lipid oxidation–polymerization	Foaming during deep-fat frying
Protein denaturation	Egg white coagulation, enzyme inactivation
Protein cross-linking	Loss of nutritive value during alkali processing
Polysaccharide synthesis and degradation	In plants postharvest
Glycolytic changes	Animal postmortem, plant tissue postharvest

TABLE 1.3
Examples of Cause-and-Effect Relationships Pertaining to Food Alteration during Handling, Storage, and Processing

Primary Causative Event	Secondary Event	Attribute Influenced (see Table 1.1)
Hydrolysis of lipids	Free fatty acids react with protein.	Texture, flavor, nutritive value
Hydrolysis of polysaccharides	Sugars react with protein.	Texture, flavor, color, nutritive value
Oxidation of lipids	Oxidation products react with many other constituents.	Texture, flavor, color, nutritive value; toxic substances can be generated
Bruising of fruit	Cells break, enzymes are released, and oxygen is accessible.	Texture, flavor, color, nutritive value
Heating of horticultural products	Cell walls and membranes lose integrity, acids are released, and enzymes become inactive.	Texture, flavor, color, nutritive value
Heating of muscle tissue	Proteins denature and aggregate, and enzymes become inactive.	Texture, flavor, color, nutritive value
cis → *trans* conversion in lipids	Enhanced rate of polymerization during deep-fat frying.	Excessive foaming during deep-fat frying, diminished nutritive value and bioavailability of lipids, solidification of frying oil

substrates or reactants is similar for all foods. Thus, nonenzymic browning involves reaction of carbonyl compounds, which can arise from existing reducing sugars or from diverse reactions, such as oxidation of ascorbic acid, hydrolysis of starch, or oxidation of lipids. Oxidation may involve lipids, proteins, vitamins, or pigments and, more specifically, oxidation of lipids may involve triacylglycerols in one food or phospholipids in another. Discussion of these reactions in detail will occur in subsequent chapters of this book.

The reactions listed in Table 1.3 cause the alterations listed in Table 1.1. Integration of the information contained in both tables can lead to an understanding of the causes of food deterioration. Deterioration of food usually consists of a series of primary events followed by secondary events, which, in turn, become evident as altered quality attributes (Table 1.1). Examples of sequences of this type are shown in Table 1.3. Note particularly that a given quality attribute can be altered as a result of several different primary events.

The sequences in Table 1.3 can be applied in two directions. Operating from left to right, one can consider a particular primary event, the associated secondary events, and the effect on a quality attribute. Alternatively, one can determine the probable cause(s) of an observed quality change (column 3, Table 1.3) by considering all primary events that could be involved and then isolating, by appropriate chemical tests, the key primary event. The utility of constructing such sequences is that they encourage one to approach problems of food alteration in an analytical manner. The physical and chemical properties of major food constituents, that is, proteins, carbohydrates, and lipids, are invariably altered during processing. These changes involve both intra- and intercomponent interactions/reactions. Major reactions that proteins, carbohydrates, and lipids undergo during the processing and handling of foods are summarized in Figures 1.1 through 1.3. These complex sets of reactions/interactions play a crucial role in the development of both desirable and undesirable sensory and nutritional properties of foods.

Figure 1.4 is a simplistic summary of reactions and interactions of the major constituents of food that lead to deterioration of food quality. Each class of compound can undergo its own characteristic type of deterioration. Noteworthy is the role that carbonyl compounds play in many deterioration processes. They arise mainly from lipid oxidation and carbohydrate degradation and can lead to the

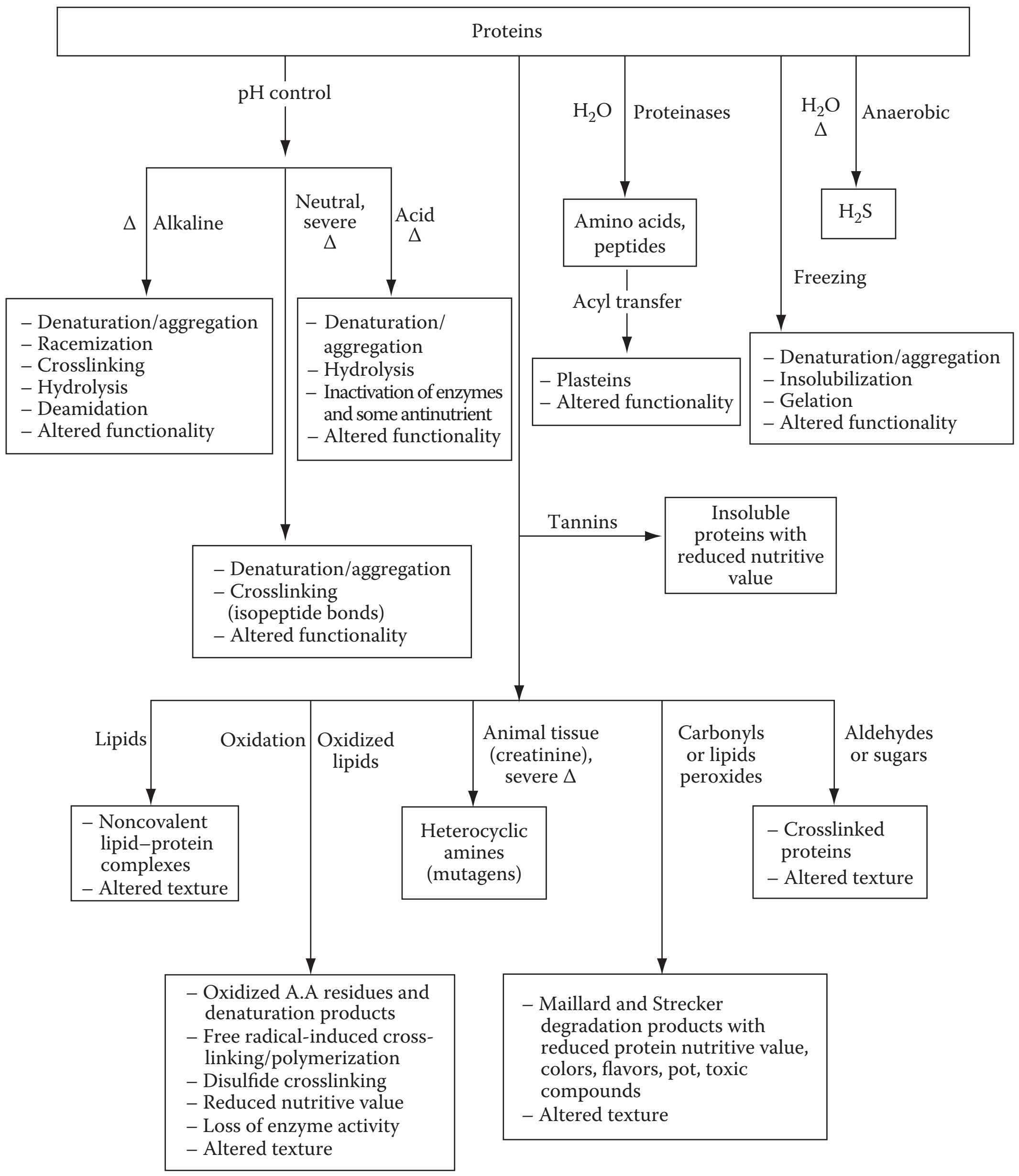


FIGURE 1.1 Major reactions that proteins can undergo during the processing and handling of foods. (From Taoukis, P. and Labuza, T.P., in: *Food Chemistry*, 3rd edn., Fennema, O., ed., Marcel Dekker, New York, 1996, p. 1015.)

destruction of nutritional value, to off-colors, and to off-flavors. Of course, these same reactions lead to desirable flavors and colors during the cooking of many foods.

1.3.1 Analysis of Situations Encountered during the Storage and Processing of Food

Having before us a description of the attributes of high-quality, safe foods, the significant chemical reactions involved in the deterioration of food, and the relationship between the two, we can

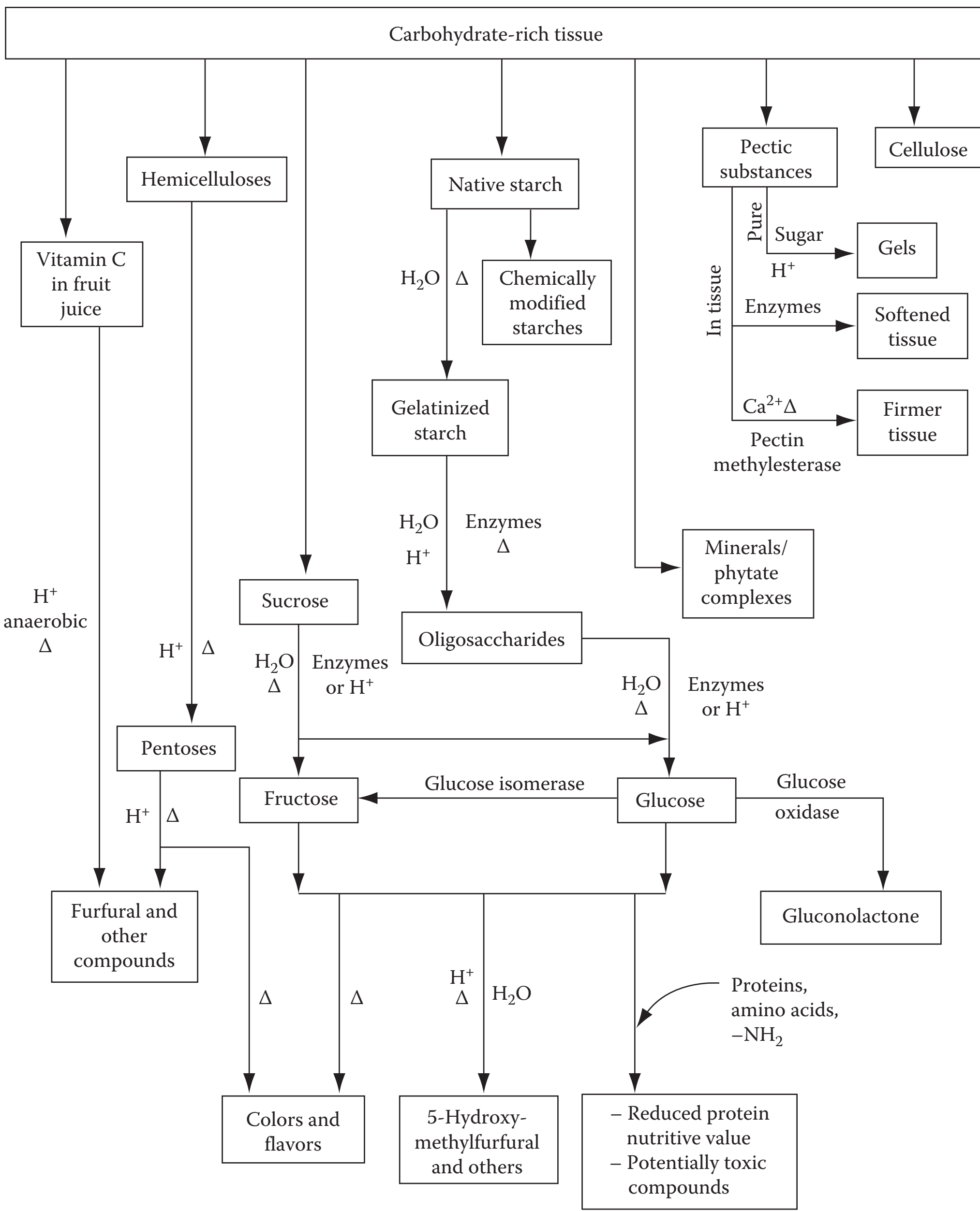


FIGURE 1.2 Major reactions that carbohydrates can undergo during the processing and handling of foods. (From Taoukis, P. and Labuza, T.P., in: *Food Chemistry*, 3rd edn., Fennema, O., ed., Marcel Dekker, New York, 1996, p. 1016.)

now begin to consider how to apply this information to situations encountered during the storage and processing of food.

The variables that are important during the storage and processing of food are listed in Table 1.4. Temperature is perhaps the most important of these variables because of its broad influence on all types of chemical reactions. The effect of temperature on an individual reaction can be estimated from the Arrhenius equation, $k = Ae^{-\Delta E/RT}$. Data conforming to the Arrhenius equation yield a straight line when log k is plotted vs. $1/T$. The parameter ΔE is the activation energy, which represents the

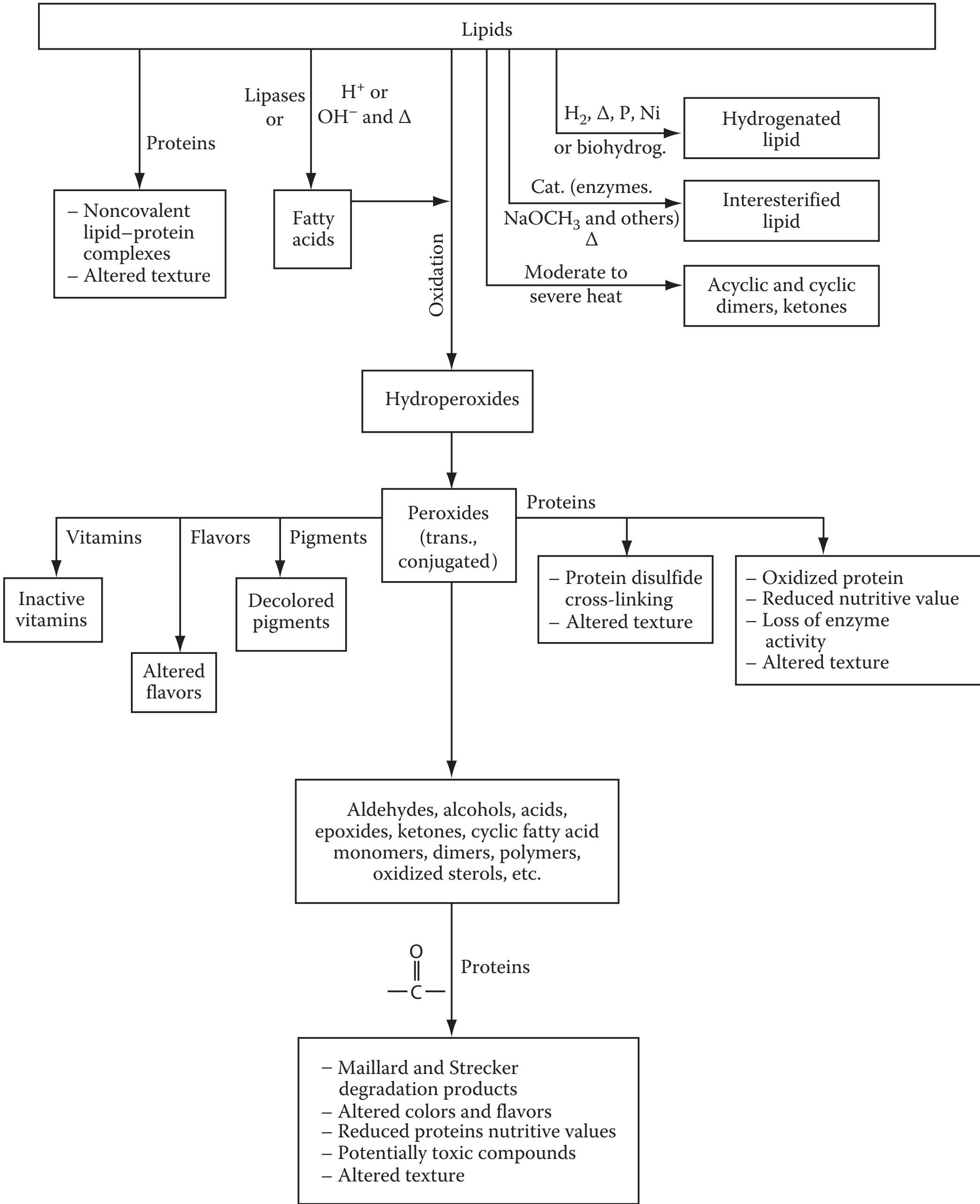


FIGURE 1.3 Major reactions that lipids can undergo during the processing and handling of foods. (From Taoukis, P. and Labuza, T.P., in: *Food Chemistry*, 3rd edn., Fennema, O., ed., Marcel Dekker, New York, 1996, p. 1017.)

free energy change required to elevate a chemical entity from a ground state to a transition state, whereupon reaction can occur. Arrhenius plots in Figure 1.5 represent reactions important in food deterioration. It is evident that food reactions generally conform to the Arrhenius relationship over a limited intermediate temperature range but that deviations from this relationship can occur at high or low temperatures [12]. Thus, it is important to remember that the Arrhenius relationship for food systems is valid only over a range of temperature that has been experimentally verified.

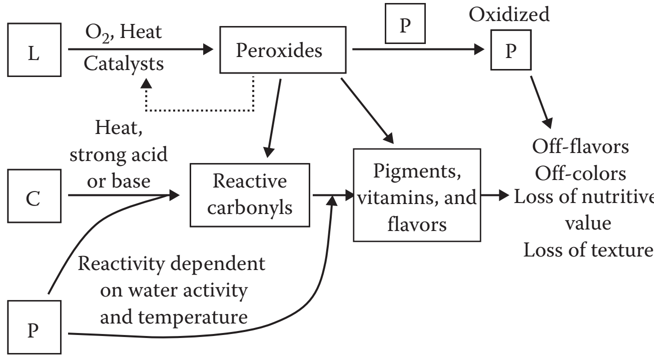


FIGURE 1.4 Summary of chemical interactions among major food constituents: L, lipid pool (triacylglycerols, fatty acids, and phospholipids); C, carbohydrate pool (polysaccharides, sugars, organic acids, etc.); P, protein pool (proteins, peptides, amino acids, and other N-containing substances).

TABLE 1.4
Important Factors Governing the Stability of Foods during Handling, Processing, and Storage

Product Factors	Environmental Factors
Chemical properties of individual constituents (including catalysts), oxygen content, pH water activity, T_g, and W_g	Temperature (T); time (t); composition of the atmosphere; chemical, physical, or biological treatments imposed; exposure to light; contamination; physical abuse

Note: Water activity = p/p_o, where p is the partial pressure of water vapor above the food and p_o is the vapor pressure of pure water; T_g is the glass transition temperature; W_g is the product water content at T_g.

Deviations from the Arrhenius relationship can occur because of the following events, most of which are induced by either high or low temperatures: (1) enzyme activity may be lost, (2) the reaction pathway or rate-limiting step may change or may be influenced by a competing reaction(s), (3) the physical state of the system may change (e.g., by freezing), or (4) one or more of the reactants may become depleted.

Another important factor in Table 1.4 is time. During storage of a food product, one frequently wants to know how long the food can be expected to retain a specified level of quality. Therefore, one is interested in time with respect to the integral of chemical and/or microbiological changes that occur during a specified storage period, and in the way these changes combine to determine a specified storage life for the product. During processing, one is often interested in the time it takes to inactivate a particular population of microorganisms or in how long it takes for a reaction to proceed to a specified extent. For example, it may be of interest to know how long it takes to produce a desired brown color in potato chips during frying. To accomplish this, attention must be given to temperature change with time, that is, dT/dt. This relationship is important because it allows the determination of the extent to which the reaction rate changes as temperature of the food matrix changes during the course of processing. If ΔE of the reaction and temperature profile of the food are known, an integrative analysis affords a prediction of the net accumulation of a reaction product. This is also of interest in foods that deteriorate by more than one means, such as lipid oxidation and nonenzymic browning. If the products of the browning reaction are antioxidants, it is important to know whether the relative rates of these reactions are such that a significant interaction will occur between them.

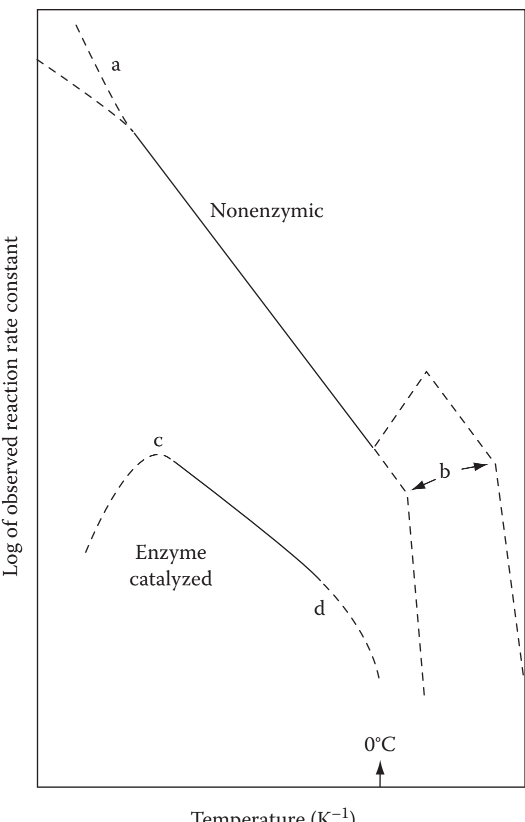


FIGURE 1.5 Conformity of important deteriorative reactions in food to the Arrhenius relationship. (a) Above a certain value of *T*, there may be deviations from linearity due to a change in the path of the reaction. (b) As the temperature is lowered below the freezing point of the system, the ice phase (essentially pure) enlarges, and the fluid phase, which contains all the solutes, diminishes. This concentration of solutes in the unfrozen phase can decrease reaction rates (supplement the effect of decreasing temperature) or increase reaction rates (oppose the effect of declining temperature), depending on the nature of the system (see Chapter 2). (c) For an enzymic reaction there is a high temperature at which the enzyme is denatured, resulting in loss of its activity, and (d) in the vicinity of the freezing point of water where subtle changes, such as the dissociation of an enzyme complex, can lead to a sharp decline in reaction rate.

Another variable, pH, influences the rates of many chemical and enzymic reactions. Extreme pH values are usually required for severe inhibition of microbial growth or enzymic processes, and these conditions can result in acceleration of acid- or base-catalyzed reactions. In contrast, even a relatively small pH change can cause profound changes in the quality of some foods, for example, muscle.

The composition of the product is important since this determines the reactants available for chemical transformation. Also important is how cellular vs. noncellular and homogeneous vs. heterogeneous food systems influence the disposition and reactivity of reactants. Particularly important from a quality standpoint is the relationship that exists between composition of the raw material and composition of the finished product. For example, (1) the manner in which fruits and vegetables are handled postharvest can influence sugar content, and this, in turn, influences the degree of browning obtained during dehydration or deep-fat frying; (2) the manner in which animal tissues are handled postmortem influences the extents and rates of glycolysis and ATP degradation, and these in turn can influence storage life, water-holding capacity, toughness, flavor, and color; and (3) the blending

of raw materials may cause unexpected interactions, for example, the rate of oxidation can be accelerated or inhibited depending on the amount of salt present.

Another important compositional determinant of reaction rates in foods is water activity (a_w). Numerous investigators have shown a_w to strongly influence the rate of enzyme-catalyzed reactions [13], lipid oxidation [14,15], nonenzymic browning [14,16], sucrose hydrolysis [17], chlorophyll degradation [18], anthocyanin degradation [19], and others. As is discussed in Chapter 2, most reactions tend to decrease in rate below an a_w corresponding to the range of intermediate moisture foods (0.75–0.85). Oxidation of lipids and associated secondary effects, such as carotenoid decoloration, are exceptions to this rule, that is, these reactions accelerate at the lower end of the a_w scale.

More recently, it has become apparent that the glass transition temperature (T_g) of food and the corresponding water content (W_g) at T_g are causatively related to rates of diffusion-limited events in the food. Thus, T_g and W_g have relevance to the physical properties of frozen and dried foods; to conditions appropriate for freeze drying; to physical changes involving crystallization, recrystallization, gelatinization, and starch retrogradation; and to those chemical reactions that are diffusion limited (see Chapter 2).

In fabricated foods, the composition can be controlled by adding approved chemicals, such as acidulants, chelating agents, flavors, or antioxidants, or by removing undesirable reactants, for example, removing glucose from dehydrated egg albumen.

Composition of the atmosphere is important mainly with respect to relative humidity and oxygen content, although ethylene and CO_2 are also important during storage of living plant foods. Unfortunately, in situations where exclusion of oxygen is desirable, this is almost impossible to achieve completely. The detrimental consequences of a small amount of residual oxygen sometimes become apparent during product storage. For example, early formation of a small amount of dehydroascorbic acid (from oxidation of ascorbic acid) can lead to Maillard browning during storage.

For some products, exposure to light can be detrimental, and it is then appropriate to package the products in light-impervious material or to control the intensity and wavelengths of light, if possible.

Food chemists must be able to integrate information about quality attributes of foods, deteriorative reactions to which foods are susceptible, and the factors governing kinds and rates of these deteriorative reactions in order to solve problems related to food formulation, processing, and storage stability.

1.4 SOCIETAL ROLE OF FOOD CHEMISTS

1.4.1 Why Should Food Chemists Become Involved in Societal Issues?

Food chemists, for the following reasons, should feel obligated to become involved in societal issues that encompass pertinent technological aspects (technosocietal issues):

- Food chemists have had the privilege of receiving a high level of education and of acquiring special scientific skills, and these privileges and skills carry with them a corresponding high level of responsibility.
- Activities of food chemists influence adequacy of the food supply, healthfulness of the population, cost of foods, waste creation and disposal, water and energy use, and the nature of food regulations. Because these matters impinge on the general welfare of the public, it is reasonable that food chemists should feel a responsibility to have their activities directed to the benefit of society.
- If food chemists do not become involved in technosocietal issues, the opinions of others—scientists from other professions, professional lobbyists, persons in the news media, consumer activists, charlatans, and antitechnology zealots—will prevail. Many of these individuals are less qualified than food chemists to speak on food-related issues and some are obviously unqualified.

- Food chemists have a role and opportunity to help resolve controversies that impact, or are perceived to impact, on public health and how the public views developments in science and technology. Examples of some current controversies include the safety of cloned organisms and GMOs, the use of animal growth hormones in agricultural production, and the relative nutritive value of crops produced through organic and conventional agricultural methods.

1.4.2 Types of Involvement

The societal obligations of food chemists include good job performance, good citizenship, and guarding the ethics of the scientific community, but fulfillment of these very necessary roles is not enough. An additional role of great importance, and one that often goes unfulfilled by food chemists, is that of helping determine how scientific knowledge is interpreted and used by society. Although food chemists and other food scientists should not have the only input to these decisions, they must, in the interest of wise decision making, have their views heard and considered. Acceptance of this position, which is surely indisputable, leads to the obvious question, "What exactly should food chemists do to properly discharge their responsibilities in this regard?" Several activities are appropriate:

1. Participate in pertinent professional societies.
2. Serve on governmental advisory committees, when invited.
3. Undertake personal initiatives of a public service nature.

The third point can involve letters to newspapers, journals, legislators, government regulators, company executives, university administrators, and others, and speeches or dialog with civic groups, including sessions with K-12 students and all other stakeholders.

The major objectives of these efforts are to educate and enlighten the public with respect to food and dietary practices. This involves improving the public's ability to intelligently evaluate information on these topics. Accomplishing this will not be easy because a significant portion of the populace has ingrained false notions about food and proper dietary practices and because food has, for many individuals, connotations that extend far beyond the chemist's narrow view. For these individuals, food may be an integral part of religious practice, cultural heritage, ritual, social symbolism, or a route to physiological well-being—attitudes that are, for the most part, not conducive to acquiring an ability to appraise foods and dietary practices in a sound, scientific manner.

One of the most contentious food issues and one that has eluded appraisal by the public in a sound, scientific manner is the use of chemicals to modify foods. *Chemophobia*, the fear of chemicals, has afflicted a significant portion of the populace, causing food additives, in the minds of many, to represent hazards inconsistent with fact. One can find, with disturbing ease, articles in the popular literature whose authors claim the American food supply is sufficiently laden with poisons to render it unwholesome at best and life threatening at worst. Truly shocking, they say, is the manner in which greedy industrialists poison our foods for profit while an ineffectual Food and Drug Administration watches with placid unconcern. Should authors holding this viewpoint be believed? The answer to this question resides largely with how credible and authoritative the author is regarding the scientific issue at the center of debate. Credibility is founded on formal education, training, and practical experience and scholarly contributions to the body of knowledge to which a particular dispute is linked. Scholarly activity can take the form of research, discovery of new knowledge, and the review and/or interpretation of a body of knowledge. Credibility is also founded on the author making all attempts to be objective, which requires consideration of alternative points of view and as much as the existing knowledge on the subject as feasible, instead of only pointing out facts and interpretations that are supportive of a preferred viewpoint. Knowledge accumulates through the publication of results of studies in the scientific literature, which is subject to peer review and is

held to specific professional standards of protocol, documentation, and ethics, thereby making them more authoritative than publications in the popular press.

Closer to the daily realm of the student or developing food science professional, a contemporary issue regarding the credibility of information deals with the expanse of information (including that of scientific nature) that is readily and easily accessible through the World Wide Web. Some such information is rarely attributed to any author, and the website may be void of obvious credentials to be regarded as a credible, authoritative source. Some information may be posted to advance a preferred point of view or cause or be part of a marketing campaign to influence the viewer's thinking or purchasing habits. While some information on the web is as authoritative as media disseminated by trained scientists and scientific publishers, the student is encouraged to carefully consider the source of information obtained from the World Wide Web and not simply defer to the expedience in accessing it.

Despite the current and growing expanse of knowledge in food science, disagreement about the safety of foods and other food science issues still occurs. The great majority of knowledgeable individuals support the view that our food supply is acceptably safe and nutritious and that legally sanctioned food additives pose no unwarranted risks [20–30], although continued vigilance for adverse effects is warranted. However, a relatively small group of knowledgeable individuals believe that our food supply is unnecessarily hazardous, particularly with regard to some of the legally sanctioned food additives.

Scientific debate in public forums has more recently expanded to include the public and environmental safety of GMOs, the relative nutritive value of organic and conventionally grown crops, and the appropriateness of marketing-driven statements that the public may construe as health claims accompanying dietary supplements, among others. The incremental nature and rate by which scientific knowledge develops is rarely sufficient to fully prepare us for the next debate. It is the scientists' role to be involved in the process and encourage the various parties to focus objectively on the science and knowledge, enabling fully informed policy makers to reach an appropriate conclusion.

In summary, scientists have greater obligations to society than do individuals without formal scientific education. Scientists are expected to generate knowledge in a productive and ethical manner, but this is not enough. They should also accept the responsibility of ensuring that scientific knowledge is used in a manner that will yield the greatest benefit to society. Fulfillment of this obligation requires that scientists not only strive for excellence and conformance to high ethical standards in their day-to-day professional activities, but that they also develop a deep-seated concern for the well-being and scientific enlightenment of the public.

REFERENCES

1. Browne, C. A. (1944). *A Source Book of Agricultural Chemistry*, Chronica Botanica Co., Waltham, MA.
2. Ihde, A. J. (1964). *The Development of Modern Chemistry*, Harper & Row, New York.
3. Filby, F. A. (1934). *A History of Food Adulteration and Analysis*, George Allen & Unwin, London, U.K.
4. Davy, H. (1813). *Elements of Agricultural Chemistry, in a Course of Lectures for the Board of Agriculture*, Longman, Hurst, Rees, Orme and Brown, London, U.K. Cited by Browne, 1944 (Reference 1).
5. Davy, H. (1936). *Elements of Agricultural Chemistry*, 5th edn. Longman, Rees, Orme, Brown, Green and Longman, London, U.K.
6. Chevreul, M. E. (1824). *Considérations générales sur l'analyse organique et sur ses applications*, F.-G. Levrault. Cited by Filby, 1934 (Reference 3).
7. Beaumont, W. (1833). *Experiments and Observations of the Gastric Juice and the Physiology of Digestion*, F. P. Allen, Plattsburgh, NY.
8. Liebig, J. von (1847). *Researches on the Chemistry of Food*, edited from the author's manuscript by William Gregory; Londson, Taylor and Walton, London, U.K. Cited by Browne, 1944 (Reference 1).
9. Accum, F. (1966). *A Treatise on Adulteration of Food, and Culinary Poisons, 1920*, Facsimile reprint by Mallinckrodt Chemical Works, St. Louis, MO.
10. Anonymous (1831). *Death in the Pot*. Cited by Filby, 1934 (Reference 3).

11. McCollum, E. V. (1959). The history of nutrition. *World Rev. Nutr. Diet. 1*:1–27.
12. McWeeny, D. J. (1968). Reactions in food systems: Negative temperature coefficients and other abnormal temperature effects. *J. Food Technol. 3*:15–30.
13. Acker, L. W. (1969). Water activity and enzyme activity. *Food Technol. 23*:1257–1270.
14. Labuza, T. P., S. R. Tannenbaum, and M. Karel (1970). Water content and stability of low-moisture and intermediate-moisture foods. *Food Techol. 24*:543–550.
15. Quast, D. G. and M. Karel (1972). Effects of environmental factors on the oxidation of potato chips. *J. Food Sci. 37*:584–588.
16. Eichner, K. and M. Karel (1972). The influence of water content and water activity on the sugar-amino browning reaction in model systems under various conditions. *J. Agric. Food Chem. 20*:218–223.
17. Schoebel, T., S. R. Tannenbaum, and T. P. Labuza (1969). Reaction at limited water concentration. 1. Sucrose hydrolysis. *J. Food Sci. 34*:324–329.
18. LaJollo, F., S. R. Tannenbaum, and T. P. Labuza (1971). Reaction at limited water concentration. 2. Chlorophyll degradation. *J. Food Sci. 36*:850–853.
19. Erlandson, J. A. and R. E. Wrolstad (1972). Degradation of anthocyanins at limited water concentration. *J. Food Sci. 37*:592–595.
20. Clydesdale, F. M. and F. J. Francis (1977). *Food, Nutrition and You*, Prentice-Hall, Englewood Cliffs, NJ.
21. Hall, R. L. (1982). Food additives, in *Food and People* (D. Kirk and I. K. Eliason, Eds.), Boyd & Fraser, San Francisco, CA, pp. 148–156.
22. Jukes, T. H. (1978). How safe is our food supply? *Arch. Intern. Med. 138*:772–774.
23. Mayer, J. (1975). *A Diet for Living*, David McKay, Inc., New York.
24. Stare, F. J. and E. M. Whelan (1978). *Eat OK—Feel OK*, Christopher Publishing House, North Quincy, MA.
25. Taylor, R. J. (1980). *Food Additives*, John Wiley & Sons, New York.
26. Whelan, E. M. (1993). *Toxic Terror*, Prometheus Books, Buffalo, NY.
27. Watson, D. H. (2001). *Food Chemical Safety*. Vol. 1: Contaminants, Vol. 2: Additives, Woodhead Publishing Ltd., Cambridge, U.K.
28. Roberts, C. A. (2001). *The Food Safety Information Handbook*, Oryx Press, Westport, CT.
29. Riviere, J. H. (2002). *Chemical Food Safety—A Scientist's Perspective*, Iowa State Press, Ames, IA.
30. Wilcock, A., M. Pun, J. Khanona, and M. Aung (2004). Consumer attitudes, knowledge and behaviour: A review of food safety issues. *Trends Food Sci. Technol. 15*:56–66.
31. Taoukis, P. and T. P. Labuza (1996). Summary: Integrative concepts (shelf life testing and modeling), in: *Food Chemistry*, 3rd edn. (O. Fennema, ed.), Marcel Dekker, New York, pp. 1013–1042.

Section I

Major Food Components

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

2 Water and Ice Relations in Foods

Srinivasan Damodaran

CONTENTS

2.1 Introduction ... 20
2.2 Physical Properties of Water ... 20
2.2.1 Phase Relationship of Water ... 22
2.2.2 Summary ... 25
2.3 Chemistry of Water Molecule ... 26
2.3.1 Hydrogen Bonds ... 26
2.3.1.1 Summary ... 29
2.4 Structures of Ice and Liquid Water ... 29
2.4.1 Structure of Ice ... 29
2.4.2 Structure of Liquid Water ... 31
2.4.2.1 Summary ... 34
2.5 Aqueous Solutions ... 34
2.5.1 Water–Solute Interactions ... 34
2.5.2 Interaction of Water with Ions ... 35
2.5.3 Interaction of Water with Neutral Polar Groups ... 38
2.5.4 Interaction of Water with Nonpolar Solutes ... 39
2.5.5 The Hydrophobic Effect ... 40
2.5.6 Concept of “Bound Water” ... 43
2.5.7 Colligative Properties ... 46
2.5.7.1 Summary ... 48
2.6 Water Activity ... 48
2.6.1 Definition and Measurement of Water Activity ... 48
2.6.1.1 Summary ... 51
2.6.2 Moisture Sorption Isotherms ... 51
2.6.3 Interpretation of Moisture Sorption Isotherms ... 52
2.6.3.1 Summary ... 55
2.6.4 Water Activity and Food Stability ... 55
2.6.5 Intermediate-Moisture Foods ... 55
2.6.6 BET Monolayer Determination ... 62
2.6.6.1 Summary ... 65
2.6.6.2 Temperature and Pressure Dependence ... 65
2.6.7 Hysteresis ... 67
2.7 Technological Challenges in Intermediate-Moisture Foods ... 70
2.7.1 Moisture Migration in Composite Foods ... 70
2.7.2 Phase Transitions in Foods ... 73
2.8 Molecular Mobility and Food Stability ... 73
2.8.1 Glass Transition ... 75
2.8.2 Molecular Mobility and Reaction Rates ... 77

2.8.3 Reaction Rate in the Glassy State 78
2.8.4 State Diagram 80
2.8.5 Limitations of the WLF Equation 82
2.8.6 Applicability of State Diagrams to Food Systems 83
2.8.7 T_g Determination 83
2.8.8 Molecular Weight Dependency of T_g 84
2.8.9 Relationship between a_w, Water Content, and Molecular Mobility Approaches to Understanding Water Relations in Foods 87
References 88

2.1 INTRODUCTION

Water is the most abundant substance on earth, and depending on the local temperature, it exists in solid, liquid, and vapor states in various regions of the earth. Current scientific theories proclaim that the genesis of life on earth would not have been possible without the presence of water: The very formation of organized biological macromolecular structures, such as biomembranes and proteins/enzymes, and the actual functioning of these biological structures are often orchestrated by liquid water. In addition, water performs various other functions, such as modulation of body temperature, as a solvent and carrier of nutrient and waste products, and participation as a reactant in hydrolysis reactions.

The water content of biological tissues varies from 50% to 90% [1]. Since fresh foods are derived mainly from plant and animal tissues, their water content is also in the range of 50%–95% on wet weight basis. Water is a major component even in fabricated food products, such as foam, emulsion, and gel-type products, and the state of water in such products strongly influences their texture, appearance, and flavor. Interaction of water with other components, such as lipids, carbohydrates, and proteins, in a food system profoundly alters their physical and chemical properties, which in turn impacts the sensorial properties and consumer acceptability of foods. On the other hand, foods containing high water content are good breeding grounds for microbes, which makes them highly susceptible to microbial spoilage. Food preservation techniques, such as freezing and dehydration, involve transformation of liquid water into ice or its removal as vapor, respectively. Since the economics of these processes are influenced by the physical properties of water under various pressure–temperature conditions, a fundamental understanding of the structure and properties of water in the liquid and solid states is quintessential for understanding water's influence on food stability in a broader context.

2.2 PHYSICAL PROPERTIES OF WATER

Water is a simple compound containing two hydrogen atoms covalently linked to an oxygen atom. Yet, its physical properties, both in the liquid and solid states, exhibit 41 anomalies compared to other substances (Box 2.1) of similar molecular size. Some of these anomalies are so critical that life on earth would not have been theoretically possible without them. For instance, the density of a substance in the liquid state at its melting temperature is usually about 5%–15% lower than its solid at the same temperature because of increased distance (volume expansion) between molecules in the liquid state. However, this is not the case for water. The density of liquid water at 0°C is greater than that of ice at 0°C. Furthermore, the density of water in the temperature range 0°C–100°C remains greater than that of ice, with a maximum at 3.984°C (Figure 2.1). As a result, ice floats on water. If ice had a higher density than liquid water, the ice in the Arctic and Antarctic oceans would have sunk and the oceans and seas would have slowly turned into solid ice over a period of time, which would have made the planet uninhabitable for life.

BOX 2.1 ANOMALOUS PROPERTIES OF WATER

1. Unusually high melting point.
2. Unusually high boiling point.
3. Unusually high critical point.
4. Unusually high surface tension.
5. Unusually high viscosity.
6. Unusually high heat of vaporization.
7. Water shrinks on melting.
8. Water has high density that increases on heating (up to 3.984°C).
9. The number of nearest neighbors increases on melting.
10. The number of nearest neighbors increases with temperature.
11. Pressure reduces its melting point (13.35 MPa gives a melting point of −1°C).
12. Pressure reduces the temperature of maximum density.
13. D_2O and T_2O differ from H_2O in their physical properties much more than might be expected from their increased mass; for example, they have increasing temperatures of maximum density (11.185°C and 13.4°C, respectively).
14. Water shows an unusually large viscosity increase but a diffusion decrease as the temperature is lowered.
15. Water's viscosity decreases with pressure (at temperatures below 33°C).
16. Water has unusually low compressibility.
17. The compressibility drops as temperature increases down to a minimum at about 46.5°C. Below this temperature, water is easier to compress as the temperature is lowered.
18. Water has a low coefficient of expansion (thermal expansity).
19. Water's thermal expansivity reduces increasingly (becomes negative) at low temperatures.
20. The speed of sound increases with temperature (up to a maximum at 73°C).
21. Water has over twice the specific heat capacity of ice or steam.
22. The specific heat capacity (C_P and C_V) is unusually high.
23. The specific heat capacity C_P has a minimum and C_V has a maximum.
24. NMR spin-lattice relaxation is very small at low temperatures.
25. Solutes have varying effects on properties such as density and viscosity.
26. None of its solutions even approach thermodynamic ideality; even D_2O in H_2O is not ideal.
27. X-ray diffraction shows an unusually detailed structure.
28. Supercooled water has two phases and a second critical point at about −91°C.
29. Liquid water may be supercooled, in tiny droplets, down to about −70°C. It may also be produced from glassy amorphous ice between −123°C and −149°C.
30. Solid water exists in a wider variety of stable and unstable crystal and amorphous structures than other materials.
31. Hot water may freeze faster than cold water: the Mpemba effect.
32. The refractive index of water has a maximum value at just below 0°C.
33. The solubilities of nonpolar gases in water decrease with temperature to a minimum and then rise.
34. At low temperatures, the self-diffusion of water increases as the density and pressure increase.
35. The thermal conductivity of water rises to a maximum at about 130°C and then falls.
36. Proton and hydroxide ion mobilities are anomalously fast in an electric field.

37. The heat of fusion of water with temperature exhibits a maximum at −17°C.
38. The dielectric constant is high and behaves anomalously with temperature.
39. Under high pressure, water molecules move further away from each other with increasing pressure.
40. The electrical conductivity of water rises to a maximum at about 230°C and then falls.
41. Warm water vibrates longer than cold water.

Source: Adapted from Chaplin, M., Anomalous properties of water. http://www.lsbu.ac.uk/water/anmlies.html, 2003.

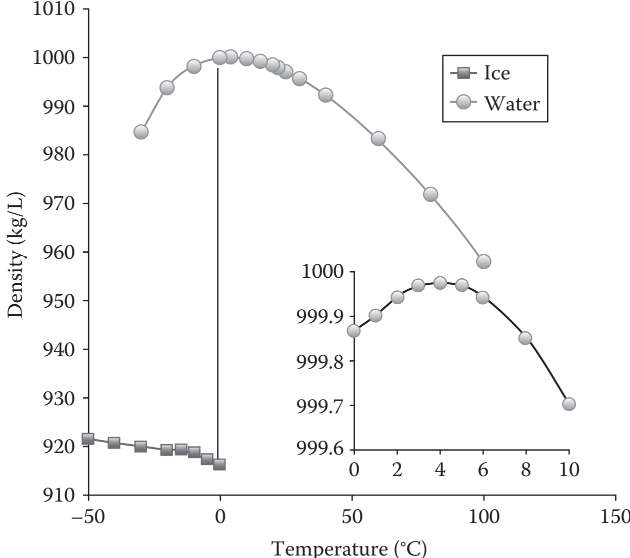


FIGURE 2.1 Density of water (-○-) and ice (-■-) as a function of temperature. The inset shows an expanded view of the data for water in the 0°C–10°C range.

Water also exhibits several other abnormal properties that are very relevant to food processing. These include abnormal boiling and melting points, a high dielectric permittivity, high surface tension, abnormal thermal properties (i.e., heat capacity, thermal conductivity, thermal diffusivity, and heats of fusion and vaporization), and high viscosity (in relation to its low molecular weight) (Table 2.1). For instance, the thermal conductivity of water and ice is large compared to other liquids and nonmetallic solids, and more importantly the thermal conductivity of ice at 0°C is fourfold greater than that of water at 0°C. Similarly, the thermal diffusivity of ice is ninefold greater than that of water and the heat capacity of ice is about one-half that of liquid water. Because of the higher thermal conductivity and diffusivity and lower heat capacity, the rate of temperature change in ice is much greater than that in water when water and ice are exposed to a given temperature gradient. The fact that foods freeze much faster than they thaw when subjected to a given positive or negative temperature gradient is primarily due to the difference mentioned earlier in the thermal properties of ice and water.

2.2.1 Phase Relationship of Water

Water exists in all three phases, that is, vapor, liquid, and solid, in the normal temperature and pressure ranges found on earth. Water is a liquid at ambient temperature and pressure; it is vaporized

TABLE 2.1
Physical Properties of Water and Ice

Property	Value
Molecular weight	18.0153
Melting point (at 101.3 kPa)	0.00°C
Boiling point (at 101.3 kPa)	100.00°C
Critical temperature	373.99°C
Critical pressure	22.064 MPa
Triple point temperature	0.01°C
Triple point pressure	611.73 Pa
ΔH_{vap} at 100°C	40.647 kJ/mol
ΔH_{sub} at 0°C	50.91 kJ/mol
ΔH_{fus} at 0°C	6.002 kJ/mol

Other Temperature-Dependent Properties	Temperature (°C)			
	Ice		Water	
	–20	0	0	+20
Density (g/cm^3)	0.9193	0.9168	0.99984	0.99821
Vapor pressure (kPa)	0.103	0.6113	0.6113	2.3388
Heat capacity (J/g/K)	1.9544	2.1009	4.2176	4.1818
Thermal conductivity (W/m/K)	2.433	2.240	0.561	0.5984
Thermal diffusivity (m^2/s)	11.8×10^{-7}	11.7×10^{-7}	1.3×10^{-7}	1.4×10^{-7}
Compressibility (Pa^{-1})		2	4.9	
Permittivity	98	90	87.9	80.2

Source: Lide, D.R. (ed.), *Handbook of Chemistry and Physics*, 74th edn., CRC Press, Boca Raton, FL, 1993/1994.

when the temperature is raised to 100°C and becomes a solid when the temperature is cooled to below 0°C at ambient atmospheric pressure. The solid lines in the phase diagram shown in Figure 2.2 depict the temperature–pressure combinations where water can exist in equilibrium between vapor/liquid, liquid/solid, and solid/vapor phases. At these phase boundaries, two phases of water (i.e., liquid/vapor, liquid/solid, and solid/vapor) coexist, such that its chemical potential in both phases is equal. The meeting point of these three phase boundaries is known as a "triple point." For water, there is only one vapor/liquid/solid triple point. At the triple point, the gas, liquid, and solid phases of water coexist in perfect equilibrium, meaning that the chemical potentials of water in the vapor, liquid, and solid phases are equal at the triple point. For water, this triple point occurs at 273.16 K temperature and 611.73 Pa (0.0060373 atm) pressure (Figure 2.2). A slight change in either the temperature or pressure away from the triple point will revert water into a two-phase system. At temperature and pressure combinations below the triple point, water exists either in solid or vapor state. Under these conditions, when the solid ice is heated at a constant pressure below the triple point, it is transformed directly into vapor, and when the vapor is subjected to high pressure at constant temperature, it is directly converted to solid ice. This property, known as sublimation, is the basis of the *freeze-drying* process used in the food industry. Freeze-drying of food materials, as compared to normal drying at high temperatures, retains the nutritional value and other quality attributes of foods. The typical temperature and pressure combination used in the freeze-drying process is −50°C and 13.3–26.6 Pa, respectively.

Another anomalous behavior of water is that while the slope of the solid–liquid equilibrium line in Figure 2.2 is positive for almost all substances, it is negative for water. As a result, when pressure

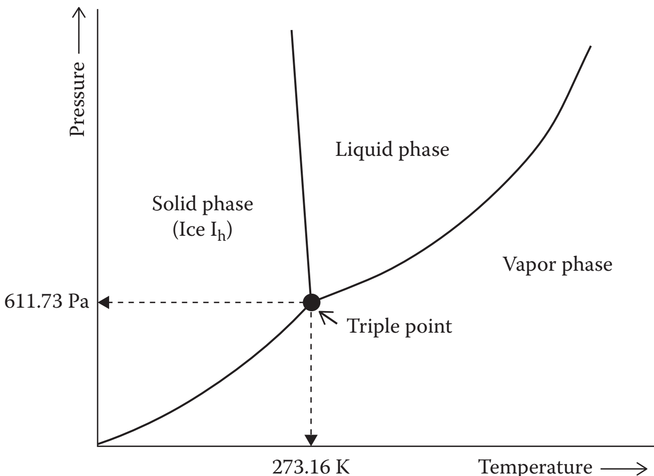


FIGURE 2.2 Phase diagram of water showing the triple point where the solid (ice I_h), liquid, and vapor states are at equilibrium.

is gradually increased at a constant temperature slightly below the triple point, the state of water is transformed from vapor → solid → liquid, whereas all other substances follow the order vapor → solid. In other words, while the melting (or solidification) temperature of most substances increases with increase of pressure, the melting temperature of ice decreases with increase of pressure. This anomalous behavior of ice is related to its unique crystal lattice structure.

Ice exists in at least 13 different structural forms depending on the temperature and pressure. As a result, the phase diagram of water exhibits several triple points, among which there is only one vapor/liquid/solid triple point and the rest are liquid/solid/solid and solid/solid/solid triple points (Figure 2.3). Among these, only the vapor/liquid, liquid/ice I_h, and ice I_h/vapor equilibrium lines (Figure 2.2) are of interest to biology and food science. While the vapor/ice I_h region of the phase diagram is useful in freeze-drying operations in food processing, the liquid/ice I_h region of the phase diagram (Figure 2.2) is relevant to freezing and thawing of frozen foods.

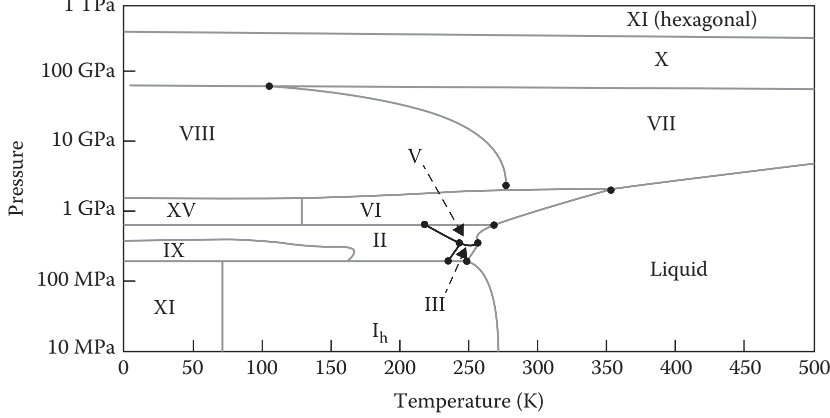


FIGURE 2.3 Pressure–temperature phase diagram of water showing various forms of ice and multiple liquid/solid/solid and solid/solid/solid triple points and a single liquid/solid/vapor triple point.

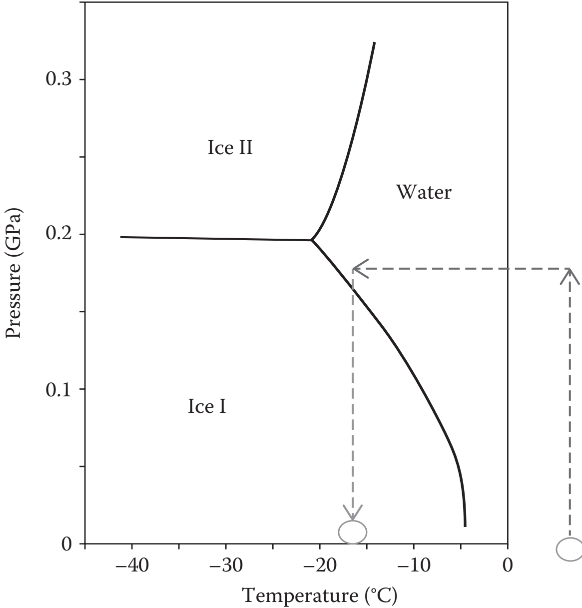


FIGURE 2.4 Details of the ice I_h/ice III/liquid triple point of water. The arrows indicate sequential pressure–temperature shift employed in the high-pressure-shift-freezing process.

In addition to the vapor/liquid/solid (ice I_h) triple point, the water/ice I_h/ice III triple point region, which occurs at high pressures, shown in the partial phase diagram in Figure 2.4, is also of considerable interest to food science. Note that the melting point of ice I_h (freezing point of water) decreases as the pressure is increased up to about 200 MPa. This anomalous behavior is exploited in a food processing operation known as high-pressure-shift-freezing process [2,3]. In this process, the food material at ambient temperature is pressurized to about 180–200 MPa and then it is cooled below 0°C (typically to −10°C to −20°C), which keeps water in the material in the liquid state. After the material is cooled to the desired temperature, the pressure is decreased rapidly to ambient pressure, which results in very rapid freezing (transformation of water to ice) of water in the material. The advantage of the pressure-shift-freezing process is that the rapid and uniform supercooling results in the formation of very small ice crystals, which helps in retaining the integrity of tissues and textural properties of the frozen food.

Another utility of the water/ice I_h/ice III phase diagram is that it can be used to device a process for quick thawing of frozen food materials. In this case, when a frozen food material at a given frozen temperature is subjected to high pressure, it will instantaneously melt at that temperature. Subsequent increase of temperature to above 0°C, followed by release of the pressure, will keep the food material in the thawed state.

2.2.2 Summary

- Water exhibits 41 anomalous physical properties. Among these anomalous density, high dielectric permittivity, high surface tension, abnormal thermal properties (i.e., heat capacity, thermal conductivity, thermal diffusivity, and heats of fusion and vaporization), and high viscosity are particularly important in food science.
- Water has 13 triple points, of which only the vapor/liquid, liquid/ice I_h, and ice I_h/vapor equilibrium lines of the vapor/liquid/solid triple point are of interest to biology and food science.

2.3 CHEMISTRY OF WATER MOLECULE

The numerous anomalous properties of water implicitly suggest that its structure, both in the liquid and solid states, is quite abnormal compared to other substances. At the molecular level, a single molecule of water has a simple chemical structure with two hydrogen atoms covalently attached to an oxygen atom. The oxygen atom is in an sp^3-hybridized state with bonding orbitals in a tetrahedral orientation. Two of the orbitals share electrons with the 1*s* orbitals of hydrogen atoms and the other two orbitals are occupied by the two lone pairs of electrons (Figure 2.5a).

In an isolated water molecule, the H–O–H angle is about 104.5° (Figure 2.5b), which is slightly lower than the tetrahedral angle 109.5°. However, in the liquid and ice states, the H–O–H angle is higher than 104.5°, presumably due to water–water interaction in the condensed state. The O–H bond length is about 0.96 Å and the van der Waals radius of the oxygen atom is about 1.4 Å. A water molecule is not perfectly spherical in shape and the molecular model shown in Figure 2.5b indicates that its diameter, as determined from its center of revolution, is about 3.12 Å. However, the *mean* van der Waals diameter of water is considered to be about 2.8 Å.

2.3.1 Hydrogen Bonds

Many of the anomalous properties of water can be traced back to its simple but unique structure. Water is a dihydride of oxygen. In this molecular structure, the highly electronegative oxygen atom attracts and dislocates the electrons of the O–H bonds more toward it, and as a result, the hydrogen atoms acquire a partial positive charge and the oxygen atom assumes a partial negative charge. The partial charge is about −0.72 on the oxygen atom and it is about +0.36 on each of the two hydrogen atoms. This asymmetrical charge distribution with an H–O–H angle of 104.5° imparts a permanent dipole character to the water molecule. The dipole moment of water is about 1.85 Debye units (D) (=6.2375×10^{-30} C m). This permanent dipole moment enables water molecules to engage in hydrogen bonding via dipole–dipole interactions. Since a water molecule has two protons and two lone pairs of electrons oriented along the axes of a tetrahedron, each water molecule can form four hydrogen bonds with four other water molecules. In this configuration, the O–H orbitals act as hydrogen-bond donors and the two-lone-pair electron orbitals of the oxygen atom act as hydrogen-bond acceptors.

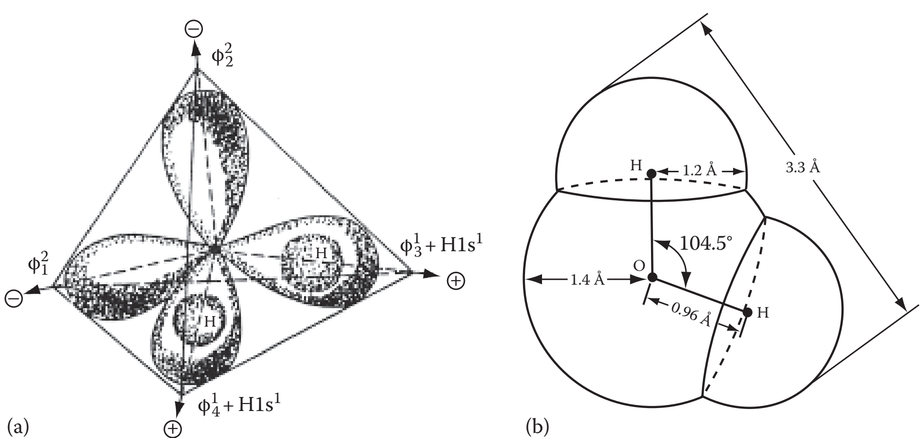


FIGURE 2.5 Schematic model of a single water molecule. (a) SP^3 configuration of water and (b) van der Waals radii of a HOH molecule in vapor state. (From Fennema, O.R., Water and ice, in: *Food Chemistry*, 3rd edn., Fennema, O.R. (ed.), Marcel Dekker, Inc., New York, 1996.)

The strong attractive interaction between water molecules mainly arises from the presence of an equal number of hydrogen-bond donors and acceptors oriented in tetrahedral geometry and to a lesser degree from the electronegativity of the oxygen atom. This equal distribution of hydrogen-bond donors and acceptors enables water to form an extended three-dimensional hydrogen-bonded network structure in the condensed state. This situation does not occur in other hydrogen-bonded liquids. For instance, hydrogen fluoride (HF) also can engage in hydrogen-bonding interactions, but it does not exhibit any anomalous behavior as water does. The fluorine atom in HF also has four bonding orbitals in a tetrahedral arrangement, but unlike in water, three orbitals are occupied by three lone pairs of electrons (hydrogen-bond acceptors) and only one hydrogen-bond donor. This uneven donor/acceptor distribution does not permit formation of a three-dimensional network in liquid HF. A similar situation also is present in liquid NH_3. The tetrahedral geometry of ammonia has three hydrogen atoms (hydrogen-bond donors) attached to nitrogen and one-lone-pair electrons, which permits only formation of a two-dimensional hydrogen-bonded network. On the other hand, among the hydrides of electronegative elements, such as O, S, Se, Te, and Po, water and H_2Po are the only ones in the liquid state, while the other hydrides are gaseous at ambient temperature, even though these hydrides also have two-lone-pair electron orbitals (hydrogen-bond acceptors) and two hydrogens (hydrogen-bond donors) (Figure 2.6). This is attributable to differences in the electronegativity of these elements, which follows the order

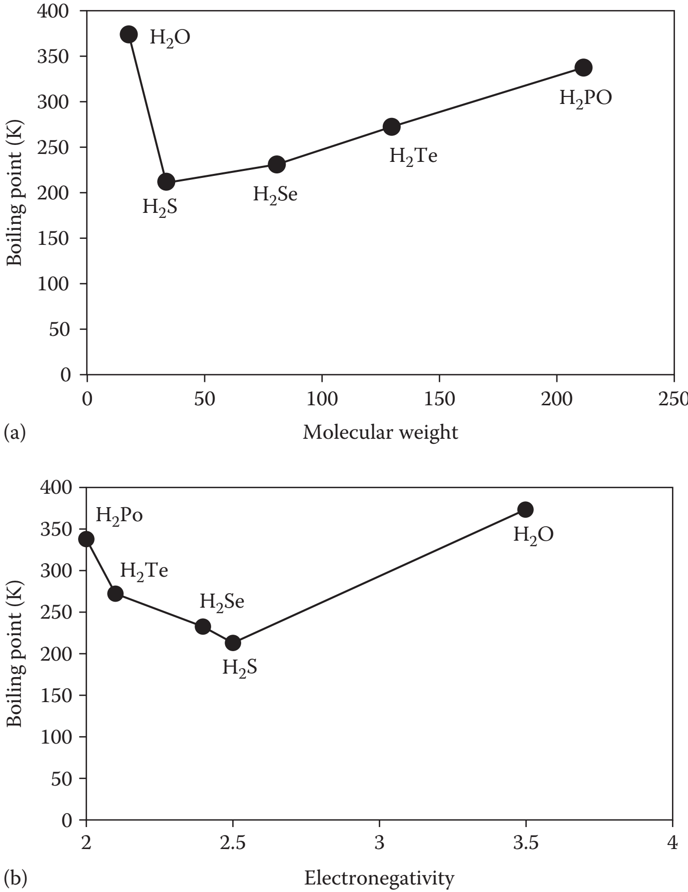


FIGURE 2.6 Variation of boiling point of hydrides of various elements as a function of (a) molecular weight and (b) electronegativity of elements.

O > S > Se > Te > Po. While the electronegativity of oxygen is 3.5, that of S, Se, Te, and Po is 2.5, 2.4, 2.1, and 2.0, respectively, compared to 2.2 for hydrogen. Furthermore, whereas the H–O–H bond angle is 104.5°, the H–X–H bond angle in other hydrides is about 90°. As a result, the extent of electron dislocation and polarization is very negligible in the hydrides of the latter elements. The departure from tetrahedral orientation of the bonding orbitals also diminishes intermolecular attractive forces in these hydrides. It should be noted that while the boiling points of hydrides of S, Se, Te, and Po decrease linearly with increase of electronegativity of these elements, water strikingly deviates from this linear trend (Figure 2.6b). This anomalous behavior indicates that the atomic size, electronic structure, and bonding orbital angle of the oxygen atom are inexplicably and inextricably involved in creating a three-dimensional network structure with several anomalous properties.

Hydrogen bond refers to interaction between an electronegative atom (such as oxygen) and a hydrogen atom covalently attached to another electronegative atom. The strength of a hydrogen bond, which is noncovalent, is typically in the range of 2–6 kcal/mol compared to about 80–120 kcal/mol for a covalent bond. However, it is significantly higher than van der Waals interaction energy, which is about 0.1–0.3 kcal/mol, and certainly much greater than the thermal energy RT, which is 0.59 kcal/mol at 25°C. Because a hydrogen bond is about 4–10 times greater than the average kinetic (thermal) energy of molecules at ambient temperature, intermolecular complexes formed via hydrogen bonds are very stable against thermal motions.

As stated earlier, hydrogen bonds in water arise because water is a dipole. The strength of water–water hydrogen bond depends on the orientation of water molecules with respect to each other. The optimum water–water configuration that confers maximum strength to the hydrogen bond is shown in Figure 2.7: The angle θ in Figure 2.7 refers to the hydrogen-bond acceptor

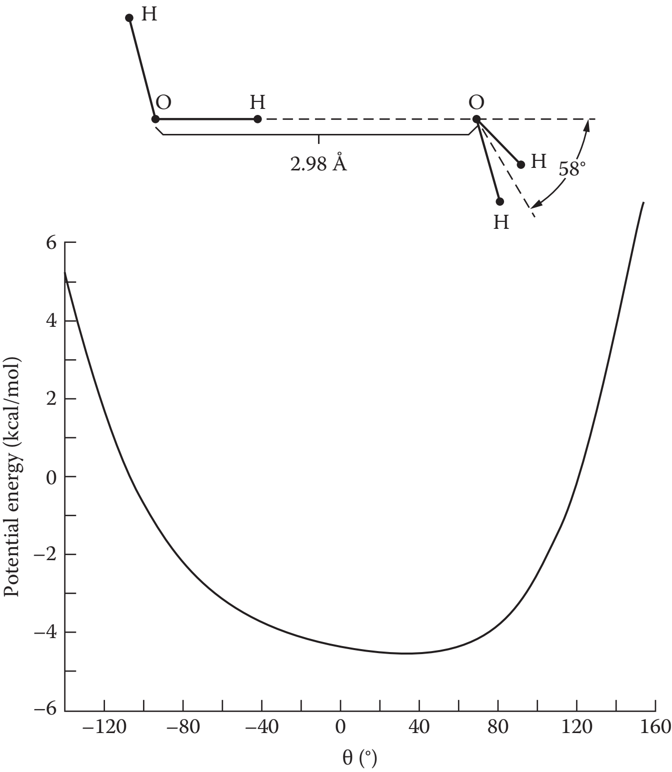


FIGURE 2.7 Potential energy of water dimer as function of hydrogen-bond acceptor bend. (From Stillinger, F.H., *Science*, 209, 451, 1980.)

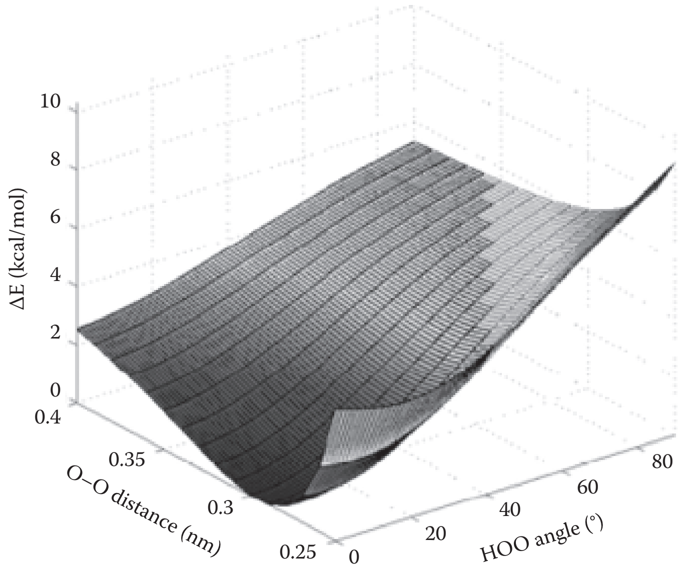


FIGURE 2.8 Water–water hydrogen-bond energy as a function of oxygen–oxygen distance and H–O···O angle. (From Scott, J.N. and Vanderkooi, J.M., *Water*, 2, 14, 2010.)

bend with respect to the axis of the hydrogen bond. The potential energy of the hydrogen-bonded dimer reaches the lowest value when β is bout 58°. In this orientation, one of the lone pairs of electrons of the oxygen atom falls in line with the O–H axis of the other water molecule. It should be noted that the potential energy of the hydrogen bond does not change very significantly when β oscillates from about 58° to −40° (Figure 2.7), indicating that fluctuation in orientation within this range is admissible without any significant energy penalty. Because of this high degree of orientational freedom, water molecules in liquid water are believed to be in a high entropy state.

The strength of the hydrogen bond is also dependent on the O–H···O distance. The potential energy of the hydrogen-bonded water dimer reaches a minimum when the O–H···O distance is about 2.9 Å. Above and below this distance, the potential energy increases in a nonlinear fashion, as shown in Figure 2.8, denoting that hydrogen bonds are short-range interactions [4].

2.3.1.1 Summary

- Water is a dipolar molecule.
- Each water molecule has two hydrogen-bond donors and two hydrogen-bond acceptors arranged in a tetrahedral orientation. This enables water to form an extended three-dimensional hydrogen-bonded network structure.
- The anomalous properties of water are related to its unique hydrogen-bonded network structure.

2.4 STRUCTURES OF ICE AND LIQUID WATER

2.4.1 Structure of Ice

Ice exists in at least 13 different phases (structural states) depending on temperature and pressure (Figure 2.3). At the typical temperature and pressure ranges found on earth, ice exists only in the hexagonal I_h form. In ice I_h, each water molecule is hydrogen bonded to four water

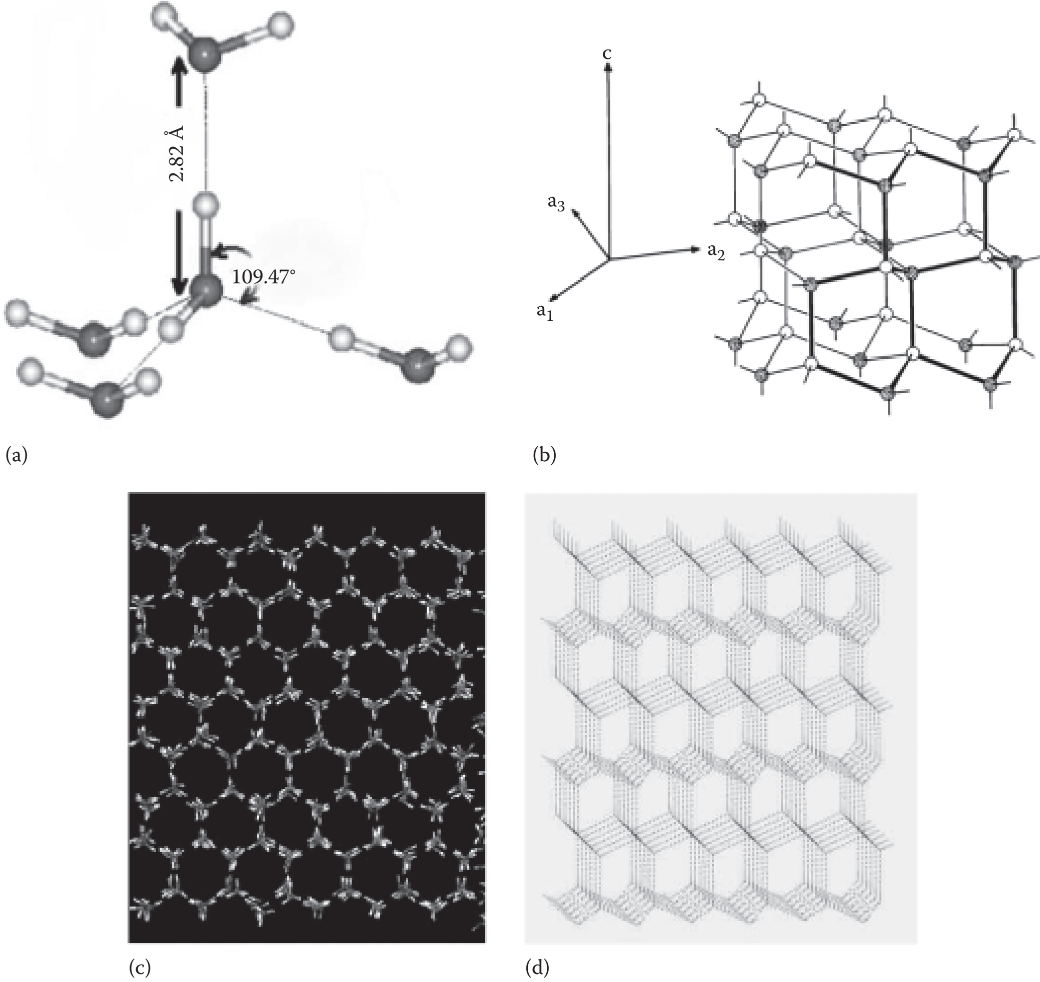


FIGURE 2.9 (a) Hydrogen bonding of water molecules in a tetrahedral configuration. (b) The structure of ice I_h. Open and shaded circles represent, respectively, oxygen atoms in upper and lower layers of basal plane. (c) Basal plane, viewed from the c-axis. (d) Prism plane.

molecules (nearest neighbors) in a tetrahedral orientation, as shown in Figure 2.9a. The O–O distance between the nearest neighbor water molecules is 2.76 Å and the O–O distance between the second nearest neighbors is 4.5 Å. Extension of this tetrahedral array creates a hydrogen-bonded three-dimensional network. Because of this unique spatial ordering of atoms in the network, ice I_h has an open structure with hexagonal crystal symmetry (Figure 2.9b). More specifically, ice belongs to the dihexagonal bipyramidal class of crystals. In this hexagonal symmetry, the oxygen atoms of six hydrogen-bonded water molecules form a hexagonal ring in a chair-like geometry. This can be seen when the ice I_h structure is viewed down the c-axis (Figure 2.9c). A two-dimensional array of these hexagonal rings, hydrogen bonded to each other, constitutes the "basal plane" of ice. In the extended three-dimensional ice structure, these basal planes are stacked over the other in a perfect alignment, connected by hydrogen bonds perpendicular to the basal planes. An ice I_h crystal is characterized by two surfaces: the basal plane when viewed down the c-axis and the prism faces when viewed from the a-axis (Figure 2.9d). The basal plane is monorefringent and therefore is the optical axis of ice, whereas the prism faces are birefringent.

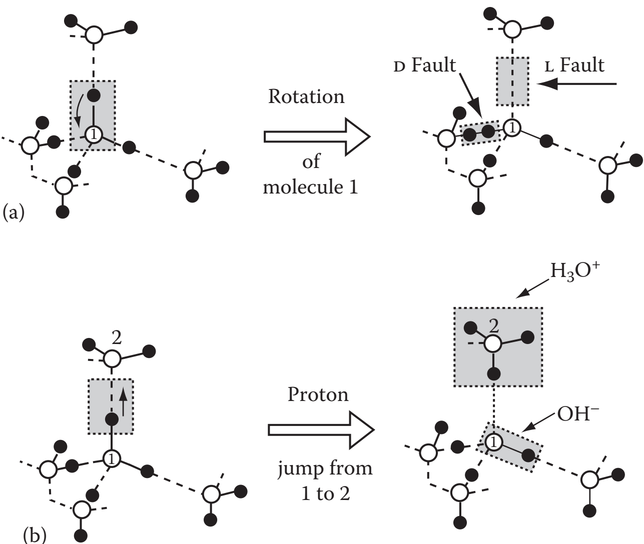


FIGURE 2.10 Schematic representation of proton defects in ice. (a) Formation of orientational defects and (b) formation of ionic defects. Open and filled circles represent, respectively, oxygen and hydrogen atoms. Solid and dashed lines represent, respectively, chemical bonds and hydrogen bonds. (From Fennema, O.R., Water and ice, in: *Food Chemistry*, 3rd edn., Fennema, O.R. (ed.), Marcel Dekker, Inc., New York, 1996.)

Because of the open hydrogen-bonded network structure, the atoms of water molecules physically occupy only about 42% of the total volume of ice I_h. The remaining 58% of the volume is merely empty space, which accounts for its low density. However, the empty space between water molecules in ice I_h is not large enough to accommodate any other molecule. Thus, when an aqueous solution, for example, sucrose or salt solution, is frozen, water crystallizes as pure ice I_h, leaving the solute behind in the unfrozen liquid phase. This property is the basis of the freeze-concentration process used in the food industry for concentrating liquid food products, such as milk and juices.

The ice structure is not static, but a dynamic one. The hydrogen bonds in ice are in a constant flux as a result of rotation/oscillation of water molecules in the crystal lattice and proton dissociation/association (which results in the formation of H_3O^+ and OH^-) (Figure 2.10). These molecular events cause “defects” in ice crystals. The extent of these defects is temperature dependent: All the hydrogen bonds in ice crystal are static and unbroken only at or below −180°C. As the temperature is increased gradually toward 0°C, molecular vibrations in the lattice structure and proton dissociation/dislocation increase. At or near 0°C, the vibrational energy of some of the water molecules is large enough for them to escape from the crystal lattice. For instance, it is estimated that the average amplitude of vibration of each water molecule in ice crystal lattice is about 0.4 Å at −10°C [5]. The high thermal diffusivity of protons in ice (Table 2.1) and only a small decrease in electrical conductivity when water is transformed from liquid to solid state are essentially related to these structural defects in ice.

2.4.2 Structure of Liquid Water

Because liquid water is the primary solvent in all biological systems and formation of organized biological macromolecular structures, such as biomembranes and proteins/enzymes, and the very functioning of these biological structures is often orchestrated by liquid water, there has been tremendous interest in the elucidation of the structure of liquid water. Unlike organic liquids, where molecules are in a relatively random state and held together mainly by short-range van der Waals interactions, liquid water is believed to possess some local order in the form of hydrogen-bonded

clusters, where the relative orientation and mobility of a water molecule is controlled and/or influenced by the neighboring water molecules. These structured clusters of various sizes, probably ranging from 3 to >200 water molecule [6,7] (Figure 2.11), are believed to rapidly break and reform but exist in a thermodynamic equilibrium, such that the populations of these associated structures are sustained at all times. In liquid water, these various clusters may assemble in various configurations via weak van der Waals forces.

The evidence for the "flickering cluster" model of water comes from various physical properties of water. As indicated earlier, water molecules in ice occupy only about 42% of the total volume of ice. The remaining space is empty, which makes ice to assume an open structure. When ice at 0°C is melted to liquid water at 0°C, the volume physically occupied by water molecules is only about 60% of the theoretically possible value for a randomly close packed molecules in a liquid. Although this partly accounts for its higher density than ice, it nevertheless suggests that liquid water has an open structure similar to ice structure. That is, much of water molecules in the liquid state are still engaged in a hydrogen-bonded tetrahedral network clusters as in ice. The empirical evidence for this is as follows: The latent heat of fusion of ice and the latent heat of sublimation of ice at 0°C is 334 and 2838 J/g, respectively. If we assume that the heat of sublimation represents the energy needed to break all hydrogen bonds in ice in order to liberate water from the solid phase to vapor

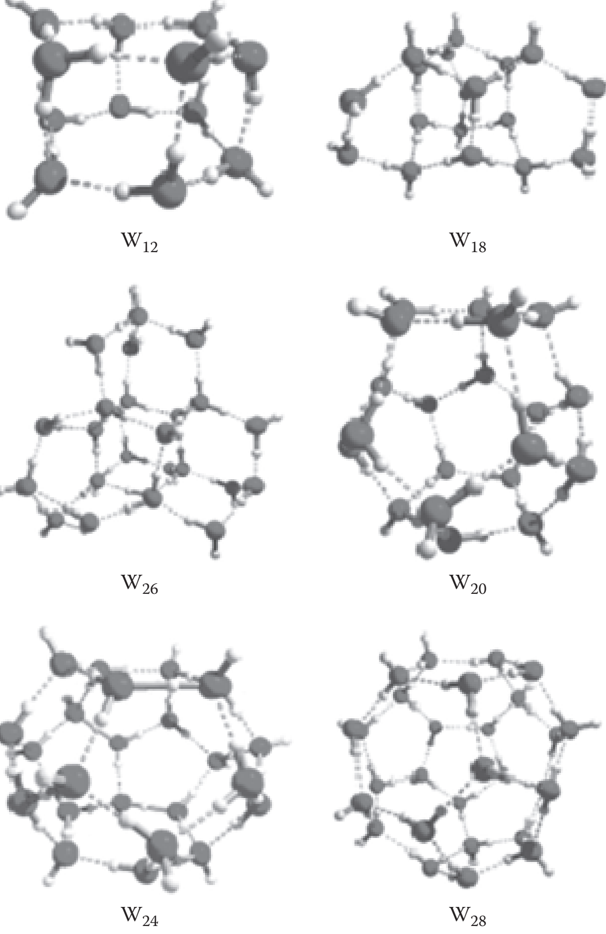


FIGURE 2.11 Icelike water clusters of sizes ranging from 12 to 28. The size of water clusters in liquid water is believed to range up to 200 water molecules. (From Ludwig, R., *Angew. Chem. Int. Ed.*, 40, 1808, 2001.)

phase, then the number of hydrogen bonds needed to be broken to melt ice at 0°C into water at 0°C is only about 12% (i.e., 334/2838) of the total hydrogen bonds in ice. A much more rigorous analysis suggests that each water molecule in liquid water is hydrogen bonded to about 3.4 water molecules compared to 4 in ice, which translates to breaking of about 15% of the hydrogen bond for the ice–water phase transition at 0°C. This implies that about 85% of the hydrogen bonds in ice at 0°C are left intact in liquid water at 0°C. However, unlike in ice, most of the hydrogen bonds in liquid water are distorted (bent, rotated, or stretched) as a result of greater thermal motions. Thus, the structure of liquid water may be viewed as partially melted ice crystal lattice in which local order is maintained but the long-range order is lost.

The oxygen radial distribution function (i.e., the probability of finding another oxygen atom at a radial distance r from the central oxygen atom) of water at 4°C, determined from x-ray diffraction, is shown in Figure 2.12 [8–10]. The profile indicates that the first layer of the nearest neighbors is present at a radial distance of 2.82 Å from the central water molecule (compared to 2.76 Å in ice), and the number of the nearest neighbors is 4.4 (instead of 4 in ice). The second layer of the nearest neighbors is at a radial distance of 4.5 Å, which is similar to that in ice. The third layer of the nearest neighbors is at 7 Å. Beyond the third layer, there is no evidence from x-ray diffraction for long-range order. When the experiment is done at 50°C, the peaks at 4.5 and 7 Å distance disappear and the number of the nearest neighbors at 2.9 Å increases to 5. These data support the contention that in the liquid state water exists as hydrogen-bonded clusters and the size of these clusters depends on the temperature but is mainly hexamers, pentamers, and tetramers at around room temperature. On a mole fraction basis, hexamers and pentamers are more predominant than the other species. All these species are in a dynamic thermodynamic equilibrium. In these clusters, each water molecule is hydrogen bonded to four water molecules and the relative orientation of water molecules in the hydrogen-bonded state is similar to that found in ice (Figure 2.7). The anomalous low viscosity of water is essentially due to very rapid interconversion between these hydrogen-bonded species, which essentially prevents long time order in water. The anomalous high heat capacity of water is also related to this hydrogen-bonding dynamics, which requires a large amount of heat energy to break the hydrogen bonds.

When ice at 0°C is melted to liquid water at 0°C, the hydrogen-bond distance between the first nearest neighbors increases from 2.76 to 2.82 Å, and it increases further to 2.9 Å as the temperature is increased to 50°C. As a consequence of this increase in the nearest neighbor distance, one would

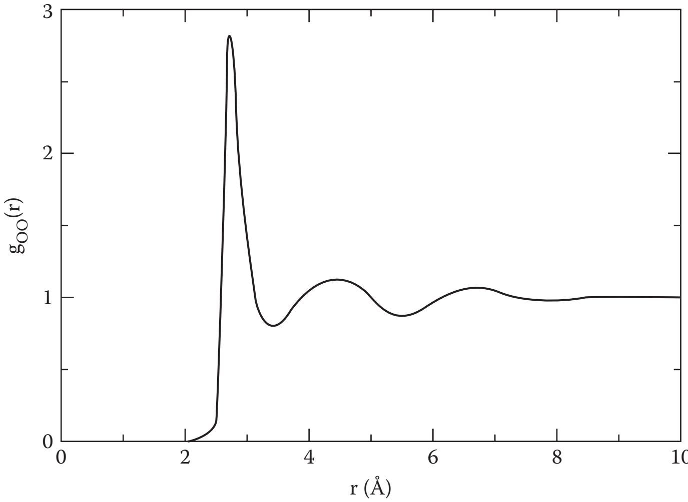


FIGURE 2.12 Radial distribution function of water at 4°C as determined from x-ray diffraction. (From Clark, G.N.I. et al., *Mol. Phys.*, 108, 1415, 2010.)

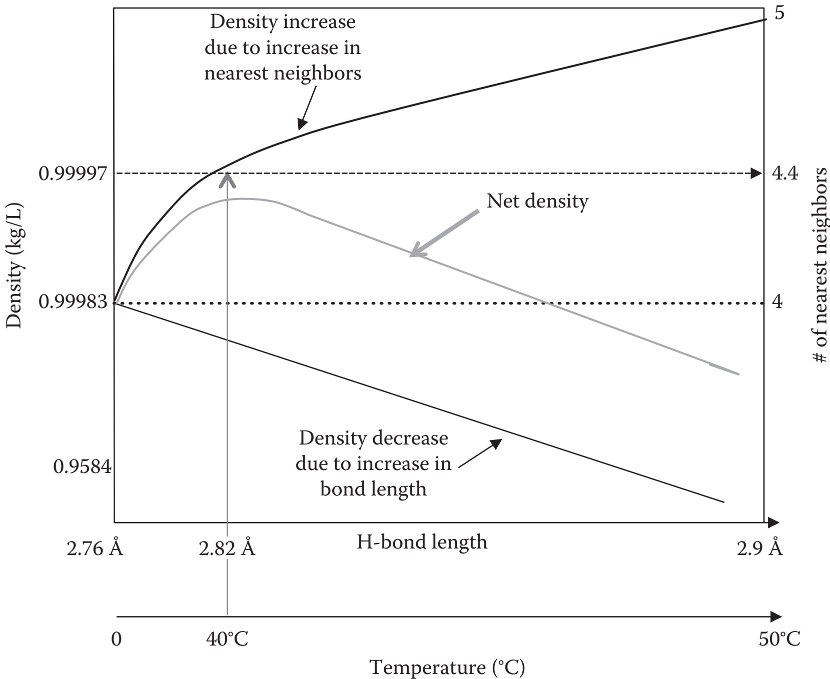


FIGURE 2.13 Schematic representation of the relative contributions of bond length and the nearest neighbors to temperature–density relationship of water.

expect a decrease in density as the temperature is increased. However, as ice is melted and the temperature is increased from 0°C to 50°C, the number of water molecules in the first layer of the nearest neighbors increases from 4 to 5. This would cause an increase in density. The interplay of these two opposing events, that is, the increase in the nearest neighbor distance and the increase in the number of the nearest neighbors, is the reason for the density of water passing through a maximum at about 3.98°C, as shown in Figure 2.13. It should be stressed that the temperature–density profile of water is influenced more by the increase in the nearest neighbor population than by the increase in the hydrogen-bond length as ice is transformed into water.

2.4.2.1 Summary

- The low density of ice is related to its open architecture with empty spaces.
- The ice structure is not static; the hydrogen bonds are in a constant flux. Several anomalous properties, for example, high thermal diffusivity of protons in ice, are related to these hydrogen-bond dynamics.
- Liquid water exists as hydrogen-bonded clusters of various sizes that exist in a thermodynamic equilibrium. In the liquid state, each water molecule has more nearest neighbors than in ice, and it ranges from 4.4 at 4°C to about 5 at 50°C. This is the major reason for the density of water being higher than that of ice.

2.5 AQUEOUS SOLUTIONS

2.5.1 Water–Solute Interactions

Since the structure of liquid water is in a dynamic equilibrium between various tetrahedrally hydrogen-bonded clusters, the introduction of a solute into liquid water will invariably cause a shift in the equilibrium structure of water. Thus, when a solute is dissolved in water, even in the absence

TABLE 2.2
Classification of Types of Water–Solute Interactions

Type	Example	Strength (kJ/mol)	Comments
Charge–dipole	Water–free ion	40–600	Depends on ion size and charge
Dipole–dipole	Water–water	5–25	
	Water–protein NH	5–25	
	Water–protein C=O	5–25	
	Water–OH groups	5–25	
Dipole–induced dipole (hydrophobic hydration)	Water–hydrocarbon (water + R → R(hyd))	Low	
Dipole–induced dipole (hydrophobic interaction)	2R(hyd) → R_2 (hyd)	4–12	

of any specific interaction between water and the solute, the entropy of mixing alters the thermodynamic and structural properties of water. The extent of these changes becomes more significant in the event of specific molecular interactions between water and the solute. Since water is a dipolar molecule, it invariably interacts with almost all dissolved solutes via charge–dipole, dipole–dipole, and dipole–induced-dipole interactions. The relative strength of various noncovalent interactions between water and functional groups of solutes is summarized in Table 2.2. Depending on the chemical nature of the solute, these interactions may either enhance or destabilize the tetrahedrally hydrogen-bonded water structure. Such changes in liquid water structure can influence the structure and stability of biological molecules, such as proteins/enzymes (see Chapter 5).

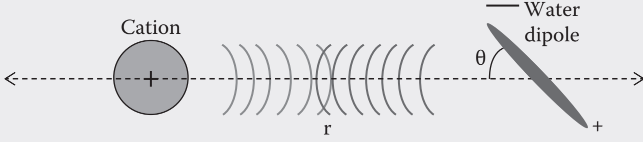
2.5.2 Interaction of Water with Ions

Charge–dipole interaction is the strongest among all noncovalent interactions listed in Table 2.2. In aqueous solutions, this occurs between water and mobile ions (such as salt ions) or immobilized ionic groups in proteins and polysaccharides (Box 2.2). The potential energy of this attractive charge–dipole interaction is given by

$$E_{ion-dipole} = -\frac{(ze)\mu}{4\pi\varepsilon_0\varepsilon}\frac{\cos\theta}{r^2} \tag{2.1}$$

where
- z is the number of charges on the ion and e is the charge of an electron (=1.602 × 10^{-19} C)
- μ is the dipole moment of water (=1.85 Debye units or 6.137 × 10^{-30} C m)
- ε_0 is permittivity of vacuum (=8.854 × 10^{-12} $C^2/N\ m^2$)
- ε is the dielectric constant of the medium (=1 for air or vacuum)
- r is the center-to-center distance between the ion and the dipole
- θ is the dipole angle, which is typically zero for freely mobile water molecule

BOX 2.2 SCHEMATIC REPRESENTATION OF ION-DIPOLE INTERACTION



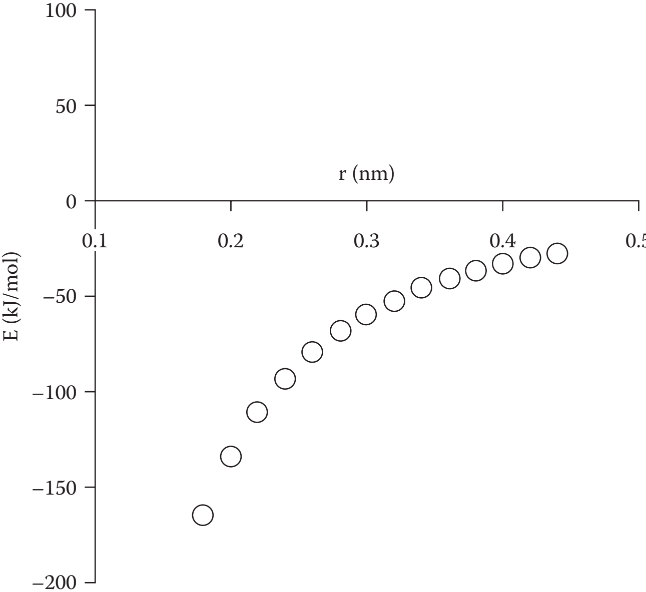


FIGURE 2.14 Theoretical charge–dipole interaction between a monovalent ion and a water molecule as a function of separation distance in gas phase ($\varepsilon = 1$).

The potential energy of interaction between a monovalent ion (e.g., Na^+, K^+) and a water molecule as a function of separation distance in the gas phase (where $\varepsilon = 1$), determined from Equation 2.1, is shown in Figure 2.14. Since the closest distance of separation (r) between an ion and water molecule is the sum of their van der Waals radii, the strength of ion–water interaction is largely dependent on the charge and size of the ion. For ions of similar charge, the ion–water interaction energy decreases with increase of ion radius.

In aqueous solutions, the ion–dipole interaction leads to formation of a hydration shell containing n water molecules around the ion. The Gibbs free energy of hydration ($\Delta_{hyd}G$) of ions is a complex function of the ion size and the number of water molecules participating in the first hydration layer and beyond [11]. The Gibbs free energy of hydration of various ions is given in Table 2.3. It should be noted that the hydration free energies of ions are very strong, suggesting that water molecules in the hydration shell might have restricted mobility. Various regions of a typical hydration shell, where changes in the dielectric permittivity of water are believed to follow a step function, are shown in Figure 2.15. The hydration shell of an ion consists of two regions: The inner hydration shell is highly ordered and most likely tightly bound (chemisorbed) to the ion. The outer shell, defined as the cybotactic region, consists of semiordered water molecules in a structurally perturbed state under the influence of the ion's electric field on the one side and the tetrahedrally hydrogen-bonded bulk water on the other side. Beyond this region, water essentially exists in the free bulk state. The number of water molecules in the inner hydration shell of monovalent cations and anions is given in Table 2.3. The hydration number is dependent on the size and therefore to the surface charge density of the ion: The higher the surface charge density, the greater is the hydration number. However, the hydration free energy, $\Delta_{hyd}G$, of an ion is not just confined to water molecules in the inner layer but is related to the total interaction of the ion's electric field with all water molecules in the inner and the cybotactic regions.

There is a strong evidence that ions affect the tetrahedrally hydrogen-bonded structure of bulk phase water. In this regard, ions fall into two categories: Ions with small radius and high surface charge density (charge/surface area), such as Li^+, Na^+, Ca^{2+}, Ba^{2+}, Mg^{2+}, and F^-, enhance the overall tetrahedrally hydrogen-bonded structure of water, whereas large ions with low surface charge

TABLE 2.3
Gibbs Free Energies of Hydration of Ions

Ion	r (nm)	Δr (nm)	n	$\Delta_{hyd}G$ (kJ/mol)	Born Self-Energy of Ions (kJ/mol)
Li^+	0.069	0.172	5.2	−475	1006
Na^+	0.102	0.116	3.5	−365	681
K^+	0.138	0.074	2.6	−295	503
NH_4^+	0.148	0.065	2.4	−285	469
Mg^{2+}	0.072	0.227	10.0	−1830	3859
Zn^{2+}	0.075	0.220	9.6	−1955	3704
Ca^{2+}	0.100	0.171	7.2	−1505	2778
F^-	0.133	0.079	2.7	−465	522
Cl^-	0.181	0.043	2.0	−340	383
Br^-	0.196	0.035	1.8	−315	354
I^-	0.220	0.026	1.6	−275	315
SCN^-	0.213	0.029	1.7	−280	326
SO_4^{2-}	0.230	0.043	3.1	−1080	1208
HCO_2^-	0.169	0.050	2.1	−395	411

Source: Marcus, Y., *J. Chem. Soc. Faraday Trans*., 87, 2995, 1991.
r is ion radius; Δr is the thickness of the first hydration shell; n is the number of water molecules in the first layer of hydration shell; $\Delta_{hyd}G$ is the free energy of hydration of the ion, which includes water molecules in the cybotactic region.

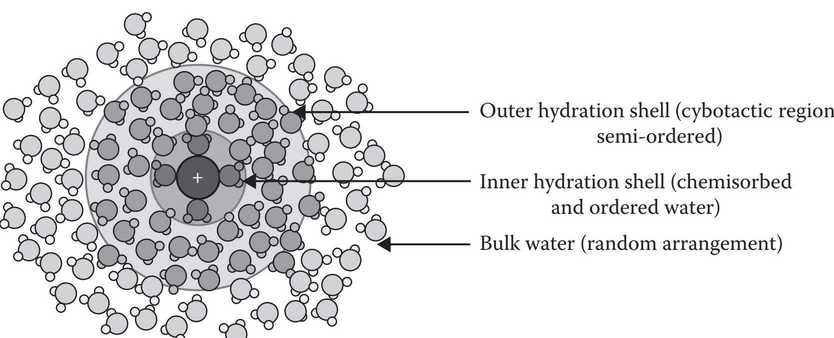


FIGURE 2.15 Schematic representation of a hydration shell around a monovalent cation. (From Lower, S., A gentle introduction to water and its structure, 2016. http://www.chem1.com/acad/sci/aboutwater.html, accessed January 27, 2015.)

density, such as Rb^+, Cs^+, Br^-, I^-, ClO_4^-, SCN–, and NO_3^-, breakdown water structure. The former group is known as "kosmotropes" and the latter is known as "chaotropes." The relative effects of these ions on bulk water structure follow a ranking order known as the Hofmeister series. Ions such as Cl^- and K^+ have minimal effect on water structure and therefore they are mostly regarded as neutral ions in the Hofmeister series. As the structure and stability of proteins in aqueous solutions is dependent on the state of bulk water structure, chaotropic salts generally cause denaturation of proteins and increase the solubility of nonpolar substances, whereas kosmotropic salts enhance the stability of protein structure and decrease the solubility of nonpolar substance in aqueous solutions.

2.5.3 Interaction of Water with Neutral Polar Groups

Water can interact with several neutral polar (hydrophilic) solutes via dipole–dipole interaction as depicted in Box 2.3.

The potential energy of this interaction is given by

$$E_{dipole-dipole} = -\frac{\mu_1\mu_2}{4\pi\varepsilon_0\varepsilon}\frac{\cos\theta}{r^3} \quad (2.2)$$

where

μ_1 and μ_2 are the dipole moments of water and the polar molecule, respectively
r is the center-to-center distance between the dipoles
θ is the angle between the dipoles

While Equation 2.2 is quite applicable to most cases of dipole–dipole interactions, it provides a lower than −5 to −6 kcal/mol estimated for hydrogen bonding in water dimer and interaction of water with other hydrogen-bonding polar groups in polysaccharides and proteins. This anomaly is due to the fact that interaction of water with another water molecule or with a polar (OH) group typically follows a multipole interaction rather than a dipole–dipole interaction [12,13]. The strength of the hydrogen bond between a polar solute and water is typically as strong as that of water–water hydrogen bond. Thus, water interacts as strongly with polar groups in food components, such as proteins and carbohydrates, as it does with itself. However, this interaction does not result in formation of a hydration shell around polar molecules as it does with ions.

As a general rule, when a solute is dissolved in water, it is bound to change the bulk water structure. This is true for all solutes, including neutral polar solutes as well as ionic solutes. However, whether or not a neutral polar solute enhances or destroys bulk water structure depends on the spatial and orientational compatibility of the solute–water hydrogen bonds with those of the tetrahedrally hydrogen-bonded bulk water. In this respect, polyols, such as sugars and glycerol, enhance the tetrahedrally hydrogen-bonded units in bulk water, whereas hydrogen bonding of urea with water destroys the tetrahedrally hydrogen-bonded bulk water structure [14–17]. Evidence for this comes from neutron diffraction studies, which have shown that although urea mixes well and substitutes for water in the hydrogen-bonded water network, its large molecular volume disrupts water–water hydrogen bonding, as evidenced from the complete disappearance of the second nearest neighbor peak at 4.5 Å in the radial distribution function (refer to Figure 2.11). In other words, urea destroys, whereas polyols enhance, the long-range order in bulk water structure. This does not mean that the total number of hydrogen bonds per mole of water is either decreased or increased, respectively, by these two classes of solutes, but it only implies that the long-range hydrogen-bonded cluster state of bulk water is altered.

Several food components, such as proteins and polysaccharides, contain several neutral polar groups, such as amino, hydroxyl, amide, and carbonyl groups that can form hydrogen bonds with

BOX 2.3 SCHEMATIC REPRESENTATION OF DIPOLE-DIPOLE INTERACTION

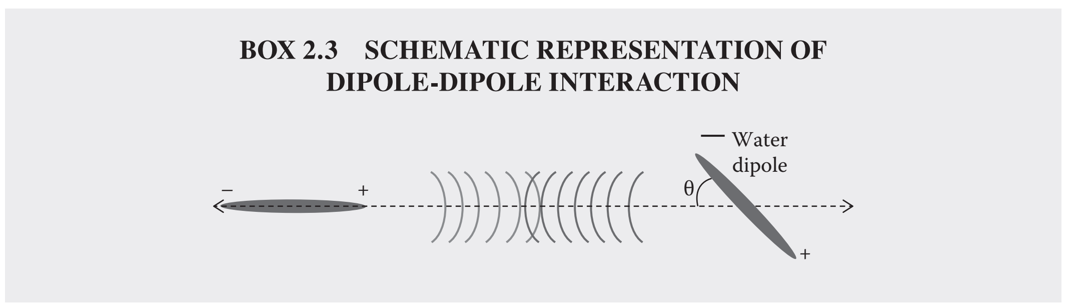


FIGURE 2.16 Examples of hydrogen-bonding interaction between water and various functional groups in proteins and carbohydrates.

water (Figure 2.16). As indicated earlier, since the strength of these hydrogen bonds is similar to that of the water–water hydrogen bond, it is believed that there is no preferential interaction of water with these groups in an aqueous medium.

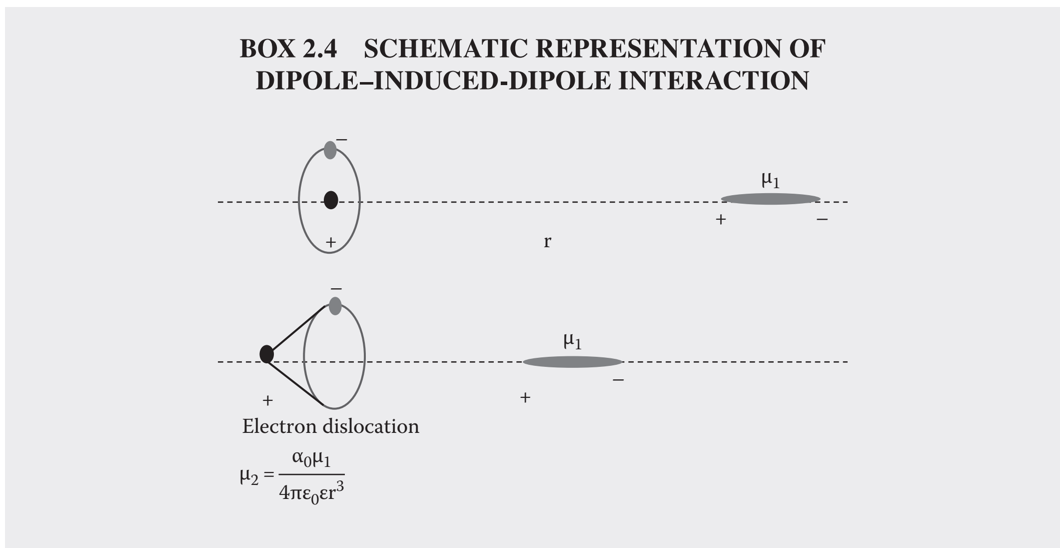
2.5.4 Interaction of Water with Nonpolar Solutes

Even though most nonpolar substances are not soluble and/or do not mix with water, at the molecular level, water does interact with nonpolar solutes via dipole–induced-dipole interaction.

Nonpolar substances do not possess a permanent dipole moment. However, when a dipolar molecule (such as water) with a dipole moment of μ_1 approaches a nonpolar molecule, it causes dislocation of the electron cloud of the nonpolar molecule (as shown in Box 2.4). This imparts an induced-dipole moment of $\mu_2 = \alpha_0\mu_1/4\pi\varepsilon_0\varepsilon$, where α_0 is the polarizability of the nonpolar molecule (in m^3). The potential energy function for dipole–induced-dipole interaction is given by

$$E_{induced-dipole} = -\frac{\alpha_0\mu_1^2}{(4\pi\varepsilon_0\varepsilon)r^6} \tag{2.3}$$

where r is the center-to-center distance between the dipoles. The dipole–induced-dipole interaction between water and a nonpolar molecule is always attractive, which implies that, at the molecular level, there is no "phobia" between water and nonpolar substances [18]. If this is the case, it raises a fundamental question as to why, on a macroscopic scale, nonpolar substances are not soluble or miscible in water.



2.5.5 The Hydrophobic Effect

Two explanations have been put forth to explain this phenomenon. According to the first school of thought, consider two immiscible liquids, such as water and n-octane. The interfacial energy between the liquids is given by

$$\gamma_{12} = \gamma_1 + \gamma_2 - W_{adh} \tag{2.4}$$

where
- γ_1 and γ_2 are the surface tensions of the liquids
- γ_{12} is the interfacial tension
- W_{adh} is the "work of adhesion" between two immiscible liquids

For most immiscible liquids, the work of adhesion is positive (e.g., 43.76 ergs/cm^2 between water and n-octane), meaning that it is attractive and therefore there is no phobia between water and hydrocarbons [18]. However, the energy of this attractive interaction (the origin of which is the dipole–induced-dipole interaction between water and hydrocarbon) is not strong enough to break apart the hydrogen bonds of water in order for the hydrocarbon to go into solution [18]. For instance, assuming that the concentration of water at the air–water interface is about 5.7×10^{-10} mol/cm^2 [19], the work of adhesion of 43.76 ergs/cm^2 between water and octane at the water–octane interface corresponds to attractive interaction energy of only about −1.85 kcal/mol. On the other hand, the average hydrogen-bond energy of bulk water is about −6 kcal/mol. Thus, the attractive interaction energy between water and octane is not large enough to break hydrogen bonds in bulk water, and this energy inequality limits the solubility of octane (and similar nonpolar substances) in water.

The second line of thought stems from experimental data of thermodynamic changes that occur when a nonpolar solute (such as cyclohexane or methane) is transferred from the gas phase or from a nonpolar solvent to an aqueous medium, as shown in Figure 2.17. The enthalpy change (ΔH) for the transfer process is either negative or zero, depending on whether the transfer is from the gas phase or a liquid phase, but the free energy change (ΔG) for this process is always positive in both cases, meaning that it is thermodynamically unfavorable. Since $\Delta G = \Delta H - T\Delta S$ (where ΔS is the entropy change), it follows that when a hydrocarbon is transferred from a nonpolar medium to an aqueous medium, a large negative (unfavorable) change in entropy occurs in the aqueous phase, which more

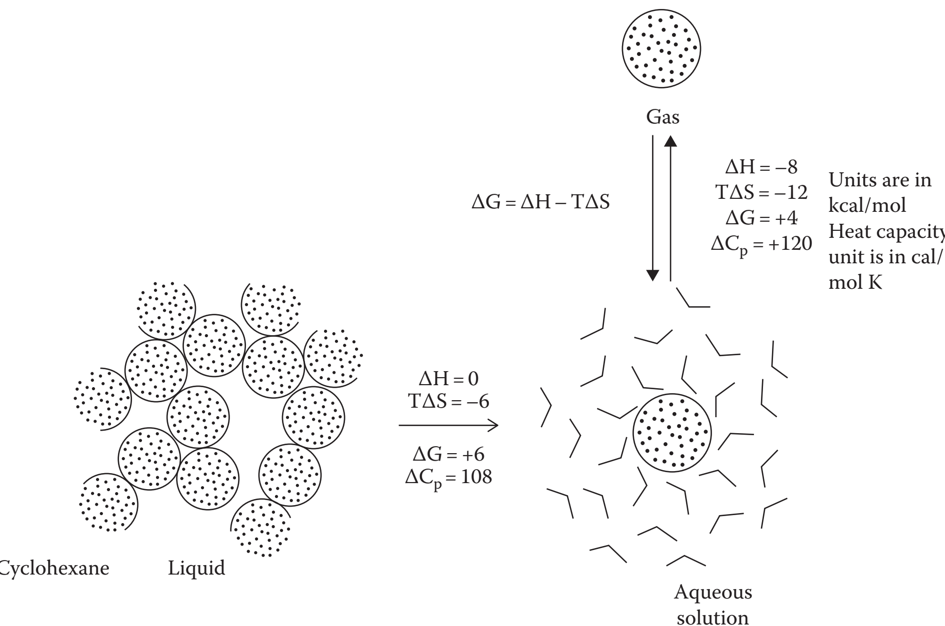


FIGURE 2.17 Typical thermodynamics of transfer of a nonpolar molecule the size of cyclohexane between the gas and liquid phases and aqueous solution at 20°C (293 K). The values of ΔH, TΔS, and ΔG are in units of kcal/mol and that of ΔC_p in units of cal/(K mol). (Adapted from Creighton, T.T., *Proteins: Structures and Molecular Properties*, 2nd edn., W.H. Freeman & Co., New York, 1996, p. 157.)

than offsets any negative (favorable) change in enthalpy, so that the net free energy change of the process is positive, that is, $\Delta G > 0$ [20,21]. The negative entropy change denotes that by its mere presence in an aqueous medium, a nonpolar solute imposes an increase in "order" or "structuring" of water. More importantly, the water–water geometry (orientation) in this structured water is quite different from that of the normal hydrogen-bonded water clusters.

When a nonpolar solute is introduced into an aqueous solution, water interacts with the nonpolar surface via dipole–induced-dipole interaction. However, in order to maintain its hydrogen-bonding interactions with other water molecules in the vicinity of the nonpolar molecule, water is forced to straddle the nonpolar surface and rearrange its orientation so that the maximum number of its hydrogen-bonding orbitals (both donors and acceptors) is pointed away from the nonpolar surface [22] (Figure 2.18). This reorganization, known as "hydrophobic hydration," is distinctly different from ionic hydration or hydration of polar solutes, where no such orientation requirement is imposed. The nonpolar solute with this type of hydration shell is known as "clathrate hydrate," and the water molecules associated with this hydration shell completely lose their rotational freedom. Clathrate hydrates are stable at low temperatures and at very high pressures (e.g., at the bottom of oceans and in thermofrost in the artic and the antartic), but very unstable at ambient conditions.

A major consequence of this structural reorganization of water around the nonpolar solute is that the relative hydrogen-bonded water–water orientation in the clathrate structure is very different from those found in hydrogen-bonded water clusters in bulk water and in ice: The water–water orientation in ice and in bulk water is in a staggered configuration, whereas in the clathrate hydrate it is in an eclipsed configuration, as shown in Figure 2.19. The eclipsed configuration differs from the staggered configuration by about 60° rotation of the hydrogen-bond dihedral angle. In the eclipsed configuration, the lone pairs of electrons of oxygen atoms come closer to each other than in the staggered configuration, and the increased repulsive interaction

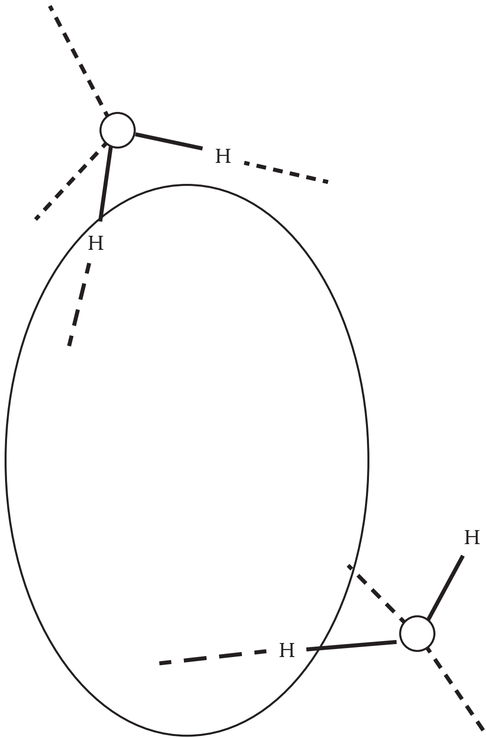


FIGURE 2.18 Orientational preference for water molecules next to a nonpolar solute. In order to maintain its hydrogen-bonding interactions with other water molecules in the vicinity of the nonpolar molecule, water is forced to straddle the nonpolar surface and rearrange its orientation so that the maximum number of its hydrogen-bonding orbitals (both donors and acceptors) is pointed away from the nonpolar surface. (From Stillinger, F.H., *Science*, 209, 451, 1980.)

between the lone pairs exerts a strain on the hydrogen bond. Together, the loss of rotational freedom of the hydrogen bond's dihedral angle and the strain on the hydrogen bond decrease the entropy of water, which renders the presence of the nonpolar solute thermodynamically unfavorable. To restore its entropy, it becomes imperative for water to minimize its association with the nonpolar solute. To accomplish this, water forces nonpolar solutes to aggregate/associate with each other so that the water released from the clathrate shells could return to their original higher entropy state (Figure 2.20). This process, which is the reversal of hydrophobic hydration with free energy change of $\Delta G < 0$, is known as "hydrophobic interaction." It should be emphasized that the interaction between nonpolar solutes under these conditions is driven not by the innate van der Waals attraction between nonpolar solutes but by the entropic force from water structure, and therefore the energy of hydrophobic interaction is considerably stronger than van der Waals interaction.

There is a consensus among biologists/biochemists that the second explanation is more appealing and probably the correct one for explaining thermodynamic incompatibility between water and nonpolar solutes. The imposition by nonpolar solutes on water to reorganize its structure and the water's proclivity to regain its higher entropy state are at the core of evolution of biological structures, such as proteins, biomembranes, and other cellular structures, and perhaps the evolution of carbon-based life itself. For example, phospholipids contain both hydrophilic (phosphate head group) and hydrophobic (long fatty acyl chains) moieties. The thermodynamically unfavorable interaction of water with the fatty acyl chains forces phospholipids

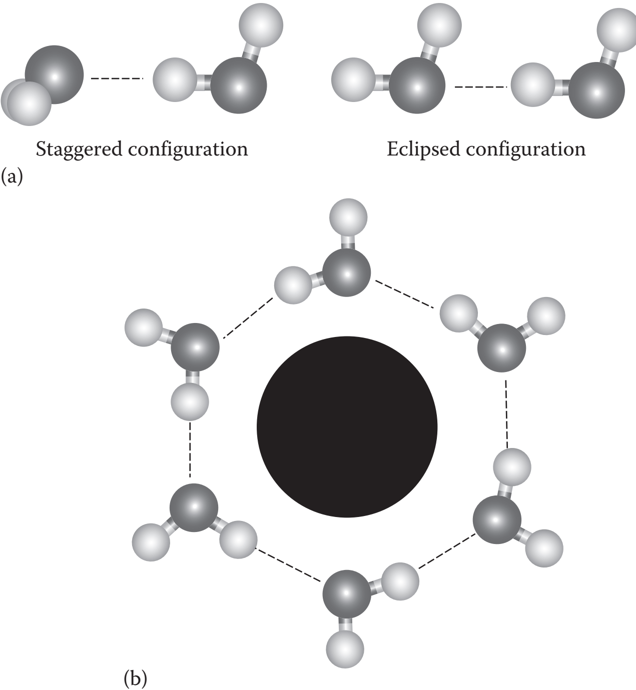


FIGURE 2.19 (a) The staggered and eclipsed configurations of hydrogen-bonded water dimers. (b) Schematics of water–water orientation (eclipsed configuration) at a hydrophobic surface.

to aggregate in the form of micelles or as lipid bilayer structures in which the acyl chains are removed from direct contact with the aqueous phase, while the hydrophilic phosphate head groups are exposed to the aqueous phase (Figure 2.21). Likewise, proteins contain both polar and nonpolar amino acid residues. Because of the thermodynamic need to avoid contact with nonpolar amino acid residues and to maximize interaction with polar amino acid residues, water forces the protein chain to fold and adopt a three-dimensional structure in which a majority of the nonpolar residues are buried deep in the interior and the polar residues are exposed to water on the surface (Figure 2.22).

2.5.6 Concept of "Bound Water"

The earlier discussions clearly indicate that water has the potential to interact with a wide array of ionic, polar, and nonpolar groups in food materials. The strength of these interactions varies from about $0.5k_BT$ (where k_B is the Boltzmann constant and T is the temperature) for dipole–induced-dipole interactions, to about $10k_BT$ for dipole–dipole interactions, and to about $25k_BT$ for ion–dipole interactions. Since k_BT represents the thermal (kinetic) energy of a molecule at temperature T, interactions that are severalfold greater than k_BT are essentially physically bound to each other. Thus, water associated with charged ionic groups in food materials could be regarded as "bound water" with restricted mobility. However, there is heated (often unnecessary) debate among food scientists about the functional definition of the term "bound water."

The equivocality arises because interaction of water with chemical groups in food materials does not involve one-to-one interaction as depicted by Equations 2.1 through 2.3 but involves interaction of several water molecules with each chemical group. This is particularly true in the case of ionic groups where ion–water interaction involves formation a hydration shell.

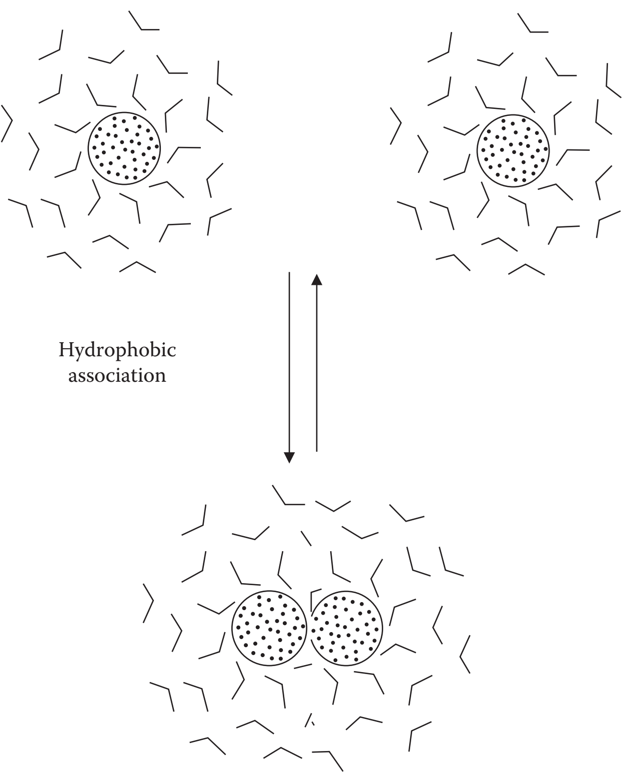


FIGURE 2.20 Schematic representation of hydrophobic association of nonpolar substances in aqueous solutions. The association is facilitated by the release of water from the low-entropy hydration shells to high-entropy free state.

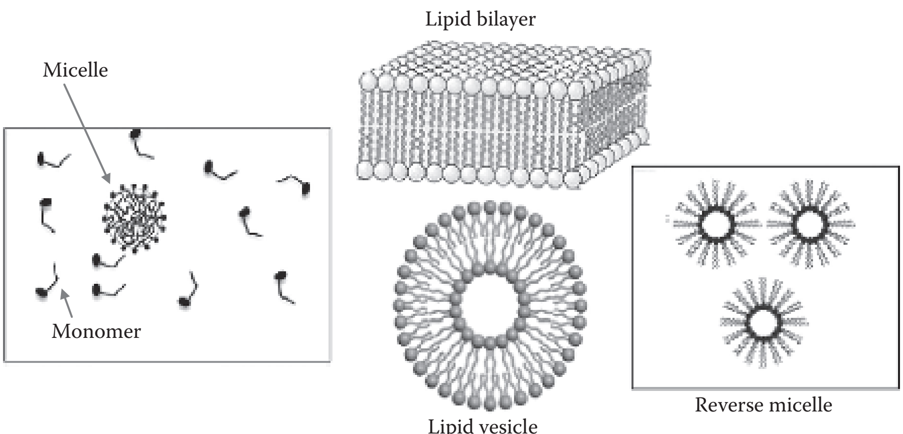


FIGURE 2.21 Formation of various organized phospholipid (or surfactant) structures (e.g., micelles, bilayer sheets, bilayer vesicles) as a result of the hydrophobic effect. (From Israelachvili, J.N., *Intermolecular and Surface Forces*, 2nd edn., Academic Press, New York, 1992, 344pp.)

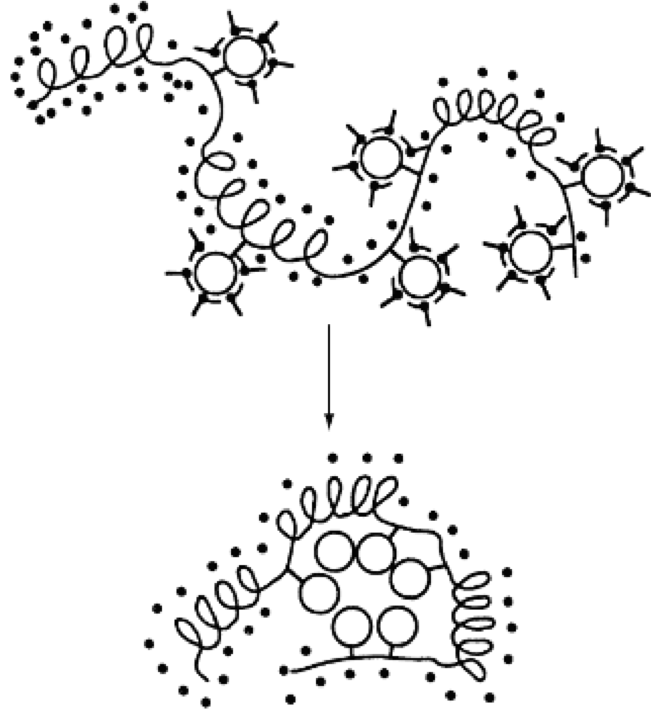


FIGURE 2.22 Schematic illustration of folding of a globular protein, driven by hydrophobic interactions. Open circles are hydrophobic groups, L-shaped entities are water molecules in the hydration shell of hydrophobic groups, and dots represent water molecules associated with polar groups. (From Fennema, O.R., Water and ice, in: *Food Chemistry*, 3rd edn., Fennema, O.R. (ed.), Marcel Dekker, Inc., New York, 1996.)

For example, in the case of a monovalent ion, such as Na^+, the Born self-energy of the ion in the unhydrated state is given by

$$E_{self} = \frac{(ze)^2}{8\pi\varepsilon_0\varepsilon a} \tag{2.5}$$

where a is bare ion radius. According to Equation 2.5, the self-energy of Na^+ (whose radius is 0.102 nm) in the unhydrated state is 681 kJ/mol (Table 2.3). When Na^+ is introduced into water, formation of a hydration shell via ion–dipole interaction reduces its self-energy by about 365 kJ/mol (Table 2.3). This large energy reduction occurs as a result of interaction of the ion with several water dipoles. If only four water molecules were involved in the hydration shell, then the average binding energy of each water molecule would be about −91 kJ/mol (or about $31k_BT$). In this situation, these four water molecules would truly represent "bound water." On the other hand, if we assume that there were 50 water molecules in the hydration shell, including those in the cybotactic region (Figure 2.15), then the *average* binding energy of each water molecule in the hydration shell would be about 7 kJ/mol (or about $3k_BT$). In this situation, the water molecules in the hydration shell are weakly bound to the ion. In reality, however, the interaction energy of water molecules in the hydration shell follows a negative exponential gradient, where the water molecules in the inner most layer of the hydration shell are tightly bound and those in the outer most layer are weakly bound to the ion. In addition, water molecules in the hydration shell are not "static" or "immobilized." They rapidly exchange with other water molecules within the hydration shell as well with bulk water at nano- to picosecond time scales. Thus, under a given set of environmental conditions of temperature and pressure, there is a

dynamic population of water in the vicinity of solute molecules whose thermodynamic properties and molecular mobility are significantly different from those far away from the solute. Since the boundary between the bulk water and the "bound water" is impossible to predict and to quantify, it would be more meaningful to use changes in the average thermodynamic properties of water as a yardstick to understand the impact of solutes on water structure and function in food systems.

2.5.7 Colligative Properties

Colligative properties refer to those properties of dilute solutions that are affected by the concentration of the solute, but not by the chemical nature of the solute. The solution properties that fall under this category are lowering of vapor pressure, depression of freezing point, elevation of boiling point, and osmotic pressure. In ideal solutions, the impact of a nonvolatile solute on these properties is essentially due to entropy of mixing, which, for a binary system, is given by

$$\Delta S_{mix} = -R(n_w \ln X_w + n_s \ln x_s) \tag{2.6}$$

where

n_w and n_s are the numbers of moles of water and solute molecules, respectively
X_w and x_s are the mole fractions of water and solute, respectively

Since the enthalpy of mixing (ΔH_{mix}) is zero for ideal solutions, the free energy change (ΔG_{mix}) for mixing arises solely from the entropy term $-T\Delta S_{mix}$. That is to say, when a solute is mixed with water, the free energy of water decreases by $n_w RT \ln X_w$ and that of the solute by $n_s RT \ln x_s$. This decrease in free energy is responsible for the freezing point depression and boiling point elevation of water in ideal solutions.

The molal freezing point depression constant of a solvent is given by

$$K_f = \frac{RT_f^2 M}{\Delta H_f} \tag{2.7}$$

where

R is the gas constant (J/mol/K)
T_f is the freezing point of pure solvent (K)
ΔH_f is the latent heat of fusion of the solvent (J/mol)
M is the molecular weight of the solvent (kg/mol)

The units of K_f is in K kg/mol. The freezing point depression constant for water is 1.86 K/m, where m is the molality (mol/kg) of the solution. Most fresh fruits and vegetables freeze at −2°C to −5°C due the presence of dissolved solutes. If ΔT is the freezing point depression of a solution, then the mole fraction x_s, of solute in that solution can be determined from

$$x_s = \frac{\Delta H_f}{RT_f^2} \Delta T \tag{2.8}$$

In the case of ionizable solutes, such as NaCl and $CaCl_2$, the freezing point depression is given by

$$\Delta T_f = iK_f m \tag{2.9}$$

where

m is the molality of the solution
i is the van't Hoff factor, which is given by

$$i = \alpha n + (1 - \alpha) \tag{2.10}$$

where α is the fraction of the solute that has dissociated into n ions. For instance, in the case of NaCl, in dilute solution it completely dissociates to Na^+ and Cl^- ions. Therefore, n = 2 and α = 1, in which case i = 2. Thus, according to Equation 2.8, the freezing point depression of a one molal solution of NaCl will be −3.72°C.

In a similar manner, nonvolatile dissolved solutes elevate the boiling point of water. The molal boiling point elevation constant is given by

$$K_B = \frac{RT_B^2 M}{\Delta H_v} \tag{2.11}$$

where

T_B is the boiling point of pure solvent (K)

ΔH_v is the latent heat of evaporation of pure solvent

The K_B value of water is 0.51 K kg/mol.

Equations 2.8 and 2.11 are valid only for ideal solutions. Deviation from ideality would imply that $\Delta H_{mix} \neq 0$. For instance, the boiling points of sucrose solutions as a function of sucrose concentration are shown in Figure 2.23 along with the linear curve predicted by Equation 2.11. It should be noted that even at very low solute concentration the experimental curve deviates from the ideal curve. This deviation from ideality is essentially due to specific solute–solvent (hydrogen bonding) interaction between sucrose and water, which further reduces the chemical potential of water over and above that resulting from entropy of mixing alone. This would require additional thermal energy to drive water from the solution phase to the vapor phase.

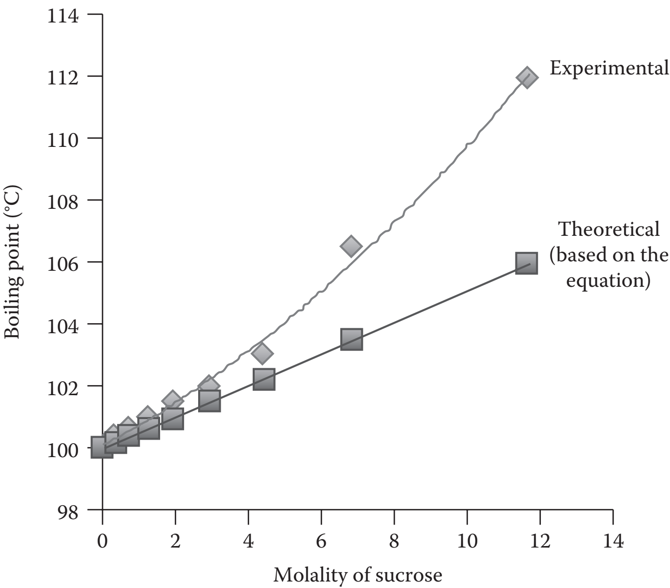


FIGURE 2.23 Boiling point elevation of water. (◆) experimental and (■) predicted by Equation 2.10.

2.5.7.1 Summary

- Water interacts with various solutes via ion–dipole, dipole–dipole, and dipole–induced-dipole interactions. Among these, the ion–dipole interaction is the strongest; it leads to formation of a strong hydration shell around an ion. Water in this shell has restricted mobility.
- Ions affect bulk water structure: Those that increase tetrahedral hydrogen-bonded structure are called kosmotropes and that that breakdown this structure are called chaotropes.
- The hydrophobic effect arises as a result of negative entropy change in water when it forms a hydration shell (clathrate hydrate) around a nonpolar substance. The hydrogen-bonded water–water orientation in the clathrate hydrate is different from those of the water clusters in bulk water, which curtails their rotational freedom and causes a loss of entropy of water.
- When water–solute interaction energy is far greater than the thermal energy (k_BT), then the fraction of water involved in such interactions can be tentatively regarded as "bound water." However, it is difficult to easily quantify bound water.
- Colligative properties are those properties of dilute solutions that are affected by the concentration of the solute, but not the chemical nature of the solute. These are lowering of vapor pressure, depression of freezing point, elevation of boiling point, and osmotic pressure. However, aqueous solutions deviate strongly from this ideal behavior even at low solute concentrations. This is due to solute-specific water–solute interactions.

2.6 WATER ACTIVITY

Water is quintessential for all living organisms. It acts as a solvent for biological reactions and transport processes, as well as a reactant in several biological reactions. Although high water content is necessary for living cells, it is not desirable for preserving foods against microbial spoilage and other nonmicrobiological degradations during storage. However, it has been observed that various foods containing the same water content differed significantly in their perishability, suggesting that it might not be the water content *per se* but the "state" or the thermodynamic "activity" of water in foods that might determine their perishability. At the same water content, the thermodynamic activity of water in various foods might be different depending of the chemical composition of foods and the intensity and/or the extent of ion–dipole, dipole–dipole, and dipole–induced-dipole interactions of water with various chemical groups in foods. Implicit in this notion is that water "bound" to chemical groups in foods might not be readily available to support growth of microorganisms or as a reactant for various hydrolytic reactions that cause quality deteriorations in foods compared to the "free" water. Thus, "water activity" of a food material reflects the thermodynamic capacity (energy status) or the effective concentration of water in a food material that can actually participate as a chemical agent in various biological and chemical processes.

2.6.1 Definition and Measurement of Water Activity

According to classical thermodynamics, the activity of water in an aqueous system is related to its effective concentration in the system. The activity of water in the pure state is unity, and in an *ideal* solution, the water activity a_w is equal to the mole fraction of water, X_{H_2O}, in the solution. That is,

$$a_w = X_{H_2O} = \frac{n_{H_2O}}{n_{H_2O} + n_{solute}} \tag{2.12}$$

where

n_{H_2O} is the number of moles of water
n_{solute} is the number of moles of dissolved solute in the system

For aqueous solutions, such as sugar syrups or salt solutions, when the concentration is expressed in molal (m) units, Equation 2.12 reduces to

$$a_w = X_{H_2O} = \frac{55.5}{55.5 + n_{solute}} \quad (2.13)$$

In ideal solutions, the ideality means that there is no solute–solvent interaction or that the solute–solvent, solvent–solvent, and solute–solute interaction energies are of equal magnitude, so that the enthalpy of mixing (ΔH_{mix}) is zero and the entropy of mixing is ideal (see Equation 2.6).

Since the Gibbs free energy change of mixing is

$$\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix} \quad (2.14)$$

and since $\Delta H_{mix} = 0$ for ideal solutions, the free energy of mixing is solely derived from the entropy of mixing, that is,

$$\Delta G_{mix} = -T\Delta S_{mix} \quad (2.15)$$

Real solutions often deviate from ideality, and this deviation arises because of either attractive or repulsive interaction between the solute and the solvent molecules. Attractive solute–solvent (water) interactions lead to negative deviations from ideality, meaning that the measured activity of water is lower than the actual mole fraction of water in the system, that is, $a_w < X_w$. This situation is predominantly encountered in foods, where strong ion–dipole and dipole–dipole interaction of water with ionic and hydrogen-bonding groups in proteins and polysaccharides causes a fraction of water in foods to become bound to the food matrix, resulting in a decrease in the effective concentration of water available for chemical and biological processes. Any deviation from ideality can be accounted for by modifying Equation 2.12 as

$$a_w = \gamma_w X_w \quad (2.16)$$

where γ_w is the activity coefficient of water in the system. γ_w defines the extent of interaction of water with the solute (i.e., food constituents) and therefore it is solute dependent (i.e., dependent on the composition of food). Taking logarithm of Equation 2.16 and multiplying it by RT, it can be rewritten as

$$RT \ln a_w = RT \ln \gamma_w + RT \ln X_w \quad (2.17)$$

that is,

$$\Delta G_w = RT \ln \gamma_w + RT \ln X_w \quad (2.18)$$

The comparison of Equation 2.18 with Equation 2.5 suggests that while RT ln X_w is the free energy change resulting from entropy of mixing, the term RT ln γ_w represents the excess free energy change resulting from enthalpy of mixing.

Hildebrand and Scott [23] showed that the activity coefficient of the solvent (i.e., water) in a solution could be calculated from the mole fraction of the solute using the equation

$$\ln \gamma = K_s X_s^2 \quad (2.19)$$

where

X_s is the mole fraction of the solute

K_s is a constant related to the chemical nature of the solute

Substitution of Equation 2.19 in Equation 2.16 provides

$$a_w = X_w e^{(K_s X_s^2)} \tag{2.20}$$

Since $a_w < X_w$ in most cases, K_s is typically a negative number. Equation 2.20 is referred to as the Norrish equation [24]. It is, however, functionally identical to Equation 2.16, but by comparing the values of K_s of various solutes, it is possible to obtain some insight into the nature of interaction between water and various chemical groups in solutes.

Direct measurement of the "effective concentration" of water in a food material is difficult, if not impossible. However, it can be measured indirectly as follows: As discussed earlier, water activity reflects the thermodynamic state of water in a system. When an aqueous system is in equilibrium with its vapor phase, the chemical potential of water at any point in the system is

$$\mu_w = \mu_w^0 + RT \ln\left(\frac{f_w}{f_w^0}\right) \tag{2.21}$$

where
- μ_w is the chemical potential of water in the system at temperature T
- μ_w^0 is the chemical potential of pure water (standard state) at that temperature
- R is the gas constant
- f_w is the fugacity of water in the system
- f_w^0 is the fugacity of pure water

Fugacity refers to the escaping tendency of a substance (water in this case) from the solution state. In Equation 2.21, water activity is defined as

$$a_w = \left(\frac{f_w}{f_w^0}\right) \tag{2.22}$$

Since the vapor pressure of water in a closed system at equilibrium arises because of the tendency of water to escape from the solution state, it is logical to assume that fugacity is closely related to vapor pressure and therefore,

$$a_w = \left(\frac{f_w}{f_w^0}\right) = \left(\frac{p_w}{p_w^0}\right) \tag{2.23}$$

where
- p_w is the partial water vapor pressure above a food material at equilibrium
- p_w^0 is the partial vapor pressure of pure water at equilibrium at the same temperature and pressure

According to Raoult's law, for an ideal solution, the ratio p_w/p_w^0 is equal to the mole fraction of that component in the solution. However, in a nonideal system, the ratio p_w/p_w^0 is equal to $\gamma_w X_w$, where γ_w is defined as the activity coefficient. This is due to the fact that attractive interactions of water molecules with chemical groups in the food material decrease their tendency to escape into the vapor phase.

The equality shown in Equation 2.23 is valid only at low pressures ($\leq$1 atm), where the difference between f_w/f_w^0 and p_w/p_w^0 is typically less than 1%, and therefore, for all practical purposes, the water activity of a food material can be determined by measuring p_w/p_w^0. The ratio p_w/p_w^0 is

also known as relative vapor pressure (RVP). Another useful expression of a_w or RSV is the percent equilibrium relative humidity (%ERH):

$$a_w = RVP = \frac{\%ERH}{100} \quad (2.24)$$

The reliability of using a_w (or p_w/p_w^0) to predict safety and stability of foods depends on two important assumptions: First, a true thermodynamic equilibrium between water in the food material and the vapor phase over the food material has been established in a closed system. Second, none of the nonaqueous components of the food material undergo phase change thereafter during storage. While these assumptions can be easily met in liquid products, this might not be possible in complex solid or semisolid food products, where the establishment of a true equilibrium might require several days and the solutes might slowly and continuously undergo phase change from an amorphous state to crystalline state. In the case of the latter situation, which is highly solute specific, a_w is not a reliable indicator of chemical, physical, and microbiological stabilities of foods, because phase change in any of the components in a food product will alter its a_w status.

2.6.1.1 Summary

- In ideal systems (solutions) water activity is the mole fraction of water in the system. In nonideal systems however, water activity is a measure of the "effective" concentration (not the mole fraction) of water in a system. It reflects the average energy status of water in a system.
- The fugacity principle is used to measure water activity in a food sample. In practical applications, water activity of a sample is defined as p/p^0 where p is the partial water vapor pressure of the food sample and p^0 is the partial vapor pressure of pure water at equilibrium at the same temperature and pressure.

2.6.2 Moisture Sorption Isotherms

Since $\mu_w - \mu_w^0 = \Delta G$, Equations 2.21 and 2.23 imply that the water activity of a food material is a measure of the change in free energy of water in a food material. This change in free energy arises both from the entropy of mixing (ΔS_{mix}) and the enthalpy (ΔH_{mix}) of water–solute interactions in the food material. Thus, by constructing an inverse plot of the water content of a food as a function of a_w, it is possible to assess the thermodynamic status of water in a food material under various experimental conditions and relate that to chemical and physical changes as well as to microbial spoilage of foods. Such plots are known as "moisture sorption isotherms" (MSIs).

MSIs are usually constructed by the *resorption* (or adsorption) method, in which a completely dry food material is incubated in controlled humidity chambers at constant temperature. Various saturated salt solutions (Table 2.4) are typically used to create various humidity atmospheres inside the chambers. The sample is kept in the humidity chamber until it reaches a constant weight (typically several days). The net gain in weight of the sample at equilibrium at a given a_w (or relative humidity) represents the water content of the sample (g water/g dry sample) at that a_w. The shapes and positions of MSIs of food materials are dependent on the composition of the food material and the phase states of the components. The MSIs usually fall into three categories. Food materials rich in crystalline materials, such as sugars and hard candies, exhibit a J-type isotherm, which is characterized by a flat isotherm with very low water content until about $a_w \approx 0.8$, followed by a sharp vertical increase in water content at $a_w > 0.8$ (Type 1, Figure 2.24). In this type of isotherm, the sharp inflection point at $a_w \approx 0.8$ is known as the deliquescence point, where the food material begins to dissolve into solution. Foods containing highly hygroscopic components, such as anticaking agents and certain types of salts (e.g., $CaCl_2$ and $MgCl_2$), exhibit the type 3 isotherm (Figure 2.24), which is characterized by a sharp increase in water content even at very low water activity values. A majority

TABLE 2.4
Water Activity of Saturated Salt Solutions

Lithium chloride	0.120
Potassium acetate	0.225
Magnesium chloride	0.336
Potassium carbonate	0.440
Magnesium nitrate	0.550
Ammonium nitrate	0.625
Sodium chloride	0.755
Lithium sulfate	0.850
Potassium sulfate	0.970

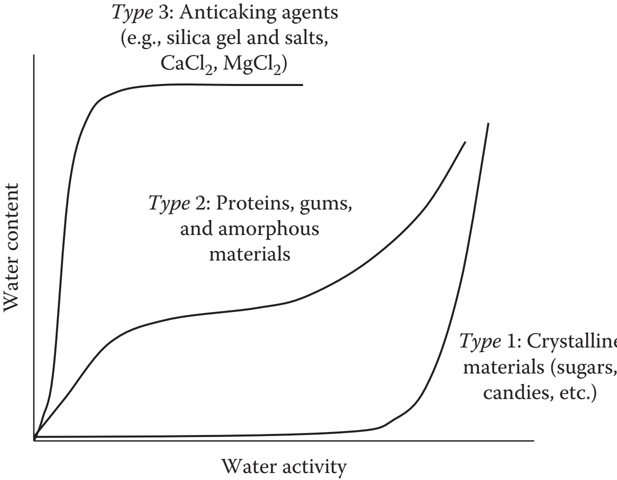


FIGURE 2.24 Schematic representation of the three types of moisture sorption isotherms commonly displayed by food materials.

of complex foods containing polymeric materials, such as proteins and polysaccharides, and amorphous components usually exhibit a sigmoidal-type isotherm (Type 2, Figure 2.24). The sigmoidal shape arises partly because of the presence of different classes of chemical groups (i.e., ionic and hydrogen-bonding groups) with varying binding affinity for water. Examples of water sorption isotherms of various food materials that exhibit both sigmoidal and J-type isotherms are shown in Figure 2.25. Note that crystalline sucrose and cellulose fiber exhibit J-type isotherms, whereas xanthan gum, ready-to-eat (RTE) cereal, whey protein, and oat bran exhibit sigmoidal shape isotherms.

2.6.3 Interpretation of Moisture Sorption Isotherms

Since water activity represents the energy status of water and the chemical and physical changes and microbial growth in foods are affected by the energy status of water in the food, an in-depth understanding of the fundamental physical principles underpinning water relations in foods is desirable. The nonlinear relationship between water content and water activity, which gives rise to the sigmoidal shape of the isotherm, suggests that water exists in different coupled states in foods at different water content levels. Conceptually, the sigmoidal shaped sorption isotherm can be divided into three regions (zones), as shown in Figure 2.26, representing three different

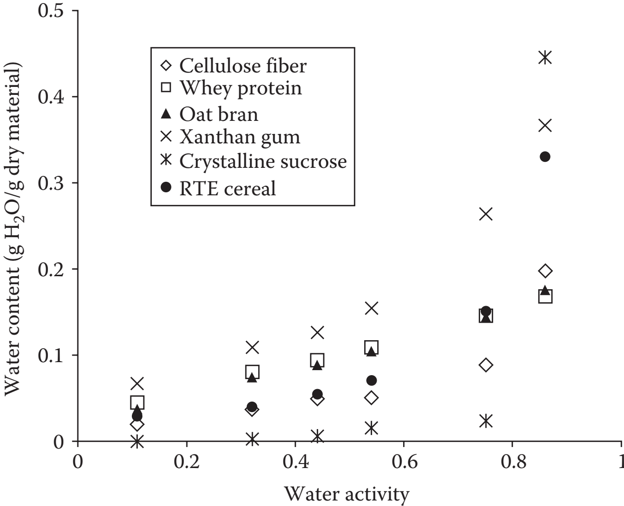


FIGURE 2.25 Moisture sorption isotherms of various food ingredients. RTE cereal refers to ready-to-eat cereal.

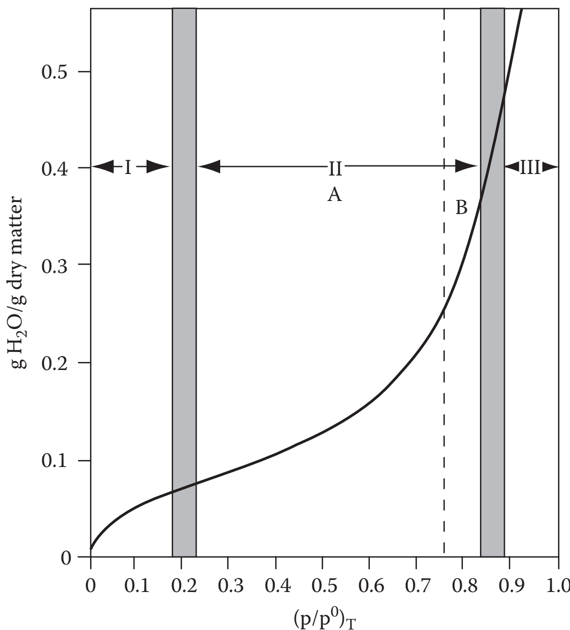


FIGURE 2.26 Generalized moisture sorption isotherm for the low-moisture segment of a food at 20°C. (From Fennema, O.R., Water and ice, in: *Food Chemistry*, 3rd edn., Fennema, O.R. (ed.), Marcel Dekker, Inc., New York, 1996.)

populations or coupled states of water. Zone I represents the region up to the first inflection point (commonly referred to as the "knee") in the sorption curve. This inflection point occurs typically when the water activity of the food reaches about 0.2–0.25. The energy status of water in zone I varies as the water activity (and water content) of the food moves from very low initial value (~0.02) in dry food to about 0.2–0.25. Since $\Delta G_w = RT \ln a_w$, the free energy change of water at the

water content corresponding to $a_w = 0.02$ is about −9.68 kJ/mol at 25°C. This water can be deemed to be tightly bound to the food material as its ΔG_w is about 3.9 times k_BT. At the high-moisture end of zone I, where $a_w = 0.2$, the free energy change is about −3.98 kJ/mol (or about $1.6k_BT$). Thus, even within zone I, water molecules in a food have different energy levels ranging from −9.68 kJ/mol to −3.98 kJ/mol. Nevertheless, the average energy status suggests that the amount of water corresponding to zone I is fairly tightly bound to the food material. This water is most likely bound to ionic groups via ion–dipole interactions (especially at the lower end of zone I) and also to some polar groups via dipole–dipole interactions (at the higher end of zone I). The water content of foods in this region is typically about 7% (g H_2O/g dry food). A food material in zone I is essentially dry and free flowing. Because of limited translational and rotational motions (required for ice formation), water in zone I remains unfreezable even at −40°C.

The water content corresponding to the high-moisture end of zone I is known as "BET monolayer" water, named after Brunauer, Emmett, and Teller [25]. At this water content, not all polar groups but only a fraction of polar groups that have high affinity and steric accessibility to water in a food are hydrated. Thus, the BET monolayer represents an unsaturated monolayer of water confined only to high-affinity binding sites. Hydration of the remaining polar groups in the food material commences when the water content (or water activity) is further increased to levels corresponding to zone II. In zone II, as the moisture content is increased, the water activity increases from 0.2 to up to 0.85. The ΔG_w increases (becomes more positive) from −3.98 kJ/mol at the lower end of zone II to about −0.4 kJ/mol at the high-moisture end of zone II at 25°C. The zone II potentially has two subpopulations: The water population in zone II-A is mainly associated with food molecules via hydrogen-bonding interactions, and the water population corresponding to zone II-B is water weakly interacting with nonpolar surfaces on food molecules via dipole–induced-dipole interactions.

As in the case of zone I population, most of the water in zone II also is unfreezable at −40°C even though its average free energy is higher than that of zone I population. When the total water content of a food is close to the boundary of zone II (which also includes zone I water), the water is primarily in the form of a saturated monolayer on food molecules (e.g., proteins and polysaccharides), covering all ionic, polar, and nonpolar surfaces. Water molecules can exchange from one binding site to another binding site across zones I and II, but the saturated monolayer contains two distinct subpopulations of water at all times, one corresponding to zone I and the other corresponding to zone II. The thermodynamic properties of these two water populations remain distinct at all times. Because zone II water population is weakly bound to food molecules, it is more mobile than zone I population but significantly less mobile than bulk water. This high mobility enables zone II water population to act as a plasticizer, causes swelling of food matrix (and thereby causing exposure of buried hydrogen-bonding sites to water), and decreases the glass transition temperature (T_g) of food materials.

As the water content is gradually increased above the zone I–zone II boundary, the glass transition temperature (T_g) of the food materials gradually decreases, and at the water content close to the boundary between zone II and zone III, the T_g of the sample becomes equal to the sample (ambient) temperature. Thus, the boundary between zone II and zone III is the critical water content at which glass–rubber transition of the material commences at ambient temperature. The glass–rubber transition is characterized by a large decrease in viscosity, and, as a result, the food material begins to flow (melt). As the water content moves further into zone III, the molecular mobility (which is inversely proportional to viscosity) of water and food constituents increases by several orders of magnitude. The critical water activity at which this quantum leap in molecular mobility occurs in most foods is about 0.75–0.85. The rates of chemical reactions and changes in physical (textural) properties, which were subdued in zones I and II because of constrained molecular mobility, increase in zone III. Some of these changes may be desirable and others may not. The greater mobility of water also promotes growth of microorganism in zone III as water becomes available to take part in biological processes. As the water content is increased further beyond the

lower end of zone III, multilayers of water are formed around food molecules (such as proteins) and macromolecules begin to dissolve into solution as the water activity approaches close to 1.

The physical properties of water at various zones in the water sorption isotherm are summarized in Table 2.5 [26]. It should be emphasized that although water activity (and therefore the free energy of water) increases in a sigmoidal (nonlinear) manner as a function of the water content of foods, populations of water with low free energies (corresponding to zones I and II) do exist even at very high moisture content. However, the amounts of these "bound" water populations constitute only a small fraction of the total water content, so that the average thermodynamic property of water in a food essentially approaches that of bulk water at high water content.

2.6.3.1 Summary

- MSI is the relationship between water activity and the moisture content (g water/g dry matter) at equilibrium of a food material at constant temperature and pressure.
- Most food materials exhibit sigmoidal-type MSI, which can be divided into three regions. The energy status water varies in these three regions. The water associated with the food materials in region I is unfreezable at −40°C and is not available for chemical reactions. The water in region II is also unfreezable but more mobile than in region I and therefore can initiate glass–rubber transitions in foods. At the water content corresponding to the high end of region II and beyond, the greater mobility of water favors chemical, physical, and microbiological changes in foods.

2.6.4 Water Activity and Food Stability

A considerable amount of studies have convincingly shown that food stability (both physical/chemical and microbiological) is influenced by a_w. By understanding the relationship between rates of these processes and water activity, we can use water activity as a technological tool to control chemical/physical/biological changes in foods.

With respect to food safety and stability, we can identify two critical threshold points in the moisture absorption isotherm. These are the zone I/zone II and zone II/zone III boundaries. The water activity of foods at these boundaries is typically 0.20–0.25 and 0.75–0.85, respectively. At $a_w \leq 0.25$ (zone I) food materials are dry and essentially free flowing dry powders; the lack of molecular mobility inhibits the rates of most of the chemical reactions (except lipid oxidation), and unavailability of water to take part in biological processes arrests growth of microorganisms. Thus, at $a_w \leq 0.25$ foods are very safe and stable, but most of them would not be edible (excluding crackers and chips). On the other hand, at $a_w \geq 0.8$, foods enter into the high-moisture/rubbery phase (zone III), where molecular mobility of water and other food constituents increase exponentially, favoring increase in the rates of undesirable chemical reactions and microbial growth, and therefore foods are chemically very unstable and microbiologically unsafe at $a_w \geq 0.8$. Thus, the intermediate water activity region, that is, at $0.25 < a_w < 0.8$, which is also known as the intermediate-moisture range, is the only region where one can manipulate the rates of chemical and physical changes and microbiological safety by fine-tuning the water content and water activity of foods. Foods that fall within this region are known as "intermediate-moisture foods."

2.6.5 Intermediate-Moisture Foods

Some examples of water activity versus food stability relationships in common foods are shown in Figures 2.27 through 2.31. Shown in Figure 2.27a is the effect of water activity on the rate of lipid oxidation in potato chips at 35°C. The data show that the rate of lipid oxidation is relatively high at very low and very high a_w but reaches a minimum value at $a_w \approx 0.4$. This abnormal behavior has been explained as follows [27]: In the very dry state, there is no barrier for oxygen collision with lipids leading to oxidation. However, as the water content is gradually increased

TABLE 2.5
Protein Hydration Levels

	Increasing Water Content in System					
	Constitutional Water[a]		Hydration Shell (≤3 Å from Surface)		Bulk-Phase Water	
Property					**Free[b]**	**Entrapped[c]**
Relative vapor pressure (p/p^0)	<0.02 p/p^0	0.02–0.2 p/p^0	0.2–0.75 p/p^0	0.75–0.85 p/p^0	>0.85 p/p^0	>0.85 p/p^0
Isotherm "zone"[d]	Extreme left, zone I	Zone I	Zone IIA	Zone IIB	Zone III	Zone III
Mol H_2O/mol dry protein	<8	8–56	56–200	200–300	>300	>300
g H_2O/g dry protein (h)	<0.01	0.01–0.07	0.07–0.25	0.25–0.58	>0.58	>0.58
Weight percent based on lysozyme (%)	1	1–6.5	6.5–20	20–27.5	>27.5	>27.5
Water characteristics: structure	Critical part of native protein structure	Water interacts primarily with charged groups (~2HOH/group). At 0.07 h transition in surface water organization; appearance of clusters associated with completion of charged group hydration	Water interacts primarily with polar surface groups (~1 HOH/polar site). Water clusters center on charged polar sites. Clusters fluctuate in size and arrangement. At 0.15 h long range connectivity of surface water is achieved	At 0.25 h water starts to condense on to weakly interacting unfilled protein surface patches. At 0.38 h "monolayer" of water covers the entire surface of the protein. Distinct water phase begins to appear, location of glass-rubber transition		
Water characteristics: thermodynamic transfer properties[e]						
ΔG (kJ/mol)	>|−6|	−6	−0.8	Close to bulk		
ΔH (kJ/mol)	>|−17|	−70	−2.1	Close to bulk		
Residence time (s) (approximate mobility)	10^{-2}–10^{-8}	<10^{-8}	<10^{-9}	10^{-9}–10^{-11}	10^{-11}–10^{-12}	10^{-11}–10^{-12}

(*Continued*)

TABLE 2.5 (*Continued*)
Protein Hydration Levels

	Increasing Water Content in System					
	Constitutional Water[a]		Hydration Shell (≤3 Å from Surface)		Bulk-Phase Water	
Property					**Free[b]**	**Entrapped[c]**
Freezability	Unfreezable	Unfreezable	Unfreezable	Unfreezable	Normal	Normal
Solvent power	None	None	Slight	Moderate	Normal	Normal
Protein characteristics: structure	Folded state, stable	Amorphous regions begin to be plasticized by water	Further plasticization of amorphous regions			
Protein characteristics: mobility (reflected in enzyme activity)	Enzyme activity negligible	Enzyme activity negligible	Proton exchange increases from 1/1000 at 0.04 h to full solution rater at 0.15 h. Some enzymes develop activity between 0.1 and 0.15 h	At 0.38 h lysozyme activity is 0.1 than in dilute solution	Maximum activity	Maximum activity

Sources: Data, largely on lysozyme, from Franks, F., in: *Characteristics of Proteins*, Franks, F. (ed.), Humana Press, Clifton, NJ, 1988, pp. 127–154; Lounnas, V. and Pettitt, B.M., *Proteins: Struct. Funct. Genet*., 18, 133, 1994; Rupley, J.A. and Careri, G., *Adv. Protein Chem*., 41, 37, 1991; Otting, G. et al., *Science*, 254, 974, 1991; Lounnas, V. and Pettitt, B.M., *Proteins: Struct. Funct. Genet*., 18, 148, 1994.

Note: Constitutional water is assumed to be present in the dry protein at the onset of the hydration process. Water is first absorbed at sites of ionized carboxylic and amino side chains, with about 40 mol water/mol lysozyme associating in this manner. Further water absorption results in gradual hydration of less attractive sites, mainly amide carbonyl groups of the protein backbone. At 0.38 h monolayer coverage is achieved through water associating with those surface sites that are still less attractive. At this stage in hydration of the protein, there is, on average, 1 HOH/20 $Å^2$ of protein surface. At water content above 0.58 h the protein is considered fully hydrated.

[a] Water molecules that occupy specific locations in the interior of the solute macromolecule.
[b] Macroscopic flow physically unconstrained by a macromolecular matrix.
[c] Macroscopic flow physically constrained by a macromolecular matrix.
[d] See Figure 2.26.
[e] Partial molar values for transfer of water from bulk phase to hydration shell.

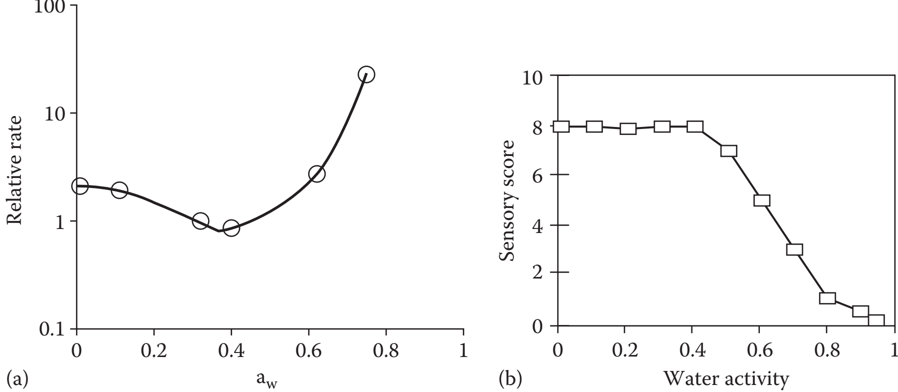


FIGURE 2.27 (a) Rate of lipid oxidation and (b) loss of sensory (crispiness) quality as a function of water activity in potato chips at 35°C. (From Quast, D.G. and Karel, M., *J. Food Sci.*, 37, 584, 1972.)

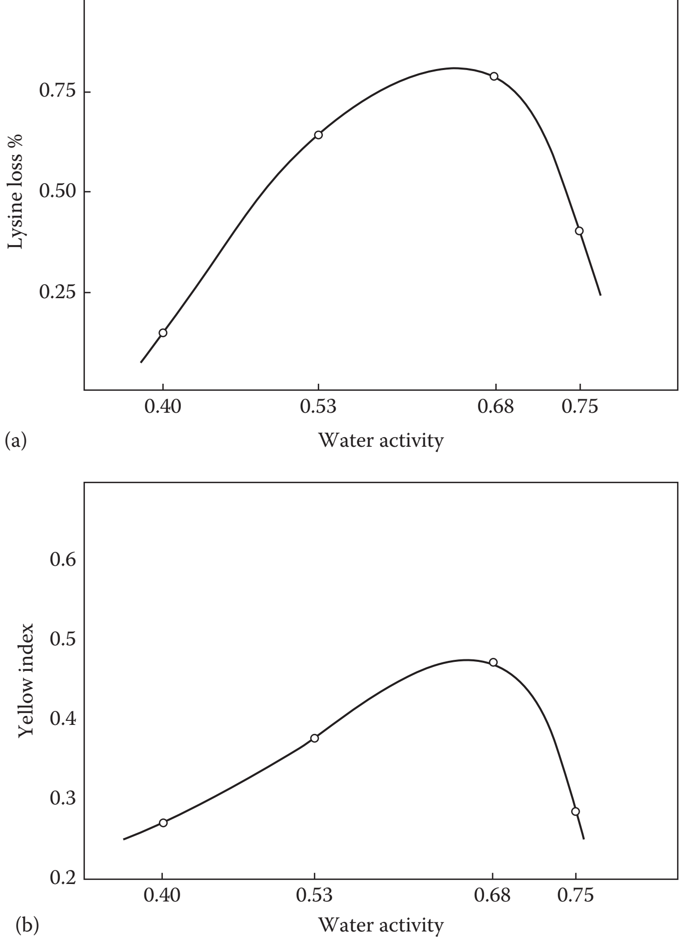


FIGURE 2.28 Effect of water activity on Maillard browning in milk powder stored at 40°C for 10 days. (a) Color change and (b) lysine loss as a result of Maillard browning. (From Loncin, M. et al., *J. Food Technol.*, 3, 131, 1968.)

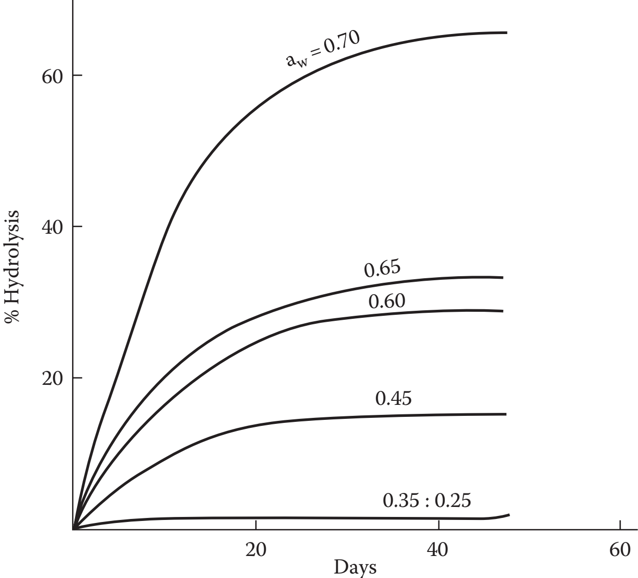


FIGURE 2.29 Effect of water activity on enzymatic hydrolysis of lecithin in barley malt. (From Acker, L., *Food Technol.*, 23, 1257, 1969.)

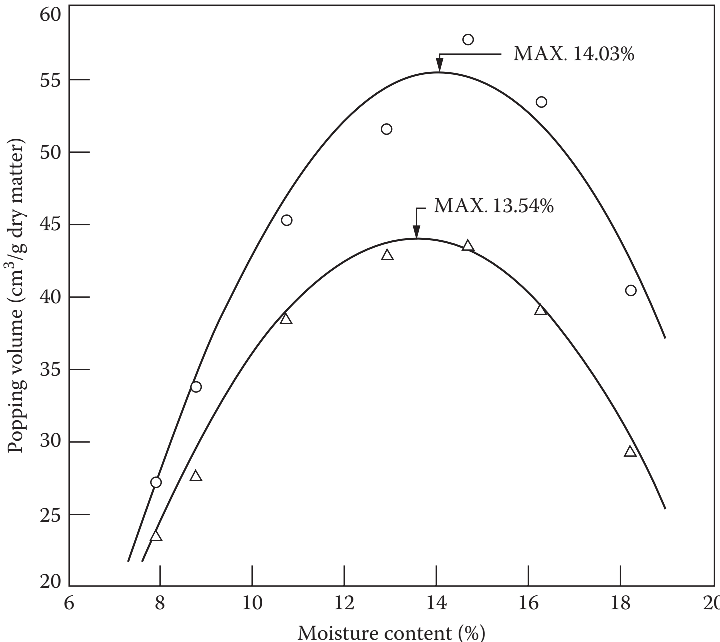


FIGURE 2.30 Effect of water content on popping volume of popcorn. ○, popping in air; △, popping in oil. (From Metzger, D.D. et al., *Cereal Chem.*, 66, 247, 1989.)

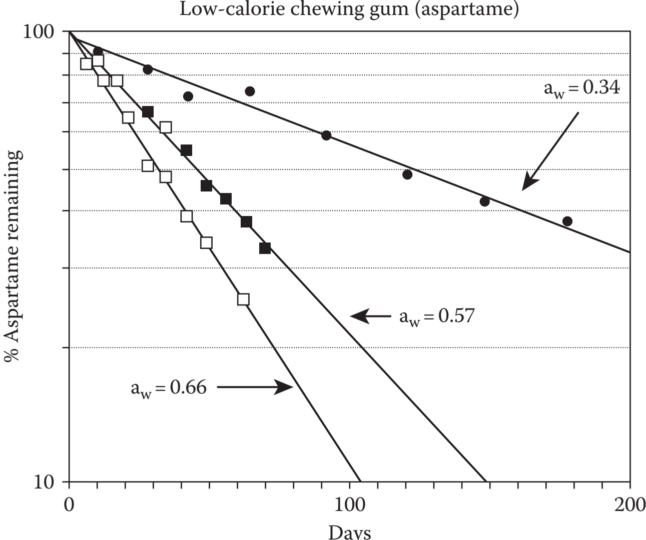


FIGURE 2.31 Effect of water activity on the rate of degradation of aspartame in chewing gum. (From Bell, L.N. and Labuza, T.P., Aspartame degradation as a function of water activity, in: *Water Relationships in Foods: Advances in the 1980s and Trends for the 1990s*, Levine, H. and Slade, L. (eds.), Springer Science and Business Media, New York, 2013, pp. 337–347.)

up to the BET monolayer coverage ($a_w \approx 0.4$), water binds to lipid hydroperoxides and interfere with their breakdown to free radicals, a step necessary for the propagation of lipid oxidation. In addition, the BET monolayer water also hydrates metal ions, such as Fe^{2+} and Cu^{+}, and decreases their effectiveness as catalysts. The increase in lipid oxidation rate at $a_w > 0.4$ is due to greater molecular mobility, which increases the collisional frequency of lipids and metal catalysts. Thus, water activity manipulates a complex set of chemical processes that cause lipid oxidation in low-moisture foods. Shown in Figure 2.27b is the effect of water activity on the crispiness (sensory score) of potato chips. It should be noted that the crispiness score also decreases above $a_w \approx 0.4$, which agrees with the fact that greater molecular mobility of water above the BET monolayer coverage (i.e., $a_w > 0.4$ in this case) causes plasticization and swelling of the microstructure of potato chip and alters its textural properties. It is interesting to note that both increase in the lipid oxidation rate and the loss of crispiness occur at about $a_w \approx 0.4$, suggesting that these two processes are interconnected.

Water activity influences the Maillard reaction in foods [28]. Shown in Figure 2.28a is loss of lysine as a function of water activity in milk powder stored at 40°C for 10 days [29]. Maximum loss of lysine occurs at $a_w \approx 0.65$. This loss is due to Maillard browning reaction (also known as carbonyl-amine reaction) between lactose, which is a reducing sugar in milk powder, and the amino group of lysine residues in milk proteins. The first reaction step in Maillard browning is Schiff base formation, which is a reversible reaction.

$$P{-}NH_2 + R{-}CHO \leftrightarrows P{-}NH{=}CH{-}R + H_2O \quad (2.25)$$

Because water is one of the products of this initial reaction step, the rate of this step is influenced by water activity of the sample. Accordingly, lysine loss is very low at $a_w < 0.4$, where collisional frequency between lactose and protein amino groups is low because of hindered molecular mobility. As a_w is increased, the increase in molecular mobility increases the reaction rate and it reaches a maximum at $a_w \approx 0.65$. At $a_w > 0.65$, the excess amount of water in the food material shifts

the equilibrium of the reaction (Equation 2.25) to the left, causing a decrease in the rate of the Maillard reaction. Shown in Figure 2.28b is the extent of brown discoloration in milk powder as a function of water activity. The correspondence between the extent of loss of lysine and increase in brown discoloration as a function of water activity confirms that these two are interrelated.

Shown in Figure 2.29 is the effect of water activity on enzymatic hydrolysis of lecithin (phospholipids) in barley malt [30]. It should be noted that the rate of enzymatic hydrolysis is negligible up to $a_w \approx 0.35$ but increases rapidly above $a_w \approx 0.4$. Other examples of water activity (or water content)–dependent popping volume of popcorn [31] and aspartame degradation in chewing gum [32] are shown in Figures 2.30 and 2.31.

Water activity affects growth of microorganisms in foods. The critical water activity needed for growth depends on the type of organism (see Table 2.6). A summary of the relationship between water activity and rates of various chemical, enzymatic, and biological processes in foods is

TABLE 2.6
Potential for Growth of Microorganisms in Food at Different Relative Vapor Pressures

Range of p/p^0	Microorganisms Generally Inhibited by Lowest p/p^0 of the Range	Foods Generally within This Range of p/p^0
1.00–0.95	*Pseudomonas*, *Escherichia*, *Proteus*, *Shigella*, *Klebsiella*, *Bacillus*, *Clostridium perfringens*, some yeasts	Highly perishable (fresh) foods, canned fruits, vegetables, meat, fish, and milk; cooked sausages and breads; foods containing up to 7% (w/w) sodium chloride or 40% sucrose
0.95–0.91	*Salmonella*, *Vibrio parahaemolyticus*, *Clostridium botulinum*, *Serratia*, *Lactobacillus*, some molds, yeasts (*Rhodotorula*, *Pichia*)	Some cheeses (Cheddar, Swiss, Muenster, Provolone), cured meats (ham), some fruit juice concentrates, foods containing up to 12% (w/w) sodium chloride or 55% sucrose
0.91–0.87	Many yeasts (*Candida*, *Torulopsis*, *Hansenula*, *Micrococcus*)	Fermented sausages (salami), sponge cakes, dry cheeses, margarine, foods containing up to 15% (w/w) sodium chloride or saturated (65%) sucrose
0.87–0.80	Most molds (mycotoxigenic penicillia), *Staphylococcus aureus*, most *Saccharomyces* (*bailii*) spp., *Debaryomyces*	Most fruit juice concentrates, sweetened condensed milk, chocolate syrup, maple and fruit syrups; flour, rice, pulses of 15%–17% moisture content; fruit cake; country style ham, fondants
0.80–0.75	Most halophilic bacteria, mycotoxigenic aspergilla	Jam, marmalade, marzipan, glace fruits, some marshmallows
0.75–0.65	Xerophilic molds (*Aspergillus chevalieri*, *A. candidus*, *Wallemia sebi*), *Saccharomyces bisporus*	Rolled oats of 10% moisture content; grained nougats, fudge, marshmallows, jelly, molasses, raw cane sugar, some dried fruits, nuts
0.65–0.60	Osmophilic yeasts (*Saccharomyces rouxii*), few molds (*Aspergillus echinulatum*, *Monascus bisporus*)	Dried fruits of 15%–20% moisture content, toffees and caramels, honey
0.60–0.50	No microbial proliferation	Pasta of 12% moisture content, spices of 10% moisture content
0.50–0.40	No microbial proliferation	Whole egg powder of 5% moisture content
0.40–0.30	No microbial proliferation	Cookies, crackers, bread crusts, etc., of 3%–5% moisture content
0.30–0.20	No microbial proliferation	Whole milk powder of 2%–3% moisture content; dried vegetables of 5% moisture content; corn flakes of 5% moisture content, country style cookies, crackers

Source: Reid, D.S. and Fennema, O., Water and ice, in: Damodaran, S., Parkin, K.L., and Fennema, O. (eds.), *Fennema's Food Chemistry*, 4th edn., CRC Press, Boca Raton, FL, 2008.

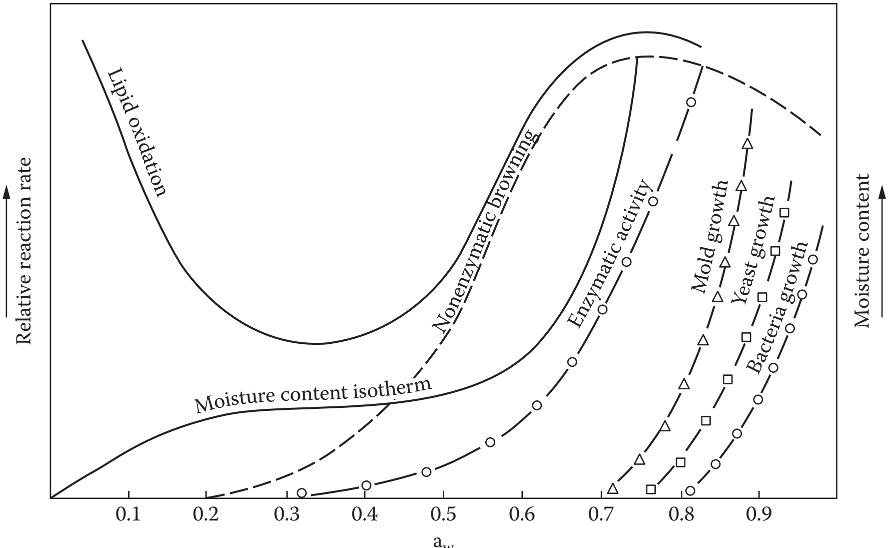


FIGURE 2.32 Relationships among relative water vapor pressure, food stability, and sorption isotherm. (From Labuza, T.P. et al., *J. Food Sci*., 37, 154, 1972.)

presented in Figure 2.32. In general, the rates of chemical and enzymatic reactions that require water as a reactant (e.g., aspartame degradation, lecithin hydrolysis, and other hydrolytic degradations) gradually increase once the food material enters the intermediate water activity range (zone II) and accelerate in zone III where highly mobile water population is available. On the other hand, when water is one of the products of the reaction (as in the case of Maillard reaction), the rates of those chemical reactions exhibit a maximum in the intermediate water activity range (zone II) as a result of mutually competing processes. When water is neither a product nor a reactant (e.g., lipid oxidation), the rates of those reactions are mainly dependent on molecular mobility, and therefore the rates of those reactions gradually increase in zone II and accelerate in zone III. In the case of microorganisms (mold, yeast, and bacteria) that require water population with molecular mobility close to that of free water, their growth in a food material occurs only at $a_w > 0.7$.

From the earlier discussions, chemical, physical, and microbiological stability of foods is maximum in the range of $a_w = 0.2 - 0.4$. However, food materials are essentially dry and gritty at this water activity range and therefore they are not edible. On the other hand, a_w in the range of 0.6–0.8, the moisture content is high enough to make foods edible. Foods having water activity in the range of 0.6–0.8 (approximately 15%–30% moisture content on dry weight basis) are often referred to as "intermediate-moisture foods." These foods are shelf stable without refrigeration, possess desirable texture, and require less packaging protection. Growth of bacteria and yeast is essentially inhibited, but some mold might grow at this water activity range. The mold growth could be controlled by adjusting the pH to <4 and/or by adding antifungal agents, such as potassium sorbate.

2.6.6 BET Monolayer Determination

As discussed earlier, the BET monolayer of water in most foods occurs in the water activity range of 0.2–0.4. Because foods are very stable at and below the critical water content corresponding to the BET monolayer, it can be used as a reference point to predict food stability. Two empirical methods are available to estimate the BET monolayer value of a food from its MSI.

The first is the BET equation [25]

$$\frac{a_w}{m(1-a_w)} = \frac{1}{m_m C_B} + \frac{(C_B - 1)}{m_m C_B} a_w \tag{2.26}$$

where

a_w is the water activity

m is the water content at that water activity

m_m is the water content at the BET monolayer

C_B is an energy constant related to the difference between the chemical potential of pure water in the bulk state and in the monolayer

According to the linear form of the BET equation (Equation 2.26), a plot of $a_w/m(1 - a_w)$ versus a_w should be a straight line with a slope of $(C_B - 1)/m_m C_B$ and an intercept of $1/m_m C_B$. The BET monolayer water content m_m can be determined from the slope and intercept values as

$$m_m = \frac{1}{\text{Intercept} + \text{Slope}} \tag{2.27}$$

However, one of the shortcomings of the BET equation (Equation 2.26) is that in most cases it is linear only up to $a_w \approx 0.4$ and abruptly deviates from linearity above 0.4. Since the linear portion of the curve constitutes data set from only a limited region of the MSI, the m_m value determined from the BET equation is not reliable although it provides a reasonable estimate in some cases.

As an example, the sorption isotherm of wheat gluten is shown in Figure 2.33 [33] and the BET plot of the data is shown in Figure 2.34. It should be noted that the BET plot is linear only up to about $a_w \approx 0.5$, and at $a_w > 0.5$ it deviates from linearity with an upswing in the plot. In the example shown in Figure 2.34, the m_m value determined from the slope and intercept of the plot is 0.052 g water/g dry gluten. The water activity of gluten at this BET monolayer water content is about 0.3.

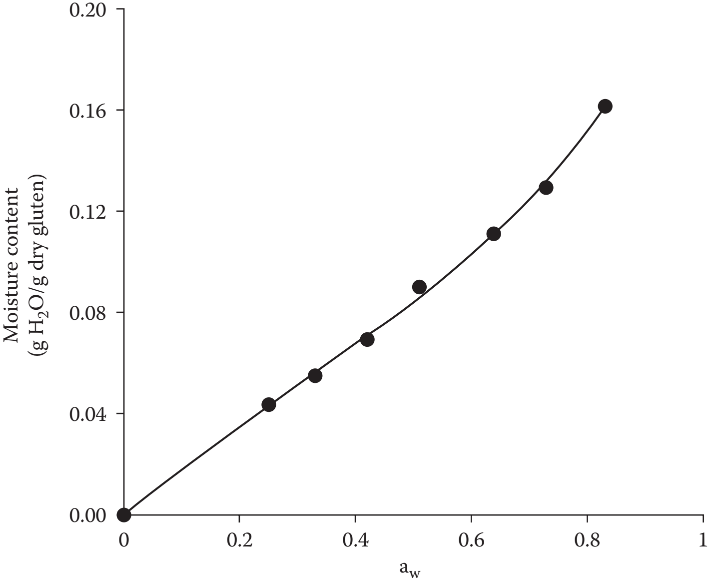


FIGURE 2.33 Moisture sorption isotherm of gluten at 25°C. (From Bock, J.E. and Damodaran, S., *Food Hydrocolloid.*, 31, 146, 2013.)

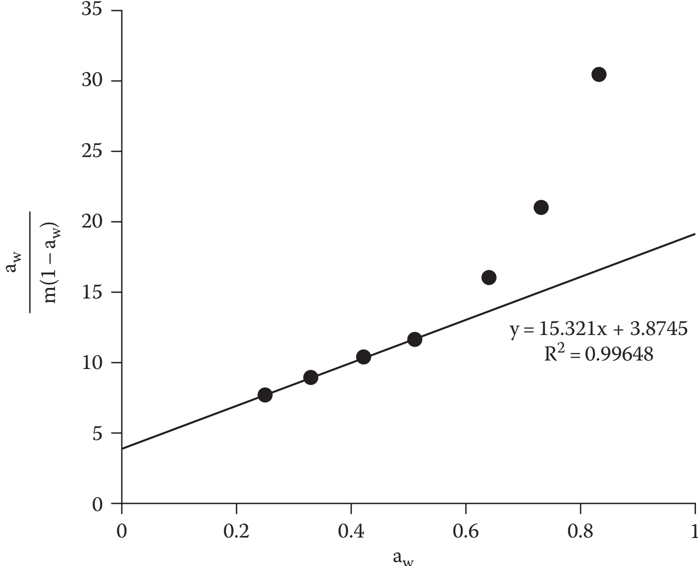


FIGURE 2.34 BET plot of the data shown in Figure 2.33. Note that deviation from linearity of the plot occurs at about $a_w = 0.5$.

It should be noted that these values would be different for different food materials, depending on their MSIs, which is dictated by their composition.

Another model, known as the GAB model, developed by Guggenheim [34], Anderson [35], and De Boer [36], to predict the critical water content is a modified version of the BET equation. It introduces a second energy constant to account for multilayer adsorption at higher water content. The linear form of the GAB equation is

$$\frac{a_w}{m(1-ka_w)} = \frac{1}{m_{1,G}kC_G} + \frac{(C_G-1)}{m_{1,G}C_G}a_w \tag{2.28}$$

where k and C_G (the subscript G denotes the GAB model) are the energy constants. A plot of the function on the left-hand side of Equation 2.28 against a_w should be linear, provided a suitable k-value is chosen so that it provides a linear fit with the best correlation coefficient (R^2) of experimental isotherm data [37]. The value of k lies between 0.5 and 0.9 for most food materials. When $k = 1$, the GAB equation becomes the BET equation. Shown in Figure 2.35 is a GAB plot of the MSI data of wheat gluten at three k-values.

It should be noted that, based on the R^2 values, the best linear fit of the data occurs when $k = 0.7$. Above and below this value, the GAB plot takes an upswing and downswing, respectively. At the correct k-value, the values of C_G and $m_{1,G}$ can be obtained from the relation

$$C_G = \frac{x}{ky} + 1 \tag{2.29}$$

and

$$m_{1,G} = \frac{1}{kC_Gy} \tag{2.30}$$

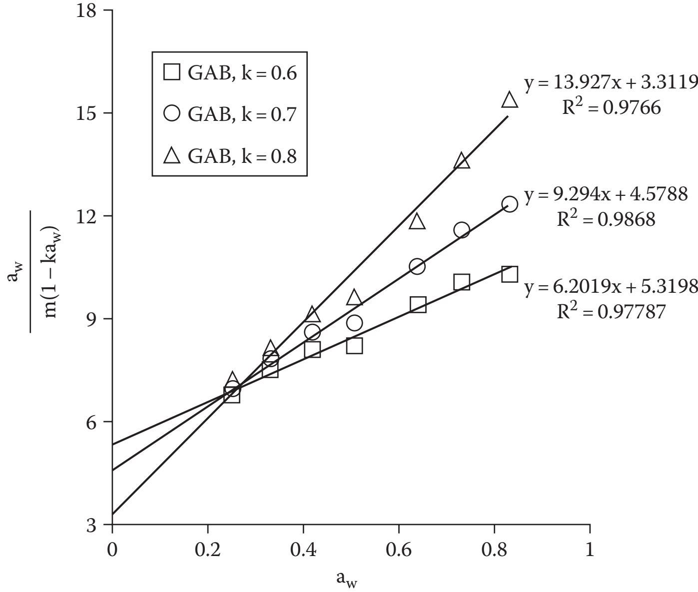


FIGURE 2.35 GAB plot of the data shown in Figure 2.33 at three values of k (see Equation 2.27). The best fit of the data occurs at k = 0.7 (R^2 = 0.9868).

where x and y are the slope and intercept, respectively, of the GAB plot. In the example shown in Figure 2.35 for wheat gluten, the value of $m_{1,G}$ for gluten is 0.08 g H_2O/g dry gluten, which is higher than that derived from the BET plot, and the computed value of C_G is 3.9 at k = 0.7.

Several modifications to the GAB equation have been proposed to improve the goodness of fit of experimental isotherm data up to close to $a_w = 1$ [38–40]; however, for all practical purposes, the original GAB equation provides reliable BET monolayer value.

2.6.6.1 Summary

- The BET monolayer represents the water content at the high-moisture end of zone I. It represents an unsaturated monolayer of water bound to high-affinity groups, for example, ionic groups, in food materials. Foods at or below this moisture content are very stable. Thus, the BET monolayer value of a food is often used as a reference point to predict the stability of foods.

2.6.6.2 Temperature and Pressure Dependence

Water activity of foods is temperature dependent. In most food products, water activity increases with temperature at constant moisture content. This generally causes a shift to the right in the MSI, as shown in Figure 2.36 for potatoes [41]. It should be noted that at constant moisture content, for example, at 0.1 g/g dry matter, the water activity of potato starch shifts from about 0.32 at 20°C to about 0.42 at 40°C and to about 0.67 at 80°C. This is conceivable, because since ion–dipole (water) and dipole–dipole interactions are exothermic in nature, the escaping tendency (fugacity) of water in the food material increases as the temperature is increased. The extent of the shift in the MSI for a given change in temperature reflects the food materials response to temperature fluctuations. This has important practical consequences. For instance, if the initial water activity of a food material at 20°C is 0.7, the product will be stable against microbial growth. However, if the temperature of the product fluctuates between 25°C and 45°C in a warehouse where the product is stored or during transit, the product's water activity could easily move up above 0.8, potentially leading to microbial growth and acceleration of chemical and enzymatic reactions in the product.

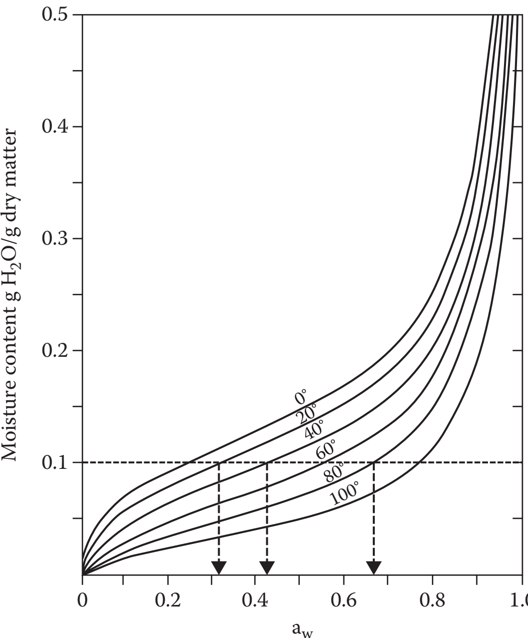


FIGURE 2.36 Moisture sorption isotherms for potatoes at various temperatures. The arrows indicate water activity values at three different temperatures at constant water content. (From Gorling, P., Physical phenomena during the drying of foodstuffs, in: *Fundamental Aspects of the Dehydration of Foodstuffs*, Society of Chemical Industry, London, U.K., pp. 42–53, 1958.)

The relationship between water activity of a food at constant moisture content and temperature is best described by the following Clausius–Clapeyron equation:

$$\frac{d(\ln a_w)}{d(1/T)} = \frac{-\Delta H_s}{R} \tag{2.31}$$

where

T is the temperature
ΔH_s is the isosteric heat of sorption
R is the gas constant (8.314 J/mol/K)

According to Equation 2.31, a plot of ln a_w versus 1/T should be linear at constant water content and the heat of water sorption (ΔH_s) of the food material can be determined from the slope of linear regression of the data. The ln a_w versus 1/T plot is usually linear over a significant temperature range at constant moisture content for most foods. However, ΔH_s is a function of moisture content; it decreases as the moisture content is increased (Figure 2.37). It represents the energy needed to desorb water from a food material. Variations in ΔH_s with moisture content of a food material reflect the difference between the water–food material interaction and the water–water interaction energies. On the other hand, differences in ΔH_s of various food materials at same moisture content would reflect differences in the magnitude of water–food material interaction energies. Upon integration, Equation 2.31 takes a more useful form:

$$\ln\left(\frac{a_{w2}}{a_{w1}}\right) = \frac{-\Delta H_s}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{2.32}$$

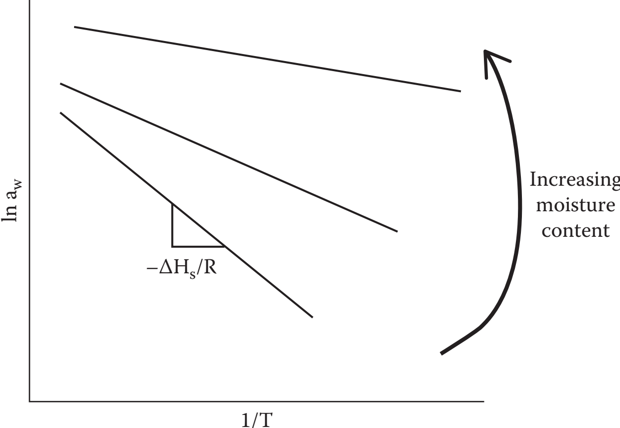


FIGURE 2.37 Typical plot of ln a_w versus 1/T (according to Equation 2.30) for food materials. Note that the slope of the plot decreases with the increase of the moisture content.

where a_{w1} and a_{w2} are the water activities at temperatures T_1 and T_2, respectively. This is a useful form of the Clausius–Clapeyron equation to predict temperature-dependent changes in a_w of a food at constant moisture content. If ΔH_s of a food material at a given water content is known and if a_{w1} is the initial water activity at temperature T_1, then the water activity of the sample at any other temperature T_2 can be predicted using Equation 2.32.

Pressure also affects the water activity of a food at constant moisture content; however, compared to the temperature effect, the pressure effect is negligible under practical situations encountered during food handling. The pressure–water activity relationship at constant moisture content and temperature is given by

$$\ln\left(\frac{a_{w2}}{a_{w1}}\right) = \frac{\overline{V_L}}{RT}(P_2 - P_1) \tag{2.33}$$

where

$\overline{V_L}$ is the molar volume of water

a_{w1} and a_{w2} are the water activities at pressures P_1 and P_2, respectively

2.6.7 Hysteresis

MSIs of food materials can be determined following two different approaches: One involves exposing a high-moisture food material to various atmospheres of decreasing relative humidity and measuring the equilibrium water content and water activity at each relative humidity (a_w). This is known as moisture desorption isotherm. In the other approach, a completely dry material is exposed to increasing relative humidity (a_w) atmospheres and measuring equilibrium water content after the exposure. This is known as moisture resorption isotherm. Although ideally the shapes of desorption and resorption (or adsorption) isotherms are supposed to be identical, for a majority of food materials, these two isotherms are not superimposable. This non-superimposability of desorption and resorption isotherms is known as "hysteresis." The desorption isotherm lies above the resorption isotherm for most foods, without exception, as shown in Figure 2.38.

Several qualitative theories have been proposed to explain the hysteresis, which include capillary condensation, chemisorption, phase changes, and morphological changes in cell structure [42,43]. Regardless of the actual mechanism, which may vary depending on the type of food material,

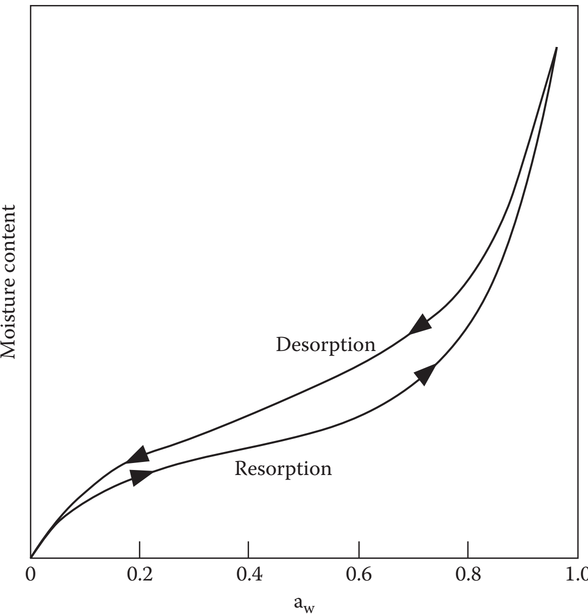


FIGURE 2.38 Hysteresis of moisture sorption isotherm. (From Fennema, O.R., Water and ice, in: *Food Chemistry*, 3rd edn., Fennema, O.R. (ed.), Marcel Dekker, Inc., New York, 1996.)

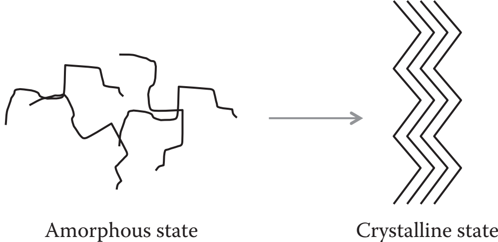


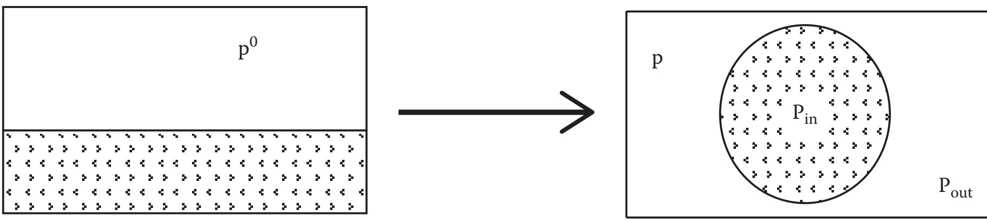
FIGURE 2.39 Schematic representation of transformation of an amorphous material to crystalline state during the desorption process.

the fundamental reason for hysteresis is collapse of capillaries and cellular structures (and possibly phase change in some of the components) during the desorption process (Figure 2.39). The role of capillaries in hysteresis can be explained using the Kelvin equation. Consider a volume of water with a flat surface existing in equilibrium with its vapor phase at vapor pressure p^0. When this water is transformed into a spherical droplet as shown below, because of unfavorable excess interfacial energy, the droplet will tend to shrink to minimize the interfacial area. As a result, the pressure inside (P_{in}) the droplet will increase compared to the outside pressure (P_{out}). At equilibrium, the pressure difference between the inside and outside of the droplet is given by the Laplace equation

$$P_{in} - P_{out} = \Delta P = \frac{2\gamma}{r} \tag{2.34}$$

where

γ is the surface tension of water
r is the radius of droplet



This pressure difference increases the tendency of water to escape from the liquid phase to the vapor phase and therefore the vapor pressure of the system increases from p^0 (for a flat liquid surface) to p (over a convexly curved liquid surface). The free energy change in the vapor (ΔG_v) phase for this transformation process is

$$\Delta G_v = RT\ln\left(\frac{p}{p^0}\right) \tag{2.35}$$

and the free energy change in the liquid phase is

$$\Delta G_L = \int_{P(o)}^{P(o)+\Delta P} V_L dP = V_L \Delta P \tag{2.36}$$

Combining Equations 2.34 and 2.36, we get

$$\Delta G_L = \frac{2\gamma V_L}{r} \tag{2.37}$$

where V_L is the molar volume of water. Since $\Delta G_v = \Delta G_L$ at equilibrium,

$$RT\ln\left(\frac{p}{p^0}\right) = \frac{2\gamma V_L}{r} \tag{2.38}$$

Equation 2.38 is the Kelvin equation for convexly curved liquid surfaces. For a concave liquid surface, that is, liquid meniscus in a capillary, the Kelvin equation can be expressed with a negative sign, that is,

$$RT\ln\left(\frac{p}{p^0}\right) = -\frac{2\gamma V_L}{r} \tag{2.39}$$

According to Equation 2.39, if the radius of curvature of a liquid meniscus is small (i.e., if the capillary diameter in a food material is very narrow), the vapor pressure above the meniscus will be low, and vice versa. This indicates that if capillaries in a food material collapse into large capillaries during the desorption process, then the vapor pressure above those large capillaries will be high, meaning that water condensation on food materials during the resorption process will take place at higher vapor pressure, that is, at higher water activity than during the desorption process, giving rise to hysteresis.

Knowledge of sorption hysteresis of a food material is important for ensuring product safety and stability during storage. For instance, the sorption hysteresis of rice is shown in Figure 2.40 [44]. Note that at any given moisture content, the water activity of rice prepared by desorption is lower than that prepared by resorption. This situation is highlighted in Figure 2.40 for the 15%

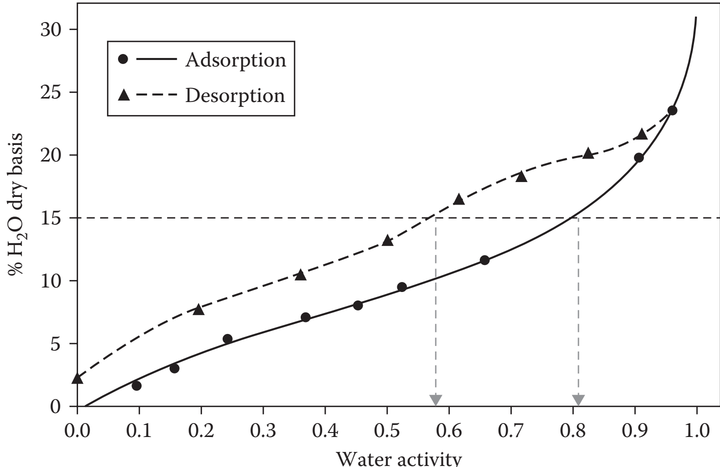


FIGURE 2.40 Moisture sorption hysteresis of rice [44]. The arrows indicate different water activity values at the same moisture content during desorption and resorption processes.

moisture content sample: whereas the water activity of rice in the desorption case is about 0.58, it is about 0.81 in the resorption case. Accordingly, while mold growth is not possible in the sample at $a_w = 0.58$, it will occur at $a_w = 0.81$. Thus, knowledge of moisture content alone is not sufficient, but knowledge of the method used, that is, desorption versus resorption, and the actual water activity of the sample also are important for assessing the microbial safety of a product. In terms of chemical stability, foods prepared by desorption method are less stable than those prepared by resorption because of their inflated matrix and higher moisture content at a given water activity. Labuza et al. [45] reported that the rate of lipid oxidation in several meat products prepared by desorption was much faster than that in products prepared by resorption at a given water activity value. Thus, even though water activity control by the resorption method is expensive than by the desorption method because it involves first complete dehydration of the food followed by resorption to the final desired water activity level, it provides better chemical and physical stability to the food product and therefore the higher cost is justified [45].

2.7 TECHNOLOGICAL CHALLENGES IN INTERMEDIATE-MOISTURE FOODS

2.7.1 Moisture Migration in Composite Foods

While it is fairly easy to control moisture content and water activity of a homogeneous food (such as cookies, crackers, and cheese), it is very complicated in multidomain foods (such as ice-cream cookies and cheese crackers) or in mixtures containing two different food components (such as raisin cereal). Moisture migration from one component to another in multidomain foods and food mixtures can cause chemical changes, alter sensory and physical properties of the components, and thereby affect their stability during storage.

Moisture migration in multidomain foods is not driven by differences in the moisture content but by the differences in water activities of food domains [46]. This also implies that if all the domains in a multidomain food have the same initial water activity, then there would not be moisture migration even if the initial moisture content of each domain were different. The thermodynamic driving force emanates from free energy differences of water in various domains of the food. This is schematically shown in Figure 2.41. If $a_{w,A}$ and $a_{w,B}$ are the initial water activities of domain A (cream) and domain B (cookie), respectively, and if $a_{w,A} > a_{w,B}$, then the free energy of water in domain A will be greater than that in domain B. In a closed system at constant temperature, this free energy

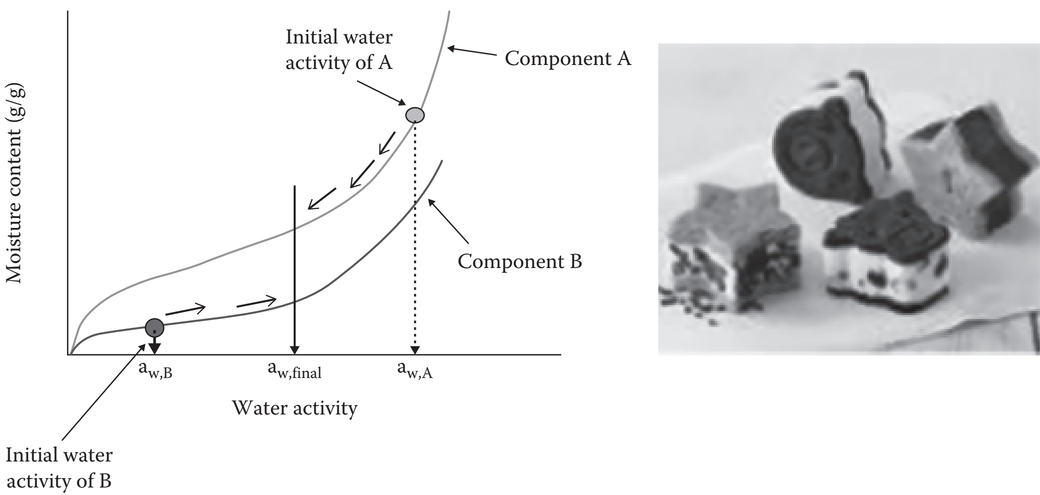


FIGURE 2.41 Schematic illustration of moisture migration in multidomain foods from the high-water-activity region (component) to low-water-activity region.

difference will drive migration of water from the high-water-activity region to the low-water-activity region until the water activity at all locations of the product is same. In other words, during storage, the moisture content and water activity of domain B will slowly increase, whereas those of domain A will decrease with time and both will reach a final water activity as shown in Figure 2.41 at equilibrium. The trajectories of these moisture–water activity movements of A and B will follow the footprints of the MSIs of domains A and B, respectively, as shown in Figure 2.41.

Moisture migration in multidomain foods can cause undesirable changes in foods. For instance, if domain B in Figure 2.41 represents a cookie and if the initial moisture content and water activity confer crispiness to the cookie domain, a shift in water activity from the initial low level to a higher final level at equilibrium might adversely affect the crispiness of the cookie domain. Thus, the ability to predict the final equilibrium water activity of a product and the final moisture content of the domains in the product after equilibrium is useful in developing product formulation strategies to retain product quality during storage.

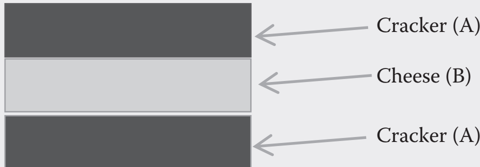
If f_A and f_B are weight fractions of domains A and B in a food system, m_A and m_B are the initial moisture contents (wet basis) of domains A and B, and $a_{w,A}$ and $a_{w,B}$ are the initial water activities of domains A and B, respectively, then the final water activity, $a_{w,final}$, of the two domain food at equilibrium in a closed system is

$$\ln a_{w,final} = \frac{f_A m_A \ln a_{w.A} + f_B m_B \ln a_{w,B}}{f_A m_A + f_B m_B} \tag{2.40}$$

The derivation of Equation 2.40 is presented in Box 2.5. If we set $a_{w,desired}$ as the final water activity target at equilibrium in order to retain certain quality attributes of the product, then one can use the initial f, m, and a_w values of domains A and B as adjustable parameters to achieve equilibrium $a_{w,desired}$. If initial m and a_w values cannot be used as adjustable parameters for practical reasons, then the final $a_{w,desired}$ can be achieved by altering the weight fractions of A and B domains using the equation

$$\frac{W_A}{W_B} = \frac{f_A}{f_B} = \frac{m_B \ln(a_{w,B}/a_{w,desired})}{m_A \ln(a_{w,desired}/a_{w,A})} \tag{2.41}$$

BOX 2.5 MOISTURE MIGRATION IN MULTIDOMAIN FOODS



Consider a cheese cracker sandwich, where A is the cracker domain and B is the cheese domain. If $a_{w,A}$ and $a_{w,B}$ are the initial water activities of A and B, respectively, then the free energy change of water in A and B is

$$\Delta G_{w,A} = \mu_{w,A} - \mu_w^0 = RT \ln a_{w,A} \quad \text{(B2.5.1)}$$

$$\Delta G_{w,B} = \mu_{w,B} - \mu_w^0 = RT \ln a_{w,B} \quad \text{(B2.5.2)}$$

If n_A and n_B are the number of moles of water in A and B, respectively, then

$$n_A \Delta G_{w,A} = n_A RT \ln a_{w,A} \quad \text{(B2.5.3)}$$

$$n_B \Delta G_{w,B} = n_B RT \ln a_{w,B} \quad \text{(B2.5.4)}$$

In a closed system at equilibrium,

$$(n_A + n_B) RT \ln a_{w,Eq} = RT(n_A \ln a_{w,A} + n_B \ln a_{w,B}) \quad \text{(B2.5.5)}$$

Therefore,

$$\ln a_{w,Eq} = \frac{(n_A \ln a_{w,A} + n_B \ln a_{w,B})}{(n_A + n_B)} \quad \text{(B2.5.6)}$$

If W_T is the total weight of the product, W_A and W_B are the weights of A and B, respectively, and m_A and m_B are the moisture contents of A and B (on wet weight basis), then $n_A = (W_A m_A)/18$ and $n_B = (W_B m_B)/18$, and Equation B.2.5.6 becomes

$$\ln a_{w,Eq} = \frac{(W_A m_A \ln a_{w,A} + W_B m_B \ln a_{w,B})}{(W_A m_A + W_B m_B)} \quad \text{(B2.5.7)}$$

Dividing the numerator and the denominator of the right-hand side of the equation by W_T and defining $f_A = W_A/W_T$ and $f_B = W_B/W_T$,

$$\ln a_{w,Eq} = \frac{(f_A m_A \ln a_{w,A} + f_B m_B \ln a_{w,B})}{(f_A m_A + f_B m_B)} \quad \text{(B2.5.8)}$$

where f_A and f_B are the wet weight fractions of A and B in the product. Knowing the initial moisture contents, water activities, and weight fractions of A and B, the equilibrium water activity can be predicted from Equation B.2.5.8. Equation B.2.5.8 can be rearranged as

$$\frac{W_A}{W_B} = \frac{f_A}{f_B} = \frac{m_B \ln(a_{w,B}/a_{w,final})}{m_A \ln(a_{w,final}/a_{w,A})} \tag{B2.5.9}$$

Equation B.2.5.9 is useful in food product development. If the final water activity of a food product is fixed based on sensory and safety criteria, then Equation B.2.5.9 can be used to calculate the weight ratio of A and B in the product required to achieve the final a_w. (Note: In Equations B2.5.8 and B2.5.9, if the weight fractions are expressed on dry weight basis, then the moisture contents also should be expressed on dry weight basis).

where W_A and W_B are dry weights (g) of domains A and B, respectively, in the formulated product. If the individual MSIs of domains A and B are known, then the final moisture content of domains A and B at $a_{w,final}$ can be determined.

Although moisture migration in multidomain foods is driven by water activity gradient in the food, it is a kinetic process and the time to reach equilibrium depends on several factors that influence the rate of water transport in the food system. For multidomain food systems in which the final equilibrium water activity is within the range where chemical, physical, and microbiological stabilities are not a concern, the rate of moisture transport within the system is not critical. However, if the final equilibrium water activity lies in the range where the extent of chemical and physical changes and microbiological safety are unacceptable, then water transport dynamics will affect the shelf life of the product [47].

2.7.2 Phase Transitions in Foods

One of the fundamental assumptions in the water activity concept of food stability is that foods are equilibrium systems, that is, the components of food do not undergo any physical change during storage. This assumption is questionable for a majority of food products, especially intermediate-moisture foods, where some of the components of food products might be in a nonequilibrium state and might be continuously undergoing phase transition during storage. For example, sugars, such as sucrose and lactose, in a food product are more often than not in an amorphous (glassy) state soon after the product has been made. In the amorphous state, sugars exhibit a sigmoidal water sorption isotherm (as shown in Figure 2.42). However, during storage in a closed environment, sugars will undergo spontaneous phase change from the high-energy (unstable) amorphous state to the low-energy (stable) crystalline state. The rate of this phase transition is dependent on the initial water activity of the food product. If the water content of the product is below the BET monolayer ($a_w \approx 0.2$–0.3), the rate of this phase transition will be extremely slow and it may not be of a huge concern in terms of product quality. At high water activity, however, the rate of this phase transition is very fast and may take only few minutes for sugars to undergo complete transformation from an amorphous state to the crystalline state, as shown in Figure 2.42. When the sugar is completely converted to the crystalline state, the shape of the MSI will change from a sigmoidal shape to a J-type isotherm. If the moisture content of the food remains the same, the water activity of the product will increase as some of the water previously bound to the amorphous state is released to free state. This might affect physical and chemical and microbial stabilities of the product.

2.8 MOLECULAR MOBILITY AND FOOD STABILITY

As discussed earlier, typical foods are essentially nonequilibrium systems that continuously, but slowly, undergo chemical and physical changes during storage. These changes include, but not limited to, phase transition in sugars and polymeric materials, protein–protein and protein–polysaccharide

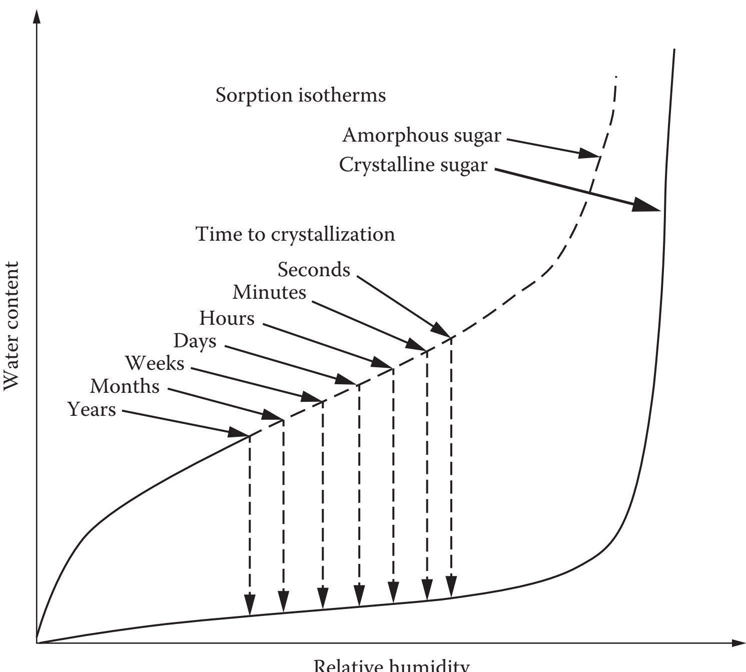


FIGURE 2.42 Schematic illustration of the effect of water activity (and water content) on the rate of phase transition from amorphous state to crystalline state in sucrose–water glass. (From Roos, Y.H., *Phase Transitions in Foods*, Academic Press, New York, 1995.)

association/dissociation reactions, and conformational changes in proteins as a result of chemical reactions with small molecules (such as reducing sugars). Such phase transitions continuously alter the thermodynamic state of water in foods, and therefore water sorption isotherms of real food systems are not true equilibrium isotherms. Thus, food quality and stability predictions based on water activity of a food material alone are not totally reliable as the water activity in the product could change with time even in a closed environment.

At the fundamental level, physical and chemical changes in food materials occur as a result of diffusion of components in the food matrix. Water as a carrier plays a role in this process. In this respect, the water sorption isotherm simply provides information on the critical thermodynamic state of water above which the rate of diffusion of components in a food matrix becomes great enough to cause undesirable chemical and physical changes in the food. If this indeed is the underlying operating principle in the water activity concept, then any other concept that can also predict rates of diffusion-limited chemical and physical processes in foods that are in a nonequilibrium state, especially amorphous (glass) and frozen food materials, would be a better alternative than the equilibrium water activity concept for predicting food quality.

The molecular mobility concept pertains only to rotational and translational motions in a material. It is mainly dependent on the temperature and viscosity of the material: It is directly proportional to temperature and inversely proportional to viscosity. However, since the viscosity of a food material is dependent on water content and its interaction with and plasticizing effect on food constituents, the water content is also one of the primary drivers of molecular mobility in food materials.

2.8.1 Glass Transition

Generally, matter exists in three states: vapor, liquid, and crystalline solid states. When the vapor is cooled, it condenses into a liquid state as a result of van der Waals, hydrogen-bonding, and other noncovalent interactions between molecules (Figure 2.43). In most cases, liquids do not possess a structure and the molecules are in random orientation due to constant kinetic motions driven by thermal energy. When a liquid is cooled slowly, the kinetic motions of molecules slow down, the molecules reorient such that their interaction potential is maximized, and at a particular temperature (freezing point) the liquid is transformed into a crystalline solid. In the crystalline state, molecules are regularly ordered and it represents the lowest energy state of matter. On the other hand, when the liquid is cooled at a faster rate (i.e., faster than the rate of molecular reorientation needed for crystal lattice formation), the liquid suddenly sets into a solid-like state at a temperature much below the freezing point, in which molecules are oriented in a random order lacking any kind of lattice symmetry typical of a crystalline solid. This state of matter is known as glass or amorphous solid, and technically it is a supercooled viscous liquid with highly constrained molecular mobility. Because intermolecular interactions are not fully maximized, the glassy state has a higher free energy than the crystalline solid state, and therefore it is considered to be in a metastable state.

The physics of glass (amorphous solid) formation is presented in Figure 2.44. The line ABCD describes the entropy–temperature relationship during phase transition of a substance from the liquid to crystalline solid state. As the liquid is cooled, at point B (freezing point) the liquid is isothermally converted to the solid state (BC) with a sudden drop in entropy. Continued cooling of the crystalline solid further decreases its entropy (CD). On the other hand, when the liquid is supercooled, the entropy–temperature curve takes a different trajectory (BE), in which

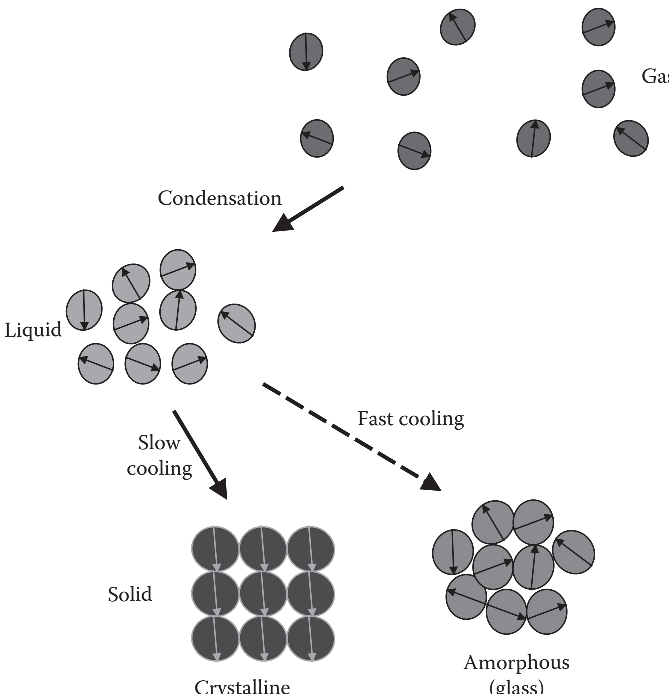


FIGURE 2.43 Various states of matter.

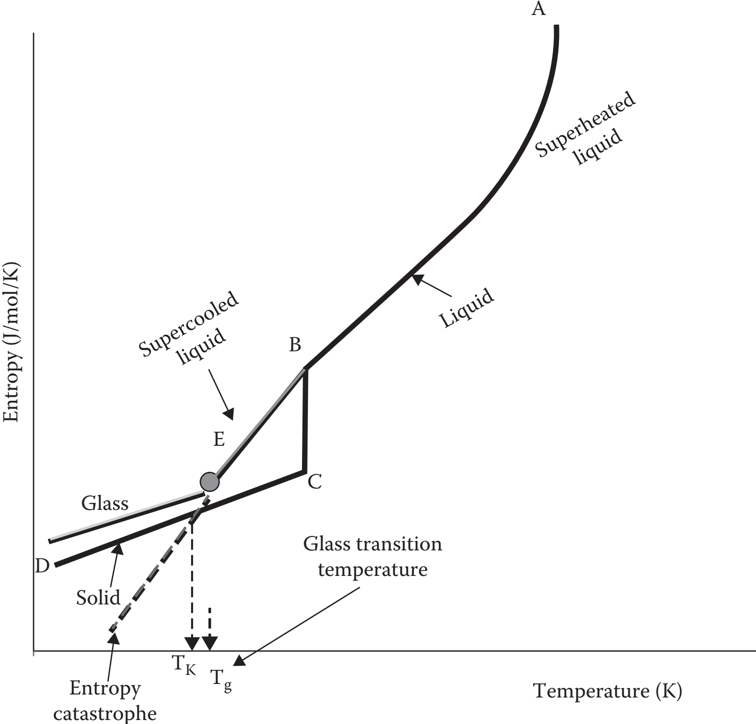


FIGURE 2.44 Generalized isobaric entropy–temperature diagram of a material.

the entropy of the supercooled liquid remains higher than that of the crystalline solid. When the trajectory of the supercooling curve is extended it intersects with the crystal line. At this intersection point, known as the Kauzmann temperature (T_K), the entropy of the supercooled liquid is same as the crystalline solid. Since this situation is not possible, T_K is often referred to as the Kauzmann paradox. Further extension of the supercooling curve below T_K leads to an even more dire situation known as "entropy catastrophe," where the entropy of the supercooled liquid would be lower than the entropy of the crystalline solid, which is a violation of the laws of nature. To avoid this catastrophe, the supercooled liquid transforms itself into a glass at a temperature T_g above the Kauzmann temperature. As the temperature of the glass is decreased, its entropy remains above that of the crystalline solid at all temperatures below T_g, thus avoiding the entropy catastrophe situation.

Glass formation is a common phenomenon in dried, semidried, and frozen food materials. In the case of frozen foods, for example, as the temperature of a food material is slowly lowered, water crystallizes at the freezing point and separates from the solution. As a result, the solute concentration in the remaining unfrozen solution increases, as shown in Figure 2.45. As the temperature is further lowered, this process continues until the solute concentration in the liquid phase reaches a saturated level. Beyond this stage, as the temperature is further lowered the solute fails to crystallize due to its low diffusivity in a highly viscous solution but water continues to crystallize owing to its high diffusivity. The system finally reaches a stage at which the maximally freeze-concentrated liquid phase sets into a glass (this point corresponds to E in Figure 2.44). Hence, a slowly frozen food material typically contains a mixture of ice and aqueous glass phases. In contrast, when the original food material is quick frozen at a cooling rate faster than the rate of ice crystal growth, the entire food material is converted into an aqueous glass at a glass transition temperature (Figure 2.45).

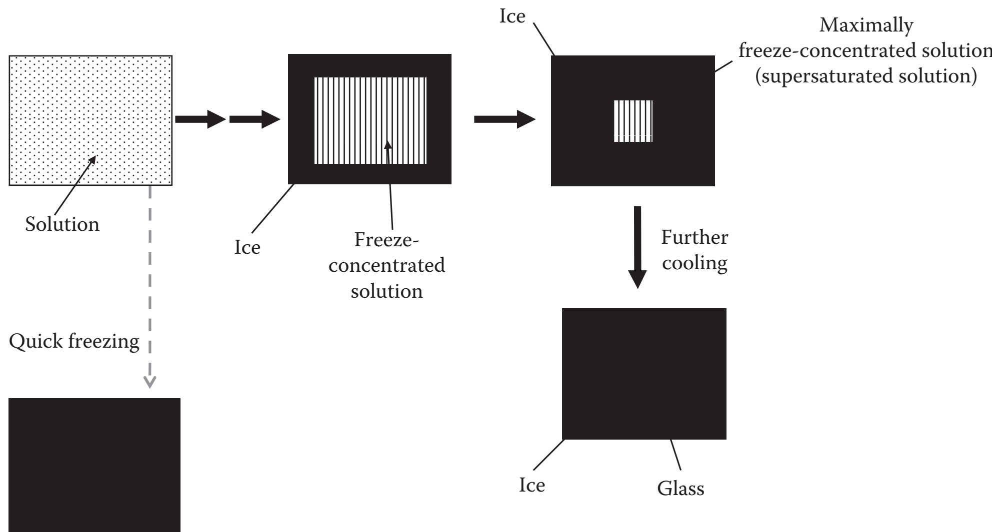


FIGURE 2.45 Schematic representation of phase changes during slow and quick freezing of an aqueous solution.

2.8.2 Molecular Mobility and Reaction Rates

Consider a bimolecular reaction between reactants A and B leading to the formation of product C. The rate of this bimolecular reaction is given by

$$\frac{dC}{dt} = k[A][B] \tag{2.42}$$

where
  k is the second-order rate constant
  [A] and [B] are the concentrations of reactants A and B, respectively

For the chemical reaction to occur, molecules must diffuse in the medium and collide with each other. However, the rates of a majority of chemical reactions are not diffusion limited, that is, every collision between reactants does not result in product formation. For a reaction to occur, the collisions must have sufficient energy to cause bond distortions that elevate the reactants from the ground state to the “activated” or “transition” state. Such reactions are known as activation energy barrier–limited reactions, and the rate constant of such reactions are described by the Arrhenius equation

$$k = Ae^{-E_a/RT} \tag{2.43}$$

where
  A is the preexponential factor
  E_a is the activation energy barrier
  R is the gas constant
  T is the absolute temperature

The preexponential factor A is related to the frequency of collisions (Z) between reactants and the probability (ρ) of the collision leading to product formation, that is, A = Zρ. The ρ factor is related to the probability of having the correct orientation of the reaction centers of the reactants at the point

of collision. The exponential factor $e^{-E_a/RT}$ describes the fraction of molecules that possess enough kinetic energy at temperature T to overcome the energy barrier of the reaction. As the temperature is increased, the fraction of molecules having more than sufficient energy to overcome the energy barrier also increases, which increases the reaction rate. When the exponential term approaches unity, that is, when E_a of a reaction is close to zero, Equation 2.43 reduces to

$$k = A \tag{2.44}$$

If the probability factor ρ = 1, then the rate constant is simply equal to the collisional frequency, that is, $k_{diff} = Z$, between molecules and such reactions are termed as diffusion-limited reactions. The diffusion-limited reactions usually have no or very low activation energy and they reach the theoretically possible maximum rate. Since the diffusion coefficient of molecules is in the order of 10^{-9} to 10^{-10} m^2/s, the rate constants of diffusion-controlled bimolecular reactions are usually in the range of 10^{10}–10^{11} M^{-1} s^{-1}. Reactions exhibiting rate constants lower than these values are usually energy barrier limited or limited by the steric (probability) factor ρ.

The rate constant of diffusion-limited reactions is given by the a modified form of Smoluchowski's equation

$$k_{diff} = \frac{4\pi N_A}{1000}(D_1 + D_2)r \tag{2.45}$$

where

D_1 and D_2 are the diffusion coefficients (m^2/s) of reactants 1 and 2
r is the closest distance of approach (sum of the radii of reactants 1 and 2)
N_A is Avogadro's number

For spherical reactant particles, the diffusion coefficient is given by the Stokes–Einstein equation

$$D = \frac{k_B T}{6\pi\eta a} \tag{2.46}$$

where

k_B is the Boltzmann constant
T is the absolute temperature (K)
η is the viscosity (N s/m^2) of the medium
a is the radius of the particle

If particles 1 and 2 have the same radius, then using Equations 2.45 and 2.46 it can be shown that

$$k_{diff} = \frac{8k_B N_A}{3000}\left(\frac{T}{\eta}\right) = \frac{8R}{3000}\left(\frac{T}{\eta}\right) \tag{2.47}$$

According to Equation 2.47, the rate constant of a diffusion-controlled reaction is directly proportional to temperature and inversely proportional to the viscosity of the medium.

2.8.3 Reaction Rate in the Glassy State

Diffusion-controlled reactions usually obey Equation 2.47 under normal temperature and pressure. Under normal conditions, the extent of decrease in viscosity of the medium (water) as the temperature is increased is minimal. For instance, when the temperature of water is increased from 20°C to 40°C, the viscosity decreases from 10^{-3} Pa s (centipoise) to 0.653 × 10^{-3} Pa s (Table 2.1).

However, this is not the case in the glassy state. At temperatures below T_g, the translational and rotational motions of molecules in a glassy material are nearly immobile [48]. For instance, at 50 K below T_g, molecular relaxation times in aqueous glasses of sorbitol, sucrose, and trehalose are in the range of 3–5 years [49], which corresponds to a viscosity of $>10^{14}$ Pa s. Thus, rates of all chemical and physical changes in a glassy material are nearly zero. However, at the glass transition temperature T_g, that is, the temperature at which a glass melts into a rubber and exists at equilibrium with its rubbery state, the molecular relaxation time decreases to about 100 seconds, which corresponds to a viscosity of about 10^{12} Pa s for most glasses [50]. This decrease in viscosity permits molecular mobility to some extent, resulting in the initiation of physical and chemical changes in the material. When the temperature is increased 20 K above T_g (i.e., $T - T_g = 20$ K), the viscosity of the material decreases by 10^5-fold from 10^{12} Pa s to about 10^7 Pa s. As a consequence, rates of diffusion-controlled reactions in glassy/rubbery materials increase by several orders of magnitude over a small change in temperature. It should be emphasized, however, that the dramatic increase in the rate constant often observed in glassy materials is largely due to viscosity changes and only to a minor extent due to the temperature alone. As a result, for glassy/rubbery materials, Equation 2.47 can be simplified as

$$k_{diff} \propto \frac{T}{\eta} \tag{2.48}$$

The viscosity change as a function of temperature in amorphous polymers is given by the Williams–Landel–Ferry (WLF) equation [51]

$$\log\left(\frac{\eta_T}{\eta_g}\right) = -\frac{C_1(T - T_g)}{C_2 + (T - T_g)} \tag{2.49}$$

where
C_1 is a dimensionless constant
C_2 is a constant in Kelvin
η_T is the viscosity at temperature T
η_g is the viscosity at the glass transition temperature T_g

C_1 and C_2 are the universal constants with values of 17.44 and 51.6 K, respectively, for all amorphous polymers. Since η_g of most amorphous materials is about 10^{12} Pa s [50], knowing the glass transition temperature T_g of an amorphous material, the viscosity η_T of the amorphous material at any temperature T above T_g can be estimated using Equation 2.49 and the rate constant of a diffusion-limited reaction can then be determined from Equation 2.47. Alternatively, by invoking Equation 2.48, the relative rate constant of a reaction in an amorphous material at temperature T compared to that at T_g can be determined from

$$\log\left(\frac{k_g}{k_T}\right) = -\frac{C_1(T - T_g)}{C_2 + (T - T_g)} \tag{2.50}$$

where k_T and k_g are the reaction rate constants at temperatures T and T_g, respectively. It is debatable, however, whether the universal values of C_1 and C_2, which were determined from studies on amorphous synthetic polymers, can be applied to complex systems of aqueous food glasses. Regardless of this ambiguity and uncertainty, the basic premise that molecular mobility in amorphous food materials increases by several orders of magnitude for a small increase in the temperature above their glass transition temperature is noncontroversial, and therefore the T_g of a food material can still be used as the reference point to predict reaction rates at temperatures above T_g.

Because the viscosity is very high and reaction rates are extremely slow at T_g, it is often impossible to experimentally determine viscosity and k_g at T_g. However, instead of using T_g as the reference

temperature, the WLF equation does allow one to use a temperature other than T_g as a reference where the rate of a reaction in as well as the viscosity of a glassy material can be experimentally measured. This also allows one, if desired, to determine the product-specific C_1 and C_2 values. If k_r is the rate constant at a reference temperature other than T_g and k_T is the rate constant at any given temperature T, then a reciprocal plot of Equation 2.50, that is, $1/\log(k_r/k_T)$ versus $(T - T_r)$, will be linear with a slope of $1/C_1$ and a slope of C_2/C_1.

2.8.4 State Diagram

Foods are typically multicomponent systems in which nonaqueous solids are combined with water. Additionally, almost all foods exist in a nonequilibrium state in which one or more components, including water, might be in phase transition from amorphous to crystalline state. Thus, the phase behavior of foods cannot be understood using conventional phase diagrams that are suitable only for systems under true equilibrium. However, they can be studied using a state diagram, which provides information on the state of a food under both nonequilibrium and equilibrium situations.

In the food context, the state diagram essentially describes various phases (stable, metastable, and unstable phases) of a given material as the temperature and composition of the material are altered. This is shown in Figure 2.46 for a simple binary system containing water and sucrose. However, even in cases of complex food materials containing polymeric components such as proteins and polysaccharide, those systems can be still approximated as a binary system in which all the nonaqueous components are grouped as a single solute [26]. This approach is valid only if none of the nonaqueous components in the food material undergo phase separation and/or are thermodynamically incompatible with other components and that water is the only component that can crystallize [26]. If phase separation occurs in a system containing more than one dominant polymer component, then one would need to identify the polymer component whose glass transition (T_g) is more relevant to controlling the critical property of the given food material [26]. For instance, if starch is the dominant component in a food, such as bakery products, then the state diagram of starch is the most relevant one to predict quality changes in that food.

As an example, the state diagram of sucrose + water binary mixture is shown in Figure 2.46. There are a couple of ways to construct the state diagram. In the first case, consider a 10% (w/w) solution of sucrose dissolved in water at room temperature (position A in the diagram). When the solution is cooled slowly, its temperature will decrease without any change in its composition until it reaches the freezing point of the solution, which will be below 0°C due to the freezing point depression (see Section 2.5.7). At the freezing point, some of the water will separate out as ice and, as a consequence, the sucrose concentration will increase in the remaining solution phase. This process will repeat itself as the system is continually subjected to slow cooling and the composition of the solution phase will move along the line T_m until it reaches a point T_E at which the solubility of sucrose reaches the saturation limit (C_E) at that temperature. The solid line, denoted as the T_m line, is the equilibrium melting (or freezing) curve of ice, where sucrose solution exists in equilibrium with ice. To increase the solubility of sucrose in water beyond the saturation limit at T_E, one needs to increase the temperature, as shown by the equilibrium solubility curve T_S. The point T_E, where the melting curve of ice and solubility curve meet, is known as the eutectic point, at which the saturated solution coexists with crystalline solvent (ice) and crystalline solute. It is also the lowest melting point of ice and the lowest solubility of the solute. The solid lines T_m and T_S and the point T_E represent true equilibrium situations. Another way to construct the T_m and the T_S line is to take a series of sucrose solution with increasing concentration and cool them at slow rate and determine their freezing temperature to determine the T_m line and the solubility at higher temperatures to determine the T_S line.

The T_g line in Figure 2.46 represents the glass transition temperature of aqueous sucrose glasses as a function of the composition of the glasses. The T_g curve is constructed by supercooling a series of sucrose solutions of increasing concentration. The rate of supercooling is chosen such that neither water nor sucrose can crystallize from the solution, but set into a homogeneous sugar–water glass

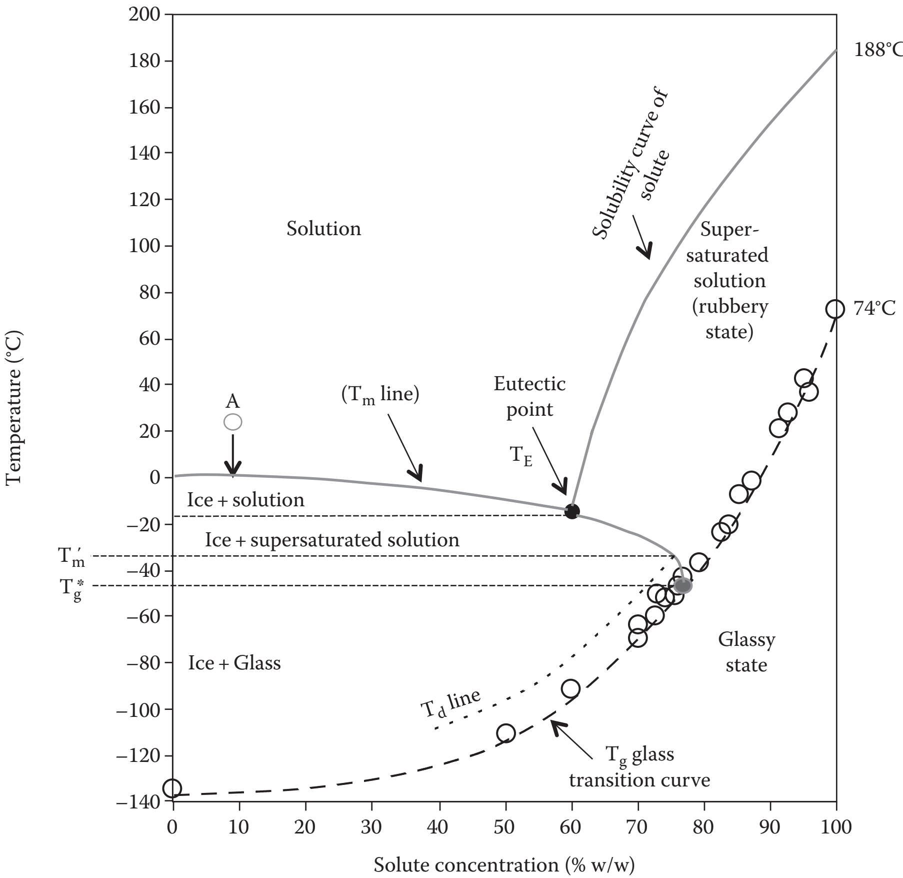


FIGURE 2.46 Annotated temperature composition state diagram for sucrose solution. The assumptions are maximal freeze concentration, no solute (sucrose crystallization), constant pressure, and no time dependence. T_m line is the melting point curve of ice. T_E is the eutectic point, and T_g is the glass transition curve. T_d is the glass devitrification curve. T_g^* is the solute specific glass transition temperature of the maximally freeze-concentrated solution, and T_m' (also known as T_g') is the onset of separation of water from molten glass in the form of ice. (Adapted from Reid, D.S. and Fennema, O., Water and ice, in: *Fennema's Food Chemistry*, 4th edn., Damodaran, S., Parkin, K.L., and Fennema, O. (eds.), CRC Press, Boca Raton, FL, 2008.)

at the glass transition temperature. Pure water forms a glass when supercooled to −135°C, whereas pure molten sucrose (melting point 188°C) forms a glass at 74°C when supercooled. The glass transition temperature of water–sucrose glasses ranges from −135°C to 74°C depending on the sucrose concentration, as shown by the T_g line (Figure 2.46).

When the solution at the concentration and temperature corresponding to the eutectic point (C_E and T_E, respectively, which represents the minimum solubility of the solute) is further cooled, under ideal situation one would expect crystallization of both ice and the solute at a constant ratio corresponding to the weight ratio of solute to water at C_E, so that the composition of the remaining solution phase remains at C_E, but the temperature declines vertically, as the heat removed is mostly the latent heat of fusion of ice plus solute. However, in real situations, because the viscosity of the solution at C_E is considerably high, the solute often fails to crystallize, whereas water, being small with high mobility, continues to crystallize. As a result, the solution phase becomes supersaturated and the system follows the trajectory denoted by the $T_E \rightarrow T_g^*$ line. As the system moves along the $T_E \rightarrow T_g^*$ line, the solution becomes increasingly supersaturated and the viscosity continuously

increases and reaches a point, denoted by T'_m, where the molecular mobility of water in the remaining solution phase drops drastically and, as a result, water also fails to crystallize, and upon further cooling, the system sets into a glass at T_g^*. Thus, the region between T_E and T_g^* represents an unstable nonequilibrium state. The T_g^* is defined as the glass transition temperature of a maximally freeze-concentrated solution, and this situation is often encountered in frozen food products.

While the solid lines in Figure 2.46 represent equilibrium situations, the dotted lines represent nonequilibrium situations. The state diagram of a binary system can be divided into various regions corresponding to different stable, metastable, and unstable phases, as depicted in Figure 2.46. The region above the T_m and T_S curves represents the stable solution state. Since the molecular mobility is high in the solution state, chemical stability is the least in this region. The region below the T_g line represents metastable glassy (amorphous) state, where the rotational and translational motions are so slow (but not zero) that no significant change in the state of matter occurs over a long period of time. Thus, rates of chemical and physical changes in food materials are negligible in this region. The region between the T_m and T_g lines at solute concentrations below C_E (the concentration at the eutectic point) represents a nonequilibrium amorphous state of frozen materials. Similarly, the region between the T_g line and the T_S line at solute concentrations above C_E represents a nonequilibrium amorphous state in which the material is in a supersaturated or rubbery state. Both these nonequilibrium regions are inherently unstable, and if the temperature–composition state of a food material lies in these regions, physical and chemical changes will occur with time. The rates of these changes, however, will depend on how far above is its temperature (T) from its T_g. In other words, molecular mobility at the glass transition temperature can be used as the reference point to predict rates of chemical and physical changes in a material at any temperature T between T_g and T_m and between T_g and T_S lines.

For example, consider ice cream at a temperature below its glass transition temperature T_g (typically about −32°C). The viscosity of ice cream in the glassy state, at $T < T_g$, will be in the neighborhood of 10^{15} Pa s. As the temperature is increased, its viscosity at the glass transition temperature, that is, at $T = T_g$, will decrease to about 10^{12} Pa s, as is the case for most amorphous polymers at their T_g [50]. As the temperature is increased further, the change (decrease) in viscosity as a function of the temperature difference $T - T_g$ will follow the WLF equation (Equation 2.49), assuming that the values of universal constants C_1 and C_2 are valid for ice cream as well. Since molecular mobility, and thereby reaction rate, is inversely proportional to viscosity of the material, the rates of chemical and physical changes, such as ice crystal growth and lipid oxidation, in ice cream at any temperature T within the temperature range T_m and T_g can be estimated using Equation 2.49 or 2.50.

2.8.5 Limitations of the WLF Equation

A basic assumption in the WLF equation is that the concentration of reactants in a system is constant at all times and the reaction kinetics is dependent only on large changes in viscosity as a function of $T - T_g$. This underlying assumption is violated in some regions of the state diagram. For instance, consider a frozen food located at any temperature T in the region between the T_g and the T_m lines. As the temperature is increased above T_g, even though the aqueous glass melts at T_g, water in the molten glass does not separate and crystallize until the temperature raises to a point on the T_d line known as the devitrification temperature. The line T_d is known as the devitrification line. As water is removed in the form of ice at $T > T_d$, the solute concentration in the remaining solution phase increases with time and the food system moves horizontally to the right in the state diagram under isothermal conditions. As a result, the temperature difference $T - T_g$ is no longer constant but continuously narrows with time. This necessitates inclusion of the effect of concentration on the reaction rate, especially for biomolecular reactions. If we assume that a bimolecular reaction follows a pseudo first order, then the change in concentration would have no effect on the relative reaction rate, but the change in the temperature difference $T - T_g$ will result in underestimation of relative reaction rate.

In contrast, consider a food whose concentration is above C_g^* and located at a temperature T in the region between the T_g and the T_S lines. In this region, the material is in a rubbery state and therefore crystallization of both water and solute is not possible. As the temperature is increased from T_g toward T_S, the viscosity of the material drops by several orders of magnitude and the molecular mobility and reaction rates increase rapidly. Many physical changes in food products have been shown to truly follow the WLF equation in this region.

2.8.6 Applicability of State Diagrams to Food Systems

Food systems are very complex. They contain several low-molecular-weight ingredients and high-molecular-weight polymers. However, if the dominant component that affects the quality of a food is known, then the quality changes in that product can be inferred using the state diagram of the dominant component. For instance, since sucrose is the major component of cookies, the sucrose–water state diagram is adequate to predict changes in quality attributes of cookies. On the other hand, if a food product consists of more than one domain, such as cheese crackers or dual-textured cookies, then it would be appropriate to use state diagrams of the dominant components in each domain of the product. However, in most complex food systems, while it is relatively easy to determine the T_m and the T_g lines, it is not the case with the T_S line because solutes in a complex food do not readily crystallize at saturation concentration. Thus, while it is relatively simple to construct a state diagram for frozen foods, it is challenging in the case of intermediate-moisture foods.

2.8.7 T_g Determination

The glass transition temperature T_g of a simple food system is usually determined using a differential scanning calorimeter (DSC). For more complex foods, however, dynamic mechanical thermal analyzer (DMTA) is the instrumentation of choice. The glass/rubber transition occurs as a second-order transition in these thermograms. A typical DSC thermogram of a binary system is shown in Figure 2.47. When the temperature of the sample is gradually increased, first the glass melts into a

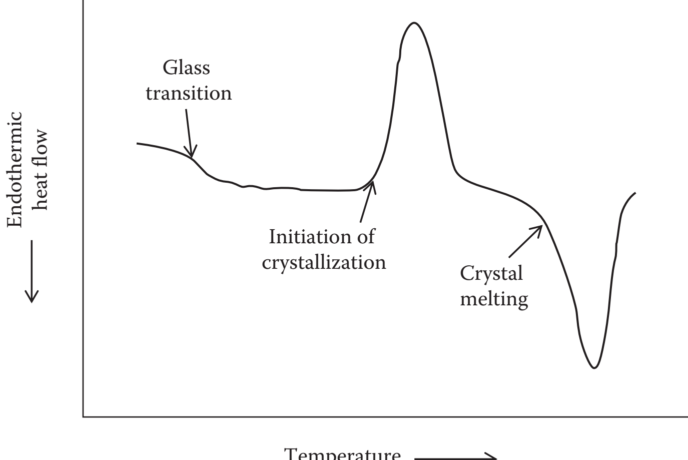


FIGURE 2.47 Schematic representation of a DSC thermogram that is typical of freeze-dried amorphous sugars. First, the amorphous (glass) material melts. As the molecular mobility becomes sufficiently high in the molten state, the solute crystallizes with release of heat (exothermic peak). Upon further heating, the crystals melt (endothermic peak) at the typical melting temperature of the material. (From Roos, Y.H., *Phase Transitions in Foods*, Academic Press, New York, 1995.)

highly viscous rubbery state at T_g. The viscosity of the rubber decreases greatly as the temperature is increased further, and above a particular temperature, the molecular mobility in the melt reaches a critical point where molecules can reorient and interact with each other to form a crystalline structure. Crystallization is indicated by the exothermic heat flow, and the temperature at the exothermic peak is the crystallization temperature of the material. As the temperature is increased further, the crystal melts by absorbing heat and the endothermic peak corresponds to the melting temperature of the material.

Determination of T_g of complex food materials is not easy, because the second-order transition is very weak and it can be easily missed in a DSC thermogram. For simple food materials that contain only a few components, for example, a binary system, the theoretical T_g value of the material can be determined using the Gordon–Taylor equation [52]

$$T_{g,mix} = \frac{w_1 T_{g1} + K w_2 T_{g2}}{w_1 + K w_2} \tag{2.51}$$

where
- w_1 and w_2 are the weight fractions of components 1 and 2, respectively
- T_{g1} and T_{g2} are the glass transition temperatures of components 1 and 2, respectively
- K is a constant, which is related to [53]

$$K = \frac{\rho_1 T_{g1}}{\rho_2 T_{g2}} \tag{2.52}$$

where ρ_1 and ρ_2 are densities of components 1 and 2, respectively. Equation 2.51 assumes no specific interaction between the components. It has been shown that the $T_{g,mix}$ of aqueous glasses of starch, lactose, and sucrose nearly followed the ideal behavior stipulated by Equation 2.51 [53].

Water is one of the most effective plasticizers of amorphous polymeric materials. It reduces the T_g of amorphous materials even at very low concentrations. As shown in Figures 2.48 and 2.49, the T_g of both amorphous wheat gluten and starch decreases as the moisture content is increased. The fact that water's effect on the T_g of both gluten and starch follows the profile predicted by Equation 2.51 suggests that, like any other small molecule, water simply acts as a plasticizer in these amorphous materials and not through any other specific process [53].

2.8.8 Molecular Weight Dependency of T_g

At a given temperature, translational mobility of molecules decreases with increase of molecular size. As a consequence, the T_g (as well as the T_g^*) increases with increasing molecular weight of the solute. In the case of polysaccharides and synthetic polymers, the relationship between T_g and the number average molecular weight M_n of the solute follows the empirical relation

$$T_g = T_{g(\infty)} - \frac{K}{M_n} \tag{2.53}$$

where
- $T_{g(\infty)}$ is the T_g of polymer with infinite molecular weight
- K is a constant

However, in the case of maltodextrins, it has been shown that the T_g^* (and T_g as well) reaches a constant value at molecular weights greater than 3000 Da (Figure 2.50).

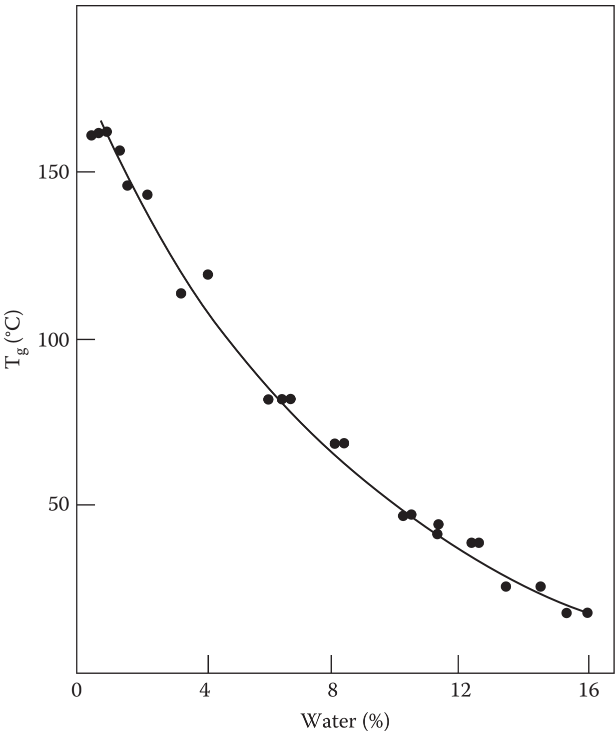


FIGURE 2.48 T_g of wheat gluten as a function of water content. (From Hoseney, R.C. et al., *Cereal Chem.*, 63, 285, 1986.)

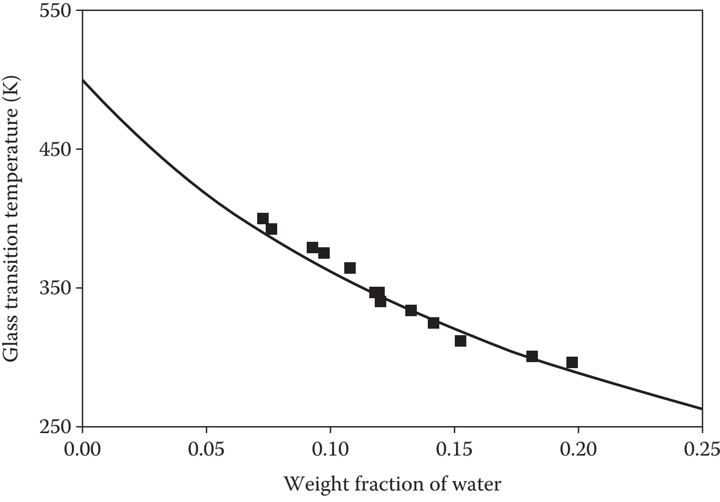


FIGURE 2.49 T_g of starch as a function of water content. The solid line is from Equation 2.51. (From Hancock, B.C. and Zografi, G., *Pharm. Res.*, 11, 471, 1994.)

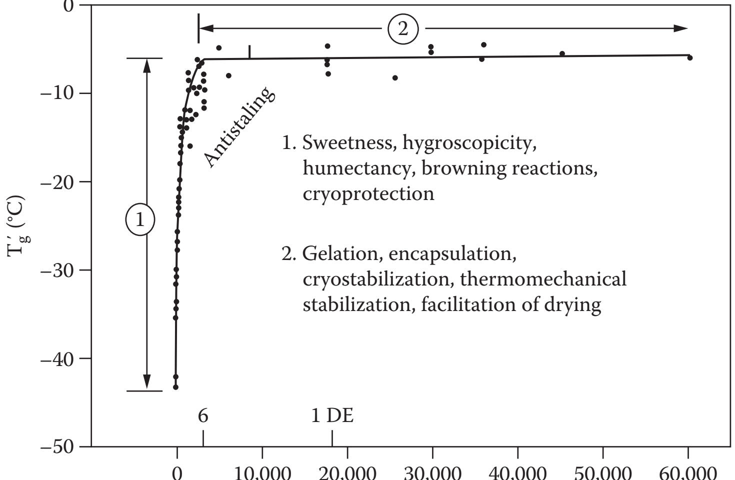


FIGURE 2.50 Typical results on the influence of dextrose equivalent (DE) and number average molecular weight of commercial starch hydrolysis products on T'_g (also known as T'_m). (From Reid, D.S. and Fennema, O., Water and ice, in: *Fennema's Food Chemistry*, 4th edn., Damodaran, S., Parkin, K.L., and Fennema, O. (eds.), CRC Press, Boca Raton, FL, 2008.)

The T_g values of mono- and disaccharides and maltodextrins are listed in Table 2.7. It should be noted that even though the molecular weight of the monosaccharides glucose, galactose, and fructose is the same, the T_g value of fructose is significantly lower than those of glucose and galactose. This difference might be related to the predominant structural forms of these sugars: while glucose and galactose are aldoses with pyranose configuration, fructose is a ketose with a furanose configuration. Thus, in addition to molecular weight, other molecular characteristics of sugars also play a role in T_g.

TABLE 2.7
Glass Transition Temperatures (T_g) of Some Common Mono- and Disaccharides and Maltodextrins

Carbohydrate	Molecular Weight	T_g (°C)
Xylose	150.1	6
Ribose	150.1	−20
Glucose	180.2	31
Fructose	180.2	5
Galactose	180.2	30
Sorbitol	182.1	−9
Mannose	180.2	25
Sucrose	342.3	62
Maltose	342.3	87
Trehalose	342.3	100
Lactose	342.3	101
Maltotriose	504.5	349
Maltopentose	828.9	398–438
Maltohexose	990.9	407–448
Maltoheptose	1153.0	412

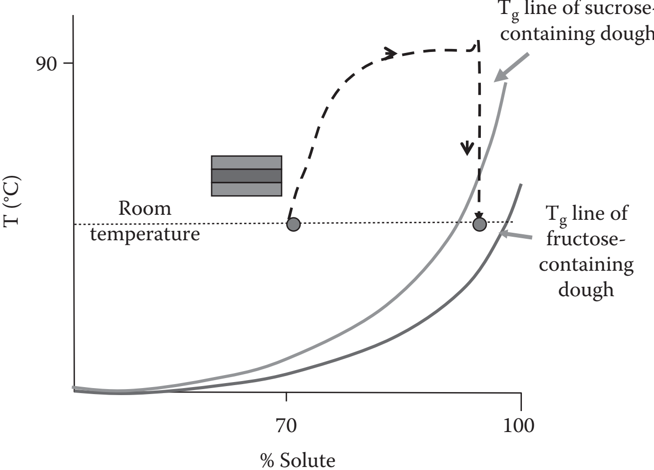


FIGURE 2.51 State diagram of a typical dual-textured cookie product (made with two different sugars, e.g., fructose and sucrose). The dotted line represents the path during baking, cooling, and final resting state. The solid lines represent the relative positions of T_g lines of fructose and sucrose.

The dependence of T_g on molecular weight of solutes can be exploited in the fabrication of dual-textured food products, such as cookies with a soft interior and a hard exterior. The state diagram of a dual-textured cookie made by coextruding two different doughs, one containing fructose (the interior part of the cookie) and the other containing sucrose (the exterior part of the cookie), is shown in Figure 2.51. When the cookie is baked and cooled to room temperature, the final state of the product sits below the T_g line of sucrose, but above the T_g line of fructose (Figure 2.51). As a result, the part of the cookie that contains sucrose will be in a glassy state and therefore would be crunchy, whereas the fructose containing part (interior) of the cookie will be in a rubbery state and therefore would be soft in texture.

2.8.9 Relationship between a_w, Water Content, and Molecular Mobility Approaches to Understanding Water Relations in Foods

While the MSI of a food material depicts the relationship between moisture content (MC) and a_w of a food at equilibrium, the relationship between T_g and MC reflects water-dependent molecular mobility in the food. Since both T_g and a_w are related to the water content, a relationship also exists between T_g and a_w. Similar to the MC–a_w relationship, the T_g–a_w relationship is also product specific. By constructing a T_g–a_w–MC diagram, the interrelationship between the equilibrium and kinetic (molecular mobility) properties of a food material and their impact on food quality can be understood.

An example of the T_g–a_w–MC relationship is shown in Figure 2.52 for spray-dried Borojo powder [54]. This type of diagram can be used to predict the critical MC and critical a_w to maintain the quality of a food product at a given storage temperature. For example, if the product (Borojo powder) is to be stored at 20°C, then the critical a_w and MC at which the glass transition temperature T_g of the product is same as the storage temperature is about 0.319 and 0.046 g water/g product, respectively, as shown in Figure 2.52. At this critical a_w and MC, the molecular mobility of water and other constituents in the product will be close to zero (since the viscosity η_g is approximately about 10^{12} Pa s). If the temperature of the product were raised to $T_g + 20$, then the orders-of-magnitude drop in η_g would cause greater molecular mobility in the product, resulting in an increase in a_w and initiation of undesirable chemical and physical changes in the product. Thus, the T_g–a_w relationship provides

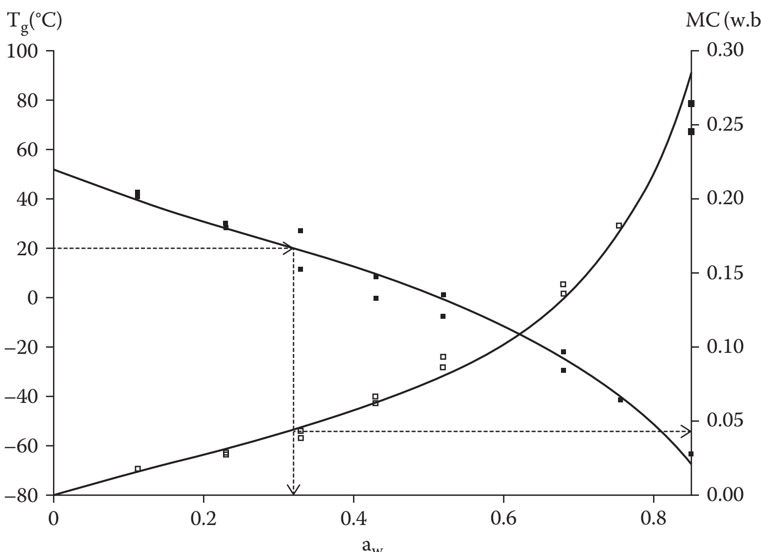


FIGURE 2.52 Glass transition temperature (T_g)–water activity (a_w, black square) and moisture content (MC g water/g product)–water activity (white square) relationships of spray-dried Borojo powder. The solid lines are GAB and Gordon and Taylor model fitted curves of the experimental data. (From Mosquera, L.H. et al., *Food Biophys*., 6, 397, 2011.)

the link between equilibrium and kinetic aspects of food products and this enables one to predict the critical MC needed to maintain a product's quality during storage.

REFERENCES

1. Fennema, O.R. (1996) Water and ice. In *Food Chemistry*, 3rd edn., Fennema, O.R. (Ed.), Marcel Dekker, Inc., New York.
2. Fernandez, P.P., Otero, L., Guignon, B., and Sanz, P.D. (2006) High-pressure shift freezing versus high-pressure assisted freezing: Effects on the microstructure of a food model. *Food Hydrocolloid*. 20, 510–522.
3. Otero, L. and Sanz, P.D. (2000) High-pressure shift freezing. Part 1. Amount of ice instantaneously formed in the process. *Biotechnol. Prog.* 16, 1030–1036.
4. Scott, J.N. and Vanderkooi, J.M. (2010) A new hydrogen bond angle/distance potential energy surface of the quantum water dimer. *Water* 2, 14–28.
5. Hobbs, P.V. (1974) *Ice Physics*, Clarendon Press, Oxford, U.K.
6. Ludwig, R. (2001) Water: From clusters to the bulk. *Angew. Chem. Int. Ed.* 40, 1808–1827.
7. Roy, R., Tiller, W.A., Bell, I., and Hoover, M.R. (2001) The structure of liquid water: Novel insights from materials research—Potential relevance to homeopathy. *Mater. Res. Innov.* 9–4, 577–608.
8. Narten, A.H. and Levy H.A. (1971) Liquid water: Molecular correlation functions from x-ray diffraction. *J. Chem. Phys.* 55, 2263–2269.
9. Clark, G.N.I., Cappa, C.D., Smith, J.D., Saykally, R.J., and Head-Gordon, T. (2010) The structure of ambient water. *Mol. Phys.* 108, 1415–1433.
10. Hura, G., Sorenson, J.M., Glaeser, R.M., and Head-Gordon, T. (2000) A high-quality x-ray scattering experiment on liquid water at ambient conditions. *J. Chem. Phys.* 113, 9140–9148.
11. Marcus, Y. (1991) Thermodynamics of solvation of ions. *J. Chem. Soc. Faraday Trans.* 87, 2995–2999.
12. Barnes, P., Finney, J.L., Nicholas, J.D., and Quinn, J.E. (1979) Cooperative effects in simulated water. *Nature* 282, 459–464.
13. Stillinger, F.H. and Lemberg, H.L. (1975) Symmetry breaking in water molecule interactions. *J. Chem. Phys.* 62, 1340–1346.

14. Idrissi, A., Gerard, M., Damay, P., Kiselev, M., Puhovskuy, Y., Dinar, E., Lagenat, P., and Vergoten, G. (2010) The effect of urea on the structure of water: A molecular dynamics simulation. *J. Phys. Chem. B* 114, 4731–4738.
15. Soper, A.K., Castner, E.E., and Luxar, A. (2003) Impact of urea on water structure: A clue to its properties as a denaturant? *Biophys. Chem.* 105, 649–666.
16. Walraten, G.E. (1966) Raman spectral studies of the effects of urea and sucrose on water structure. *J. Chem. Phys.* 44, 3726–3727.
17. Guo, F. and Friedman, J.M. (2009) Osmolyte-induced perturbations of hydrogen bonding between hydration layer waters: Correlation with protein conformational changes. *J. Phys. Chem. B* 114, 4731–4738.
18. Hildebrand, J.H. (1979) Is there a "hydrophobic effect"? *Proc. Natl. Acad. Sci. USA* 76, 194.
19. Damodaran, S. (1998) Water activity at interfaces and its role in regulation of interfacial enzymes: A hypothesis. *Colloids Surf. B: Biointerfaces* 11, 231–237.
20. Tanford, C. (1978) Hydrophobic effect and organization of living matter. *Science* 200, 1012–1018.
21. Tanford, C. (1991) *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd edn., Krieger, Malabar, FL.
22. Stillinger, F.H. (1980) Water revisited. *Science* 209, 451–457.
23. Hildebrand, J.H. and Scott, R.L. (1962) *Regular Solutions*, Prentice Hall, Englewood Cliffs, NJ.
24. Norrish, R.S. (1966) An equation for the activity coefficients and equilibrium relative humidities of water in confectionery syrups. *J. Food Technol.* 1, 25–39.
25. Burnauer, S., Emmett, H.P., and Teller, E. (1938) Adsorption of gases in multimolecular layers. *J. Am. Chem. Soc.* 60, 309–319.
26. Reid, D.S. and Fennema, O. (2008) Water and ice. In *Fennema's Food Chemistry*, 4th edn., Damodaran, S., Parkin, K.L., and Fennema, O. (Eds.), CRC Press, Boca Raton, FL.
27. Karel, M. and Yong, S. (1981) Autoxidation-initiated reactions in foods. In *Water Activity: Influences Food Quality*, Rockland, L.B. and Stewart, C.F. (Eds.), Academic Press, New York, pp. 511–529.
28. Gonzales, A.S.P., Naranjo, G.B., Leiva, G.E., and Malec, L.S. (2010) Maillard reaction kinetics in milk powder: Effect of water activity and mild temperatures. *Int. Dairy J.* 20, 40–45.
29. Loncin, M., Bimbenet, J.J., and lenges, J. (1968) Influence of the activity of water on the spoilage of food stuffs. *J. Food Technol.* 3, 131–142.
30. Acker, L. (1969) Water activity and enzyme activity. *Food Technol.* 23, 1257–1270.
31. Metzger, D.D., Hsu, K.H., Ziegler, K.E., and Bern, C.J. (1989) Effect of moisture content on popcorn popping volume for oil and hot-air popping. *Cereal Chem.* 66, 247–248.
32. Bell, L.N. and Labuza, T.P. (2013) Aspartame degradation as a function of water activity. In *Water Relationships in Foods: Advances in the 1980s and Trends for the 1990s*, Levine, H. and Slade, L. (Eds.), Springer Science and Business Media, New York, pp. 337–347.
33. Bock, J.E. and Damodaran, S. (2013) Bran-induced changes in water structure and gluten conformation in model gluten dough studied by attenuated total reflectance Fourier transformed infrared spectroscopy. *Food Hydrocolloid.* 31, 146–155.
34. Guggenheim, E.A. (1966) *Applications of Statistical Mechanics*, Clarendon Press, Oxford, U.K., pp. 186–206.
35. Anderson, R.B. (1946) Modifications of the Brunauer, Emmett and Teller equation. *J. Am. Chem. Soc.* 68, 686–691.
36. De Boer, J.H. (1968) *The Dynamical Character of Adsorption*, 2nd edn., Clarendon Press, Oxford, U.K., pp. 200–219.
37. Timmermann, E.O., Chirife, J., and Iglesias, H.A. (2001) Water sorption isotherms of foods and foodstuffs: BET or GAB parameters. *J. Food Eng.* 28, 19–31.
38. Lewicki, P.P. (1998) A three parameter equation for food moisture sorption isotherms. *J. Food Process Eng.* 21, 127–144.
39. Peleg, M. (1993) Assessment of a semi-empirical four parameter general model for sigmoid moisture sorption isotherms. *J. Food Process Eng.* 16, 21–37.
40. Blahovec, J. and Yanniotis, S. (2008) GAB generalized equation for sorption phenomena. *Food Bioprocess Technol.* 1, 82–90.
41. Gorling, P. (1958) Physical phenomena during the drying of foodstuffs. In *Fundamental Aspects of the Dehydration of Foodstuffs*, Society of Chemical Industry, London, U.K., pp. 42–53.
42. Kapsalis, J.G. (1981) Moisture sorption hysteresis. In *Water Activity: Influences on Food Quality*, Rockland, L.B. and Stewart, G.F. (Eds.), Academic Press, New York, pp. 143–177.

43. Kapsalis, J.G. (1987) Influences of hysteresis and temperature on moisture sorption isotherms. In *Water Activity: Theory and Applications to Food*, Rockland, L.B. and Beuchat, L.R. (Eds.), Marcel Dekker, Inc., New York, pp. 173–213.
44. Wolf, M., Walker, J.E., and Kapsalis, J.G. (1972) Water vapor sorption hysteresis in dehydrated food. *J. Agric. Food Chem.* 20, 1073–1077.
45. Labuza, T.P., McNally, L., Gallagher, D., Hawkes, J., and Hurtado, F. (1972) Stability of intermediate moisture foods. 1. Lipid oxidation. *J. Food Sci.* 37, 154–159.
46. Labuza, T.P. and Hyman, C.R. (1998) Moisture migration and control in multi-domain foods. *Trends Food Sci. Technol.* 9, 47–55.
47. Risbo, J. (2003) The dynamics of moisture migration in packaged multi-component food systems I: Shelf life predictions for a cereal-raisin system. *J. Food Eng.* 58, 239–246.
48. Zhou, D. (2002) Physical stability of amorphous pharmaceuticals: Importance of configurational thermodynamic quantities and molecular mobility. *J. Pharm. Sci.* 91, 1863–1872.
49. Shamblin, S.L., Tang, X., Chang, L., Hancock, B.C., and Pikal, M.J. (1999) Characterization of the time scales of molecular motion in pharmaceutically important glasses. *J. Phys. Chem.* 103, 4113–4121.
50. Yue, Y. (2004) Fictive temperature, cooling rate, and viscosity of glasses. *J. Chem. Phys.* 120, 8053–8059.
51. Williams, M.L., Landel, R.F., and Ferry, J.D. (1955) The temperature dependence of relaxation mechanism in amorphous polymers and other glass-forming liquids. *J. Am. Chem. Soc.* 77, 3701–3707.
52. Gordon, M. and Taylor, J.S. (1952) Ideal co-polymers and the second order transitions of synthetic rubbers. 1. Non-crystalline copolymers. *J. Appl. Chem.* 2, 493–500.
53. Hancock, B.C. and Zografi, G. (1994) The relationship between the glass transition temperature and the water content of amorphous pharmaceutical solids. *Pharm. Res.* 11, 471–477.
54. Mosquera, L.H., Moraga, G., de Cordoba, P.F., and Martinez-Navarrete, N. (2011) Water content–water activity–glass transition temperature relationships of spray-dried Borojo as related to changes in color and mechanical properties. *Food Biophys.* 6, 397–406.
55. Creighton, T.T. (1996) *Proteins: Structures and Molecular Properties*, 2nd edn., W.H. Freeman & Co., New York, p. 157.
56. Israelachvili, J.N. (1992) *Intermolecular and Surface Forces*, 2nd edn., Academic Press, New York, p. 344.
57. Quast, D.G. and Karel, M. (1972) Effects of environmental factors on the oxidation of potato chips. *J. Food Sci.* 37, 584–588.
58. Roos, Y.H. (1995) *Phase Transitions in Foods*, Academic Press, New York.
59. Hoseney, R.C., Zeleznak, K., and Lai, C.S. (1986) Wheat gluten: A glassy polymer. *Cereal Chem.* 63, 285–286.
60. Lide, D.R. (Ed.) (1993/1994) *Handbook of Chemistry and Physics*, 74th edn., CRC Press, Boca Raton, FL.
61. Franks, F. (1988) In *Characteristics of Proteins*, Franks, F. (Ed.), Humana Press, Clifton, NJ, pp. 127–154.
62. Lounnas, V. and Pettitt, B.M. (1994) A connected-cluster of hydration around myoglobin: Correlation between molecular dynamics simulation and experiment. *Proteins: Struct. Funct. Genet.* 18, 133–147.
63. Rupley, J.A. and Careri, G. (1991) Protein hydration and function. *Adv. Protein Chem.* 41, 37–172.
64. Otting, G. et al. (1991) Protein hydration in aqueous solution. *Science* 254, 974–980.
65. Lounnas, V. and Pettitt, B.M. (1994) Distribution function implied dynamics versus residence times and correlations: Solvation shells of myoglobin. *Proteins: Struct. Funct. Genet.* 18, 148–160.
66. Lower, S. (2016) A gentle introduction to water and its structure. http://www.chem1.com/acad/sci/aboutwater.html.

3 Carbohydrates

Kerry C. Huber and James N. BeMiller

CONTENTS

3.1 Monosaccharides 92
3.1.1 Monosaccharide Isomerization 96
3.1.2 Monosaccharide Ring Forms 96
3.1.3 Glycosides 99
3.1.4 Monosaccharide Reactions 100
3.1.4.1 Oxidation to Aldonic Acids and Aldonolactones 100
3.1.4.2 Reduction of Carbonyl Groups 101
3.1.4.3 Uronic Acids 102
3.1.4.4 Hydroxyl Group Esters 103
3.1.4.5 Hydroxyl Group Ethers 103
3.1.4.6 Nonenzymic Browning 105
3.1.4.7 Caramelization 109
3.1.4.8 Formation of Acrylamide in Food 110
3.1.5 Summary 113
3.2 Oligosaccharides 113
3.2.1 Maltose 114
3.2.2 Lactose 114
3.2.3 Sucrose 116
3.2.4 Trehalose 117
3.2.5 Cyclodextrins 117
3.2.6 Summary 119
3.3 Polysaccharides 119
3.3.1 Polysaccharide Chemical Structures and Properties 119
3.3.2 Polysaccharide Crystallinity, Solubility, and Cryostabilization 120
3.3.3 Polysaccharide Solution Viscosity and Stability 122
3.3.4 Gels 131
3.3.5 Polysaccharide Hydrolysis 132
3.3.6 Starch 132
3.3.6.1 Amylose 133
3.3.6.2 Amylopectin 134
3.3.6.3 Starch Granules 135
3.3.6.4 Granule Gelatinization and Pasting 136
3.3.6.5 Uses of Unmodified Starches 137
3.3.6.6 Starch Gelatinization within Vegetable Tissues 138
3.3.6.7 Retrogradation and Staling 140
3.3.6.8 Starch Complexes 140
3.3.6.9 Hydrolysis of Starch 141
3.3.6.10 Modified Food Starches 143
3.3.6.11 Pregelatinized Starch 148
3.3.6.12 Cold-Water-Swelling Starch 148

3.3.7 Cellulose: Forms and Derivatives ... 149
3.3.7.1 Microcrystalline Cellulose ... 149
3.3.7.2 Carboxymethylcelluloses ... 150
3.3.7.3 Methylcelluloses (MCs) and Hydroxypropylmethylcelluloses (HPMCs) ... 150
3.3.8 Guar and Locust Bean Gums ... 151
3.3.9 Xanthan ... 153
3.3.10 Carrageenans, Agar, and Furcellaran ... 154
3.3.11 Algins ... 157
3.3.12 Pectins ... 158
3.3.13 Gum Arabic ... 160
3.3.14 Gellan ... 161
3.3.15 Konjac Glucomannan ... 161
3.3.16 Inulin and Fructooligosaccharides ... 162
3.3.17 Polydextrose ... 162
3.3.18 Summary ... 163
3.4 Dietary Fiber, Prebiotics, and Carbohydrate Digestibility ... 163
3.4.1 Summary ... 165
Chapter Problems ... 166
References ... 166
Further Reading ... 169

Carbohydrates comprise more than 90% of the dry matter of plants. As a result, they are abundant, widely available, and inexpensive. Carbohydrates are common constituents of foods, both as inherent natural components and as added ingredients. Both the quantities consumed and the variety of products in which they are found are large. They have many different molecular structures, sizes, and shapes; exhibit a variety of chemical and physical properties; and differ in their physiological effects on the human body. They are amenable to chemical, biochemical, and in some cases physical modifications, which are employed commercially to improve their properties and extend their use.

Starch, lactose, and sucrose are generally digested by humans, and they, along with D-glucose and D-fructose, are energy sources, providing 70%–80% of the calories in the human diet worldwide. In the United States, they supply less than that percentage, with amounts varying widely from individual to individual. Aside from strictly a caloric contribution, carbohydrates also occur in nature in less digestible forms, providing a beneficial source of dietary fiber to the human diet.

The term "carbohydrate" suggests a general elemental composition, namely $C_x(H_2O)_y$, which signifies molecules containing carbon atoms along with hydrogen and oxygen atoms in the same ratio as they occur in water. However, the great majority of naturally occurring carbohydrate compounds produced by living organisms do not have this simple empirical formula. Rather, most natural carbohydrate is in the form of oligomers (oligosaccharides) or polymers (polysaccharides) comprised of simple and modified sugars, with low-molecular-weight carbohydrates most often produced by depolymerization of natural polymers. However, this chapter begins with a presentation of the simple sugars and builds from there to larger and more complex structures.

3.1 MONOSACCHARIDES [7,21]

Carbohydrates contain *chiral carbon atoms*, each of which has four different, chemically distinct atoms or chemical groups attached to it, giving rise to two different spatial arrangements of atoms around a given chiral center. The two different arrangements of the four atoms or groups in space (configurations) are nonsuperimposable mirror images of each other (Figure 3.1). In other words,

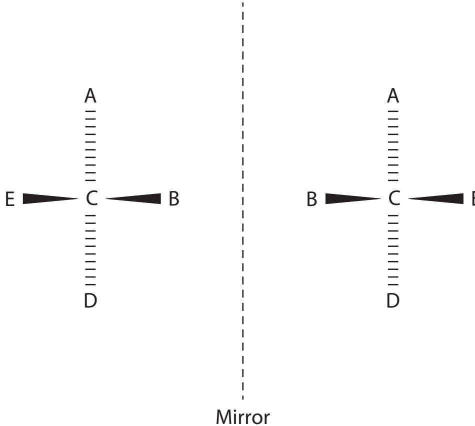


FIGURE 3.1 A chiral carbon atom. A, B, D, and E represent different atoms, functional groups, or other groups of atoms attached to carbon atom C. Wedges indicate chemical bonds projecting outward from the plane of the page; dashes indicate chemical bonds projecting into or below the plane of the page.

one is the reflection of the other that one would see in a mirror, with the atoms depicted on the right of the molecule in one configuration depicted on the left in the other, and vice versa.

D-Glucose, the most abundant carbohydrate and the most abundant organic compound in nature (if its presence in all combined forms of carbohydrates is considered), belongs to the class of carbohydrates called *monosaccharides*. Monosaccharides are the most basic carbohydrate molecules that cannot be broken down to simpler carbohydrate molecules by hydrolysis and are sometimes referred to as *simple sugars*. They are the monomeric units that are joined together to form larger carbohydrate structures, viz., oligosaccharides and polysaccharides (see Sections 3.2 and 3.3), which themselves can be converted into their constituent monosaccharides by hydrolysis.

D-Glucose is both a polyalcohol and an aldehyde. It is classified as an *aldose*, a designation for sugars containing an aldehydic group (Table 3.1). The suffix "-ose" signifies a sugar; the prefix "ald-" signifies an aldehydic group. When D-glucose is written in an open or vertical straight-chain fashion (Figure 3.2), known as an acyclic structure, the aldehydic group (carbon atom 1) and the primary hydroxyl group (carbon atom 6) are depicted at the top and bottom of the chain, respectively. In this scenario, each carbon atom possessing a secondary hydroxyl group (carbon atoms 2, 3, 4, and 5) has four different substituent groups attached to it and is, therefore, chiral.

TABLE 3.1
Classification of Monosaccharides

	Kind of Carbonyl Group	
Number of Carbon Atoms	**Aldehyde**	**Ketone**
3	Triose	Triulose
4	Tetrose	Tetrulose
5	Pentose	Pentulose
6	Hexose	Hexulose
7	Heptose	Heptulose
8	Octose	Octulose
9	Nonose	Nonulose

H — C ═ O	HC ═ O	C-1
H — C — OH	HCOH	C-2
HO — C — H	HOCH	C-3
H — C — OH	HCOH	C-4
H — C — OH	HCOH	C-5
H — C — OH	CH_2OH	C-6
H		

FIGURE 3.2 D-Glucose (open-chain or acyclic structure).

Since each chiral carbon atom has a mirror image (two possible arrangements per chiral carbon atom), there is a total of 2^n (where n designates the number of chiral carbon atoms in the molecule) different arrangements of atoms around chiral carbon centers. Therefore, in a six-carbon aldose such as D-glucose with its four chiral carbon atoms, there are 2^4 or 16 different arrangements of secondary hydroxyl groups about the chiral carbon centers, with each individual arrangement representing a unique sugar (isomer). Eight of these six-carbon-atom aldoses belong to the D series (Figure 3.3); the other eight are their mirror images and belong to the L series. All sugars that have the hydroxyl group of the highest numbered chiral carbon atom (C-5 in this case) positioned to the right-hand side of the molecule are arbitrarily called D sugars, while all with a left-hand positioned hydroxyl group on the highest numbered chiral carbon atom are designated L sugars. Naturally occurring glucose is represented as the D form, specifically D-glucose, while

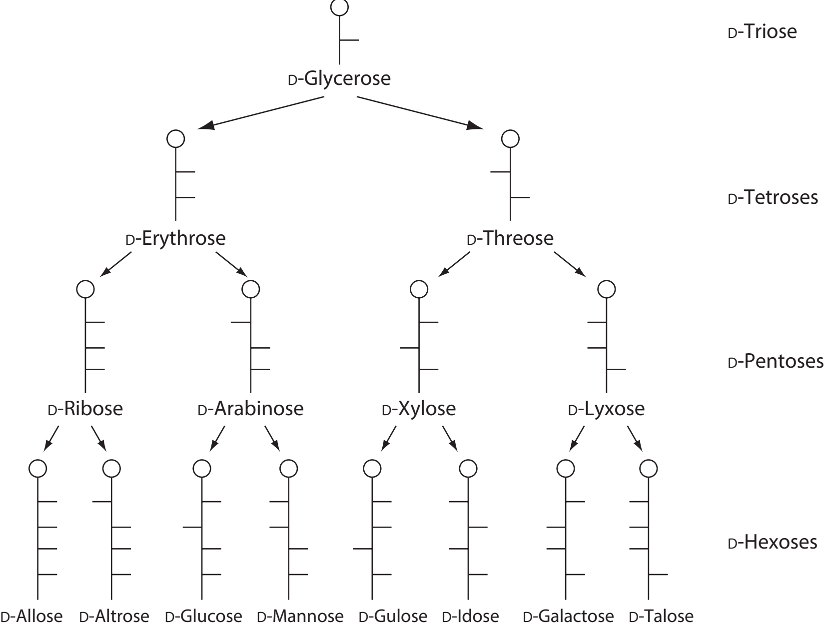


FIGURE 3.3 Rosanoff structures of the D-aldoses containing 3–6 carbon atoms.

its molecular mirror image is termed L-glucose. Two structures of D-glucose in open-chain, acyclic form (called the Fischer projection) with the carbon atoms numbered in the conventional manner are given in Figure 3.2. In this convention, each horizontal bond projects outward from the plane of the page, while each vertical bond projects into the plane of the page. (It is customary to omit the horizontal lines for covalent chemical bonds to the hydrogen atoms and hydroxyl groups as in the structure on the right.) Because the lowermost carbon atom (C-6) is nonchiral, it is meaningless to designate the relative positions of the atoms and groups attached to it. Thus, it is written as $-CH_2OH$.

D-Glucose and all other aldose sugars containing six carbon atoms are called *hexoses* (Table 3.1), which represent the group of aldoses most abundant in nature. The categorical names are often combined, a six-carbon-atom aldehydic sugar being termed an *aldohexose*.

There are two aldoses containing three carbon atoms. They are D-glyceraldehyde (D-glycerose) and L-glyceraldehyde (L-glycerose), each possessing only one chiral carbon atom. Aldoses with four carbon atoms, the tetroses, have two chiral carbon atoms; aldoses with five carbon atoms, the pentoses, have three chiral carbon atoms and comprise the second most common group of aldoses. Extending the series above six carbon atoms gives heptoses, octoses, and nonoses, which is the practical limit for naturally occurring sugars. Development of the eight D-hexoses from D-glyceraldehyde is shown in Figure 3.3. In this figure, the circle in each molecular representation depicts the aldehydic group, the horizontal lines designate the positions of each hydroxyl group in relation to its chiral carbon atom, and the bottom of the vertical lines is the terminal nonchiral primary hydroxyl group ($-CH_2OH$). This shorthand way of representing monosaccharide structures is called the *Rosanoff method*. D-Glucose, D-galactose, D-mannose, D-arabinose, and D-xylose are commonly found in plants, predominantly in combined forms, that is, in glycosides, oligosaccharides, and polysaccharides (discussed later). D-Glucose is the primary free aldose usually present in natural foods, and then only in small amounts.

L-Sugars are less numerous and less abundant in nature than are the D-forms; nevertheless, they have important biochemical roles. Two L-sugars found in foods are L-arabinose and L-galactose, both of which occur as monomeric units in carbohydrate polymers (polysaccharides).

In addition to aldoses, there is another type of monosaccharide, in which the carbonyl function is a ketone group. These sugars are called *ketoses*. (The prefix "ket-" signifies the ketone group.) The suffix designating a ketose in systematic carbohydrate nomenclature is "-ulose" (Table 3.1). D-Fructose (systematically D-*arabino*-hexulose) is the prime example of this sugar group (Figure 3.4) [46,71,75]. It is one of the two monosaccharide units comprising the disaccharide sucrose (see Section 3.2.3), and makes up ~55% of a common high-fructose syrup and about 40% of honey. D-Fructose has only three chiral carbon atoms (C-3, C-4, and C-5). Thus, there are but 2^3 or 8 ketohexose isomers. D-Fructose is the only commercial ketose and the only one found in free form in natural foods, but like D-glucose, only in small amounts.

Structure	Carbon
CH_2OH	C-1
C=O	C-2
HOCH	C-3
HCOH	C-4
HCOH	C-5
CH_2OH	C-6

FIGURE 3.4 D-Fructose (open-chain or acyclic structure).

3.1.1 Monosaccharide Isomerization

Simple aldoses and ketoses containing the same number of carbon atoms are isomers of each other, that is, an aldohexose and a hexulose both have the empirical formula $C_6H_{12}O_6$ and can be interconverted by isomerization. Isomerization of monosaccharides involves both the carbonyl group and the adjacent or α-hydroxyl group. By this reaction, an aldose is converted into another aldose (with the opposite configuration at C-2) and the corresponding ketose, while a ketose is converted into the corresponding two aldoses. Therefore, by isomerization, D-glucose, D-mannose, and D-fructose can be interconverted (Figure 3.5). Isomerization can be catalyzed by either a base or an enzyme.

3.1.2 Monosaccharide Ring Forms

Carbonyl groups of aldehydes are reactive and readily undergo nucleophilic attack by the oxygen atom of a hydroxyl group to produce a hemiacetal. The hydroxyl group of a hemiacetal can react further (by condensation) with a hydroxyl group of an alcohol to produce an acetal (Figure 3.6). The carbonyl group of a ketone reacts similarly.

Hemiacetal formation almost always occurs within the same aldose or ketose sugar molecule: that is, the carbonyl group of a sugar molecule can react with one of its own hydroxyl groups, as illustrated in Figure 3.7 for D-glucose. The six-membered sugar ring that results from reaction of an aldehydic group with the hydroxyl group at C-5 is called a *pyranose* ring. Notice that, for the oxygen atom of the hydroxyl group at C-5 to react to form the ring, C-5 must rotate to bring its oxygen atom upward. This rotation brings the hydroxymethyl group (C-6) to a position above the ring. The representation of the D-glucopyranose ring in Figure 3.7 is termed a *Haworth projection*. To avoid clutter in writing Haworth ring structures, common conventions are adopted wherein ring carbon atoms are indicated by angles in the ring and hydrogen atoms attached to carbon atoms are eliminated altogether.

D-Glucose ⇌ *trans*-Enediol ⇌ D-Fructose ⇌ *cis*-Enediol ⇌ D-Mannose

FIGURE 3.5 Interrelationship of D-glucose, D-mannose, and D-fructose via isomerization.

Hemiacetal Acetal

FIGURE 3.6 Formation of an acetal by reaction of an aldehyde with methanol.

D-Glucose (Fischer projection)

D-Glucopyranose (Haworth projection)

FIGURE 3.7 Formation of a pyranose hemiacetal ring from D-glucose.

FIGURE 3.8 L-Arabinose in the furanose ring form and α-L configuration.

Sugars also occur in five-membered (*furanose*) rings (Figure 3.8), but less frequently than they do in pyranose rings.

When the carbon atom of the carbonyl group is involved in ring formation, leading to hemiacetal (pyranose) development (Figure 3.7), it becomes chiral and is defined as the anomeric carbon atom. For D-sugars, the configuration that has the hydroxyl group located below the plane of the ring (in the Haworth projection) is the α-form (Figure 3.9). For example, α-D-glucopyranose is D-glucose in the pyranose (six-membered) ring form with the configuration of the hydroxyl group at the new chiral or anomeric carbon atom, C-1, below the plane of the ring (alpha position). When the newly formed hydroxyl group at C-1 is above the plane of the ring (in the Haworth projection), it is in the

D-Glucopyranose

α-D-Glucopyranose

β-D-Glucopyranose

FIGURE 3.9 D-Glucopyranose as a mixture of two chiral forms.

β-position, and the structure is named β-D-glucopyranose (Figure 3.9). This designation holds for all D-sugars. For sugars in the L-series, the opposite is true: that is, the anomeric hydroxyl group is up in the α-anomer and down in the β-anomer.* (See, for example, Figure 3.8.) This is so because, for example, α-D-glucopyranose and α-L-glucopyranose are mirror images of one another. Irrespective of the sugar designation (D- or L-), a mixture of chiral (anomeric*) forms is indicated by a wavy line (i.e., bond) between the anomeric carbon and its hydroxyl group (Figure 3.9).

However, pyranose rings are not actually flat with the attached hydroxyl groups sticking straight up or straight down as the Haworth representation suggests. Rather, they occur in a variety of shapes (conformations), most commonly in one of two chair conformations, so called because they are shaped somewhat like a chair. In a chair conformation, one bond on each carbon atom projects either directly up or down from the ring; these are called axial bonds or axial positions. The other bond not involved in ring formation, is either slightly up or down with respect to the axial bonds but, with respect to the ring, projects outward around the perimeter of the molecule in what is called an *equatorial position* (Figure 3.10).

Using β-D-glucopyranose as an example, C-2, C-3, C-5, and the ring oxygen atom occur in the same plane, but C-4 is raised slightly above the plane and C-1 is positioned slightly below the plane as in Figures 3.10 and 3.11. This conformation is designated $^4C_1$. The notation C indicates that the ring is chair-shaped; the superscript number indicates that C-4 is above the plane of the ring and the subscript number indicates that C-1 is below the plane. (There are two chair forms. The second, $^1C_4$, has all the axial and equatorial groups reversed.) The six-membered ring distorts the normal carbon and oxygen atom bond angles less than do rings of other sizes. The strain is further lessened when the bulky hydroxyl groups are maximally separated from each other by the ring conformation that arranges the greatest number of them in equatorial, rather than axial, positions. The equatorial position is energetically favored, and rotation of carbon atoms takes place about their connecting bonds to swivel the bulky groups to equatorial positions in so far as possible.

As noted, β-D-glucopyranose has all its hydroxyl groups in the equatorial arrangement, but each is positioned either slightly above or slightly below the true equatorial position (Figure 3.11). In β-D-glucopyranose ($^4C_1$ conformation), the hydroxyl groups, all of which are in an equatorial position, alternate in a slight up-and-down arrangement, with that at C-1 positioned slightly up, that on C-2 slightly down, and continuing with an alternating up-and-down arrangement. The bulky

FIGURE 3.10 A pyranose ring showing the equatorial (solid line) and axial (dashed line) bond positions.

FIGURE 3.11 β-D-Glucopyranose in the $^4C_1$ conformation. All bulky groups are in equatorial positions and all hydrogen atoms in axial positions.

* The α- and β-ring forms of a sugar are known as *anomers*. The two anomers comprise an anomeric pair.

TABLE 3.2
Equilibrium Distribution of Cyclic and Anomeric Forms of Some Monosaccharides in Aqueous Solution at Room Temperature (~20°C)

	Pyranose Ring Forms		Furanose Ring Forms	
Sugar	**α-**	**β-**	**α-**	**β-**
Glucose	36.2	63.8	0	0
Galactose	29	64	3	4
Mannose	68.8	31.2	0	0
Arabinose	60	35.5	2.5	0.5
Ribose	21.5	58.5	6.5	13.5
Xylose	36.5	63	<1	<1
Fructose	4	75	0	21

hydroxymethyl group, C-6 in hexoses, is almost always in a sterically free equatorial position. If β-D-glucopyranose were in a $^1C_4$ conformation, all the bulky groups would be axial. Being a much higher energy form, very little of D-glucopyranose exists in the $^1C_4$ conformation.

Six-membered sugar rings are then quite stable if bulky groups such as hydroxyl groups and the hydroxymethyl group are in equatorial positions. Thus, β-D-glucopyranose dissolves in water to give a rapidly equilibrating mixture containing the open-chain form and its five-, six-, and seven-membered ring forms. At room temperature (~20°C), the six-membered (pyranose) ring forms predominate, followed by the five-membered (furanose) ring forms. The configuration of the anomeric carbon atom (C-1 of aldoses) of each ring may be α or β. The equilibrium ratio of the ring forms varies with the sugar and the temperature. This interconversion between the tautomeric forms of a given sugar in solution is termed *mutarotation*, and may be catalyzed by either an acid or a base. Examples of the distribution of cyclic and anomeric forms for different monosaccharides in aqueous solution are given in Table 3.2.

The open-chain aldehydic form constitutes only about 0.003% of the total forms; but because of rapid interconversion among the ring forms, a sugar can readily and rapidly react as if it were entirely in the free aldehyde form (Figure 3.12).

3.1.3 Glycosides

The hemiacetal form of sugars can react with an alcohol to produce a full acetal; the product is called a *glycoside*. In the laboratory, the reaction occurs under anhydrous conditions in the presence of an acid (as a catalyst) at elevated temperatures, but glycosides are most commonly made in nature, that is, in aqueous environments, by enzyme-catalyzed reactions in pathways involving several intermediates. The acetal linkage at the anomeric carbon atom is called a *glycosidic linkage* (or bond). Glycosides are named by changing the "-ose" suffix to "-ide." In the case of D-glucose reacting with methanol, the product is mainly methyl α-D-glucopyranoside, with less methyl β-D-glucopyranoside (Figure 3.13). The two anomeric forms of the five-membered-ring furanosides are also formed; but being higher energy structures, they reorganize into more stable forms under the conditions of formation and are present at equilibrium in only low amounts. The methyl group in this case, and any other group bonded to a sugar to make a glycoside, is termed an *aglycon*.

Glycosides undergo hydrolysis in acidic aqueous environments to yield a reducing sugar and a hydroxylated compound. Acid-catalyzed hydrolysis becomes more rapid as the temperature is raised.

α-D-Glucopyranose

α-D-Glucofuranose

CHO
HCOH
HOCH
HCOH
HCOH
CH_2OH

Aldehydo-D-glucose

β-D-Glucopyranose

β-D-Glucofuranose

FIGURE 3.12 Interconversion of the acyclic and cyclic forms of D-glucose.

(a) (b)

FIGURE 3.13 Methyl α-D-glucopyranoside (a) and methyl β-D-glucopyranoside (b).

3.1.4 Monosaccharide Reactions

All carbohydrate molecules have hydroxyl groups available for reaction. Simple monosaccharide and most other low-molecular-weight carbohydrate molecules also have carbonyl groups available for reaction. Formation of pyranose and furanose rings (cyclic hemiacetals) and glycosides (acetals) of monosaccharides has already been presented. As mentioned in Section 3.1.2, although aldoses in solution exist almost entirely in pyranose or furanose ring forms, they can react as if they had a free aldehydic group, because of the rapid interconversion among open-chain and ring forms (Figure 3.12). Addition of a chemical group to the aldehydic group, or exhaustive substitution of primary hydroxyl groups, will limit a monosaccharide to the open chain or acyclic form.

3.1.4.1 Oxidation to Aldonic Acids and Aldonolactones

Aldoses are readily oxidized to aldonic acids by oxidation of the aldehydic group to a carboxyl/carboxylate group. This reaction is commonly used for quantitative determination of sugars. One of the earliest methods for the detection and measurement of sugars employed the

FIGURE 3.14 Oxidation of D-glucose catalyzed by glucose oxidase.

Fehling solution. It is an alkaline solution of copper(II), which oxidizes an aldose to an aldonate and in the process is reduced to copper(I), which precipitates as brick-red Cu_2O (see Equation 3.1).

$$2Cu(OH)_2 + R-\overset{\displaystyle H}{\overset{|}{C}}=O \longrightarrow R-\overset{\displaystyle O}{\overset{\|}{C}}-OH + Cu_2O + H_2O \tag{3.1}$$

Variations (the Nelson–Somogyi and Benedict reagents) are still used for determining the amounts of reducing sugars in foods and other biological materials. Because in the process of oxidizing the aldehydic group of an aldose to the salt of a carboxylic acid group the oxidizing agent is reduced, that is, the sugar reduces the oxidizing agent, aldoses are called *reducing sugars*. α-Hydroxy ketoses (e.g., fructose) are also termed *reducing sugars* because, under the alkaline conditions of the Benedict, Fehling, and Tollens tests, they are first isomerized to aldoses, although the formation of an aldonic acid is relatively less efficient for ketoses than aldoses.

A simple and specific method for quantitative oxidation of D-glucose to D-gluconic acid uses the enzyme glucose oxidase, the initial product being the 1,5-*lactone* (an intramolecular ester) of the acid (Figure 3.14). The reaction is commonly employed to measure the amount of D-glucose in foods and other biological materials, including the D-glucose concentration in blood and urine. D-Gluconic acid is a natural constituent of fruit juices and honey.

The reaction given in Figure 3.14 is also used for the manufacture of commercial D-gluconic acid and its lactone (D-glucono-delta-lactone [GDL]; D-glucono-1,5-lactone according to systematic nomenclature). GDL undergoes hydrolysis (largely to completion) in water in ~3 h at room temperature, effecting a decrease in pH. Its slow hydrolysis, producing slow acidification, and mild taste make GDL unique among food acidulants. It is used in meats and dairy products, but particularly in refrigerated dough as a chemical leavening component (see also Chapter 6).

3.1.4.2 Reduction of Carbonyl Groups [21,46,48,75]

Hydrogenation is the addition of hydrogen to a double bond. When applied to carbohydrates, it entails the addition of hydrogen to the double bond between the oxygen atom and the carbon atom of the carbonyl group of an aldose or ketose. Hydrogenation is readily accomplished with hydrogen gas under pressure in the presence of Raney nickel as a catalyst. The hydrogenation product of D-glucose is D-glucitol (Figure 3.15), commonly known as *sorbitol*, the "-itol" suffix denoting a sugar alcohol (an *alditol*). Alditols are also known as *polyols* and *polyhydroxy alcohols*. As a consequence of hydrogenation, these polyhydroxy compounds no longer possess a carbonyl group necessary to promote formation of a ring structure, and therefore exist in the open-chain (i.e., acyclic) form. Because it is derived from a hexose, D-glucitol (sorbitol) is specifically a hexitol. Sorbitol is widely distributed in plants, ranging from algae to higher plants where it is found in fruits and berries; but the amounts present are generally small. It is about half as sweet as sucrose, is sold both

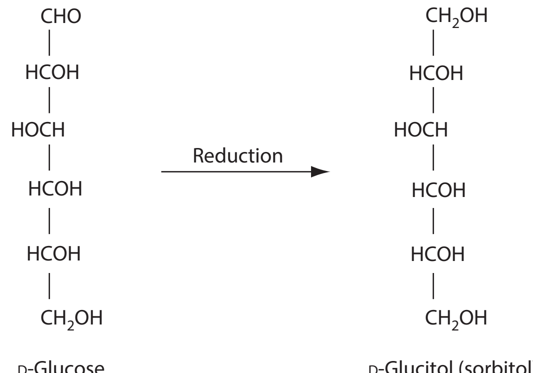


FIGURE 3.15 Reduction of D-glucose.

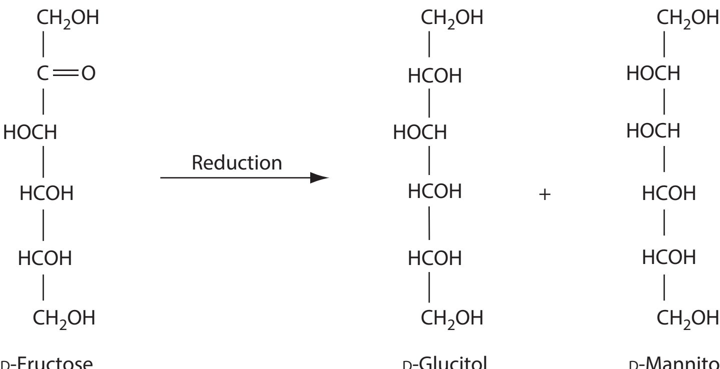


FIGURE 3.16 Reduction of D-fructose.

as a syrup and in the form of crystals, and is used as a general humectant, that is, a substance that will hold/retain moisture in a product.

D-Mannitol can be obtained by the hydrogenation of D-mannose. Commercially, it is obtained along with sorbitol from hydrogenolysis of sucrose. It is a product of the hydrogenation of the D-fructose (Figure 3.16) component of sucrose or also from isomerization of D-glucose (to D-fructose), which can be controlled by the alkalinity of the solution undergoing catalytic hydrogenation. D-Mannitol, unlike sorbitol, is not a humectant. Rather, it crystallizes easily and is only moderately soluble. It has been used as a nonsticky coating on candies. It is 65% as sweet as sucrose and is used in sugar-free chocolates, pressed mints, cough drops, and hard and soft candies.

Xylitol (Figure 3.17) is produced from the hydrogenation of D-xylose obtained from hemicelluloses, especially from birch trees. Its crystals have a high negative heat of solution. This endothermic heat of solution of crystalline xylitol produces a cooling sensation in the mouth, which makes xylitol desirable as an ingredient in mint candies and in sugarless chewing gums. Its sweetness is nearly equal to that of sucrose. Xylitol is noncariogenic because it is not metabolized by the microflora of the mouth that produce dental plaques.

3.1.4.3 Uronic Acids

The terminal carbon atom (at the opposite end of the carbon chain from the carbonyl group) of a monosaccharide unit may occur in an oxidized (carboxylic acid) form. Such an aldohexose with C-6 in the form of a carboxylic acid group is called a *uronic acid*. When the chiral carbon atoms of a

CH_2OH
|
HCOH
|
HOCH
|
HCOH
|
CH_2OH

FIGURE 3.17 Xylitol.

COOH
O
HO
OH
OH
OH

FIGURE 3.18 D-Galacturonic acid.

uronic acid are in the same configuration, as they occur in D-galactose, for example, the compound is D-galacturonic acid (Figure 3.18), the principal component of pectin (see Section 3.3.12). Uronic acid forms of monosaccharides most commonly occur in various oligo- and polysaccharides.

3.1.4.4 Hydroxyl Group Esters

The hydroxyl groups of carbohydrates, like the hydroxyl groups of simple alcohols, may form esters with organic and some inorganic acids. Reaction of hydroxyl groups with an activated form of a carboxylic acid, primarily a carboxylic acid anhydride, in the presence of a suitable base produces an ester.

$$\mathrm{ROH} + \mathrm{R'{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}O{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}R'} \quad \text{or} \quad \mathrm{R'{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}Cl} \rightarrow \mathrm{R{-}O{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}R'} + \mathrm{HO{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}R'} \quad \text{or} \quad \mathrm{HCl} \qquad (3.2)$$

Acetates, succinate half-esters, and other carboxylic acid esters of carbohydrates occur in nature. They are especially found as components of polysaccharides. Sugar phosphates are common metabolic intermediates (Figure 3.19).

Monoesters of phosphoric acid are also found as constituents of polysaccharides. For example, potato starch contains a small percentage of phosphate ester groups, while corn starch contains even less. In producing modified food starch, corn starch is often derivatized to form mono- and di-starch esters or both (see Section 3.3.6.10). Other esters of starch, most notably acetate, succinate and substituted succinate half-esters, and distarch adipates, are components of modified food starches (see Section 3.3.6.10). Sucrose (see Section 3.2.3) fatty acid esters are produced commercially for use as water-in-oil emulsifiers. The family of carrageenan polysaccharides (see Section 3.3.10), which are isolated from red seaweed, contain sulfate groups (half-esters of sulfuric acid, $R{-}OSO_3^-$).

3.1.4.5 Hydroxyl Group Ethers

The hydroxyl groups of carbohydrates, like the hydroxyl groups of simple alcohols, can form ethers, as well as esters, though ethers of carbohydrates are not as common in nature as esters. However,

CHO
HCOH
HOCH
HCOH
HCOH
$CH_2OPO_3H^-$

$CH_2OPO_3H^-$
O
OH
OH
HO
OH

(a)

$CH_2OPO_3H^-$
C=O
HOCH
HCOH
HCOH
$CH_2OPO_3H^-$

$^-HO_3POCH_2$
O
OH
HO
$CH_2OPO_3H^-$
HO

(b)

FIGURE 3.19 Examples of sugar phosphate metabolic intermediates. (a) D-Glucose 6-phosphate. (b) D-Fructose 1,6-bisphosphate.

polysaccharides are etherified commercially to modify their properties and make them more useful for various end-use applications. Examples are the production of methyl ($-O-CH_3$), sodium carboxymethyl ($-O-CH_2-CO_2^-$ Na^+), and hydroxypropyl ($-O-CH_2-CHOH-CH_3$) ethers of cellulose and hydroxypropyl ethers of starch, all of which are approved for food use (see Sections 3.3.6.10, 3.3.7.2, and 3.3.7.3).

A special type of ether, an internal ether linkage between carbon atoms 3 and 6 of a D-galactosyl unit (Figure 3.20), is found in the red seaweed polysaccharides, specifically agar, furcellaran, κ-carrageenan, and ι-carrageenan (see Section 3.3.10). Such an internal ether is known as a *3,6-anhydro ring*, the name deriving from the fact that it can be viewed as the product formed by the net removal of a water (HOH) molecule from the hydroxyl groups on C-3 and C-6.

Members of a family of nonionic surfactants based on sorbitol (D-glucitol) are used in foods as water-in-oil emulsifiers and as defoamers. They are produced by esterification of sorbitol with

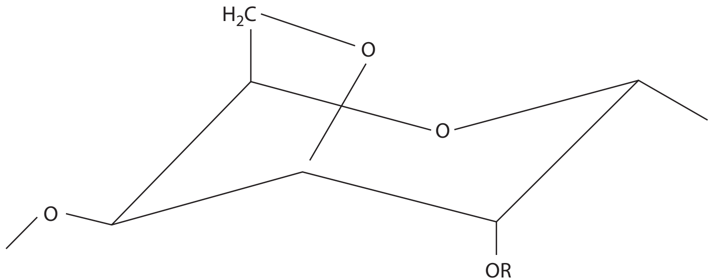


FIGURE 3.20 A 3,6-anhydro-α-D-galactopyranosyl unit found in red seaweed polysaccharides.

FIGURE 3.21 Anhydro-D-glucitols (sorbitans). Numbering refers to the carbon atoms in the original molecule of D-glucose (and of sorbitol).

fatty acids. Cyclic dehydration accompanies esterification (primarily at a primary hydroxyl group, i.e., C-1 or C-6) so that the carbohydrate (hydrophilic) portion is not only sorbitol but also its mono- and dianhydrides (internal cyclic ethers of sorbitol called sorbitans) (Figure 3.21). The products are known as *sorbitan esters* (Spans) and include mono-, di-, and tri-ester derivatives. (The designations mono-, di-, and tri- simply indicate the ratio of fatty acid ester groups to sorbitan.) The product called *sorbitan monostearate* is actually a mixture of partial stearic (C_{18}) and palmitic (C_{16}) acid esters of sorbitol (D-glucitol), 1,5-anhydro-D-glucitol (1,5-sorbitan), 1,4-anhydro-D-glucitol (1,4-sorbitan), and 1,4:3,6-dianhydro-D-glucitol (isosorbide), an internal dicyclic ether. Sorbitan fatty acid esters, such as sorbitan monostearate, sorbitan monolaurate, and sorbitan monooleate, are sometimes modified by reaction with ethylene oxide to produce the so-called ethoxylated sorbitan esters called Tweens that are also nonionic detergents approved by the U.S. Food and Drug Administration (FDA) for food use.

3.1.4.6 Nonenzymic Browning [3,21,40,50,77]

The common browning of foods on heating or during storage is usually due to a chemical reaction between reducing sugars, mainly D-glucose, and a primary amino group (a free amino acid or amino group on a side chain of a protein molecule.) This reaction is called the *Maillard reaction* and the overall process is sometimes designated *Maillard browning*. It is also called *nonenzymic* or *nonenzymatic browning* to differentiate it from enzyme-catalyzed browning (see Section 6.5.2.1) commonly observed in freshly cut fruits and vegetables, such as apples and potatoes.

When aldoses or ketoses are heated with amines, a variety of reactions ensue, producing numerous compounds, some of which are flavors, aromas, and dark-colored polymeric materials; but both reactants disappear only slowly. The generated flavors, aromas, and colors may be either desirable and important for some foods or unintended and undesirable in others. They may be produced slowly during storage or much more rapidly at the high temperatures encountered during frying, roasting, or baking. Good examples of foods in which desirable colors, flavors, and aromas are formed by Maillard browning reactions are French fries and baked bread (most notably within the outer crusts in both cases). In short, the Maillard reaction can be considered as a multifaceted reaction occurring in three primary stages:

1. Initial condensation of a carbonyl compound (e.g., reducing sugar) with an amine, followed by a series of reactions leading to formation of the Amadori product (presuming an aldose).
2. Rearrangement, dehydration, decomposition, and/or reaction of Amadori intermediates to form furfural compounds, reductones/dehydroreductones (and their decomposition products), and Strecker degradation products.
3. Reaction of Maillard intermediary products to form heterocyclic flavor compounds and red/brown to black colored, high-molecular-weight melanoidin pigments.

The first stage starts with the addition of a nonprotonized amine to the electrophilic carbon atom of an aldehydic group of an acyclic sugar to form a Schiff base (an imine, RHC=NHR′), which

FIGURE 3.22 Products of reaction of D-glucose with a primary amine (RNH_2).

may cyclize (in the same way that an aldose cyclizes) to form a *glycosylamine* (sometimes called an *N*-glycoside), as illustrated with D-glucose (Figure 3.22). The Schiff base undergoes a reversible reaction called *Amadori rearrangement* to give, in the case of D-glucose, a derivative of 1-amino-1-deoxy-D-fructose, the so-called *Amadori compound*. Conversely, the *N*-ketosylamine of a ketose sugar would be converted to its respective 2-amino-2-deoxyaldose derivative via the Heyns rearrangement. Amadori and Heyns reaction products are early intermediates in the Maillard browning reaction sequence.

The second stage of the Maillard reaction involves multiple dehydrations of various intermediate products and, in some cases, cleavage of the saccharide chains and a subsequent Strecker degradation of some of the products. Thus, the Amadori compounds undergo significant transformation to form a complex mixture of intermediates and products. A primary class of intermediates formed by rearrangements and eliminations comprises 1-, 3-, and 4-deoxydicarbonyl compounds, commonly designated by their common names as 1-, 3-, and 4-deoxyosones, respectively. Formation of these intermediates occurs most readily in the pH range 4–7, with the specific deoxyosone intermediate formed influenced by the pH. The most prevalent of these intermediates is usually the 3-deoxyosone, more properly called a *3-deoxyhexosulose* (Figure 3.23), which is the favored intermediate (via 1,2-enolization route) under acidic conditions. Conversely, at neutral pH, the 1-deoxyhexosulose (via 2–3-enolization) is most prevalent.

Osones will undergo rapid dehydration, especially at high temperature, and may also cyclize in the same way that aldoses and ketoses do. As the reaction proceeds, most notably under acidic pH conditions (i.e., 3-deoxyosone route), the dehydrated intermediate may cyclize to form a furan derivative—that from a hexose being 5-hydroxymethyl-2-furaldehyde, commonly known as

FIGURE 3.23 Conversion of the Amadori product into HMF.

hydroxymethylfurfural (HMF) (Figure 3.23), and that formed from a pentose is furfural (furaldehyde). When high concentrations of compounds containing primary amino groups (such as proteins [see Chapter 5] containing higher proportions of L-lysine) are present, the primary products are pyrroles (products in which the ring oxygen atom of HMF and furfural is a nitrogen atom, N−R). Under neutral or mildly alkaline conditions, intermediates called reductones are formed from 1-deoxyosones, with many of the intermediate products formed via this pathway similar to those formed during caramelization reactions (Section 3.1.4.7). Reductones are antioxidants, and because reductones can be involved in redox reactions, other intermediates can be formed from them (Figure 3.24). For example, furanone compounds, including 4-hydroxy-5-methyl-3(2*H*)-furanone—a component of cooked meat flavor produced from reactions of ribose with amines, are derived via the 2,3-enolization pathway. Maltol and isomaltol (Figure 3.25), both of which contribute to the flavor and aroma of

FIGURE 3.24 Two of several types of structures of reductones.

FIGURE 3.25 Maltol and isomaltol.

bread, have been proposed to be potentially formed from the 1-deoxyosone via direct dehydration, though 4-O substitution of the 1-deoxyosone with either a glucosyl or galactosyl unit is reported to enhance maltol formation.

Osones (Figure 3.23) will also undergo cleavage, either between the two carbonyl groups or at the site of an enediol (−COH=COH−), forming shorter chain products, primarily aldehydes that can undergo various reactions. An important reaction of dicarbonyl compounds (osones and deoxyosones) is the Strecker degradation. Reaction of one of these compounds with an α-amino acid ($R{-}CHNH_2{-}CO_2H$) results first in a Schiff base being formed, and then decarboxylation (releasing CO_2), dehydration, and elimination to produce an aldehyde that is one carbon atom shorter than the original amino acid. Aldehydes produced from amino acids often are major contributors to the aroma produced during nonenzymic browning, with the pool of amino acids impacting the nature of the flavor compounds produced. Among the important aroma compounds produced in this way are 3-methylthiopropanal (methional, $CH_3{-}S{-}CH_2{-}CH_2{-}CHO$) from L-methionine, phenylacetaldehyde ($Ph{-}CH_2{-}CHO$) from L-phenylalanine, methylpropanal ($(CH_3)_2{-}CH{-}CHO$) from L-valine, 3-methylbutanal ($(CH_3)_2{-}CH{-}CH_2{-}CHO$) from L-leucine, and 2-methylbutanol ($(CH_3{-}CH_2)(CH_3){-}CH{-}CHO$) from L-isoleucine.

The third stage of the Maillard reaction involves the formation of high-molecular-weight brown or black pigments and heterocyclic flavor and aroma compounds from the various intermediates. Reactive carbonyl compounds (HMF, furfural, and other carbonyl compounds) and compounds containing amino groups (primarily amino acids) polymerize to form a mixture of dark-colored, insoluble, nitrogen-containing polymers, collectively called *melanoidins*. The variety of pigments formed arises from the diversity of intermediates and the array of possible condensation reactions. Some intermediates contain nitrogen; others contain only carbon, hydrogen, and oxygen atoms. All melanoidins contain aromatic rings and conjugated double bonds, but vary in color (brown to black), molecular weight, nitrogen content, and solubility.

Other products of the Maillard browning reaction are modified proteins. Protein modification primarily is the result of their reaction (especially reaction of the side chains of their L-lysine and L-arginine units) with carbonyl group-containing compounds such as reducing sugars, osones, furfural, HMF, and pyrrole derivatives. For example, reaction of the ε-amino group of a unit of L-lysine in a protein molecule followed by the Amadori rearrangement converts the L-lysine unit into a unit of *N*-fructofuranosyl-lysine. Further reactions result in substituted furan and pyrrole rings, being formed from the fructofuranosyl unit that is attached to the protein molecule. Reactions of this kind destroy the amino acid. Since L-lysine is an essential amino acid, its destruction in this way reduces the nutritional quality of the food. Losses of lysine and arginine of 15%–40% in baked and roasted foods are common.

The Maillard product mixture formed is a function of temperature, time, pH, the nature of the reducing sugar, and the nature of the amino compound for the following reasons. Different sugars undergo nonenzymic browning at different rates. The reactivity of the carbonyl group differs according to the following rules: Aldoses are generally more reactive with amino acids than are ketoses, while α-dicarbonyl compounds are even more reactive than aldoses. However, some studies have shown that D-fructose undergoes the Maillard browning reaction faster than D-glucose [16a]. Reactivity follows the order trioses > tetroses > pentoses > hexoses > disaccharides. Though sucrose

is a nonreducing sugar (Section 3.2.3), it may be degraded to fructose and glucose during heating and still measurably contribute to Maillard browning reactions. Amino compounds exhibit variable reactivity according to their basicity. Ammonium ions react with reducing sugars more readily than amines, while secondary amines give different reaction products than primary amines. While proteins, peptides, and amino acids may all participate in the Maillard reaction, the reactivity of proteins is primarily due to the ε-amino group of lysine, though the guanidyl group of arginine and the thiol group of cysteine may likewise react.

Protonation of the oxygen atom of the carbonyl group increases its reactivity, while protonation of the amino group reduces its reactivity; thus, pH is important in controlling the extent of reaction. The reaction rate is maximum in a slightly acidic medium for a reaction with amines and in a slightly basic medium for reaction of amino acids (see Section 5.2). Because the reaction has a relatively high energy of activation, application of heat is generally required. The rate of the Maillard reaction is also a function of the water activity (a_w) of a food product, reaching a maximum at a_w values in the range 0.6–0.7. Thus, for some foods, Maillard browning can be controlled by controlling water activity, as well as by controlling reactant concentrations, time, temperature, and pH. Sulfur dioxide and bisulfite ions react with aldehyde groups, forming addition compounds, and thus will inhibit Maillard browning by removing at least some of a reactant (reducing sugar, HMF, furfural, etc.). Color, taste, and aroma are, in turn, greatly impacted by the product mixture. Reaction variables that can be controlled to increase or decrease the Maillard browning reaction are the following: (1) temperature (decreasing the temperature decreases the reaction rate) and time at the temperature; (2) pH (decreasing the pH decreases the reaction rate); (3) adjustment of the water content (maximum reaction rate occurs at water activity values of 0.6–0.7 [~30% moisture]); (4) the specific sugar; and (5) the presence of transition-metal ions that undergo a one-electron oxidation under energetically favorable conditions, such as Fe(II) and Cu(I) ions (a free radical reaction may be involved near the end of the pigment-forming process.)

In summary, Maillard browning products, including soluble and insoluble polymers, are formed where reducing sugars and amino acids, proteins, and/or other nitrogen-containing compounds are heated together, for example, in soy sauce and bread crusts. Browning is desired in baking (e.g., in bread crusts and cookies) and roasting of meats. The volatile compounds produced by nonenzymic browning (the Maillard reaction) during baking, frying, or roasting often provide desirable aromas. Maillard reaction products are also important contributors to the flavor of milk chocolate, caramels, toffees, and fudges, in which reducing sugars react with milk proteins. The Maillard reaction also produces flavors, especially bitter substances, which may be desired (e.g., in coffee). On the other hand, the Maillard reaction can result in off-flavors and off-aromas, which are commonly produced during the ultrahigh-temperature pasteurization of milk, storage of dehydrated foods, and grilling of meat or fish. Application of heat to intermediate moisture foods is generally required for nonenzymic browning to occur.

3.1.4.7 Caramelization [3,67]

Heating of carbohydrates, in particular sucrose (Section 3.2.3) and reducing sugars, without nitrogen-containing compounds effects a complex group of reactions known as caramelization. The reaction is facilitated by small amounts of acids and certain salts. Although it does not involve amino acids or proteins as reactants, caramelization is similar to nonenzymic browning. The final product—caramel—like in Maillard browning, contains a complex mixture of polymeric compounds formed from unsaturated cyclic (five- and six-membered ring) compounds; both flavor and aroma compounds are also produced. Heating causes dehydration of the sugar molecule with introduction of double bonds or formation of anhydro rings (Figure 3.20). As in Maillard browning, intermediates such as 3-deoxyosones and furans are formed. The unsaturated rings may further condense to form useful brown-colored polymers possessing conjugated double bonds. Catalysts increase the reaction rate and are used to direct the reaction to effect the specific type of caramel color produced, as well as their solubilities and acidities.

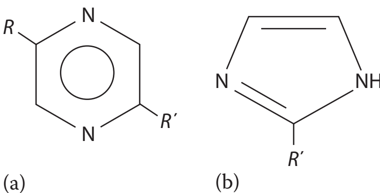


FIGURE 3.26 Pyrazine (a) and imidazole (b) derivatives formed during caramelization in the presence of ammonia $R = –CH_2–(CHOH)_2–CH_2OH$, $R' = –(CHOH)_3–CH_2OH$.

Caramel products are produced commercially as both coloring and flavoring ingredients. To make a caramel ingredient, a carbohydrate is heated alone or in the presence of an acid, a base, or a salt. The carbohydrate most often used is sucrose, but D-fructose, D-glucose (dextrose), invert sugar (see Section 3.2.3), glucose syrups, high-fructose syrups (see Section 3.3.6.9), malt syrups, and molasses may also be used. Acids that may be used are food-grade sulfuric, sulfurous, phosphoric, acetic, and citric acids. Bases that may be used are ammonium, sodium, potassium, and calcium hydroxides. Salts that may be used are ammonium, sodium, and potassium carbonates, bicarbonates, phosphates (both mono- and dibasic), sulfates, and bisulfites. So there is a very large number of variables, including temperature, in caramel manufacture. Ammonia may react with intermediates, such as 3-deoxyosones, produced by thermolysis to produce pyrazine and imidazole derivatives (Figure 3.26).

There are four recognized classes of caramel, all of which may or can employ an acid or base during preparation, in addition to the specific conditions noted below for each class. Class I caramel (also called *plain caramel* or *caustic caramel*) is prepared by heating a carbohydrate without a source of either ammonium or sulfite ions. Class II caramel (also called *caustic sulfite caramel*) is prepared by heating a carbohydrate in the presence of a sulfite but in the absence of any ammonium ions. This caramel, which is used to add color to beers and other alcoholic beverages, is reddish brown, contains colloidal particles with slightly negative charges, and has a solution pH of 3–4. Class III caramel (also called *ammonium caramel*) is prepared by heating a carbohydrate in the presence of a source of ammonium ions but in the absence of sulfite ions. This caramel, which is used in bakery products, syrups, and puddings, is reddish brown, contains colloidal particles with positive charges, and gives a solution pH of 4.2–4.8. Class IV caramel (also called *sulfite ammonium caramel*) is prepared by heating a carbohydrate in the presence of both sulfite and ammonium ions. This caramel, which is used in cola soft drinks, other acidic beverages, baked goods, syrups, candies, pet foods, and dry seasonings, is brown, contains colloidal particles with negative charges, and gives a solution pH of 2–4.5. In this case, the acidic salt catalyzes the cleavage of the glycosidic bond of sucrose, and the ammonium ion reacts with the liberated reducing sugars to further undergo the Amadori rearrangement (Heyns rearrangement in the case of ketoses) (see Section 3.1.4.6). The pigments in all four types of caramel are large polymeric molecules with complex, variable, and unknown structures. It is these polymers that make up the colloidal particles. Their rate of formation increases with increasing temperature and pH. Of course, caramelization may also occur during cooking or baking, especially when sugar is present. It may occur along with nonenzymic (Maillard) browning in food processes where both reducing sugars and amines are present, during the preparation of chocolate and fudge.

3.1.4.8 Formation of Acrylamide in Food [2,24,27,57,78,81]

The Maillard reaction has been implicated in the formation of acrylamide in many foods that have been heated to high temperatures during processing or preparation. Levels of acrylamide (typically less than 1.5 ppm) have been reported in a wide range of food products subjected to frying, baking, puffing, roasting, or other elevated-temperature process schemes associated with production or

TABLE 3.3
Ranges of Acrylamide Found in Some Common Food Products Containing High Levels

Food	ppb Acrylamide[a]
Breads	24–130
Breakfast cereals (RTE)	11–1057
Chocolates	0–74
Coffee (ground, unbrewed)	64–319
Coffee, decaffeinated (ground)	27–351
Coffee with chicory	380–609
Cookies	34–955
Crackers	26–1540
French fries	109–1325
Potato chips	117–2762[b]
Pretzels	46–386
Tortilla chips	130–196

Source: Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Silver Spring, MD. (The European Safety Authority also monitors the amounts of acrylamide in foods and exposures to it by age groups.)

[a] Extreme values, especially extremely high values, are usually representative of only a small number of sampled products.

[b] A sample of sweet potato chips contained 1570 ppb acrylamide and a sample of veggie chips contained 1970 ppb.

preparation (Table 3.3). Acrylamide is not detected in unheated or even boiled foodstuffs, such as boiled potatoes, because the temperature during boiling does not go above ~100°C. Acrylamide is undetected, or detected at only very low levels, in canned or frozen fruits, vegetables, and vegetable protein products (vegetable burgers and related products) with the exception of pitted ripe olives, in which the measured levels ranged from 0 to 1925 ppb. (*Note*: Acrylamide is a known neurotoxicant at doses much higher than are obtained from food. There is no direct evidence that acrylamide causes cancer in, or has any other physiological effects on, humans in amounts typical of dietary exposure. There are, however, efforts under way to reduce acrylamide levels in foods, efforts which begin with understanding its origin.)

Using a model system of varied sugar and amino acid composition, acrylamide was shown to be formed in a second-order reaction between reducing sugars (carbonyl moiety) and the α-amino group of free L-asparagine [81] (see Section 5.2) (Figure 3.27). The reaction requires the presence of both substrates, and most likely proceeds via a Schiff base intermediate, which then undergoes decarboxylation, followed by carbon–carbon bond cleavage to form acrylamide. The atoms of acrylamide are known to be derived solely from L-asparagine. Though acrylamide is not the favored product of this complex series of reactions (general reaction efficiency ≈ 0.1%), it is able to accumulate to detectable levels in food products subjected to prolonged heating at high temperatures.

The reaction pathways for acrylamide formation in complex food systems is more complicated, extending beyond simply the direct reaction of reducing sugars with asparagine. Fried potato products, such as potato chips and French fries, are notably susceptible to acrylamide formation because potatoes contain both free reducing sugars and free L-asparagine. (Potatoes can accumulate free sugars during storage [particularly at cold temperatures, i.e., 3°C–4°C] with starch being converted first to sucrose and subsequently to D-glucose and D-fructose. Commercially, a solution

FIGURE 3.27 A proposed mechanism of acrylamide formation in foods. (Adapted from Parker, J.K. et al., *J. Agric. Food Chem.*, 60, 9321, 2012; Zyzak, D.V. et al., *J. Agric. Food Chem.*, 51, 4782, 2003.)

of D-glucose is applied to blanched potato strips prior to initial par-frying [before freezing], either by dipping or spraying, to optimize and standardize French fry color development during finish frying.) For fried potato strips, Maillard browning product intermediates (i.e., deoxyhexosuloses, dicarbonyl compounds, etc.; see Section 3.1.4.6) generated during initial reactions of reducing sugars and amines (i.e., amino acids, peptides, proteins) are proposed to react with asparagine and contribute significantly to acrylamide formation [53]. A kinetic model accounting for substrate levels, as well as moisture and temperature gradients during frying, was developed to predict acrylamide levels in finish-fried potato strips. Only 0.6% of the total asparagine consumed in the high-temperature frying reactions of potato strips was converted to acrylamide. Also, D-glucose contributes more to color formation and less to acrylamide formation than D-fructose, which produces opposite effects [29].

Acrylamide formation requires a minimum temperature of 120°C, which means that it cannot occur in high-moisture foods and is kinetically favored with increasing temperatures approaching 200°C. With extended heating at temperatures above 200°C, acrylamide levels may actually decrease via thermal elimination/degradation reactions. Food levels of acrylamide are also impacted by pH. Acrylamide formation is favored as the pH is increased over the range 4–8.

Reduced acrylamide formation in the acid range is thought to be due in part to protonation of the α-amino group of asparagine, reducing its nucleophilic potential. Furthermore, acrylamide appears to undergo increased rates of thermal degradation as the pH decreases. Acrylamide levels increase rapidly, as does browning in general, in the latter stages of prolonged heating processes as the water at food surfaces is driven off to allow surface temperatures to increase above 120°C. Products with high amounts of surface area, such as potato chips, are among those high-temperature processed foods that are prone to acrylamide formation. Thus, the exposed surface area of a food can be an additional factor, provided that reaction substrates and processing temperatures are sufficient for acrylamide formation.

Efforts to minimize the formation of acrylamide in food generally involve one or more of three strategies: (1) elimination or removal of either one or both of the substrates, (2) alteration of processing conditions, including the addition of process aids, and (3) acrylamide removal from food following formation. Through blanching or soaking in water, it is possible to achieve up to a 60% reduction in acrylamide levels within processed potato products via removal of reaction substrates (reducing sugars and free asparagine). Reagent modification (e.g., protonation of asparagine by lowering the pH or conversion of asparagine to aspartic acid with asparaginase), addition of competing substrates that do not yield acrylamide (e.g., amino acids other than asparagine or protein), and incorporation of salts have been shown to mitigate acrylamide formation. Where possible, better control or optimization of thermal processing conditions (temperature/time relationships) may also prove beneficial to minimize acrylamide levels. It is likely that a combination of mitigation methods will be required to effectively limit acrylamide formation within food products, with employed methods likely to vary according to the nature and needs of a particular food system.

Although studies to date have uncovered no association between acrylamide consumption in foods and the risk of cancer, long-term carcinogenicity, mutagenicity, and neurotoxicity studies are still going on as are efforts to reduce acrylamide formation during food processing and preparation.

3.1.5 Summary

- Monosaccharides are carbohydrates that cannot be broken down by hydrolysis into smaller carbohydrate units.
- Monosaccharides are described as polyhydroxy aldehydes or ketones (open-chain or acyclic form), but may cyclize to form intramolecular ring structures (hemiacetal form).
- Individual monosaccharides are defined and differentiated by the
  - Number of carbon atoms (3–9 most common)
  - Nature of the carbonyl group: aldehyde (aldose) versus ketone (ketose)
  - Orientation of hydroxyl groups about chiral carbon atoms
  - Hydroxyl group orientation about the highest numbered chiral carbon atom: D- vs. L
  - Type of ring configuration (α vs. β), ring size (commonly five- or six-membered), and ring conformation (e.g., $^4C_1$ vs. $^1C_4$).
- Monosaccharides can be converted into glycosides (the acetal form), oxidized to carboxylic acids (aldoses only), reduced to alcohols, or modified to form hydroxyl group esters or ethers.
- Monosaccharides react at high temperatures to form brown pigments, flavors, and aromas within characteristic foods via nonenzymic (Maillard) browning and caramelization reactions.

3.2 OLIGOSACCHARIDES

An oligosaccharide contains 2–10 or 2–20 sugar units joined by glycosidic bonds, depending on who is defining the term. When a molecule contains more than 20 units, it is generally considered to be a polysaccharide.

Disaccharides are glycosides in which the aglycon is another monosaccharide unit. A compound containing three monosaccharide units is a *trisaccharide*. Structures containing 4–10 glycosyl units, whether linear or branched, are *tetra*-, *penta*-, *hexa*-, *octa*-, *nona*-, and *decasaccharides*, and so on. Only a few oligosaccharides occur in nature; most are produced by hydrolysis of polysaccharides into smaller units. Glycosidic bonds are acetal structures, and may undergo hydrolysis in the presence of water, an acidic pH, and heat or specific glycosidase enzymes.

3.2.1 Maltose

Maltose (Figure 3.28) is an example of a disaccharide. The reducing end unit (customarily depicted on the right-hand end of the molecule) has a potentially free aldehydic group and in solution will be in equilibrium with α and β six-membered (pyranose) ring forms, as described earlier for monosaccharides (see Section 3.1.2). Since O-4 is blocked by the attachment of the second D-glucopyranosyl unit, a furanose ring cannot form. Maltose is a reducing sugar, because its aldehydic group is free to react with oxidants and, in fact, undergoes almost all reactions that free aldoses do (see Section 3.1.4).

Maltose is produced by the hydrolysis of starch using the enzyme β-amylase (see Section 3.3.6.9). It occurs rarely in nature and only in plants as a result of partial hydrolysis of starch. Maltose is produced during malting of grains, especially barley, and commercially by the specific enzyme-catalyzed hydrolysis of starch using β-amylase from *Bacillus* species, though the β-amylases from barley seed, soybeans, and sweet potatoes may also be used. Maltose is used sparingly as a mild sweetener for foods. Maltose may also be reduced to the alditol, maltitol, which is used in sugarless chocolate (see Section 3.1.4.2).

3.2.2 Lactose

The disaccharide lactose (Figure 3.29) occurs in milk, mainly in free form, and to a small extent as a component of higher oligosaccharides. The concentration of lactose in milk varies from 2% to

FIGURE 3.28 Maltose.

FIGURE 3.29 Lactose.

8.5% depending on the mammalian source, with lactose being the primary carbohydrate source for developing mammals. Cow and goat milks contain 4.5%–4.8% lactose, human milk about 7%. In humans, lactose constitutes 40% of the energy consumed by an infant during nursing. Utilization of lactose for energy must be preceded by hydrolysis to its constituent monosaccharides D-glucose and D-galactose, because only monosaccharides are absorbed from the small intestine. Milk also contains 0.3%–0.6% of lactose-containing oligosaccharides, many of which are important as energy sources for growth of a specific variant of *Lactobacillus bifidus*, which, as a result, is the predominant microorganism of the intestinal flora of breast-fed infants.

Lactose is ingested in milk and other unfermented dairy products, such as ice cream. Fermented dairy products, such as most yogurt and cheese, contain less lactose because, during fermentation, much of the lactose is converted into lactic acid. Lactose stimulates intestinal adsorption and retention of calcium, and is not digested until it reaches the small intestine, where the hydrolytic enzyme lactase is present. Lactase (a β-galactosidase) is a membrane-bound enzyme located in the brush border epithelial cells of the small intestine. It catalyzes the hydrolysis of lactose into its constituent monosaccharides D-glucose and D-galactose, both of which are rapidly absorbed and enter the blood stream.

$$\text{lactose} \xrightarrow{\text{lactase}} \text{D-glucose} + \text{D-galactose} \tag{3.3}$$

If for some reason the ingested lactose is only partially hydrolyzed, that is, only partially digested, or is not hydrolyzed at all, a clinical syndrome called lactose intolerance may result in some individuals. If there is a deficiency of lactase, some lactose remains in the lumen of the small intestine, where its presence tends to draw fluid into the lumen by osmosis. This fluid produces abdominal distention and cramps. From the small intestine, the lactose passes into the large intestine (colon) where it undergoes anaerobic bacterial fermentation to lactic acid (present as the lactate anion) (Figure 3.30) and other short-chain acids. The increase in the concentration of molecules, that is, the increase in osmotic strength, results in still greater retention of fluid. In addition, the acidic products of fermentation lower the pH and irritate the lining of the colon, leading to an increased movement of the contents. Diarrhea is caused both by the retention of fluid and the increased movement of the intestinal contents. The gaseous products of fermentation cause bloating and cramping.

Lactose intolerance is not usually observed in children until after about 6 years of age. At this point, the incidence of lactose-intolerant individuals begins to rise and increases throughout the life span with the greatest incidence in the elderly. Both the incidence and the degrees of lactose intolerance vary by ethnic group, indicating that the presence or absence of lactase is under genetic control.

There are three ways to overcome the effects of lactase deficiency. One is to greatly reduce or eliminate lactose by fermentation of the food, as in yogurt and cultured buttermilk products. Another is to produce reduced-lactose milk by adding lactase to it (see Chapter 6). However, both

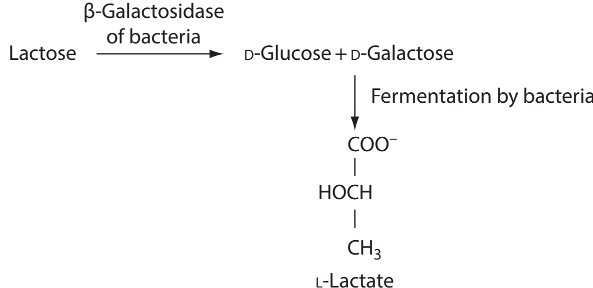


FIGURE 3.30 The fate of lactose in the large intestine of persons with lactase deficiency.

products of hydrolysis, namely D-glucose and D-galactose, are sweeter than lactose, and at about 80% hydrolysis, the taste change becomes quite evident. Therefore, most reduced-lactose milk has the lactose reduced as close as possible to the 70% government-mandated limit for a claim. The third is for the lactase-deficient individual to consume β-galactosidase along with the dairy product.

3.2.3 Sucrose [42,54]

Sucrose is composed of an α-D-glucopyranosyl unit and a β-D-fructofuranosyl unit linked head to head (reducing end to reducing end) rather than by the usual head-to-tail linkage (Figure 3.31). Since it has no free reducing end, it is classified as a nonreducing sugar.

There are two principal sources of commercial sucrose—sugar cane and sugar beets. Also present in sugar beet extract are a trisaccharide, raffinose, which has a D-galactopyranosyl unit attached to sucrose, and a tetrasaccharide, *stachyose*, which contains a second D-galactosyl unit (Figure 3.32). These oligosaccharides, also found in beans, are nondigestible. These and other carbohydrates that are not completely broken down into monosaccharides by intestinal enzymes and are not absorbed pass into the colon. There, they are metabolized by microorganisms producing lactate and gas. Diarrhea, bloating, and flatulence result.

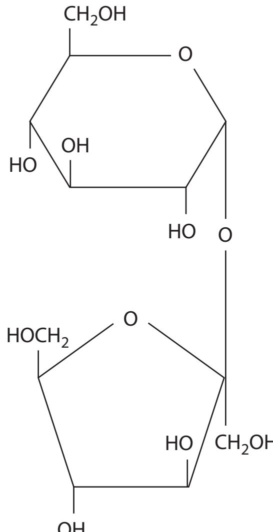


FIGURE 3.31 Sucrose.

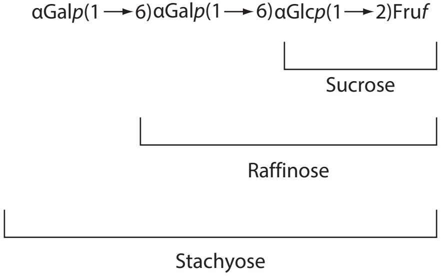


FIGURE 3.32 Sucrose, raffinose, and stachyose. (For explanation of the shorthand designations of structures, see Section 3.3.1.)

Sucrose has a specific optical rotation of +66.5°. The equimolar mixture of D-glucose and D-fructose produced by the hydrolysis of the glycosidic bond joining the two monosaccharide units has a specific optical rotation of −33.3°. Early investigators noticing this called the process *inversion* and the product *invert sugar*.

Sucrose and most other low-molecular-weight carbohydrates (e.g., monosaccharides, alditols, disaccharides, and other low-molecular-weight oligosaccharides), because of their great hydrophilicity and solubility, can form highly concentrated solutions of high osmolality. Such solutions, as exemplified by honey, need no preservatives themselves and can be used not only as sweeteners (though not all such carbohydrate syrups have such a high degree of sweetness) but also as preservatives and humectants.

A portion of the water in any carbohydrate solution is non-freezable. When the freezable water crystallizes (i.e., forms ice), the concentration of solute in the remaining liquid phase increases, and the freezing point decreases. There is a consequential increase in the viscosity of the remaining solution. Eventually, the liquid phase solidifies as a glass, in which the mobility of all molecules becomes restricted and diffusion-dependent reactions become very slow (see Chapter 2) and, because of the restricted motion, water molecules become unfreezable, that is, they cannot form crystals. In this way, carbohydrates function as cryoprotectants and protect against the dehydration that destroys the structure and texture caused by freezing.

The sucrase-isomaltase enzyme of the human intestinal tract catalyzes hydrolysis of sucrose into D-glucose and D-fructose, making sucrose one of the three non-simple carbohydrates humans can digest and utilize for energy, the other two being lactose and starch. Monosaccharides (D-glucose and D-fructose being the nutritionally significant ones in the human diet) are absorbed directly from the small intestine and pass into the blood stream.

A compound made by replacing three of the eight hydroxyl groups of sucrose with chlorine atoms (Sucralose) is a high-intensity sweetener (see Chapter 12). The process also results in the conversion of the native glucose molecule of sucrose to galactose.

3.2.4 Trehalose [48]

Trehalose is a commercially available disaccharide that is comprised of two α-D-glucopyranosyl units linked through their respective anomeric carbon atoms (similar to sucrose), and thus is a nonreducing sugar. Although not used extensively, it is claimed to have unique properties when used in processed food products, namely, the ability to stabilize and protect enzymes and other proteins from heating and freezing, to reduce the retrogradation of cooked starch and to extend the shelf-life of bakery products, to preserve cell structures during freezing, and to preserve flavors and aromas, especially during freezing.

3.2.5 Cyclodextrins [17,21,65]

Cyclodextrins, formerly known as Schardinger dextrins and cycloamyloses, are a family of cyclic oligosaccharides comprised of (1→4)-linked α-D-glucopyranosyl units (Figure 3.33). These cyclic structures are formed from soluble, partially hydrolyzed starch polymers (see Section 3.3.6.9) through action of the enzyme cyclodextrin glucanotransferase (also referred to as *cyclomaltodextrin glucanotransferase*) (see Chapter 6), which catalyzes the intramolecular cyclization of starch polymer chains. Cyclodextrins consist of six, seven, or eight glucosyl units; these cyclodextrins are referred to as α-, β-, and γ-cyclodextrins, respectively. In commercial production schemes, cyclodextrins may be isolated by selective crystallization (following treatment of the reaction broth with glucoamylase) or differential precipitation involving the addition of a substrate-specific complexing agent (typically an organic solvent). While α-, β- and γ-cyclodextrins are all permitted for use in food (self-affirmed GRAS [generally regarded as safe] regulatory status), only β-cyclodextrin is utilized to any appreciable degree due to its lower cost (relative to the other two, but still rather high) and established function.

FIGURE 3.33 Generalized chemical structures of α- (n = 6), β- (n = 7), and γ- (n = 8) cyclodextrins.

Cyclodextrins possess a truncated funnel- or doughnut-like geometry with an internal hydrophobic core or cavity and a hydrophilic external surface (Figure 3.34). The solubility of cyclodextrins in water, which is attributable to the presence of the hydroxyl groups on their outer molecular surface, is different for α-, β-, and γ-types (Table 3.4). γ-Cyclodextrin is the most water soluble, followed by α-cyclodextrin, while the β-type, due to an extensive band of intramolecular hydrogen bonds spanning the entire outer molecular perimeter, has the lowest water solubility. In contrast, the internal cavity provides a hydrophobic environment for the formation of inclusion complexes with nonpolar guest molecules through hydrophobic and other noncovalent associations. The size of the inner cavity increases as the number of cyclodextrin glycosyl units increases (γ > β > α) (Table 3.4). This complexing ability is the most significant property of cyclodextrins and is the driving force for cyclodextrin's use in almost all food and industrial applications. Within food systems, cyclodextrins may be used to complex flavors, lipids, and color compounds for an array of purposes. Cyclodextrins may be used to complex undesireable constituents (such as masking of off-flavors,

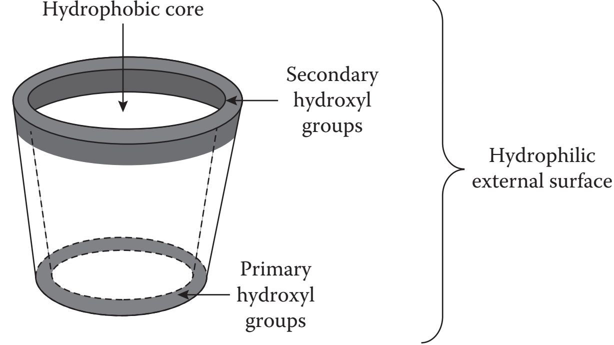


FIGURE 3.34 Depiction of the idealized geometric shape of cyclodextrins.

TABLE 3.4
Chemical Characteristics of α-, β-, and γ-Cyclodextrins

Characteristic	α	β	γ
No. of glucosyl units	6	7	8
Molecular weight	972	1135	1297
Solubility (g/100 mL at 25°C)	14.5	1.9	23.2
Cavity diameter (Å)	4.7–5.3	6.0–6.5	7.5–8.3

odors, and bitter compounds and removal of cholesterol and free fatty acids), to stabilize against chemical oxidation (e.g., protection of flavor compounds, binding of enzymic browning phenolic precursors), to enhance non-water-soluble (lipophilic) flavor compounds, and to improve the physical stability of food ingredients (encapsulation of volatiles, controlled release of flavor).

3.2.6 Summary

- Oligosaccharides contain 2–20 monosaccharide units joined via glycosidic linkages.
- The most abundant oligosaccharide is the disaccharide sucrose, which is comprised of D-glucose (an aldose) in the six-membered (pyranose) ring form joined to D-fructose (a ketose) in the five-membered (furanose) ring form via an anomeric carbon atom to anomeric carbon atom glycosidic linkage.

3.3 POLYSACCHARIDES [12,18,72]

3.3.1 Polysaccharide Chemical Structures and Properties

Polysaccharides are polymers of monosaccharides. Like oligosaccharides, they are composed of glycosyl units in linear or branched arrangements, but most are much larger than the 10- or 20-unit limit of oligosaccharides. The number of monosaccharide units in a polysaccharide, which is termed its *degree of polymerization* (DP), varies. Only a few polysaccharides have DPs less than 100; most have DPs in the range 200–3000. The larger ones, like cellulose, have DPs of 7,000–15,000. Starch amylopectin is even larger, having an average molecular weight of at least 10^7 (DP > 60,000). It is estimated that more than 90% of the carbohydrate mass in nature is in the form of polysaccharides. The general scientific term for polysaccharides is *glycans*.

As implied in the above paragraph, all polysaccharides occur in a range of molecular weights—not just those from different sources, but also those within a specific source. This noted range in molecular weight occurs because polysaccharides, unlike proteins, are synthesized by enzymes without the aid of an RNA template. The term *polydisperse* is used to describe the range of molecular weights among chains of a given polysaccharide population; thus, each molecule within a preparation of a given polysaccharide may have a molecular weight (DP) that is different from that of any other molecule in the preparation. For the same reason, that is, biosynthesis without the aid of a template, the chemical fine structures of most polysaccharides also differ from molecule to molecule. For a given polysaccharide, chemical fine structures may vary in the type, proportion, and/or distribution of monosaccharide units and linkages comprising individual chains and in the number and distribution of non-carbohydrate groups (if present). The term that describes this characteristic is *polymolecular*.

If all the glycosyl units of a given polysaccharide are of the same monosaccharide type, it is homogeneous with respect to the monomer units and is a *homoglycan*. Examples of homoglycans are cellulose (see Section 3.3.7), starch amylose (see Section 3.3.6.1), which is linear, and amylopectin (see Section 3.3.6.2), which is branched. All three are composed only of D-glucopyranosyl units.

When a polysaccharide is composed of two or more different monosaccharide units, it is a *heteroglycan*. A polysaccharide that contains two different monosaccharide units is a diheteroglycan; a polysaccharide that contains three different monosaccharide units is a triheteroglycan, and so on. Diheteroglycans often, but not always, consist of either blocks of similar monosaccharide units repeated along a linear polymer chain, or comprise a linear chain of one type of glycosyl unit, with a second type present as single-unit branches. Examples of the former type are algins (see Section 3.3.11), and of the latter type are guar and locust bean gums (see Section 3.3.8).

In the shorthand notations of oligo- and polysaccharides, the glycosyl units are designated by the first three letters of their names (with the first letter being capitalized), except for glucose, which is Glc. If the monosaccharide unit is that of a D-sugar, the D is assumed and omitted; only L-sugars are

so designated in shorthand notations: for example, LAra for L-arabinose. The size of the ring is designated by an italicized *p* for pyranose or *f* for furanose. The anomeric configuration is designated with α or β as appropriate; for example, an α-D-glucopyranosyl unit is indicated as αGlc*p*. Uronic acids are designated with a capital A; for example, an L-gulopyranosyluronic acid unit (see Section 3.3.11) is depicted as LGul*p*A. The position of linkages are designated either as, for example, 1→3 or 1,3, the latter being more commonly used by biochemists and the former more commonly used by carbohydrate chemists. Using the shorthand notation, the structure of lactose is represented as βGal*p*(1→4)Glc or βGal*p*1,4Glc and maltose as αGlc*p*(1→4)Glc or αGlc*p*1,4Glc. (Note that the reducing end cannot be designated as α or β or as being in a pyranose or furanose ring [except in the case of a crystalline product] because the ring can open and close; that is, in solutions of both lactose and maltose and other oligo- and polysaccharides, the reducing end unit will occur as a mixture of α- and β-pyranose ring forms and the acyclic form, with rapid interconversion between them, see Figure 3.12.)

3.3.2 Polysaccharide Crystallinity, Solubility, and Cryostabilization

Most polysaccharides contain glycosyl units that, on average, have three free hydroxyl groups. Each of the hydroxyl groups has the possibility of hydrogen bonding to one or more water molecules. Also, the ring oxygen atom and the glycosidic oxygen atom involved in the linkage connecting one sugar ring to another can form hydrogen bonds with water. With every sugar unit in the chain having the capacity to hold water molecules, glycans possess a strong affinity for water, and most hydrate readily when water is available. In aqueous systems, polysaccharide particles can take up water, swell, and usually undergo partial or complete dissolution.

Polysaccharides, like lower-molecular-weight carbohydrates, modify and control the mobility of water in food systems, and water plays an important role in influencing the physical and functional properties of polysaccharides. Polysaccharides and water together impact and control many functional properties of foods, including texture.

The water of hydration that is naturally hydrogen-bonded to and, thus, solvates polysaccharide molecules, is often described as non-freezable water, that is, water whose structure has been sufficiently modified by the presence of the polymer molecule such that it will not freeze. This water has also been referred to as *plasticizing water*. The motions of the molecules that make up this water are retarded; however, they are able to exchange freely and rapidly with bulk water molecules. This water of hydration makes up only a small part of the total water in gels and fresh tissue foods. Water in excess of the hydration water is entrapped in capillaries and cavities of various sizes in the gel or tissue.

Polysaccharides are cryostabilizers rather than cryoprotectants. They do not increase the osmolality or depress the freezing point of water significantly, because they are large, high-molecular-weight molecules, and osmotic strength and freezing point depression are colligative properties. When a polysaccharide solution is frozen, a two-phase system of crystalline water (ice) and a glass consisting of perhaps 70% polysaccharide molecules and 30% non-freezable water is formed. As in the case of solutions of low-molecular-weight carbohydrates, the non-freezable water is part of a highly concentrated solution in which the mobility of water molecules is restricted by the extremely high viscosity. While some polysaccharides provide cryostabilization by producing this freeze-concentrated matrix, which severely limits molecular mobility, others provide cryostabilization by restricting ice crystal growth by adsorption to nuclei or active crystal growth sites. Some polysaccharides in nature are ice crystal nucleators.

So both high- and low-molecular-weight carbohydrates are generally effective in protecting food products stored at freezer temperatures (typically −18°C) from destructive changes in texture and structure, with various degrees of effectiveness. The improvement in product quality and storage stability is a result of controlling both the amount (particularly in the case of low-molecular-weight carbohydrates) and the structural state (particularly in the case of polymeric carbohydrates) of the freeze-concentrated, amorphous matrix surrounding ice crystals.

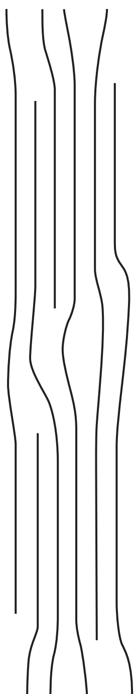


FIGURE 3.35 Crystalline regions in which the chains are parallel and ordered, separated by amorphous regions.

Most, if not all, polysaccharides, except those with very bush-like branch-on-branch structures, exist in some sort of helical shape. Certain linear homoglycans, like cellulose (see Section 3.3.7), have flat ribbon-like structures. Such uniform linear chains undergo extensive hydrogen bonding with each other so as to form crystallites separated by amorphous regions (Figure 3.35). It is these crystallites of linear chains that give cellulose fibers, like wood and cotton fibers, their great strength, insolubility, and resistance to breakdown, the latter because the crystalline regions are nearly inaccessible to enzyme penetration. These polysaccharides with flat ribbon-like structures (leading to extensive interchain orientation and associations) and crystallinity are exceptions. Most polysaccharides are not so crystalline and readily hydrate and dissolve in water.

Unbranched diheteroglycans containing nonuniform blocks of glycosyl units and most branched polysaccharides cannot form crystallites because their chain segments are prevented from becoming closely packed over extended lengths necessary to provide enough intermolecular bonding to form sizeable crystallites. Hence, these chains have a degree of solubility that increases as the chains become less able to fit closely together to form crystallites. In general, polysaccharides become more soluble in proportion to the degree of irregularity of the molecular chains, which is another way of saying that, as the ease with which molecules fit or bond together decreases, the solubility of the molecules increases.

Water-soluble polysaccharides and modified polysaccharides used in food and other industrial applications are divided into two categories: (1) native and modified starches and (2) non-starch polysaccharides, which are known as *hydrocolloids* or *food gums*. Hydrocolloids are sold as powders of varying particle size. Non-starch polysaccharides are also the major components of dietary fiber (see Section 3.4).

3.3.3 Polysaccharide Solution Viscosity and Stability [39,49,55,72]

Polysaccharides (hydrocolloids/food gums) are used in foods primarily to thicken and/or gel aqueous systems, or otherwise to modify and/or control the flow properties and textures of liquid products and the deformation properties of semisolid, that is, soft products. Polysaccharides other than starch are generally used in food products at low concentrations of 0.10%–0.50%, indicative of their great ability to produce viscosity and to form gels.

The viscosity of a polymer solution is a function of the size and shape of its molecules and the conformations they adopt in the solvent. In foods and beverages, the solvent is an aqueous solution of other solutes. The shapes of polysaccharide molecules in solution are a function of the allowable rotations about the bonds of the glycosidic linkages. The greater the internal freedom at each glycosidic linkage, the greater the number of conformations available to each individual segment. Chain flexibility provides a strong entropic drive, which generally overcomes energy considerations and induces the chain to approach disordered or random coil (Figure 3.36) states in aqueous solution. However, most polysaccharides exhibit deviations from strictly random coil states, forming stiff coils, often with helical segments, the specific nature of the coils being a function of the monosaccharide composition and linkages.

The motion of linear polymer molecules in solution results in their sweeping out a large spherical space or domain. When they collide with each other or experience overlap of their respective domains, they create friction, consume energy, and thereby produce viscosity. Linear polysaccharides produce highly viscous solutions, even at low concentrations. Viscosity depends both on the DP (which is related to molecular weight) and the shape and flexibility of the solvated polymer chain, with longer linear, more extended, and/or more rigid molecules producing the greatest viscosity. With respect to DP, carboxymethylcellulose (CMC) (see Section 3.3.7.2) preparations, for example, can have solution viscosities at 2% concentration that can vary from <5 to >100,000 mPa s. A high-viscosity-grade product would probably be used if product thickening was the needed attribute, while a low-viscosity-grade product would be used if it were desirable to have more solids in solution, such as for film formation or to provide sufficient body/mouthfeel.

A highly branched polysaccharide will sweep out much less space than a linear polysaccharide of the same molecular weight or DP (Figure 3.37). As a result, at equal concentrations in solution, highly branched molecules will collide or overlap less frequently and will produce a much lower viscosity than linear molecules of the same DP. This also implies that a highly branched polysaccharide must be significantly larger than a linear polysaccharide to produce the same viscosity at the same concentration.

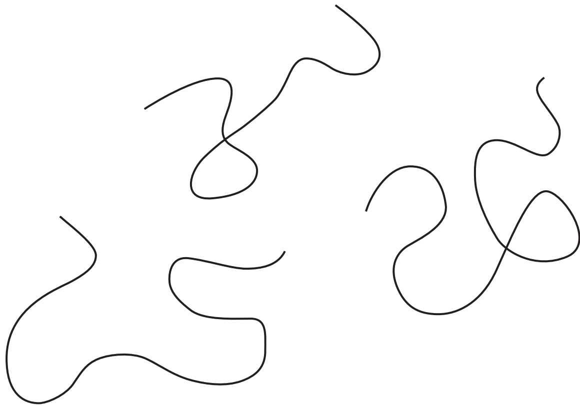


FIGURE 3.36 Randomly coiled polysaccharide molecules.

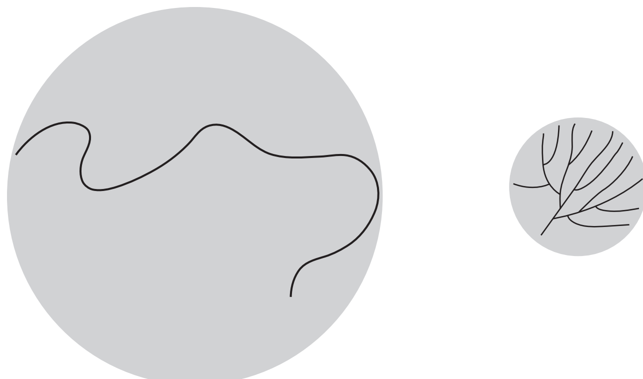


FIGURE 3.37 Relative volumes occupied by a linear polysaccharide and a highly branched polysaccharide of the same molecular weight.

Likewise, linear polysaccharide chains bearing only a single type of ionic charge (almost always a negative charge imparted by ionized carboxyl or sulfate half-ester groups) cause them to assume an extended configuration due to repulsion of the like charges, increasing the end-to-end chain distance and thus increasing the volume swept out by the polymer. Therefore, these polymers tend to produce solutions of high viscosity.

Unbranched glycans with regular repeating unit structures form unstable aqueous dispersions that precipitate or gel rapidly. This occurs as segments of the long molecules collide and form intermolecular bonds over the distance of a few units. Initial short alignments then extend in a zipper-like fashion to greatly strengthen intermolecular associations. Additional like segments of other chains collide with this organized nucleus and bind to it, increasing the size of the ordered crystalline phase. Linear molecules continue to bind to form a crystallite, which may reach a size where gravitational forces cause precipitation. For example, starch amylose, when dissolved in water with the aid of heat and then cooled to below 65°C, undergoes molecular aggregation and precipitates, a process called *retrogradation*. During cooling of bread and other baked products, amylose molecules associate to produce firming. Over longer storage times, the branches of amylopectin associate (and may partially crystallize) to produce staling (see Section 3.3.6.7).

In general, molecules of unbranched neutral homoglycans have an inherent tendency to associate and partially crystallize. However, if linear glycans are derivatized, or occur naturally derivatized, as does guar gum (see Section 3.3.8), which has single-unit glycosyl branches along a backbone chain, their chain segments are prevented from association, and stable solutions result.

Stable solutions are also formed if the linear chains contain charged groups such that coulombic repulsions prevent chain segments from approaching each other. As already mentioned, charge repulsion also causes chains to extend, which provides high viscosities. Such highly viscous, stable solutions are seen with sodium alginate (see Section 3.3.11), where each glycosyl unit is a uronic acid unit having a negatively charged, ionized carboxylate group, and for xanthan (see Section 3.3.9), where one of every five glycosyl units is a uronic acid unit and an additional carboxylate group from a cyclic acetal of pyruvic acid is present at a frequency of about one per every ten monosaccharide units. But, if the pH of an alginate solution is lowered to 3, which causes an increasing proportion of carboxylic acid groups to become protonated (pK_a values of the carboxylic acid groups are 3.38 and 3.65), the less charged nature of the molecules can allow chains to associate and precipitate or form a gel—as would be expected for an unbranched, uncharged (neutral) glycan.

Carrageenans are mixtures of linear chains of nonuniform structures that have a negative charge due to numerous ionized sulfate half-ester groups present along the chains (Section 3.3.10). These molecules do not precipitate at low pH because the sulfate group remains ionized at all practical pH values.

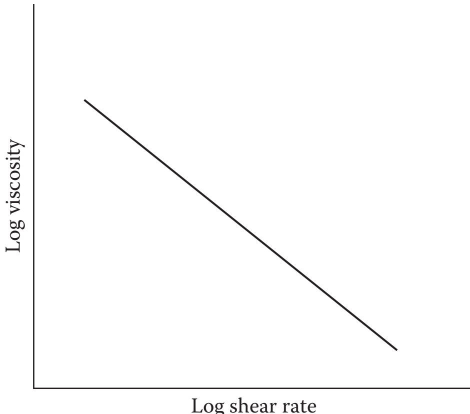


FIGURE 3.38 The logarithm of viscosity as a function of the shear rate for a pseudoplastic shear-thinning fluid.

Solutions of hydrocolloids are molecular dispersions and/or aggregates of hydrated molecules. Their flow behavior is determined by the size, shape, ease of deformation (flexibility), and the presence and magnitude of charges on the hydrated molecules and/or aggregates. There are two general kinds of rheological flow exhibited by polysaccharide solutions: pseudoplastic (by far the most common) and thixotropic; both are characterized by shear thinning.

In *pseudoplastic flow*, a more rapid flow results from an increase in shear rate, that is, the greater the applied force, the less viscous it becomes (Figure 3.38). The applied force can be that of pouring, chewing, swallowing, pumping, mixing, or anything else that induces shear. Upon removal of the applied force, the solution regains its initial viscosity instantaneously. The change in viscosity is independent of time: that is, the rate of flow changes instantaneously as the shear rate is changed. In general, high-molecular-weight linear gums form the most pseudoplastic solutions (refer to xanthan, Table 3.5), with the effect further enhanced by an increasing chain stiffness or rigidity.

Hydrocolloid solutions that are less pseudoplastic are said to give *long flow**; such solutions are generally perceived as being slimy. More pseudoplastic solutions are described as having short flow and are generally perceived as being non-slimy. In food science, a slimy material is one that is thick, coats the mouth, and is difficult to swallow. Sliminess is inversely related to pseudoplasticity: that is, to be perceived as being non-slimy, there must be marked thinning at the low shear rates produced by chewing and swallowing.

Thixotropic flow is a second type of shear-thinning flow behavior. In this case, the viscosity reduction that results from an increase in the rate of flow does not occur instantaneously. Rather, the viscosity of thixotropic solutions decreases under a constant rate of shear in a time-dependent manner, and regains the original viscosity after cessation of shear, but again only after a clearly defined and measurable time interval. This behavior is due to a gel → solution → gel transition. In other words, a thixotropic solution at rest is a weak (pourable) gel (see Section 3.3.4). Carboxymethylcellulose is an example of a gum that may possess thixotropic flow behavior (Table 3.5).

For solutions of most hydrocolloids, an increase in temperature results in a decrease in viscosity. This loss of viscosity as the temperature is raised is often an important property, for it means that

* "*Short flow*" is exhibited by shear-thinning, primarily pseudoplastic, viscous solutions and "*long flow*" by viscous solutions that exhibit little or no shear thinning. These terms were applied long before there were instruments to determine and measure rheological phenomena. They were arrived at in this way: When a gum or starch solution is allowed to drain from a pipette or a funnel, those that are not shear-thinning come out in long strings while those that shear-thin form short drops. The latter occurs because, as more and more fluid exits the orifice, the weight of the string becomes greater and greater, which causes it to flow faster and faster, which causes it to shear-thin to the point that the string breaks into drops.

TABLE 3.5
Predominantly Used, Water-Soluble, Nonstarch Food Polysaccharides

Gum	Source	Class	General Shape	Monomer Units and Linkages (Approx. Ratios)	Non-Carbohydrate Substituent Groups	Water Solubility	Key General Characteristics	Major Food Applications
Algins (alginates) (generally sodium alginate)	Brown algae	Seaweed (algal) extract; Poly (uronic acid)	Linear	Block copolymer of the following units: →4)-βMan*p*A (1.0) →4)-αLGul*p*A (0.5–2.5)		Sodium alginate soluble	Gels with Ca^{2+} Viscous, not very pseudoplastic solutions	Forms nonmelting gels (dessert gels, fruit analogs, other structured foods) Meat analogs
						Alginic acid insoluble		Alginic acid forms soft, thixotropic, nonmelting gels (tomato aspic, jelly-type bakery fillings, filled fruit-containing breakfast cereal products)
					Hydroxypropyl ester groups in propylene glycol alginate (PGA)	Soluble	Surface active; Solutions stable to acids and Ca^{2+}	Emulsion stabilization in creamy salad dressings; Thickener in low-calorie salad dressings
Carboxymethyl-cellulose (CMC)	Derived from cellulose	Modified cellulose; Cellulosic	Linear	→4)-βGlc*p*-(1→	Carboxymethyl ether (DS 0.4–0.8)[a]	High	Clear, stable solutions that can be either pseudoplastic or thixotropic	Retarder of ice crystal growth in ice creams and other frozen dessert products Thickener, suspending aid, protective colloid, and improver of mouthfeel, body, and texture in a variety of dressings, sauces and spreads Lubricant, film former, and processsing aid for extruded products

(*Continued*)

TABLE 3.5 (*Continued*)
Predominantly Used, Water-Soluble, Nonstarch Food Polysaccharides

Gum	Source	Class	General Shape	Monomer Units and Linkages (Approx. Ratios)	Non-Carbohydrate Substituent Groups	Water Solubility	Key General Characteristics	Major Food Applications
								Batter thickener and humectant in cake and related mixes Moisture binder and retarder of crystallization and/or syneresis in icings, frostings, toppings, fillings, and puddings Syrup thickener Suspending aid and thickener in dry powder, hot and cold drink mixes Gravy maker in dry pet food
Carrageenans	Red algae	Seaweed (algal) extracts Sulfated galactans	Linear		Sulfate half-ester			
				Kappa types: →3)-βGal*p* 4-SO_3^- (1→ 4)-3,6An-αGal*p* (1→		Kappa types: Na^+ salt soluble in cold water, K^+ and Ca^{2+} salts insoluble; all salts soluble at temperatures >65°C; soluble in hot milk, insoluble in cold milk	Forms stiff, brittle, thermoreversible gels with $K^+ > Ca^{2+}$; thickens and gels milk at low concentration; synergistic gelation with LBG	Secondary stabilizer in ice cream and related products Preparation of evaporated milk, infant formulas, freeze–thaw stable whipped cream, dairy desserts, and chocolate milk Meat coating Improves adhesion and increases water-holding capacity of meat emulsion products Improves texture and quality of low-fat meat products

(*Continued*)

TABLE 3.5 (*Continued*)
Predominantly Used, Water-Soluble, Nonstarch Food Polysaccharides

Gum	Source	Class	General Shape	Monomer Units and Linkages (Approx. Ratios)	Non-Carbohydrate Substituent Groups	Water Solubility	Key General Characteristics	Major Food Applications
				Iota types: →3)-βGal*p* 4-SO_3^- (1→4)-3,6An-αGal*p* 2-SO_3^- (1→		Iota types: Na^+ salt soluble in cold water, K^+ and Ca^{2+} salts insoluble; all salts soluble at temperatures > 55°C; soluble in hot milk, insoluble in cold milk	Forms soft, resilient, thermoreversible gels with Ca^{2+} > K^+; gels do not synerese and have good freeze–thaw stability	Forms elastic, syneresis-free, thermally reversible water gels that are freeze–thaw stable. Often blended with κ-carrageenan to make water dessert gels that do not require refrigeration and whipped toppings, desserts, and eggless custards and flans
				Lambda types: →3)-βGal*p* 2-SO_3^- (1→4)-αGal*p* 2,6-diSO_3^- (1→		Lambda types: all salts soluble in hot and cold water and milk	Thickens cold milk	Whipped cream, instant breakfast drinks, nondairy coffee creamers, and dry-mix hot cocoa
Gellan	Fermentation medium	Microbial poly-saccharide	Linear	→4)-αLRha*p*-(1→3)-βGlc*p*-(1→4)-βGlc*p*A-(1→4)-βGlc*p*-(1→	Native type contains an acetate and a glycerate ester group on each repeating unit	Soluble in warm water	Gels with any cation Solutions have high yield values Low-acyl types form firm, brittle, non-elastic gels High-acyl types form soft, elastic, non-brittle gels	Bakery mixes Nutrition bars Nutritional beverages Fruit toppings Sour cream and yogurt products

(*Continued*)

TABLE 3.5 (*Continued*)
Predominantly Used, Water-Soluble, Nonstarch Food Polysaccharides

Gum	Source	Class	General Shape	Monomer Units and Linkages (Approx. Ratios)	Non-Carbohydrate Substituent Groups	Water Solubility	Key General Characteristics	Major Food Applications
Guar gum	Guar seed	Seed galacto-mannan	Linear with single-unit branches (behaves as a linear polymer)	→4)-βMan*p* (~0.56) αGal*p* 1 ↓ 6 →4)-βMan*p* (~1.0) (Man:Gal = ~1.56:1)		High	Stable, opaque, very viscous, moderately pseudoplastic solutions Economical thickening	Binds water, prevents ice crystal growth, improves mouthfeel, softens texture produced by carrageenan + LBG, and slows meltdown in ice cream and ices Dairy products, prepared meals, bakery products, sauces, pet food
Gum arabic (gum acacia)	Acacia tree	Exudate gum	Branch-on-branch, highly branched	Complex, variable structure; contains polypeptide		Very high	Emulsifier and emulsion stabilizer Compatible with high concentrations of sugar Very low viscosity at high concentrations	Prevents sucrose crystallization in confections Emulsifies and distributes fatty components in confections Preparation of flavor oil-in-water emulsions Component of coating of pan-coated candies Preparation of flavor powders
Inulin	Chicory root	Plant extract	Linear	→2)-βFru*f*(1→		Soluble	Gels when hot solutions are cooled Can be used as a fat mimetic	Ingredient in nutrition, breakfast, and energy bars and vegetable patties as a source of dietary fiber and fat mimetic
Konjac mannan	Konjac tubers	Plant extract	Branched	→4)-βMan*p*(1→ →4)-βGlc*p*(1→ (Man:Glc ~1.6:1)	Acetyl groups	Native—soluble	Native—high-viscosity, shear-thinning solutions Deacetylated—strong, elastic, irreversible gels	Used in Asia in pasta/noodles, structured foods, and dessert gels Binder in meat and poultry products, including pet foods Provides fat-replacement properties in low-fat meat products

(*Continued*)

TABLE 3.5 (*Continued*)
Predominantly Used, Water-Soluble, Nonstarch Food Polysaccharides

Gum	Source	Class	General Shape	Monomer Units and Linkages (Approx. Ratios)	Non-Carbohydrate Substituent Groups	Water Solubility	Key General Characteristics	Major Food Applications
Locust bean gum (carob gum, LBG)	Locust bean (carob) seed	Seed galacto-mannan	Linear with single-unit branches (behaves as a linear polymer)	→4)-βMan*p* (~2.5) αGal*p* 1 ↓ 6 →4)-βMan*p* (~1.0) (Man:Gal = ~3.5:1)		Soluble only in hot water; requires 90°C for complete solubilization	Interacts with xanthan and κ-carrageenan to form rigid gels; rarely used alone	Provides excellent heat shock resistance, smooth meltdown, and desirable texture in ice creams and other frozen dessert products Gels with xanthan or κ-carrageenan in meat analog pet foods
Methylcelluloses (MC) and hydroxy-propylmethyl-celluloses (HPMC)	Derived from cellulose	Modified cellulose	Linear	→4)-βGlc*p*-(1→	Hydroxypropyl (MS 0.02–0.3)[a] and methyl (DS 1.1–2.2)[a] ether groups	Soluble in cold water; insoluble in hot water	Clear solutions that are thermal gelling; surface active	*MC*: Provides fat-like characteristics Reduces fat absorption in fried products Imparts creaminess through film and viscosity formation Provides lubricity Gas retention during baking Moisture retention and control of moisture distribution in bakery products (increases shelf life and imparts tenderness) *HPMC*: Nondairy whipped toppings, where it stabilizes foams, improves whipping characteristics, prevents phase separation, and provides freeze–thaw stability

(*Continued*)

TABLE 3.5 (*Continued*)
Predominantly Used, Water-Soluble, Nonstarch Food Polysaccharides

Gum	Source	Class	General Shape	Monomer Units and Linkages (Approx. Ratios)	Non-Carbohydrate Substituent Groups	Water Solubility	Key General Characteristics	Major Food Applications
Pectins	Citrus peel Apple pomace	Plant extract Poly(uronic acid)	Linear	Primarily composed of →4)-αGal*p*A units	Methyl ester groups May contain amide groups	Soluble	Forms jelly- and jam-type gels in presence of sugar and acid or with Ca^{2+}	*HM pectin*: high-sugar jellies, jams, preserves, and marmalades Acidic milk drinks *LM pectin*: dietetic jellies, jams, preserves, and marmalades
Xanthan	Fermentation medium	Microbial poly-saccharide	Linear with trisaccharide unit branches on every other main chain unit (behaves as a linear polymer)	βMan*p* 1 ↓ 4 βGlc*p*A 1 ↓ 2 αMan*p* 6-Ac 1 ↓ 3 →4)-βGlc*p*-(1→4)-βGlc*p*-(1→	Acetyl ester Pyruvyl cyclic acetal on some βMan*p* end units	High	Very pseudoplastic, high viscosity solutions; excellent emulsion and suspension stabilizer; solution viscosity unaffected by temperature; solution viscosity unaffected by pH; excellent salt compatibility; synergistic increase in viscosity upon interaction with guar gum; heat-reversible gelation with LBG	Stabilization of dispersions, suspensions, and emulsions Thickener

[a] For definitions of DS and MS, see Sections 3.3.6.10 and 3.3.7.3, respectively.

more hydrocolloid can be put into solution at a higher temperature; then the solution can be cooled for thickening. (Xanthan is an exception because the viscosity of its solutions is essentially constant at temperatures between 0°C and 100°C; see Section 3.3.9.)

3.3.4 Gels [16,72]

A *gel* is a continuous, three-dimensional network of connected molecules or particles (such as crystals, emulsion droplets, or molecular aggregates/fibrils) entrapping a large volume of a continuous liquid phase, much as does a sponge. In many food products, the gel network consists of polymer (polysaccharide and/or protein) molecules or fibrils formed from polymer molecules joined in junction zones by hydrogen bonding, hydrophobic associations (i.e., van der Waals attractions), ionic cross bridges, entanglements, and/or covalent bonds over small segments of their lengths, while the liquid phase is an aqueous solution/dispersion of low-molecular-weight solutes and segments of the polymer chains not involved in junction zones.

Gels have some characteristics of solids and some characteristics of liquids. When polymer molecules or fibrils formed from polymer molecules interact over portions of their lengths to form junction zones and a three-dimensional network (Figure 3.39), a fluid solution may be changed into a material that can retain its shape (partially or entirely). The three-dimensional network structure offers sufficient resistance to an applied stress to cause it to behave in part as an elastic solid. However, the continuous liquid phase, in which molecules are completely mobile, makes a gel less stiff than an ordinary solid, causing it to behave in some respects as a viscous liquid. Therefore, a gel is a *viscoelastic semisolid*, that is, the behavior of a gel in response to an applied stress is partly that of an elastic solid and partly that of a viscous liquid.

Although gel-like or salve-like materials can be formed by high concentrations of particles (much like tomato paste), to form a gel from dissolved hydrocolloid molecules, the polymer molecules or

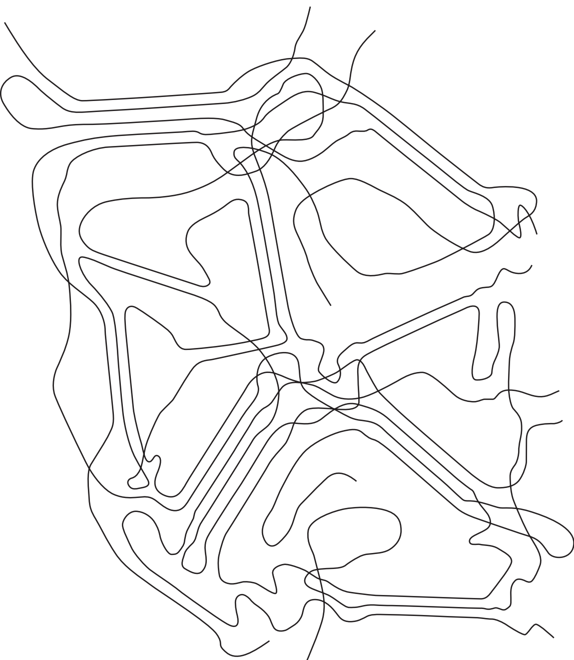


FIGURE 3.39 A diagrammatic representation of the type of three-dimensional network structure found in gels. Parallel side-by-side lines indicate the ordered, crystalline structures of a junction zone. The gaps between junction zones contain an aqueous solution of dissolved segments of polymer chains and other solutes.

aggregates of molecules must partially come out of solution over limited segments of their chains to form junction zone regions that tie them together in a three-dimensional gel network structure. In general, if the junction zones continue to grow after the formation of the gel, the network becomes more compact, the structure contracts, and syneresis results. (*Syneresis* is the expulsion of liquid from a gel.)

Although polysaccharide gels generally contain less than 2% polymer, that is, they are likely to contain as much as 98% water, they can be quite strong. Examples of polysaccharide gels are dessert gels, aspics, structured fruit pieces, structured onion rings, meat-analog pet foods, jams, jellies, and confections like gum drops.

The choice of a specific hydrocolloid for a particular application depends on the viscosity or gel strength desired, the desired rheology, the pH of the system, temperatures during processing, interactions with other ingredients, the desired product texture, and the cost of the amount needed to impart the desired properties. Consideration is also given to desired functional characteristics. These include a hydrocolloid's ability to function as a binder, bodying agent, bulking agent, crystallization inhibitor, clarifying agent, clouding agent, coating agent/film former, emulsifier, emulsion stabilizer, encapsulating agent, fat mimetic, flocculating agent, foam stabilizer, mold-release agent, suspension stabilizer, swelling agent, syneresis inhibitor, whipping agent, and water absorber and binder (to effect water retention and control water migration). Each food gum tends to have an outstanding property (or perhaps several unique properties), which is often the basis for its choice for a particular application (Table 3.5).

3.3.5 Polysaccharide Hydrolysis

Polysaccharides are relatively less stable to hydrolytic cleavage than proteins and may, at times, undergo depolymerization during food processing and/or storage of foods.* Often, food hydrocolloids are deliberately depolymerized for functional purposes. For example, hydrocolloids might be intentionally depolymerized so that a relatively high polymer concentration could be used to provide body (mouthfeel) without producing excessive viscosity.

Hydrolysis of glycosidic bonds joining monosaccharide (glycosyl) units in oligo- and polysaccharides can be catalyzed by acids (H^+) and/or enzymes. The extent of depolymerization, which has the effect of reducing viscosity, is determined by the pH, temperature, time at a given temperature and pH, and structure of the polysaccharide. Hydrolysis occurs most readily during thermal processing of acidic foods (as opposed to storage) because the elevated temperature accelerates the rate of reaction. Defects associated with depolymerization during processing can usually be overcome by using more of the polysaccharide (hydrocolloid) in the formulation to compensate for breakdown, using a higher viscosity grade of the hydrocolloid, again to compensate for any depolymerization, or using a relatively more acid-stable hydrocolloid. Depolymerization can also be an important determinant of the shelf-life.

Polysaccharides are also subject to enzyme-catalyzed hydrolysis. The rate and end products of this process are controlled by the specificity of the enzyme, pH, temperature, and time. Polysaccharides, like any and all other carbohydrates, are subject to microbial attack because of their susceptibility to enzyme-catalyzed hydrolysis. Furthermore, hydrocolloid products are very seldom, if ever, delivered sterile, a fact that must be considered when using them as ingredients.

3.3.6 Starch [5,19,20,33,55]

The unique chemical and physical characteristics and nutritional aspects of starch set it apart from all other carbohydrates. Starch is the predominant energy and food reserve substance in higher plants, and provides 70%–80% of the calories consumed by humans worldwide. Starch and starch

* On the other hand, polysaccharides do not undergo denaturation.

hydrolysis products constitute most of the digestible carbohydrate in the human diet. Also, the amount of starch used in the preparation of food products—without counting that present in flours used to make bread and other bakery products, that naturally occurring in grains, such as rice and corn eaten as such or used to make breakfast cereals, or that naturally consumed in fruits and vegetables, such as potatoes—greatly exceeds the combined use of all food hydrocolloids.

Commercial starches are obtained from cereal grain seeds, particularly from normal corn, waxy corn (waxy maize), high-amylose corn, wheat, and various rices, and from tubers and roots, particularly potato and cassava (tapioca). For example, corn starch is commercially extracted via a wet-milling process, in which dry kernels are steeped in water followed by grinding and washing steps to release and purify starch from other kernel constituents. Starches and modified starches have an enormous number of food uses, including adhesive, binding, clouding, dusting, film-forming, foam-strengthening, gelling, glazing, moisture-retaining, stabilizing, texturizing, and thickening applications.

Starch is unique among carbohydrates because it occurs in nature as discrete, partially crystalline particles called *granules*. Starch granules are insoluble, but they do hydrate to some extent in room-temperature water. As a result, they can be dispersed in water, producing low-viscosity suspensions/slurries that can be easily mixed and pumped, even at concentrations up to 40%. The viscosity-building (thickening) power of starch is realized only when a slurry of granules is cooked. Heating a 5% slurry of most unmodified starch granules to about 80°C (175°F) with stirring produces a very high viscosity dispersion called a *paste*. A second unique characteristic is that most starch granules are composed of a mixture of two polymers: an essentially linear polysaccharide called *amylose*, and a highly branched polysaccharide called *amylopectin*.

3.3.6.1 Amylose [5]

While amylose is essentially a linear chain of (1→4)-linked α-D-glucopyranosyl units, some amylose molecules contain a few branches connected to the main chain via α-(1→6) linkages, which represent the branch points. Perhaps 1 in 180–320 units, or 0.3%–0.5% of the linkages, are branch points. The branches in branched amylose molecules are either very long or very short, and most branch points are separated by large distances along the chains such that the physical properties of amylose molecules are essentially those of linear molecules. The average molecular weights of amylose molecules vary with the source of the starch. Amylose molecules from different starch botanical sources have molecular weights that, on average, range from 10^5 to 10^6.

The axial→equatorial arrangement of the glycosidic bond of (1→4)-linked α-D-glucopyranosyl units in amylose chains gives the molecules a right-handed spiral or helical shape (Figure 3.40). The interior of the helix contains a predominance of hydrogen atoms and is hydrophobic/lipophilic,

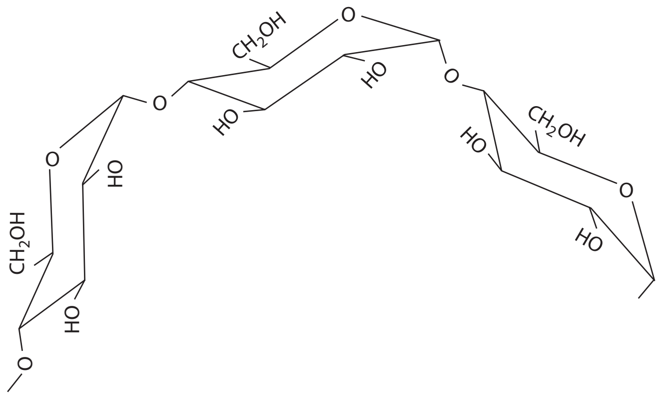


FIGURE 3.40 A trisaccharide segment of an unbranched portion of amylose or amylopectin molecule.

TABLE 3.6
General Properties of Some Starch Granules and Their Pastes

	Common Corn Starch	Waxy Maize Starch	High-Amylose Corn Starch	Potato Starch	Tapioca Starch	Wheat Starch
Granule size (major axis, μm)	2–30	2–30	2–24	5–100	4–35	2–55
% Amylose	28	<2	50–75	21	17	28
Gelatinization temp. (°C)[a]	62–80	63–72	66–170[b]	58–65	52–65	52–85
Relative viscosity	Medium	Medium high	Very low[b]	Very high	High	Low
Paste rheology[c]	Short	Long	Short	Very long	Long	Short
Paste clarity	Opaque	Very slightly cloudy	Opaque	Clear	Clear	Opaque
Tendency to gel/retrograde	High	Very low	Very high	Medium to low	Medium	High
Lipid (% dry basis)	0.8	0.2	—	0.1	0.1	0.9
Protein (% dry basis)	0.35	0.25	0.5	0.1	0.1	0.4
Phosphorus (% dry basis)	0.00	0.00	0.00	0.08	0.00	0.00
Flavor	Cereal (slight)	"Clean"		Slight	Bland	Cereal (slight)

[a] From the initial temperature of gelatinization to complete pasting.
[b] Under ordinary cooking conditions, where the slurry is heated to 95°C–100°C, high-amylose corn-starch produces essentially no viscosity. Pasting does not occur until the temperature reaches 160°C–170°C (320°F–340°F).
[c] For a description of long and short flow, see Section 3.3.3.

while the hydroxyl groups are positioned on the exterior of the coil. Looking down the axis of the helix gives a view very much like that of looking down a stack of α-cyclodextrin molecules (see Section 3.2.4) because each turn of the helix contains about 6 α-D-glucopyranosyl units linked (1→4).

On a weight basis, most starches contain about 25% amylose (Table 3.6). The two high-amylose corn starches that are commercially available have apparent amylose contents of about 52% and 70%–75%.

3.3.6.2 Amylopectin [5]

Amylopectin is a very large, very highly branched molecule, with branch point linkages constituting 4%–7% of the total linkages. Amylopectin consists of a main chain possessing the only reducing end group on the molecule, to which are attached numerous branch chains, to which are attached one to several third layer branch chains. The short branches of amylopectin molecules are clustered (Figure 3.41) and occur in granules as double helices, while the longer branch chains extend beyond a single cluster, and provide intercluster connections over the length of amylopectin molecules. Weight-average molecular weights from ~8×10^5 (DP ~ 5000) up to perhaps 6×10^9 (DP ~ 37×10^6) make amylopectin molecules among the largest, if not the largest, molecules found in nature.

Amylopectin is present in all common starches, and constitutes about 75% (weight basis) of these starches (Table 3.6). Some starches consist entirely of amylopectin and are called *waxy* or *amylopectin starches*. Waxy corn (waxy maize), the first grain recognized as one in which the starch consists only of amylopectin, is so termed because, when the kernel is cut, the new surface has a vitreous or waxy appearance. Most other all-amylopectin starches are also called "waxy" although, as in corn, there is no wax present.

Potato amylopectin is unique among the commercial starches in having more than trace amounts of phosphate ester groups. These phosphate ester groups are attached most often (60%–70%) at an

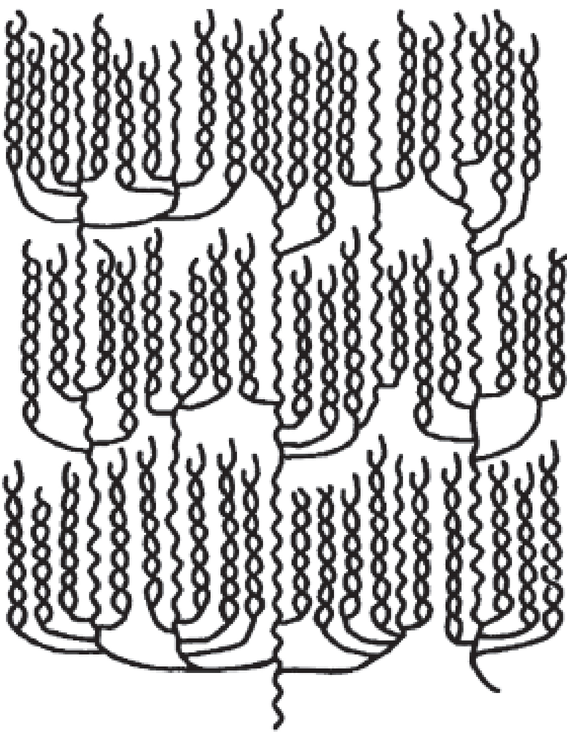


FIGURE 3.41 A diagrammatic representation of a portion of an amylopectin molecule. (Redrawn from Imberty, A. et al., *Starch/Staerke*, 43, 375, 1991. With permission.)

O-6 position, with the other third at O-3 positions. These phosphate ester groups occur about once in every 215–560 α-D-glucopyranosyl units.

3.3.6.3 Starch Granules [5]

Starch granules consist of amylose and/or amylopectin molecules arranged radially, with the reducing ends of starch chains facing inward toward the granule center. Granules contain both semicrystalline and largely noncrystalline (so-called amorphous) regions or shells in alternating layers.* The semicrystalline or more dense layers of starch granules contain greater amounts of crystalline structure. The crystalline nature of the dense shells or layers within starch granules arises from the clustered, double-helical branches of amylopectin, which pack together to form short, periodic crystalline lamellae throughout the granule (Figure 3.41). Thus, the crystalline framework and molecular order of granules is largely provided by amylopectin molecules, and is stabilized by hydrogen bonds among and within the chains. When viewed under a polarizing microscope (with polarizers set 90° to each other), the ordered, radial arrangement of starch molecules within a granule is evident from the observed birefringence, the pattern of which occurs as the characteristic polarization cross (black cross in a white background). The center of the cross is at the hilum, the origin of growth of the granule.† The precise location and arrangement of amylose within granules is still a matter of debate.

Corn starch granules, even from a single source, have mixed shapes, some being almost spherical, some angular, and some indented (for the size, see Table 3.6). Wheat starch granules have a

* Starch granules are composed of layers somewhat like the layers of an onion, except that the layers are interconnected and cannot be peeled off.

† There a several sources of good optical microscopy (taken with and without cross-polarization) and SEM pictures of starch granules. Among them are References 35 and 59.

bimodal size distribution (roughly <10 and >10 μm), with the larger granules being lenticular in shape and smaller granules being more spherical. Rice granules are the smallest of the commercial starch granules (1–9 μm), although the small granules of wheat starch are within this same size range. Many of the granules in tuber and root starches, such as potato and tapioca starches, tend to be larger than those of seed starches, and are generally less dense and easier to cook. Many potato starch granules have an oblong shape and may be as large as 100 μm along the major axis.

All commercial starches contain small amounts of ash, lipid, and protein (Table 3.6). The phosphorus content of potato starch (0.06%–0.1%, 600–1000 ppm) is due to the presence of phosphate ester groups on amylopectin molecules. The phosphate ester groups give potato starch amylopectin chains a slight negative charge, resulting in some coulombic repulsion that may contribute to the rapid swelling of potato starch granules in warm water and to several properties of potato starch pastes, namely, their high viscosities, good clarity (Table 3.6), and low rate of retrogradation (see Section 3.3.6.7). The phosphate ester groups are also responsible for the altered cooking and paste properties of potato starch in the presence of calcium ions (provide for salt bridges between adjacent chains). Cereal starch molecules either do not have phosphate ester groups or have much smaller amounts than potato starch molecules. Only the cereal starches contain endogenous lipids in the granules. These internal lipids are primarily free fatty acids (FFAs) and lysophospholipid (LPL) (see Chapter 4), largely lysophosphatidyl choline (89% in corn starch), with the ratio of FFA to LPL varying from one cereal starch to another.

3.3.6.4 Granule Gelatinization and Pasting [5,6,61]

Undamaged starch granules are insoluble in cold water, but can imbibe water reversibly: that is, they can swell slightly, and then return to their original size on drying. When heated in water, starch granules undergo a process called *gelatinization*. Gelatinization is the loss of granular and molecular order, that is, crystallite melting and unfolding of double helical structures due to the disruption of hydrogen bonds stabilizing starch chains within the native granules. Evidence for the loss of order includes irreversible granule swelling, loss of birefringence, and loss of crystallinity. Some leaching of amylose molecules from granules occurs over the course of gelatinization. Total gelatinization of a population of granules occurs over a temperature range (Table 3.6), due to the structural heterogeneity among the granules (all populations of starch granules are heterogeneous). The apparent temperature of initial gelatinization and the range over which gelatinization occurs depend on the method of measurement and on the starch-to-water ratio, granule type, and the degree of structural heterogeneity within the granule population under observation. Several aspects of gelatinization of a population of granules can be determined. These are the gelatinization onset temperature, the peak or midpoint temperature, and the conclusion temperature.

Continued heating of starch granules in excess water results in further granule swelling, additional leaching of soluble components (primarily amylose), and eventually, especially with the application of shear forces, total disruption of granules. These phenomena result in the formation of a starch paste. (In starch technology, what is called a *paste* is what results from heating a starch slurry in the presence of shear.) Granule swelling and disruption produces a viscous paste consisting of a continuous phase of solubilized amylose and/or amylopectin molecules and a discontinuous phase of granule remnants (granule *ghosts** and fragments). Complete molecular dispersion is not accomplished except, perhaps, under conditions of high temperature, high shear, and excess water, conditions seldom, if ever, encountered in the preparation of food products.

Cooling of a hot, normal corn starch paste results in a viscoelastic, firm, rigid gel.

Because gelatinization of starch is an endothermic process, differential scanning calorimetry (DSC), which measures both the temperature and the enthalpy of gelatinization, is widely used to monitor the process. Although there is no complete agreement on the interpretation of DSC

* The granule ghost is the remnant remaining after cooking under no to moderate shear. It consists of the outer portion of the granule. It appears as an insoluble, outer envelope.

data and the events that take place during gelatinization of starch granules, the following general picture is widely accepted: Water acts as a plasticizer. Its mobility-enhancing effect is first realized in the amorphous regions, which physically have the nature of a glass. When starch granules are in the presence of sufficient water (at least 60%), and a specific temperature (T_g, the glass transition temperature) is reached, the plasticized amorphous regions of the granule undergo a phase transition from a glassy state to a rubbery state.* However, the glassy to rubbery transition for hydrated granular starch may occur below room temperature, so it is not detected by DSC under usual operating conditions. So what is measured are the onset, peak, and conclusion temperatures of gelatinization and the enthalpy of crystallite melting.

Melting of lipid–amylose complexes occurs at much higher temperatures (100°C–120°C in excess water) than the melting of the amylopectin double-helical branches comprising the granule crystalline order. Lipid–amylose complexes are formed between single-helical segments of amylose molecules when a starch paste containing fatty acids or monoacyl glycerides (see Chapter 4) is cooled. A DSC peak for this event is absent in starch products containing no amylose, that is, waxy starches.

Under normal food processing conditions (heat and moisture, though many food systems contain limited water as far as starch cooking is concerned), starch granules quickly swell to an irreversible point, that is, beyond that achieved at the moment of gelatinization. Water molecules enter between chains, break interchain bonds, and establish hydration layers around the separated molecules. These hydration layers plasticize (lubricate) the chains such that they become more fully separated and solvated. Entrance of large amounts of water causes the granules to swell to several times their original size. When a 5% starch suspension is gently stirred and heated, granules imbibe water until much of the water is absorbed by them, forcing them to swell, press up against each other, and fill the container with a highly viscous starch paste with most of the water inside the swollen granules. Such a starch paste has viscosity like that of a pudding because most of the space is composed of swollen granules, which move past one another only with great difficulty. Such highly swollen granules of native starches are easily broken apart (disintegrated) by stirring, resulting in a decrease in viscosity. As starch granules swell, hydrated amylose molecules diffuse through the mass into the external continuous phase (water), a phenomenon responsible for some aspects of paste behavior. Results of starch swelling can be recorded using various instruments (e.g., Viscoamylograph or Rapid ViscoAnalyzer) that record the viscosity continuously as the temperature is increased, held constant for a time, and then decreased (Figure 3.42).

Most suspensions of starch granules are stirred during heating to prevent the granules from settling to the bottom of the container. Instruments that record changes that occur during starch pasting (i.e., paste behavior) as a function of temperature to produce curves, like those in Figure 3.42, also employ stirring. By the time peak viscosity is reached, some granules have already been broken by stirring. With continued stirring, more granules rupture and fragment, causing a further decrease in viscosity. On cooling, some starch molecules partially reassociate to form a limited precipitate or gel. This process is called *retrogradation* (see Section 3.3.6.7). The firmness of the gel depends on the extent of junction zone formation (see Section 3.3.4), which is influenced (either facilitated or hindered) by the presence of other ingredients such as fats, proteins, sugars, and acids and the amount of water present.

3.3.6.5 Uses of Unmodified Starches

Starches serve a variety of roles in food production. Principally they are used to produce desired textural qualities. Primarily, they provide body and bulk. The extent of starch gelatinization in baked goods strongly affects product properties, including storage behavior and rate of digestion. In baked products made from low-moisture doughs, many wheat starch granules remain ungelatinized,

* A glass is a mechanical solid (a supercooled liquid) capable of supporting its own weight against flow. A rubber is an undercooled liquid that can exhibit viscous flow. (See Chapter 2 for further details.)

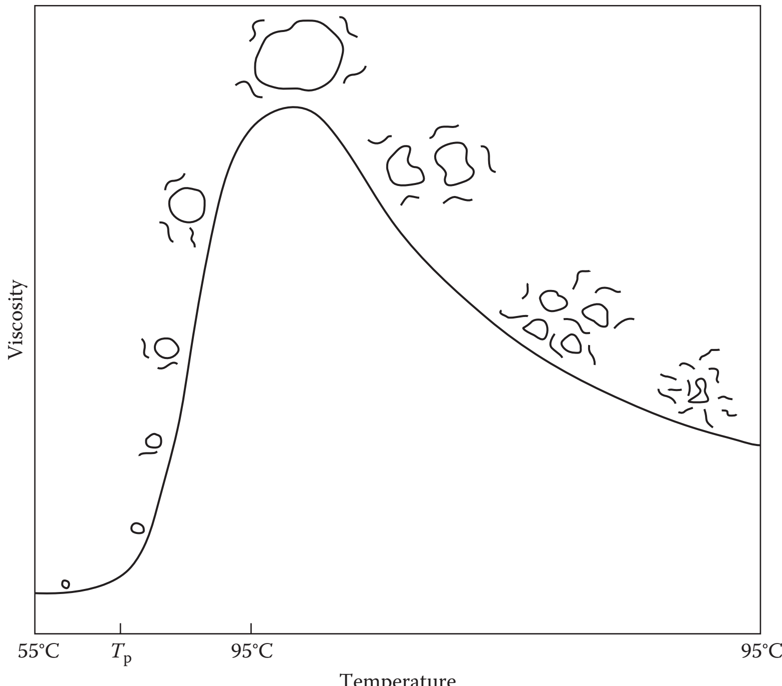


FIGURE 3.42 Representative cooking/pasting curve showing viscosity changes related to typical starch granule swelling and disintegration as a granule suspension is heated to 95°C and then held at that temperature using an instrument that imparts low shear. T_p is the pasting temperature, which is the temperature at which the rapid increase in system viscosity begins (occurs following gelatinization).

due to insufficient moisture being present to facilitate gelatinization. In higher moisture products, most or all of the granules become gelatinized.

Most starch utilized as a food ingredient is "modified food starch" (see Section 3.3.6.10) because, for the most part, textures of cooked suspensions of native starches, especially of native normal cereal starches, such as corn starch, are undesirable. The clear, cohesive pastes produced from waxy maize starch are somewhat more desirable, but even this starch is usually chemically modified to improve its functionality. Unmodified potato starch is used in extruded cereal and snack food products and in dry mixes for soups and cakes. Normal rice starch produces opaque gels useful for baby food. Waxy rice starch gels are clear and cohesive. Wheat starch gels are weak and have a slight flavor due to residual flour components. Tuber (potato) and root (tapioca) starches have weak intermolecular bonding within native granules and swell greatly to give high-viscosity pastes (Table 3.6), but if shear is applied, the viscosity decreases quickly because the highly swollen granules rupture easily.

3.3.6.6 Starch Gelatinization within Vegetable Tissues [1,36,37,52]

The majority of dietary starch occurs within grain- and vegetable-based food products that contain starch as the predominant dry matter. Thus, it is important to understand the thermal properties of starch within these native environments, as it relates to the acceptability and texture of processed foods. The degree to which starch is gelatinized within food systems is generally dependent upon both the amount of water present and the extent of the heat treatment. As already mentioned, in some baked products, starch granules may remain ungelatinized even when heated to high temperatures. In pie crust and some cookies that are high in fat and low in moisture, approximately 90% of the wheat starch granules remain ungelatinized. In bread and cakes, which have higher moisture

contents, ~96% of the wheat starch granules are gelatinized, but because they are heated without shear, the granules are still evident and can be isolated, although many are deformed.

Thermal processing (blanching, baking, boiling, steaming, frying) of vegetables is generally sufficient to induce a desired tissue softening. Following a heating process, vegetable tissue becomes more susceptible to fracture between (as opposed to through) its parenchyma cells.

Parenchyma tissue is the most abundant type of tissue in edible plants. In general, parenchyma tissue is comprised of aggregates of polygonally shaped cells, each of which contains clusters of starch granules, surrounded by a cellulosic cell wall. Adjoining cells are attached or cemented together by a middle lamella, which consists primarily of pectic substances. Water, which is the predominant constituent of most vegetable tissues, resides primarily in vacuoles and the cytoplasm within cells (84%), while the balance is associated with starch granules (13%) and cell wall components (3%).

As plant tissue is heated, the semicrystalline starch granules take up available water within cells and undergo swelling and gelatinization (Figure 3.43). The native moisture within parenchyma tissue is generally sufficient to plasticize starch granules and facilitate gelatinization, though the temperature at which these thermal events occur is slightly higher for starch granules housed within native plant cells compared to isolated starch. The higher gelatinization temperature of in situ starch has been attributed to the presence of solutes. Though starch gelatinization is complete within plant tissue (molecular order is fully lost), granule swelling is limited by the boundaries of the surrounding cell walls. Starch granules swell (with some leaching of amylose) to fill most of the entire volume of their respective cells, producing a swollen starch mass that may still possess some subtly discernable granule remnants. Granule swelling during heating has been shown to exert an observable internal pressure on parenchyma cell walls (estimated at 100 kPa). Though the magnitude of swelling pressure is itself insufficient to bring about cell rupture (cells generally remain intact), isolated potato parenchyma cells temporarily increase in size and become more spherical as a result of starch gelatinization. This phenomenon, referred to as cell *rounding off*, occurs in concert with pectin degradation by β-elimination [4] within the middle lamellae to cause softening of the parenchyma tissue. As the characteristic softening phenomenon is observed in tissues that do not contain appreciable starch contents, such as tomatoes, this effect is primarily attributed to pectin degradation within the middle lamellae.

Nevertheless, in starch-containing tissues such as potato, a high starch content and/or degree of starch granule swelling is associated with a softer, more friable cooked tissue. It is thought that the cell "rounding off" phenomenon exerts physical pressure on a partially degraded or weakened middle lamellae, contributing secondarily to cell separation or tissue sloughing. Also, the degree to which gelatinized starch swells to fill the volume of parenchyma cells is thought to influence the

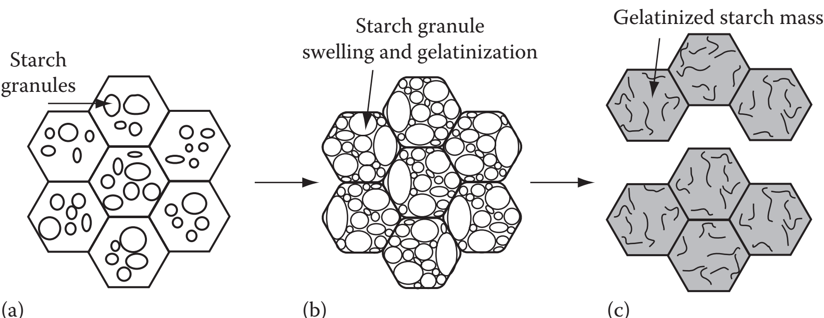


FIGURE 3.43 Within plant parenchyma tissue, starch granules (a) within cells undergo swelling and gelatinization during heating to exert a temporary "swelling pressure" on surrounding cell walls (b). With further heating, starch granules evolve into a fairly uniform gelatinized starch mass within cells (c). Heated tissue becomes prone to increased sloughing (cell separation), which is primarily attributable to pectin degradation within the middle lamella, though starch swelling pressure is thought to contribute a significant secondary role.

human perception of tissue moistness in the mouth. A high starch content and swelling capacity is generally more effective in binding up free moisture within the cooked tissue, producing a corresponding dry mouthfeel. Cooked potato texture has been traditionally classified in terms of "mealiness" and "waxiness." A mealy texture is characterized by a dry-appearing tissue that crumbles or sloughs easily. In contrast, a waxy tissue (not to be confused with a waxy starch) is defined by a moist appearance, a gummy mouthfeel, and a firm texture. Generally, mealy potatoes are traditionally deemed more suitable for the majority of processed products (French fries, mashed potatoes, etc.). Potato varieties whose tissue has a waxy texture after cooking find application in boiling and canning applications. In conclusion, starch gelatinization behavior appears to exert significant influence on cooked vegetable texture and end-use potential through its secondary role in tissue softening (cell "rounding off") and its water-binding capabilities within parenchyma tissue.

3.3.6.7 Retrogradation and Staling [28,45,47,61]

As already pointed out, cooling a hot starch paste generally produces a firm viscoelastic gel. The formation of the junction zones of a gel can be considered to be the first stage of an attempt by gelatinized starch molecules to recrystallize. As starch pastes are cooled and stored, the starch becomes progressively less soluble. In dilute solution, starch molecules will precipitate. The collective process of starch molecules in a solution or paste becoming less soluble is called *retrogradation*. Retrogradation of cooked starch involves both of the two constituent polymers, amylose and amylopectin, with amylose undergoing observable retrogradation on a much more rapid timescale (minutes to hours) than amylopectin (days to weeks to months), depending, of course, on the nature of the product and storage conditions. (Amylose chains are predominantly involved in the initial viscosity development and gel strength of pastes upon cooling.) The rate of retrogradation depends on several variables, including the molecular ratio of amylose to amylopectin; structures of the amylose and amylopectin molecules, which are determined by the botanical source of the starch; temperature; starch concentration; and the presence and concentration of other ingredients, primarily surfactants and salts. Many quality defects in food products, such as bread staling and loss of viscosity and precipitate formation in soups and sauces, are due, at least in part, to starch retrogradation.

Staling of baked goods is noted by an increase in crumb firmness and a loss in the perception of product freshness. Staling begins as soon as baking is complete and the product begins to cool. The rate of staling is dependent on the product formulation, the baking process, and storage conditions. Staling is due, at least in part, to the gradual transition of amorphous starch to a partially crystalline retrograded state. In baked goods, where there is just enough moisture to gelatinize starch granules (while retaining a granule identity), amylose retrogradation (insolubilization) may be largely complete by the time the product has cooled to room temperature. Retrogradation of amylopectin is believed to involve primarily reassociation of its outer branch chains in an intra- and/or intermolecular fashion, and requires a much longer time than amylose to undergo recrystallization, giving it prominence in the staling process in the time period after the product has cooled.

Most polar lipids with surfactant properties retard crumb firming by forming complexes with starch polymer molecules. Compounds such as glyceryl monopalmitate (GMP), other monoacylglycerols and their derivatives, and sodium stearoyl 2-lactylate (SSL) are incorporated into doughs of bread and other baked goods, in part to increase shelf-life.

3.3.6.8 Starch Complexes

Because amylose chains are helical with hydrophobic (lipophilic) interiors, they are able to form complexes with linear hydrophobic portions of molecules that can fit within the hydrophobic core. Iodine (as I_3^-) complexes with both amylose and amylopectin molecules. Again, the complexing occurs within the hydrophobic interior of helical segments. With amylose, the long helical segments allow long chains of poly(I_3^-) to complex, producing the characteristic blue color used as a diagnostic test for the presence of starch. The amylose–iodine complex contains 19% iodine, and

the determination of the amount of complexing can be used to measure the amount of apparent amylose present in a starch. The amylopectin complexes are a reddish-brown color because the branch chains of amylopectin are too short to allow formation of long chains of poly(I_3^-). The color difference between amylose and amylopectin iodine complexes is used to distinguish between nonwaxy and waxy genotypes.

A characteristic of amylose-containing cereal starches is that they contain small but functionally important amounts of FFAs, lysophospholipids, and monoacylglycerols (see Chapter 4). Wheat, rye, and barley starches contain almost exclusively lysophospholipids; other cereal starches contain mainly FFAs. Lysophosphatidylcholine is the major lipid in wheat and corn/maize starches. Starch complexes with these polar lipids and emulsifiers/surfactants form single-helical complexes, primarily after the starch in a food product is cooked or gelatinized (often pasted). Such polar lipids can affect starch pastes and starch-based foods in one or more of three ways as a result of complex formation: (1) by affecting the processes associated with starch gelatinization and pasting (i.e., the loss of birefringence, granular swelling, leaching of amylose, melting of the crystalline regions of starch granules, and viscosity increases during cooking); (2) by modifying the rheological behavior of the resulting pastes; and (3) by inhibiting the association of starch molecules to limit the retrogradation process. Here too, complexation with emulsifiers occurs much more readily with, and has a much greater effect on, the amylose component than on the amylopectin component, so emulsifiers affect normal starches much more than waxy starches.

Certain flavor and aroma compounds also can form complexes with amylose molecules and the longer chains of amylopectin. In such complexes, flavor, aroma, or other organic compounds may be incorporated as guest molecules within starch helices (similar to amylose–lipid complexes) and/or between starch helices for encapsulation purposes. Complexation aids retention of volatile compounds and can provide a protective effect against their oxidation.

3.3.6.9 Hydrolysis of Starch [5,15,32,48,58,63,69,71,74,75]

Starch molecules, like all other polysaccharide molecules, are depolymerized by hot acids. Hydrolysis of the glycosidic bonds occurs more or less randomly to produce, initially, very large fragments. Commercially, hydrochloric acid is sprayed onto well-mixed starch, or stirred slightly moistened, granular starch is treated with hydrogen chloride gas, and the mixture is then heated until the desired degree of depolymerization is obtained. The acid is neutralized, and the product is recovered, washed, and dried. The products are still granular, but break up (cook out) much more easily than the parent untreated starch. They are called *acid-modified*, *thin-boiling*, *thinned*, or *fluidity starches*. Even though only a few glycosidic bonds are hydrolyzed, the starch granules disintegrate much more easily during heating in water. Acid-modified starches form gels with improved clarity and increased strength, even though their hot pastes are less viscous. These products are used as film formers and adhesives in products such as pan-coated nuts and candies and wherever a strong gel is desired, for example, in gum candies (such as jelly beans, jujubes, orange slices, and spearmint leaves) and in processed cheese loaves. To prepare especially strong and rapidly setting gels, a high-amylose corn starch is used as the base starch.

More extensive depolymerization of granular starch with acid produces dextrins. Dextrins produce lower viscosities at equal concentrations than thin-boiling starches and can be used at high concentrations in food processing. They have film-forming and adhesive properties and are useful in products such as pan-coated roasted nuts and candy. They are also used as fillers, encapsulating agents, and carriers of flavors, especially spray-dried flavors. They are classified by their cold-water solubility and color (white or yellow). Dextrins that retain large amounts of linear chains or long chain fragments form strong gels.

Further, incomplete hydrolysis of cooked (i.e., pasted) starch dispersions with either an acid or an enzyme produces mixtures of maltooligosaccharides,* which are referred to industrially as

* Oligosaccharides from starch are known as *maltooligosaccharides*.

TABLE 3.7
Functional Properties of Starch Hydrolysis Products

Properties Enhanced by Greater Hydrolysis[a]	Properties Enhanced in Products of Less Conversion[b]
Sweetness	Ability to produce viscosity
Hygroscopicity and humectancy	Ability to provide body
Freezing point depression	Foam stabilization
Flavor enhancement	Ice crystal growth prevention
Fermentability	Sugar crystallization prevention
Browning reaction	

[a] High-conversion (high-DE) syrups.
[b] Low-DE syrups and maltodextrins.

maltodextrins. Maltodextrins are usually described on the basis of their dextrose equivalency (DE). DE is related to the degree of polymerization through the equation DE = 100/DP, where both DE and DP are average values for populations of molecules. Therefore, the DE of a product of hydrolysis is its reducing power as a percentage of the reducing power of pure D-glucose (dextrose); thus, DE is inversely related to average molecular weight. *Maltodextrins* are defined as products with DE values that are measurable, but less than 20, that is, their average DPs are more than 5. Maltodextrins of lowest DE (i.e., the highest average molecular weight) are nonhygroscopic, while those of highest DE tend to absorb moisture. Maltodextrins are bland with virtually no sweetness and are excellent for contributing body or bulk to food systems (Table 3.7).

Continued hydrolysis of starch produces a mixture of D-glucose, maltose, and other malto-oligosaccharides. Syrups with these components in various ratios are produced in enormous quantities, with one of the most common syrups having a DE of 42. These syrups are stable because crystallization does not occur easily in such complex mixtures. They are sold in concentrations of high osmolality (about 70% solids), high enough that ordinary organisms cannot grow in them. An example is waffle and pancake syrup, which is colored with caramel coloring and flavored with maple flavoring. Hydrolysis to DE values of 20–60 gives mixtures of molecules that, when dried, are called *corn syrup solids*. Corn syrup solids dissolve rapidly and are mildly sweet. The functional properties of the hydrolysis products of starches are given in Table 3.7.

Three to four enzymes are used for the industrial hydrolysis of starch to D-glucose (see Chapter 6). α-Amylase is an endo-enzyme that cleaves both amylose and amylopectin molecules internally, producing oligosaccharides. The larger oligosaccharides may be singly, doubly, or triply branched via (1→6) linkages, since α-amylase acts only on the (1→4) linkages of starch. α-Amylase also does not attack double-helical starch polymer segments or polymer segments complexed with a polar lipid (stabilized single helical segments).

Glucoamylase (amyloglucosidase) is used commercially, in combination with an α-amylase, for producing D-glucose (dextrose) syrups and crystalline dextrose. This enzyme acts upon fully gelatinized starch as an exo-enzyme, sequentially releasing single D-glucosyl units from the non-reducing ends of amylose and amylopectin molecules, even those joined through (1→6) bonds. Consequently, the enzyme can completely hydrolyze starch to D-glucose, but is always used on starch that has already been depolymerized with α-amylase to generate fragments and, thus, more nonreducing ends.

β-Amylase releases the disaccharide maltose sequentially from the nonreducing ends of starch polymer chains. When amylopectin is the substrate, it attacks the nonreducing ends, sequentially releasing maltose, but it cannot cleave the (1→6)-linkages at branch points; so it leaves a pruned amylopectin residue termed a *limit dextrin*, more specifically a β-limit dextrin.

There are several debranching enzymes that specifically catalyze hydrolysis of (1→6)-linkages in amylopectin, producing numerous linear, but low-molecular-weight, molecules. One such enzyme is isoamylase; another is pullulanase.

Cyclodextrin glucanotransferase is a unique *Bacillus* enzyme that forms rings of (1→4)-linked α-D-glucopyranosyl units called *cyclodextrins* (see Section 3.2.5) from starch polymers.

Glucose syrup, often called corn syrup in the United States, is the major source of D-glucose and D-fructose. To make a syrup, a slurry of starch in water is mixed with a thermally stable α-amylase and put through a special cooker where rapid gelatinization and enzyme-catalyzed hydrolysis (liquefaction) take place. After cooling to 55°C–60°C (130°F–140°F), hydrolysis is continued with glucoamylase. Then, the syrup is clarified, concentrated, carbon-refined, and ion-exchanged. If the syrup is properly refined and combined with seed crystals, crystalline D-glucose (dextrose) can be obtained.

For production of D-fructose, a D-glucose solution is passed through a column containing bound (immobilized) glucose isomerase. This enzyme catalyzes the isomerization of D-glucose to D-fructose (see Figure 3.5), forming an equilibrium mixture of approximately 58% D-glucose and 42% D-fructose (see Chapter 6). Higher concentrations of D-fructose are usually desired. (The high-fructose syrup [HFS] most often used as a soft drink sweetener contains approximately 55% D-fructose.) To make a syrup with a concentration of D-fructose greater than 42%, the isomerized syrup is passed through a bed of cation-exchange resin in the calcium salt form. The resin binds D-fructose, which is recovered and added to the normal syrup to produce a syrup enriched in D-fructose.

3.3.6.10 Modified Food Starches [6,74,76]

Food systems present a diverse range of demanding conditions, that is, high-temperature heating, high-acid environments, high-shear mixing/pumping, freezing/thawing operations, and extended refrigerated storage, through which a starch must be able to retain its intended functionality. Thus, food processors generally require starches with behavioral characteristics that cannot be provided by native starches. Native starches generally produce weak-bodied, cohesive, rubbery pastes when cooked and undesirable gel properties and stability when the pastes are cooled. Modification of starch is done to improve the characteristics of pastes and gels and to alter the resultant pastes to withstand the conditions of heat, shear, and acid associated with particular processing conditions; other modifications are done to introduce specific functionalities such as paste clarity, gel strength, or enhanced stability to retrogradation. Modified food starches are functional, useful, and abundant food macroingredients and additives.

Modifications can be chemical or physical. Chemical modifications include cross-linked, stabilized, oxidized, and depolymerized (acid-modified, thin-boiling; see Section 3.3.6.9) starch products. Physical modifications consist of pregelatinized (see Section 3.3.6.11) and cold-water-swelling/cold-water-soluble (see Section 3.3.6.12) starch products, as well as those with reduced or slowed digestibility (see Section 3.4). Chemical modifications have the greatest effects on functionalities, and the majority of modified food starch products have been derivatized with reagents that react with hydroxyl groups to form ethers or esters. Modifications can be of a single type, though modified starches often are prepared by combinations of two, three, and sometimes four different processes.

Chemical reactions both currently allowed and used to produce modified food starches in the United States are as follows:

- Esterification with acetic anhydride, succinic anhydride, the mixed anhydride of acetic and adipic acids, 2-octenylsuccinic anhydride, phosphoryl chloride, sodium trimetaphosphate, sodium tripolyphosphate, and monosodium orthophosphate
- Etherification with propylene oxide
- Acid modification with hydrochloric and sulfuric acids
- Bleaching with hydrogen peroxide, peracetic acid, potassium permanganate, and sodium hypochlorite

- Oxidation with sodium hypochlorite
- Some allowed combinations of these reactions

Current regulations in the United States further allow for specific combinations of these reactions to be utilized to generate dual-modified (stabilization + cross-linking) and other multiply modified starch products using these reactions. In other countries, acetylation using vinyl acetate, cross-linking with epichlorohydrin, and oxidations with other oxidants (such as hydrogen peroxide in the presence of Cu(II) ions) are practiced. U.S. approved and utilized esterified and etherified modified food starches include the following:

Stabilized starches

- Hydroxypropyl starches (starch ether)
- Starch acetates (starch ester)
- Starch octenylsuccinates (monostarch ester)
- Monostarch phosphate (ester)

Cross-linked starches

- Distarch phosphate
- Distarch adipate

Cross-linked and stabilized starches

- Hydroxypropylated distarch phosphate
- Phosphorylated distarch phosphate
- Acetylated distarch phosphate
- Acetylated distarch adipate

Cross-linked starches have higher gelatinization and pasting temperatures, increased resistance to shear, and improved stability to low pH conditions, and generally produce pastes with greater viscosities and temperature stability as compared to the base starch.

Stabilized products have lower gelatinization and pasting temperatures, are easier to redisperse when pregelatinized, and produce pastes and gels with a reduced tendency for retrogradation, that is, greater stability, improved freeze–thaw stability, and greater clarity as compared to the base starch.

Starches that have been both cross-linked and stabilized generally have lowered gelatinization and pasting temperatures, produce pastes with greater viscosity, and demonstrate the combined attributes of cross-linking and stabilization as compared to the base starch.

Hypochlorite-oxidized products are whiter, have lower gelatinization and pasting temperatures, produce a lower maximum paste viscosity, and result in softer and clearer gels as compared to the unmodified starch.

Thinned (see Section 3.3.6.9), that is, very slightly depolymerized, products have lower gelatinization and pasting temperatures and produce hot pastes with less viscosity, but exhibit increased gel strength upon cooling, as compared to the base starch.

Any starch (corn, waxy maize, potato, tapioca/cassava, wheat, rice, etc.) can be modified, but modification is practiced significantly on normal corn, waxy maize, tapioca, wheat, and potato starches. Modified waxy maize starches are especially prevalent in the U.S. food industry. Pastes of unmodified common corn starch will gel, and the gels will generally be cohesive, rubbery, and prone to syneresis (i.e., weeping or release of moisture). Pastes of waxy maize starch show little tendency to gel at room temperature, which is why waxy maize starch is generally preferred as the base starch for food starches, but pastes of waxy maize starch will

become cloudy and chunky, and exhibit syneresis when stored under refrigerated or freezing conditions; so even waxy maize starch is usually modified to improve the stability of its pastes. The most common and useful derivative employed for starch stabilization is the hydroxypropyl ether (discussed later).

Specific property improvements that can be obtained by appropriate combinations of modifications are reduction in the energy required for cooking (enhanced gelatinization and pasting), modification of cooking behaviors (reduced hot paste viscosity), increased solubility, either increased or decreased paste viscosity, increased freeze–thaw stability of pastes, enhancement of paste clarity, increased paste sheen, reduction or enhancement of gel formation and gel strength, reduction of gel syneresis, improvement of interaction with other substances, improvement in stabilizing properties, enhancement of film formation, improvement in water resistance of films, reduction in paste cohesiveness, and improvement of stability to acid, heat, and shear.

Starch, like all carbohydrates, can undergo reactions at its various hydroxyl groups. In modified food starches, only very few of the hydroxyl groups are modified. Normally, ester or ether groups are attached at very low DS (degree of substitution)* values. DS values are often <0.1 and are generally in the range 0.002–0.2, depending on the modification.† This range corresponds respectively to, on average, one substituent group on every 500-5 D-glucopyranosyl units. Low levels of derivatization change the properties of starches dramatically and greatly extend their usefulness. Starch products that are esterified or etherified with monofunctional reagents resist interchain associations, which reduces the tendency of a starch paste to gel or retrograde, and the tendency for precipitation to occur. Hence, this modification is often called *stabilization* and the products are called *stabilized starches* (see below). Use of bifunctional reagents produces cross-linked starches. Modified food starches are often both cross-linked and stabilized (i.e., dual-modified).

Acetylation of starch to the maximum allowed in foods (DS 0.09) (U.S.) lowers the gelatinization temperature, improves paste clarity, provides stability to retrogradation, and provides some freeze–thaw stability (but generally not as effectively as hydroxypropylation).

Starch phosphate monoesters (monostarch phosphates) (Figure 3.44) are made by impregnating and reacting starch granules with solutions of sodium tripolyphosphate or monosodium orthophosphate. Monostarch phosphates produce clear, stable pastes that have a long, cohesive texture.

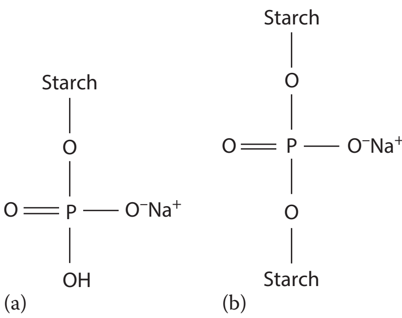


FIGURE 3.44 Structures of starch monoester phosphate (a) and diester phosphate (b). The diester joins two molecules together, resulting in cross-linked starch granules.

* The degree of substitution (DS) is defined as the average number of esterified or etherified hydroxyl groups per monosaccharide unit. Both branched and unbranched polysaccharides composed of neutral hexopyranosyl units have an average of three hydroxyl groups per monomeric unit. Therefore, the theoretical maximum DS for a starch and cellulose is 3.0, although this maximum possible is not allowed in products used as food ingredients.

† Specific limits to the amount of derivatization of a modified food starch are established by law or regulation in different countries and regions.

Paste viscosity, which is generally high, can be controlled by varying the reagent concentration, time of reaction, temperature, and pH. Increasing substitution lowers the gelatinization temperature until the products become first cold-water-swelling and then cold-water-soluble (see Section 3.3.6.12). Corn starch phosphates produce pastes with high viscosity, clarity, stability, and texture—more like those of potato starch. Starch phosphates are good emulsion stabilizers and produce pastes with improved freeze–thaw stability. In the United States, a starch phosphorylated for use as a food ingredient may contain residual phosphate in amounts not exceeding 0.4% when monosodium orthophosphate is the reagent used and 0.04% when sodium trimetaphosphate is the reagent used (both calculated as phosphorus).

Preparation of an alkenylsuccinate ester of starch attaches a hydrophobic hydrocarbon chain to its polymer molecules (Figure 3.45). Even at very low DS values, starch 2-octenylsuccinate molecules concentrate at the interface of an oil-in-water emulsion because of the hydrophobicity of the alkenyl group. This characteristic makes them useful as emulsion stabilizers. Starch 2-octenylsuccinate products can be used in a variety of food applications where emulsion stability is needed, such as in flavored beverages. The presence of the aliphatic chain tends to give the starch derivative a sensory perception of fattiness, so it is possible to use the derivative as a partial replacement for fat in certain foods. Products with higher DS are nonwetting and are used as release agents for dusting on dough sheets and as processing aids.

Hydroxypropylation is the most often used reaction to prepare a stabilized starch product. Hydroxypropylstarch (starch–O$-$CH$_2-$CHOH$-$CH$_3$) is prepared by reacting starch with propylene oxide to produce a low level of etherification (DS 0.02–0.2, 0.2 being the maximum allowed in the United States). Hydroxypropylstarch has properties similar to those of starch acetate, because it similarly has "bumps" along the starch polymer chains that prevent the interchain associations that lead to retrogradation. Hydroxypropylation reduces the gelatinization temperature, and the resultant modified starches form clear pastes that do not retrograde and withstand freezing and thawing. They are used as thickeners and extenders. To improve viscosity, particularly under acidic, high shear, or extended cooking conditions, acetylated and hydroxypropylated starches are often also cross-linked with phosphate groups.

The majority of modified food starch is cross-linked. Cross-linking occurs when starch granules are reacted with bifunctional reagents that react with hydroxyl groups on two different molecules or adjacent chains within the granule. Cross-linking is accomplished most often by producing distarch phosphate esters (Figure 3.44). Starch is reacted in alkaline slurries either with phosphorus oxychloride (phosphoryl chloride, $POCl_3$) or with sodium trimetaphosphate, with $POCl_3$ being the reagent most often used for cross-linking. The linking together of starch chains with phosphate diester or other cross-links reinforces the granule structure and reduces both the rate and the degree of granule swelling and subsequent disintegration. Thus, granules

FIGURE 3.45 Preparation of the 2-octenylsuccinate ester of starch.

exhibit reduced sensitivity to processing conditions (high temperature, extended cooking times, low pH, high shear during mixing, milling, homogenization, and/or pumping). Cooked pastes of cross-linked starches are more viscous,* heavier bodied, shorter textured, and less likely to break down during extended cooking or during exposure to low pH and/or severe agitation than are pastes of the native starches from which they are prepared. Only a small amount of cross-linking is required to produce a noticeable effect; and with lower levels of cross-linking, granule swelling is inversely proportional to DS. As cross-linking is increased, the granules become more and more tolerant to physical conditions and acidity, but less and less dispersible by cooking, and energy requirements to reach maximum swelling and viscosity are increased. For example, treatment of a starch with only 0.0025% of sodium trimetaphosphate greatly reduces both the rate and degree of granule swelling, greatly increases paste stability, and changes dramatically the pasting/paste viscosity profile and textural characteristics of its paste. Treatment with 0.08% of trimetaphosphate produces a product in which granule swelling is restricted to the point where a peak viscosity is never reached during the hot holding period. As the degree of cross-linking increases, the starch also becomes more acid-stable. Though some hydrolysis of glycosidic bonds occurs during heating of cross-linked starch in aqueous acid, the phosphate cross-links that link starch chains to one another help retain sufficiently large molecules to prevent the loss of viscosity (i.e., cross-links themselves are reasonably stable to an acid environment and help compensate for the effects of starch hydrolysis). The only other cross-link permitted in a food starch (in the United States) is the distarch ester of adipic acid.

Most cross-linked food starches contain less than one cross-link per 1000 α-D-glucopyranosyl units. Trends toward the use of continuous cooking processes require increased shear resistance and stability to hot surfaces. Storage-stable thickening is also provided by cross-linked starches. In retort sterilization of canned foods, cross-linked starches, because of their reduced rate of gelatinization and swelling, maintain a low initial viscosity long enough to facilitate the rapid heat transfer and temperature rise that are needed to provide uniform sterilization—before granule swelling brings about the desired viscosity, texture, and suspending characteristics. Cross-linked starches are used in canned soups, gravies, and puddings, and in batter mixes. Cross-linking of waxy maize starch gives the clear paste sufficient rigidity so that, when used in pie fillings, the cut pie sections hold their shape.

Oxidation with sodium hypochlorite (chlorine in an alkaline solution) results in depolymerization, viscosity reduction, and a decreased pasting temperature. Oxidation also reduces association of amylose molecules, that is, results in some stabilization via the introduction of small amounts of carboxylate and carbonyl groups to starch chains. Oxidized starches produce less viscosity and softer gels (as compared with the base starch) and are used when such properties are needed. They are also used to improve adhesion of starch batters to fish, meat, and vegetables. Mild treatment with sodium hypochlorite, hydrogen peroxide, or potassium permanganate simply bleaches the starch and reduces the count of viable microbes.

Acid modification (as described in Section 3.3.6.9) can also be done with an etherified starch. Such "thinning" may be practiced for the same reason as with native starches, that is, to reduce viscosity in order to get more solids in solution and thereby enhance the bulk, body, adhesion characteristics, and so on, of the product. Reduced viscosity can also be achieved by oxidation with sodium hypochlorite as described in the previous paragraph.

Modified food starches are tailor-made for specific applications. Properties that can be controlled by combinations of cross-linking, stabilization, and thinning of corn, waxy maize, potato, wheat, and other starches include, but are not limited to, the following: adhesion, clarity of solutions/pastes, color, emulsion stabilization ability, film-forming ability, flavor release, hydration rate, moisture-holding capacity, stability to acids, stability to heat and cold, stability to shear, temperature required

* Note in Figure 3.42 that maximum viscosity is reached when the system contains highly swollen granules. Cross-linked granules are less prone to disintegration as shear is applied. Thus, there is less loss of viscosity after the peak is reached.

to cook, and viscosity (hot paste and cold paste). Some characteristics imparted to the food product include, but are not limited to, the following: mouthfeel, reduction of oil migration, texture, sheen, stability, and tackiness.

Starches that are both cross-linked and stabilized are used in canned, frozen, baked, and dry foods. In baby foods and fruit pie fillings in cans and jars, they provide long shelf-life. They also allow frozen fruit pies, pot pies, and gravies to remain stable under long-term storage.

3.3.6.11 Pregelatinized Starch

Starch products that have been described so far are known as *cook-up starches*. In contrast, starch that has been cooked/pasted and dried with little or no retrogradation can be partially redissolved in room-temperature water, providing viscosity without the requirement of further heating or cooking. Such starch is called *pregelatinized* or *instant starch*. It has been gelatinized, but it has also been pasted, that is, many swollen granules have been destroyed; so it should more properly be called precooked or prepasted starch. There are two basic approaches to making pregelatinized products. In one, a starch–water slurry is introduced into the nip between two nearly touching and counter-rotating, steam-heated rolls, or, alternatively, is applied to the top of a single rotating, steam-heated roll. In either case, the starch slurry is gelatinized and pasted almost instantaneously, after which the resultant paste that coats the rolls dries rapidly. The dry film is scraped from the roll and ground to a powder. The resulting products are cold-water-soluble and will produce viscous dispersions when stirred into room-temperature water, although some heating or shear is often necessary to achieve maximum viscosity. The second method of preparation uses an extruder. In this process, the heat and shear generated within the extruder gelatinizes and destroys the structures of the moistened, swollen starch granules. The puffed, crispy, glassy extrudate is ground to a powder.

Both chemically modified and unmodified starches can be used to make pregelatinized starches. If chemically modified starches (Section 3.3.6.10) are used, the properties introduced by the modification(s) carry through to the pregelatinized products; thus, paste properties such as stability and freeze–thaw stability can also be imparted to pregelatinized starches. Pregelatinized, slightly cross-linked starch is useful in instant soup, pizza topping, extruded snacks, and breakfast cereals.

The primary advantage of pregelatinized starches is that they can be used without cooking. Like a water-soluble gum, finely ground pregelatinized starch can form small gel particles when added to water, but when properly dispersed and dissolved, gives solutions of high viscosity. More coarsely ground products disperse more easily, and produce dispersions of lower viscosity and with a graininess or pulpiness that is desirable in some products. Many pregelatinized starches are used in dry mixes, such as instant pudding mixes. They disperse readily with high-shear stirring or when premixed with sugar or other dry ingredients.

3.3.6.12 Cold-Water-Swelling Starch

Granular starch that swells extensively in room-temperature water is made by heating common corn starch in 75%–90% aqueous ethanol or by a special spray-drying process. This product (also called *cold-water-soluble starch*) is also categorized as a pregelatinized or instant starch by some. The difference between it and conventional pregelatinized starch is that, while the crystalline order and birefringence of the granules have been disrupted or destroyed by the initial treatment, the granules remain intact. Therefore, when added to water, granules swell as if they were being cooked. The dispersion made by incorporating cold-water-swelling starch into sugar solutions or glucose syrups by rapid stirring can be poured into molds, where it sets to a rigid gel that can be sliced. The result is a gum candy. Cold-water-swelling starch is also useful in making desserts and in muffin batters containing particles, such as blueberries, that otherwise would settle to the bottom before the batter is thickened by heating during baking.

3.3.7 Cellulose: Forms and Derivatives [79]

Cellulose is a high-molecular-weight, linear, insoluble homopolysaccharide of repeating β-D-glucopyranosyl units joined by (1→4) glycosidic linkages (Figure 3.46). The axial → equatorial (1→4)-linkages joining the α-D-glucopyranosyl units of starch polymer molecules produce a coiled structure (an α-helix) (Figure 3.40). In contrast, the equatorial → equatorial (1→4)-linkages joining the β-D-glucopyranosyl units of cellulose molecules give them a flat, ribbon-like structure, in which each glucopyranosyl unit in the chain is turned upside down as compared to the unit directly preceding and following it. Because of their flat and linear nature, cellulose molecules can associate with each other via hydrogen bonding over extended regions, forming polycrystalline, fibrous bundles. Crystalline regions are separated by, and connected to, amorphous regions. Cellulose is insoluble in water because, in order for it to dissolve, most of its very numerous hydrogen bonds would have to be released all at once. Cellulose can, however, through derivatization, be converted into water-soluble gums.

Cellulose and its modified forms serve as dietary fiber because they are undigested and do not contribute significant nourishment or calories as they pass through the human digestive system. Dietary fiber is important to human nutrition (see Section 3.4).

A purified cellulose powder is available as a food ingredient. High-quality cellulose can be obtained from wood through pulping and subsequent purification. However, chemical purity of powdered cellulose is not required for food use because cellulosic cell walls are components of all fruits and vegetables. The powdered cellulose used in foods has negligible flavor, color, and microbial contamination, and is most often added to bread to provide noncaloric bulk. Reduced-calorie baked goods made with powdered cellulose have an increased content of dietary fiber.

3.3.7.1 Microcrystalline Cellulose [51,55,63,72]

A purified, insoluble cellulose termed *microcrystalline cellulose* (MCC) is made by partial hydrolysis of purified wood pulp cellulose, with hydrolysis taking place in the amorphous regions, followed by separation of the released microcrystals. Cellulose molecules are fairly rigid and completely linear chains of about 3000 β-D-glucopyranosyl units, and associate easily in long junction zones. However, the long and unwieldy chains do not align perfectly over their entire lengths, creating breaks in the crystalline structure. These lapses in crystallinity are simply the divergence of cellulose chains away from an ordered to a more random arrangement, forming the amorphous regions. Such a structure is represented in Figure 3.35. When purified wood pulp is hydrolyzed with acid, the acid penetrates the lower density hydrated amorphous regions where the polymer chains have greater freedom of movement, and effects hydrolytic cleavage of chains in these regions, releasing individual fringed crystallites.

Two types of MCC are produced, each of which is stable to both heat and acids. Powdered MCC is a spray-dried product. Spray-drying produces porous aggregates of microcrystals. Powdered MCC is used as a flavor carrier and as an anticaking agent for shredded cheese. The second type, colloidal MCC, is water-dispersible and has functional properties similar to those of water-soluble gums.

FIGURE 3.46 Cellulose (repeating unit).

To make colloidal MCC, considerable mechanical energy is applied after initial hydrolysis to tear apart the weakened microfibrils and provide a high proportion of colloidal-sized aggregates (<0.2 μm in diameter). To prevent reassociation of the aggregates during drying, sodium carboxymethylcellulose (CMC) (Section 3.3.7.2), xanthan (Section 3.3.9), or sodium alginate (Section 3.3.11) is added. The anionic gum aids in redispersion by interacting directly with the aggregates and acting as a barrier to reassociation by giving the aggregate particles a stabilizing negative charge.

The major functions of colloidal MCC are to stabilize foams and emulsions, especially during high-temperature processing, to form gels with salve-like textures (MCC does not dissolve, nor does it form intermolecular junction zones; rather it forms a network of hydrated microcrystals), to stabilize pectin and starch gels to heat, to improve adhesion, to replace fat and oil in products like salad dressings and ice cream, and to control ice crystal growth. MCC stabilizes emulsions and foams by adsorbing at interfaces and strengthening interfacial films. It is a common ingredient of reduced fat ice cream and other frozen dessert products.

3.3.7.2 Carboxymethylcelluloses [30,33,51,55,63,73]

CMC (Table 3.5) is widely and extensively used as a food gum. Treatment of purified wood pulp with 18% sodium hydroxide solution produces alkali cellulose (99% pure). When alkali cellulose is reacted with the sodium salt of chloroacetic acid, the sodium salt of the carboxymethyl ether (cellulose–$O-CH_2-CO_2^-Na^+$) is formed. Most commercial CMC products have a degree of substitution (DS, see Section 3.3.6.10) in the range 0.4–0.8. The most widely sold type for use as a food ingredient has a DS of 0.7.

Since CMC consists of long and fairly rigid molecules that bear a negative charge due to numerous ionized carboxyl groups, electrostatic repulsion causes its molecules in solution to be extended. Also, adjacent chains repel each other. Consequently, CMC solutions tend to be both highly viscous and stable. CMC is available in a wide range of viscosity grades. CMC stabilizes protein dispersions, especially near the isoelectric pH value of the protein.

3.3.7.3 Methylcelluloses (MCs) and Hydroxypropylmethylcelluloses (HPMCs) [30,33,51,55,63,73]

To make methylcellulose products (Table 3.5), alkali cellulose is treated with methyl chloride to introduce methyl ether groups (cellulose–$O-CH_3$). Many members of this family of gums also contain hydroxypropyl ether groups (cellulose–$O-CH_2-CHOH-CH_3$). Hydroxypropylmethylcelluloses are made by reacting alkali cellulose with both propylene oxide and methyl chloride. The degree of substitution with methyl ether groups of commercial methylcelluloses ranges from 1.1 to 2.2. The molar substitution (MS)* with hydroxypropyl ether groups in commercial hydroxypropylmethylcelluloses ranges from 0.02 to 0.3. (Both the methylcellulose and hydroxypropylmethylcellulose members of this gum family are generally referred to simply as *methylcelluloses*.) Both products are cold-water-soluble because the methyl and hydroxypropyl ether group protrusions along the chains prevent the intermolecular association characteristic of cellulose.

While a few added ether groups spread along the chains enhance water solubility, they also decrease chain hydration by replacing water-binding hydroxyl groups with less polar ether groups, giving members of this family unique characteristics. The ether groups restrict solvation of the chains to the point that they are on the borderline of water solubility. When an aqueous solution is heated, the water molecules hydrating the polymer dissociate from the chain and hydration is decreased sufficiently that intermolecular associations increase (probably via van der Waals interactions) and

* The moles of substitution or molar substitution (MS) value indicates the average number of moles of a substituent attached to a glycosyl unit of a polysaccharide. Because reaction of a hydroxyl group with propylene oxide creates a new hydroxyl group with which propylene oxide can react further, poly(propylene oxide) chains, each terminated with a free hydroxyl group, can form. Because more than three moles of propylene oxide can react with a single hexopyranosyl unit, MS rather than DS is used.

gelation occurs. Lowering the temperature of the gel allows the molecules to rehydrate and redissolve, so the thermogelation is reversible.

Because of the ether groups, the hydrocolloid chains are somewhat surface active and absorb at interfaces. This helps stabilize emulsions and foams. Methylcelluloses also can be used to reduce the amount of fat in food products through two mechanisms: (1) they provide fat-like properties so that the fat content of a product can be reduced, and (2) they reduce adsorption of fat in products being fried, since the gel structure produced by thermogelation provides a barrier to oil, holds moisture, and acts as a binder.

3.3.8 Guar and Locust Bean Gums [30,33,43,51,55,63,73]

Guar and locust bean gums (LBGs) are important thickening polysaccharides (Table 3.5). Guar gum produces the highest viscosity of any natural commercial gum. Both gums are the ground endosperm of seeds. The main component of both endosperms is a galactomannan. Galactomannans consist of a main chain of β-D-mannopyranosyl units joined by (1→4) bonds with single-unit α-D-galactopyranosyl branches attached at O-6 (Figure 3.47). The specific polysaccharide that makes up most of guar gum is guaran. In guaran, about one-half of the D-mannopyranosyl main-chain units contain an α-D-galactopyranosyl unit.

The galactomannan of LBG (also called *carob gum*) has fewer branch units than guaran and its structure is more irregular, with long stretches of about 80 underivatized D-mannosyl units alternating with sections of about 50 units in which most of the main-chain units have an α-D-galactopyranosyl group glycosidically connected to their O-6 positions.

Because of the difference in structures, guar gum and LBG have different physical properties, even though both are galactomannans and are composed of long, rather rigid chains that provide high solution viscosity. Because guaran has its galactosyl units fairly evenly distributed along the chain, there are few available locations on the chains that are suitable for the formation of junction zones. However, LBG with its long "naked chain" sections can form junction zones and, as a result, requires heating in water to about 90°C for complete solubilization. Though LBG does not form a gel by itself, its molecules interact with xanthan (Figure 3.48, Section 3.3.9) and κ-carrageenan (Section 3.3.10) helices, forming junction zones and rigid gels (Figure 3.49).

Guar gum provides economical thickening to numerous food products. It is frequently used in combination with other food gums, for example, in ice cream, where it is often used in combination with carboxymethylcellulose (Section 3.3.7.2), carrageenan (Section 3.3.10), and LBG.

Typical products in which LBG is found are the same as those for guar gum. About 85% of LBG is used in dairy and frozen dessert products. It is rarely used alone; rather it is used in combination

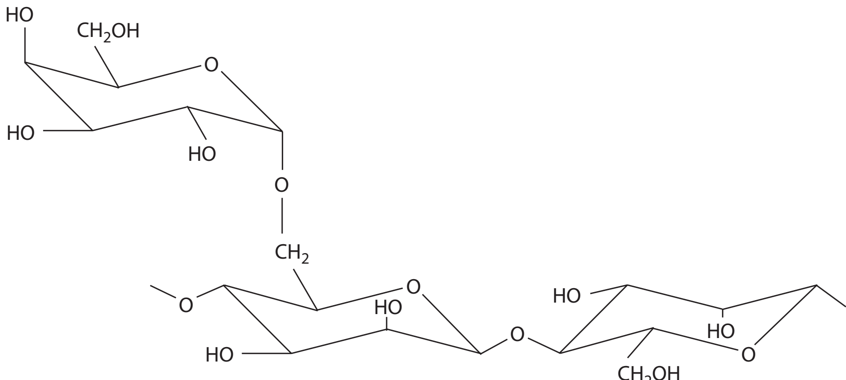


FIGURE 3.47 A representative segment of a galactomannan molecule.

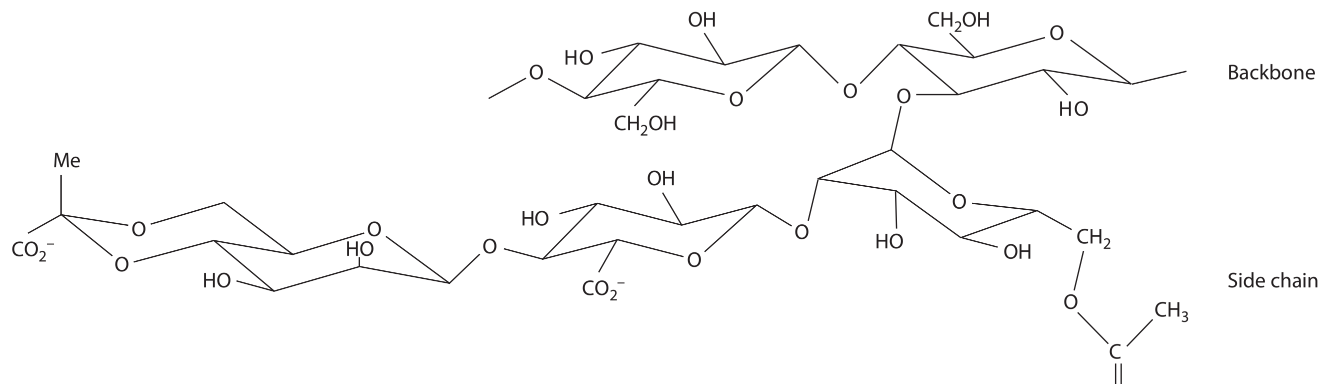


FIGURE 3.48 Structure of the pentasaccharide repeating unit of xanthan. Note the 4,6-*O*-pyruvyl-D-mannopyranosyl nonreducing end unit of the trisaccharide side chain. Normally, about one-half of the side chains are pyruvylated.

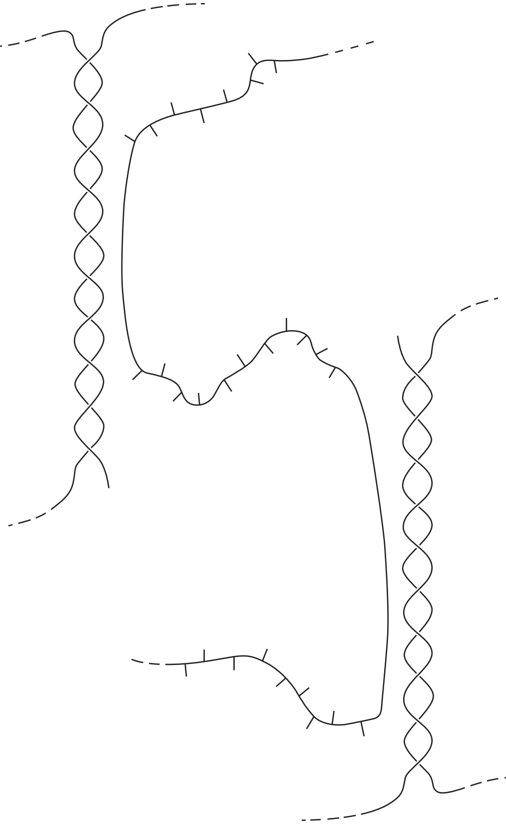


FIGURE 3.49 Representation of the hypothesized interaction of a locust bean gum molecule with double helical portions of xanthan or carrageenan molecules to form a three-dimensional network and a gel.

with other gums such as CMC, carrageenan, xanthan, and guar gum. It is used in combination with κ-carrageenan and xanthan to take advantage of the synergistic gel-forming phenomenon. A typical use level is 0.05%–0.25%.

3.3.9 Xanthan [18,30,33,49,51,55,63,73]

Xanthomonas campestris, a bacterium commonly found on the leaves of plants of the cabbage family, produces a polysaccharide, termed *xanthan*, that is produced in large fermentation vats and is widely used as a food gum. The polysaccharide is known commercially as *xanthan gum* (Table 3.5).

Xanthan has a backbone chain identical to that of cellulose (Figure 3.48). (Compare with Figure 3.46). In the xanthan molecule, every other β-D-glucopyranosyl unit in the cellulose backbone has attached, at the O-3 position, a β-D-mannopyranosyl-(1→4)-β-D-glucuronopyranosyl-(1→2)-6-*O*-acetyl-β-D-mannopyranosyl trisaccharide unit.* About half of the terminal β-D-mannopyranosyl units have pyruvic acid attached as a 4,6-cyclic acetal. The trisaccharide side chains interact with the main backbone and make the xanthan chain rather stiff. The rigidity of xanthan is further enhanced by its ordered structure, which is thought to consist of a double-stranded helix. The molecular weight is probably in the order of 2×10^6, although much larger values, presumably due to aggregation, have been reported.

* Bacterial heteroglycans, unlike plant heteroglycans, have regular, repeating-unit structures.

Xanthan interacts with guar gum synergistically to produce an increase in solution viscosity. The interaction with LBG produces a heat-reversible gel (Figure 3.49).

Xanthan is widely used as a food gum because of the following important characteristics: it is soluble in both hot and cold water; it produces high solution viscosity at low concentrations; there is no discernible change in solution viscosity in the temperature range from 0°C to 100°C, which makes it unique among food gums; it exhibits strong pseudoplastic behavior; it is both soluble and stable in acidic systems; it has excellent compatibility with salt; it forms gels when used in combination with LBG; it is a remarkable stabilizer of suspensions and emulsions; and it imparts stability to products exposed to freezing and thawing. The unusual and very useful properties of xanthan undoubtedly result from the structural rigidity and extended nature of its molecules, which in turn result from its linear cellulosic backbone, which is stiffened and shielded by the anionic trisaccharide side chains.

Xanthan is ideal for stabilizing aqueous dispersions, suspensions, and emulsions. The fact that the viscosity of its solutions changes very little with temperature, that is, its solutions do not thicken upon cooling, makes it irreplaceable for thickening and stabilizing such products as pourable salad dressings and chocolate syrup, which need to pour as easily when taken from the refrigerator as they do at room temperature, and gravies, which should neither thicken appreciably as they cool nor thin too much when hot. In regular pourable salad dressings, it serves as a thickener and as a stabilizer for both the suspension of particulate particles and the oil-in-water emulsion. It is also used as a thickener and suspending agent in no-oil (reduced-calorie) dressings. In both oil-containing and no-oil salad dressings, xanthan is almost always used in combination with propylene glycol alginate (PGA) (see Section 3.3.11). PGA decreases the viscosity of the xanthan-containing system and reduces its pseudoplasticity. Together, they give the desired pourability associated with the pseudoplastic xanthan and the creaminess sensation associated with a non-pseudoplastic solution.

3.3.10 Carrageenans, Agar, and Furcellaran [9,30,33,51,55,63,73]

The term *carrageenan* denotes a group or family of sulfated galactans extracted from red seaweeds with dilute alkaline solutions; the sodium salt of a carrageenan is normally produced. Carrageenans are mixtures of several related sulfated galactans (Table 3.4). Carrageenans are linear chains of D-galactopyranosyl units joined with alternating (1→3)-α-D- and (1→4)-β-D-glycosidic linkages, with most galactosyl units having one or two sulfate half-ester groups esterified to the hydroxyl groups at carbon atoms C-2 and/or C-6. This gives a sulfate content ranging from 15% to 40%. Units often contain a 3,6-anhydro ring. The principal structural types are termed kappa (κ), iota (ι), and lambda (λ) carrageenans (Figure 3.50). The disaccharide units shown in Figure 3.50 represent the predominate building block of each type, but are not repeating unit structures. Carrageenans, as extracted, are mixtures of nonhomogeneous polysaccharides. Carrageenan products, of which there may be more than 100 from a single supplier for different specific applications, contain different proportions of the three main behavioral types (kappa, iota, and lambda) produced by starting with mixtures of red seaweed species. Other substances, such as potassium ions and sugar (for standardization), may be added to the obtained powder.

Carrageenan products dissolve in water to form highly viscous solutions. The viscosity is quite stable over a wide range of pH values because the sulfate half-ester groups are always ionized, even under strongly acidic conditions, giving the molecules a consistent negative charge. However, carrageenans undergo depolymerization in hot acidic solutions, so these conditions are to be avoided when using a carrageenan product.

Segments of molecules of κ- and ι-type carrageenans exist as double helices of parallel chains. In the presence of potassium or calcium ions, thermoreversible gels form upon cooling a hot solution containing double-helical segments. Gelation can occur in water at gum concentrations as low as 0.5%. When κ-type carrageenan solutions are cooled in the presence of potassium ions, a stiff, brittle gel results. Calcium ions are less effective in causing gelation, though potassium and calcium

κ-Carrageenan

ι-Carrageenan

λ-Carrageenan

FIGURE 3.50 Idealized unit structures of κ-, ι-, and λ-type carrageenans.

ions together produce a high gel strength. Gels made with κ-type carrageenans are the strongest of the carrageenan gels. These gels tend to undergo syneresis as junction zones within the structure grow in length. The presence of other gums retards syneresis.

Iota-type carrageenans are a little more soluble than the κ-types, but, again, only the sodium salt form is soluble in cold water. Iota types gel best with calcium ions. The resulting gel is soft and resilient, has good freeze–thaw stability, and does not synerese, presumably because iota-type carrageenans are more hydrophilic and form fewer junction zones than κ-type carrageenans.

During cooling of solutions of κ- or ι-type carrageenans, gelation occurs because the linear molecules are unable to form continuous double helices due to the presence of structural irregularities. The linear helical portions then associate to form a three-dimensional gel in the presence of the appropriate cation (Figure 3.51). All salts of λ-type carrageenans are soluble and non-gelling.

Under conditions in which double-helical segments are present, carrageenan molecules, particularly those of the κ-type, form junction zones with the naked segments of LBG to produce rigid, brittle, syneresing gels (Figure 3.49). This gelation occurs at a concentration one-third that needed to form a pure κ-type carrageenan gel.

Carrageenans are most often used because of their ability to form gels with milk and water. Mixtures of carrageenan types are used to provide a wide range of products that are standardized with various amounts of sucrose, glucose (dextrose), buffer salts, or gelling aids such as potassium chloride. The available commercial products form a variety of gels: gels that are clear or turbid, rigid or elastic, tough or tender, heat-stable or thermally reversible, and do or do not undergo syneresis. Carrageenan gels do not require refrigeration because they do not melt at room temperature. They are freeze–thaw stable.

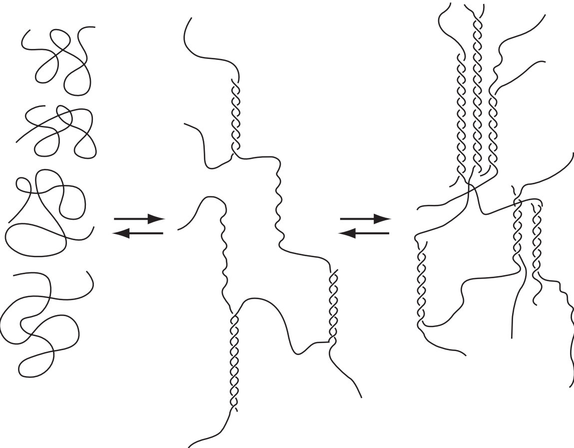


FIGURE 3.51 A representation of the hypothesized mechanism of gelation of κ- and ι-type carrageenans. In a hot solution, the polymer molecules are in a random coil state. As the solution is cooled, the molecules intertwine to form double-helical structures. As the solution is cooled further, the double helices are believed to nest together with the aid of potassium or calcium ions.

A useful property of carrageenans is their reactivity with proteins, particularly those of milk. Kappa-type carrageenans complex with κ-casein micelles of milk, forming a weak, thixotropic, pourable gel. The thickening effect of κ-carrageenans in milk is 5–10 times greater than in water. This property is used in the preparation of chocolate milk, in which the thixotropic gel structure prevents the settling of cocoa particles. Such stabilization requires only about 0.025% gum. This property is also utilized in the preparation of ice cream, evaporated milk, infant formulas, freeze–thaw-stable whipped cream, and emulsions in which milk fat is replaced with a vegetable oil.

The synergistic effect between κ-carrageenan and LBG (Figure 3.49) produces gels with greater elasticity and gel strength, and with less syneresis than gels made with κ-carrageenan (potassium salt form) alone. As compared to κ-type carrageenan alone, the κ-type carrageenan–LBG combination provides greater stabilization and air-bubble retention (overrun) in ice cream, but also a little too much chewiness, so guar gum is added to soften the gel structure.

Cold hams and poultry rolls take up 20%–80% more brine when the brine contains 1%–2% of a κ-type carrageenan. Improved slicing also results. Carrageenan coatings on meats can serve as a mechanical protection and a carrier for seasonings and flavors. Carrageenan is sometimes added to meat analogs made from casein and vegetable proteins. Carrageenan is used to hold water and maintain water content, and thereby to maintain softness of meat products, such as wieners and sausages, during the cooking operation. Addition of a κ- or ι-type carrageenan in the Na^+ form or PES/PNG carrageenan (see below) to low-fat ground beef improves the texture and quality of hamburger. Normally, fat serves the purpose of maintaining softness, but because of the binding power of carrageenan for protein and its high affinity for water, carrageenans can be used to replace in part this function of natural animal fat in lean products.

Some uses of carrageenans are indicated in Table 3.5. However, carrageenan products produced as food ingredients are often blends of carrageenan types. For example, a blend of κ- and λ-types is used in milk shakes, and blends of κ- and ι-types are used in water dessert gels that do not require refrigeration.

Also prepared and used is an alkali-modified seaweed flour that was formerly called *processed Euchema seaweed* (PES) or *Philippine natural grade* (PNG) carrageenan, but is now just called carrageenan. To prepare this form of carrageenan, red seaweed is treated with a potassium hydroxide solution. Because the potassium salts of the types of carrageenans found in these seaweeds are insoluble, the carrageenan molecules are not solubilized and not extracted out.

Primarily, low-molecular-weight soluble components are removed from the plants during this treatment. The remaining seaweed is dried and ground to a powder. This carrageenan is, therefore, a composite material that contains not only the molecules of carrageenan that would be extracted with dilute sodium hydroxide but also other cell-wall materials.

Two other food gums, agar and furcellaran (also called *Danish agar*), also come from red seaweeds and have structures and properties that are closely related to those of the carrageenans. Like gellan (Section 3.3.13), the primary use of agar is in bakery icings and frostings because it is compatible with large amounts of sugar and because products made with it neither melt at high storage temperatures nor stick to packaging materials.

3.3.11 Algins [30,33,49,51,55,63,73]

Commercial algins are salts, most often the sodium salt, of a linear poly(uronic acid), alginic acid, obtained from brown seaweeds (Table 3.5). Alginic acid is composed of two monomeric units, β-D-mannopyranosyluronic acid and α-L-gulopyranosyluronic acid. These two monomers occur in homogeneous regions or blocks (composed exclusively of one unit or the other) and in regions or blocks of mixed units. Segments containing only D-mannuronopyranosyl units are referred to as M blocks, and those containing only L-guluronopyranosyl units are referred to as G blocks. D-Mannuronopyranosyl units are in the $^4C_1$ conformation, while L-guluronopyranosyl units are in the $^1C_4$ conformation (see Section 3.1.2 and Figure 3.52), giving the different blocks quite different chain conformations. M-block regions are flat and ribbon-like, similar to the conformation of cellulose (see Section 3.3.7) because of the equatorial → equatorial bonding. G-block regions have a pleated (corrugated) conformation as a result of their axial → axial glycosidic bonds. Different percentages of the different block segments cause algins (alginates) from different seaweeds to have different properties. Algins with greater G-block contents produce gels of higher strength.

Solutions of sodium alginates are highly viscous. The calcium salt of alginates is insoluble. Insolubility results from interactions between calcium ions and the G-block regions of the chain. The voids formed between two G-block chains are cavities that bind calcium ions. The result is a junction zone that has been called an "egg box" arrangement with the calcium ions, being likened to eggs in the pockets of an egg carton (Figure 3.53). The strength of the gel depends on the content of G blocks in the alginate used and the concentration of calcium ions.

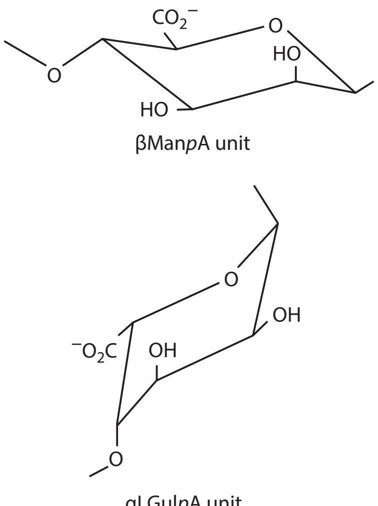


FIGURE 3.52 Units of β-D-mannopyranosyluronic acid (βMan*p*A) in the $^4C_1$ conformation and α-L-gulopyranosyluronic acid (αLGul*p*A) in the $^1C_4$ conformation.

FIGURE 3.53 A representation of the proposed formation of a junction between G-block regions of three alginate molecules promoted by calcium ions.

Propylene glycol alginates are made by reacting moist alginic acid with propylene oxide to produce a partial ester with 50%–85% of the carboxyl groups esterified. Solutions of propylene glycol alginates (PGAs) are much less sensitive to low pH values and polyvalent cations, including calcium ions and proteins, than solutions of nonesterified alginates, because esterified carboxyl groups can no longer ionize. Also, the propylene glycol group introduces a protrusion or "bump" in the chain, which prevents close association of chains. Therefore, PGA solutions are stable. Because of its tolerance to calcium ions, PGA can be used in dairy products. The hydrophobic propylene glycol groups also give the molecules mild interfacial activity: that is, foaming, emulsifying, and emulsion-stabilizing properties. PGA is used when stability to acid, non-reactivity with calcium ions (e.g., in milk products), or its surface active property is desired. Accordingly, it finds use as a thickener in salad dressings (Table 3.5). In low-calorie dressings, it is often used in conjunction with xanthan (Section 3.3.9).

Alginate salts are most often used as food ingredients because of their ability to form gels. Calcium alginate gels are obtained by diffusion setting, internal setting, and setting by cooling. Diffusion setting can be used to prepare structured foods. A good example is the structured pimento strip. In the production of pimento strips for stuffing green olives, pimento puree is first mixed with water containing a small amount of guar gum as an immediate thickener, and then with sodium alginate. The mixture is pumped onto a conveyor belt and gelled by the addition of calcium ions. The set sheet is cut into thin strips and stuffed into olives. Internal setting for fruit mixes, purees, and fruit analogs involves a slow release of calcium ions within the mixture. The slow release is obtained by the combined action of a slightly soluble organic acid and a sequestrant on an insoluble calcium salt. Setting by cooling involves mixing the components required to form a gel at a temperature above the gel's melting temperature and allowing the mixture to set on cooling. Alginate gels are reasonably heat stable and show little or no syneresis. Unlike gelatin gels, alginate gels are not thermoreversible and, like carrageenan gels, do not require refrigeration and can be used as dessert gels that do not melt, even at high ambient temperatures; but as a result, they do not melt in the mouth like gelatin gels. Calcium alginate films are used as edible sausage casing. Alginic acid, that is, an alginate solution whose pH has been lowered, with or without addition of calcium ions, is employed in the preparation of soft, thixotropic, nonmelting gels (Table 3.5).

3.3.12 Pectins [18,30,33,51,55,63,73]

Commercial pectins are galacturonoglycans [poly(α-D-galactopyranosyluronic acids)] with various contents of methyl ester groups (Table 3.5). The native parent molecules present in the cell walls and intercellular layers of all land plants, from which commercial pectins are obtained, are much more

complex molecules than those that are converted into methyl-esterified galacturonoglycans during extraction with acid. Commercial pectin is obtained from citrus peel and apple pomace, with pectin from lemon and lime peel generally being the easiest to isolate and of the highest quality. Depending on their structure, pectins have a unique ability to form spreadable gels in the presence of sugar and acid or in the presence of calcium ions, and are used primarily in these types of applications.

The compositions and properties of pectins vary with the source, the processes used during preparation, and subsequent treatments. During extraction with mild acid, some hydrolytic depolymerization and hydrolysis of methyl ester groups occurs. Therefore, the term *pectin* denotes a family of compounds, and is usually used in a generic sense to designate those water-soluble poly(galacturonic acid) (galacturonoglycan) preparations of varying methyl ester contents and degrees of neutralization that are capable of forming gels. In all native pectins, some of the carboxyl groups occur in the methyl ester form. Depending on the manufacturing conditions, the remaining free carboxylic acid groups may be partly or fully neutralized, that is, partly or fully present as sodium, potassium, or ammonium carboxylate groups. Typically, they are present in the sodium salt form.

By definition, preparations in which more than half of the carboxyl groups are in the methyl ester form ($-COOCH_3$) are classified as high-methoxyl (HM) pectins (Figure 3.54); the remainder of the carboxyl groups will be present as a mixture of free acid ($-COOH$) and salt (e.g., $-COO^-Na^+$) forms. Preparations in which less than half of the carboxyl groups are in the methyl ester form are called low-methoxyl (LM) pectins. The percentage of carboxyl groups esterified with methanol is the degree of esterification (DE) or the degree of methylation (DM). Treatment of a pectin preparation with ammonia (often dissolved in methanol) converts some of the methyl ester groups into carboxamide groups (15%–25%). In the process, an LM pectin (by definition) is formed. These products are known as *amidated LM pectins*.

The principal and key feature of all pectin molecules is a linear chain of (1→4)-linked α-D-galactopyranosyluronic acid units. Neutral sugars, primarily L-rhamnose, are also present. In citrus and apple pectins, the α-L-rhamnopyranosyl units are inserted into the polysaccharide chain at rather regular intervals. The inserted L-rhamnopyranosyl units may provide the necessary irregularities in the structure required to limit the size of the junction zones and effect gelation (as opposed to precipitation/complete insolubility). At least some pectins contain covalently attached, highly branched arabinogalactan chains and/or short side chains composed of D-xylosyl units, though many of these native branch chains are removed during commercial extraction. The presence of side chains may also be a factor that limits the extent of chain association, further enhancing gel formation and stability. Junction zones are formed between regular unbranched pectin chains when the negative charges on the carboxylate groups are removed (addition of acid), hydration of the molecules is reduced (by the addition of a co-solute, almost always sugar, to a solution of HM pectin), and/or when polymer chains are bridged by calcium cations.

HM pectin solutions gel when sufficient acid and sugar is present. As the pH of a pectin solution is lowered, the highly hydrated and charged carboxylate groups are converted into uncharged, only slightly hydrated carboxylic acid groups. As a result of losing some of their charge and hydration, the polymer molecules can now associate over a portion of their length, forming junction zones and a network of polymer chains that entraps the aqueous solution of solute molecules. Junction zone formation is assisted by the presence of a high concentration (~65%, at least 55%) of sugar, which

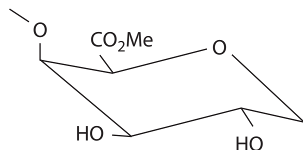


FIGURE 3.54 The most prevalent monomeric unit of an HM pectin.

competes with the pectin molecules for the water molecules and reduces hydration of the chains, allowing them to interact with one another.

LM pectin solutions gel only in the presence of divalent cations, which provide cross-bridges between chains. Increasing the concentration of divalent cations (only calcium ion is used in food applications) generally increases gel strength, as well as the temperature at which an LM pectin gel may be formed. Amidated LM pectins exhibit increased sensitivity to calcium cations, and often do not require any added calcium (beyond that present in tap water) to induce gel formation. The same general egg box model (Figure 3.53) used to describe the formation and structure of calcium alginate gels (Section 3.3.11) is used to explain gelation of solutions of LM (both standard and amidated) pectins upon addition of calcium ions. Since it does not require sugar for gelation, LM pectin is used to make low-sugar jams, jellies, and marmalades.

3.3.13 Gum Arabic [30,33,51,55,63,73]

When the bark of some trees and shrubs is injured, the plants exude a sticky material that hardens to seal the wound and give protection from infection and desiccation. Such exudates are commonly found on plants that grow in semiarid regions. Since they are sticky when freshly exuded, dust, insects, bacteria, and/or pieces of bark adhere to the exudate tears (as they are called). Gum arabic (gum acacia), gum karaya, and gum ghatti are exudates of trees; gum tragacanth is the exudate of a shrub. Of the exudate gums, only gum arabic is a major food gum today.

Gum arabic (gum acacia) (Table 3.5) is an exudate of acacia trees, of which there are many species distributed over tropical and subtropical regions. The most important growing areas for species that give the best gum are the Sudan and Nigeria. Purified, spray-dried forms of gum arabic are commonly produced.

Gum arabic is a heterogeneous material, but generally consists of two primary fractions. One, which accounts for about 70% of the gum, is composed of polysaccharide chains with little or no protein. The other fraction contains molecules of higher molecular weight that have protein as an integral part of their structures. The protein–polysaccharide fraction is itself heterogeneous with respect to protein content. The polysaccharide structures are covalently attached to the protein component by linkage to hydroxyproline and, perhaps, serine units, the two predominant amino acids in the polypeptide. The overall protein content is about 2 wt%, but fractions may contain as much as 25 wt% protein.

The polysaccharide structures, both those attached to protein and those that are not, are highly branched acidic arabinogalactans with the following approximate composition: D-galactose, 44%; L-arabinose, 24%; D-glucuronic acid, 14.5%; L-rhamnose, 13%; 4-*O*-methyl-D-glucuronic acid, 1.5%. They contain main chains of (1→3)-linked β-D-galactopyranosyl units having two- to four-unit side chains consisting of (1→3)-linked β-D-galactopyranosyl units joined to it by (1→6)-linkages. Both the main chain and the numerous side chains have attached α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl, and 4-*O*-methyl-β-D-glucuronopyranosyl units. The uronic acid units occur most often as nonreducing end units.

Gum arabic dissolves easily when stirred in water. It is unique among the food gums, except for gums that have been intentionally depolymerized to produce low-viscosity types, because of its high solubility and the low viscosity of its solutions. Solutions of 50% concentration can be made. Above this concentration, dispersions are somewhat gel-like.

Gum arabic is a fair emulsifying agent and a very good emulsion stabilizer for flavor oil-in-water emulsions. It is the gum of choice for the emulsification of citrus oils, other essential oils, and imitation flavors used as concentrates for soft drinks and baker's emulsions. In the United States, the soft drink industry consumes about 30% of the gum supply as an emulsifier and stabilizer. For a gum to have both an emulsifying and an emulsion stabilizing effect, it must have anchoring groups with a strong affinity for the surface of the oil and a molecular size large enough to cover the surfaces of dispersed droplets. Gum arabic has surface activity and forms a thick, sterically stabilizing,

macromolecular layer around oil droplets. Emulsions made with flavor oils and gum arabic can be spray-dried to produce dry flavor powders that are nonhygroscopic and in which the flavor oil is protected from oxidation and volatization. Rapid dispersion and release of flavor without affecting product viscosity are other attributes. These stable flavor powders are used in dry package products such as beverage, cake, dessert, pudding, and soup mixes.

Another important characteristic of gum arabic is its compatibility with high concentrations of sugar. Therefore, it finds widespread use in confections with a high sugar content and a low water content. More than half the world's supply of gum arabic is used in confections such as caramels, toffees, jujubes, and pastilles. In confections, it prevents sucrose crystallization, emulsifies and distributes fatty components, and helps prevent bloom (the surface whitening caused by polymorphic transitions of cocoa butter lipids). Another use is as a component of the glaze or coating of pan-coated candies.

3.3.14 Gellan [30,33,49,51,55,63,73]

Gellan, know commercially as *gellan gum* (Table 3.5), is an extracellular, anionic polysaccharide produced by the bacterium *Sphingomonas elodea* cultured in large fermentation vats. The gellan molecule is linear and is composed of β-D-glucopyranosyl, β-D-glucuronopyranosyl, and α-L-rhamnopyranosyl units in the molar ratio of 2:1:1. Native gellan (also called *high-acyl gellan*) contains two ester groups, an acetyl group and a glycerate group, both on the same glucosyl unit. On average, there is one glycerate ester group per tetrasaccharide repeat unit and one acetate ester group for every two repeat units.

Some gellan is de-esterified by treatment with alkali. Removal of the acyl groups has a dramatic effect on the gel properties of gellan. The de-esterified form is known as *low-acyl gellan*. Its tetrasaccharide repeat unit structure is →4)-αLRha*p*-(1→3)-βGlc*p*-(1→4)-βGlc*p*A-(1→4)-βGlc*p*-(1→. Three basic forms of the gum are available: high-acyl (native), low-acyl clarified, and low-acyl unclarified. The majority of gellan used in food products is the low-acyl, clarified type.

Gellan forms gels with both monovalent and divalent cations, divalent cations (Ca^{2+}) being about 10 times more effective. Gels can be formed with as little as 0.05% gum (99.95% water). Gelation is often effected by cooling a hot solution containing the required cation. Shearing during cooling of a hot gellan solution prevents the normal gelation mechanism from occurring and produces a smooth, pourable, thixotropic fluid that stabilizes emulsions and suspensions very effectively. Gentle agitation of a weak gellan gel will also disrupt the gel structure and turn the gel into a smooth, pourable, thixotropic fluid with excellent emulsion and suspension stabilizing properties.

The low-acyl types of gellan form firm, brittle, nonelastic gels (with textures similar to those of gels made with agar and κ-carrageenan). The high-acyl (native) type forms soft, elastic, nonbrittle gels (with textures similar to those made with mixtures of xanthan and LBG). A range of intermediate gel textures can be achieved by mixing the two basic types of gellan.

When gellan is used as an ingredient in bakery mixes, it does not hydrate appreciably at room temperature and thereby increase the viscosity of the batter. It does, however, hydrate upon heating and holds moisture in the baked product. Gellan is used in formulating nutrition bars because of its moisture-retaining ability. The ability of its solutions to suspend at low concentration (without producing high viscosity) makes it useful in nutritional and diet beverages.

3.3.15 Konjac Glucomannan [33,55]

Commercial konjac glucomannan (also known as *konjac mannan*) (KG) is a flour made from the tubers of *Amorphophallus* species, which are grown throughout Asia. Various grades of the flours differing in purity of the polysaccharide are available. The basic structure of the polysaccharide is that of a slightly branched chain of β-D-mannopyranosyl and β-D-glucopyranosyl units in a ratio of ~1.6:1 joined (1→4). Native KG is slightly acetylated.

KG interacts synergistically with starches, κ- and ι-carrageenans, agar, and xanthan. Strong, elastic, thermally reversible gels are formed when hot solutions of combinations of κ-carrageenan or xanthan plus KG are cooled. When solutions of these hydrocolloid combinations are heated to retort temperatures before cooling, heat-stable gels are formed. Gels formed from KG–xanthan combinations have good freeze–thaw stability.

KG–xanthan combinations produce unheated solution viscosities that are about three times those of either hydrocolloid used alone at the same total concentration. KG also interacts with starches (both native and modified) to produce increased viscosity. Heat-stable gels are formed by freezing pastes of cooked KG-starch mixtures.

Although native KG by itself does not form gels, deacetylated KG forms gels that are stable to retort temperatures. Thus, several different types of gels can be made from different combinations of KG with different starches and with different hydrocolloids and different conditions of gel formation.

3.3.16 Inulin and Fructooligosaccharides [8,11,14,22,23,25,31,38,41,55,56,66]

Inulin (Table 3.5) occurs naturally as a storage carbohydrate in thousands of plant species, including onion, garlic, asparagus, and banana. The primary commercial source is chicory (*Chicorium intybus*) root. Some is also obtained from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers.

Inulin is composed of β-D-fructofuranosyl units linked 2→1. The polymer chains are often, but not always (because of degradation, either natural or during isolation), terminated at the reducing end with a sucrose unit. The DP of inulin rarely, if ever, exceeds 60. It occurs in plants together with fructooligosaccharides, giving an overall DP range of 2–60.

Molecules containing furanosyl units, such as molecules of inulin and sucrose, undergo acid-catalyzed hydrolysis much more easily than those containing pyranosyl units. Inulin is a storage, that is, a reserve food, oligo/polysaccharide, so it is seemingly apparent that, at any time, molecules in various stages of synthesis and, perhaps, breakdown are present. As a result, inulin preparations are mixtures of fructooligosaccharide and small polysaccharide molecules.

Inulin is often deliberately depolymerized into fructooligosaccharides. Both inulin and the fructooligosaccharide products produced from it are prebiotics. (Prebiotics are nondigestible food ingredients that have a beneficial effect on the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial bacterial species already present in the colon. Prebiotics are most often used for the nutritional/health benefits they impart.)

Aqueous solutions of inulin can be made at concentrations as high as 50%. When hot solutions of inulin at concentrations greater than 25% are cooled, thermoreversible gels are formed. Inulin gels are described as particle gels (especially after shearing) with a creamy, fat-like texture. Hence, inulin can be used as a fat mimetic in reduced-fat products. It improves the texture and mouthfeel of low-fat ice creams and sauces. Inulin is an ingredient in nutrition, breakfast, meal replacement and sports/energy bars, soy beverages, and vegetable patties.

Neither inulin nor fructooligosaccharides are digested by enzymes in either the stomach or small intestine. Therefore, they are components of dietary fiber (Section 3.4). They have a glycemic index of zero, that is, they raise neither the glucose nor insulin levels in the blood.

3.3.17 Polydextrose [46,48]

When sugars are heated in the dry state (with or without an added alcohol) in the presence of an acid catalyst, glycosidic linkages (see Section 3.1.3) are formed. A product called *polydextrose* (see Chapter 12) is produced by heating together D-glucose/dextrose (at least 90%), D-glucitol/sorbitol (not more than 2%), and citric acid. A variety of glycosidic linkages are formed, and because both D-glucose and sorbitol have multiple hydroxyl groups, the polydextrose chain is highly branched. Polydextrose chains span the range of both oligo- and polysaccharides (average DP ~ 12).

It is ~90% dietary fiber (see Section 3.4), that is, it is only ~10% digestible and thus has a low caloric value. Polydextrose is also a prebiotic (see Section 3.4). It is employed as a replacer for the functional properties of sucrose, that is, as a nonsweet bulking agent (providing solids to maintain organoleptic properties in products containing high-intensity sweeteners instead of sucrose), as a humectant to prevent or reduce water migration from bakery fillings and nutrition bars, to lower the freezing point in low-sugar dairy desserts, and in other low- or no-sugar products. It has fat-sparing properties and is used in fat-free ice cream and low-fat cookies.

3.3.18 Summary

- Polysaccharides are diverse polymers comprised of >20 to >60,000 monosaccharide units joined together by glycosidic linkages.
  - As polyhydroxy polymers, all polysaccharides are either water-soluble or water-binding. Those that are water-soluble gel and/or thicken (increase the viscosity of) aqueous systems.
  - The viscosity of a polysaccharide solution is determined by the molecular size, shape, rigidity, and concentration of the polysaccharide chains.
  - Most polysaccharide solutions exhibit either pseudoplastic or thixotropic flow behavior at concentrations commonly employed in foods.
  - Polysaccharide gels are generally stabilized by junction zones formed between polymer chains.
  - Polysaccharides may undergo hydrolytic cleavage under acidic conditions, though the specific conditions that promote hydrolysis vary for each specific polysaccharide.
- The most abundant polysaccharide in food products is starch, which is the only digestible polysaccharide, supplies most of the calories in human diets worldwide by being converted into its monosaccharide units (D-glucose), and is comprised of two polysaccharides—amylose and amylopectin.
  - Starch granules are unique in that polymer chains are arranged in plants into semicrystalline, water-insoluble aggregates (1–100 μm) called *granules*. These granules must first be cooked/heated in water to solubilize the polymer chains and realize the functionality of starches.
  - Starches are often modified chemically to enhance and extend their physical properties before being used as a food ingredient.
- Other water-soluble polysaccharides from land plants, marine algae, and microorganisms and from chemical modifications of cellulose are used as food ingredients (thickeners, stabilizers, binders, gelling agents, etc.), and are known as *hydrocolloids*.

3.4 DIETARY FIBER, PREBIOTICS, AND CARBOHYDRATE DIGESTIBILITY [7,10–14,20,22,23,26,31,34,38,44,55,59,62,64,68,70,80]

The underlying bases for the benefits of soluble and insoluble dietary fibers, the prebiotic effects of carbohydrate fibers, and the possible interrelationships between carbohydrate catabolism within the small and large intestines represent key areas of interest. Nutritionists set requirements for dietary fiber at 25–50 g per day. Traditionally, dietary fiber provides multiple health benefits that aid the normal functioning of the gastrointestinal tract. Dietary fiber largely consists of hydrophilic molecules that increase intestinal and fecal bulk (primarily by means of its water-holding capacity), which lowers intestinal transit time and prevents constipation. Soluble fiber lowers blood cholesterol levels, perhaps by sweeping out bile salts and reducing their chances for reabsorption from the large intestine, lessening the chance of heart disease. Prebiotic fiber may be especially helpful in mitigating inflammatory bowel diseases, and reducing chances of colonic and rectal cancer. Through its effect on fermentation in the colon and its short-chain fatty acid fermentation products, prebiotic

fiber also provides immune system regulation. The nutritional aspects and physiological effects of carbohydrates and the role of carbohydrates in the health and well-being of humans are very active areas of food science research.

Dietary oligo- and polysaccharides may be digestible (most starch-based products), partially digestible, or nondigestible (resistant starch [see below] and essentially all other polysaccharides) as they pass through the human small intestine. Starch, once gelatinized, is the only polysaccharide that can be hydrolyzed by human enzymes, that is, broken down to D-glucose, which is absorbed by microvilli of the small intestine to supply the principal metabolic energy of humans. Only when complete digestive hydrolysis to monosaccharides occurs are the products of carbohydrate digestion able to be absorbed and catabolized. (Only monosaccharides can be absorbed through the wall of the small intestine, and only D-glucose is produced by digestion of polysaccharides in humans because only starches can be digested.)

Plant cell-wall materials, primarily cellulose, other nonstarch polysaccharides, and lignin consumed as natural components of edible vegetables, fruits, and other plant materials, as well as food gums added to prepared food products (Sections 3.3.7 through 3.3.17), are not digested in the stomach or small intestine of humans. (The acidity of the stomach is neither strong enough, nor is the residence time of polysaccharides in the stomach sufficiently long, to cause significant chemical cleavage.) Dietary fiber also encompasses substances other than polymers, including nondigestible oligosaccharides, for example, raffinose and stachyose (Section 3.2.3) in legumes. The only common feature of all these substances is that they are nondigestible (within the small intestine), which is the principal criterion for being classified as a component of dietary fiber.

Cereal brans, and kidney and navy beans are especially good sources of dietary fiber. A product based on psyllium seed hulls has high water-binding properties, leading to rapid transit time in the gastrointestinal tract, and is used to prevent constipation. A product with a methylcellulose base is sold for the same purpose. Other hydrocolloids, because they are nondigestible, also function as dietary fiber. A noteworthy component of dietary fiber is a water-soluble polysaccharide, β-glucan, that is present in oat and barley brans. Oat β-glucan has become a commercial food ingredient because it has been shown to be effective in reducing the level of serum cholesterol. The oat β-glucan molecule is a linear chain of β-D-glucopyranosyl units. About 70% are linked (1→4) and about 30% (1→3). The (1→3) linkages occur singly and are separated by sequences of two or three (1→4) linkages. Thus, the molecule is composed of (1→3)-linked β-cellotriosyl [→3)-βGlc*p*-(1→4)-βGlc*p*-(1→4)-βGlc*p*-(1→] and β-cellotetraosyl units (Figure 3.55). Such (1→4,1→3)-β-glucans are often called *mixed linkage β-glucans*.

When taken orally in foods, β-glucans reduce postprandial serum glucose levels and the insulin response, that is, they moderate the glycemic response, in both normal and diabetic human subjects. They also reduce serum cholesterol concentrations in rats, chickens, and humans. These physiological effects are typical of those of soluble dietary fiber. Other soluble polysaccharides have similar effects, but to differing degrees.

Carbohydrates not digested to monosaccharides by human enzymes in the small intestine (all others except sucrose, lactose, and products such as maltodextrins made from starch) pass into the colon or large intestine as dietary fiber. When undigested polysaccharides reach the large intestine, they come into contact with normal intestinal microorganisms, some of which produce enzymes

$$\rightarrow 3)-\beta\text{Glc}p-(1\left[\rightarrow 4)-\beta\text{Glc}p-(1\rightarrow\right]_n$$

FIGURE 3.55 Representative structure (shorthand notation) of a segment of oat and barley β-glucans where *n* usually is 1 or 2, but occasionally may be larger.

that catalyze hydrolysis of certain polysaccharides or certain parts of polysaccharide molecules. The consequence of this is that polysaccharides not cleaved in the upper intestinal tract may be broken down and utilized by bacteria within the large intestine. Sugars that are removed from polysaccharide chains are used by the microorganisms of the large intestine as energy sources in anaerobic fermentation pathways that produce lactic, acetic, propionic, butyric, and valeric acids. These short-chain acids can be partially absorbed through the human intestinal wall and metabolized, primarily in the liver, for energy. In addition, a small, though significant in some cases, fraction of the released sugars can be taken up by the intestinal wall and transported to the portal blood stream where they are conveyed to the liver and metabolized. It is calculated that, on average, ~7% of human energy is derived from sugars split from polysaccharides by microorganisms in the large intestine or from the short-chain acids produced from them via anaerobic fermentation pathways. The extent of polysaccharide cleavage depends on the abundance of the particular microorganism(s) producing the specific enzymes required. Substrates that reach the large intestine undigested and are metabolized as previously described by the microflora of the colon represent a special class of dietary fiber referred to as *prebiotics*. Prebiotics are substances that are not digested by human small intestinal enzymes, but provide favorable physiological effects and health benefits to the host by selectively stimulating the growth and/or bioactivity of beneficial microorganisms already present in the gastrointestinal tract, especially in the large intestine/colon.

Resistant starch (RS) is both a source of dietary fiber and a prebiotic of emerging significance [60]. Some of the starch in a food may exist in a form that allows it to pass through the small intestine intact, that is, undigested. RS is an especially important component of dietary fiber, because it produces greater amounts of butyric acid than do other forms of fiber as it undergoes fermentation in the colon. (Butyric acid is implicated in the prevention of colorectal cancer.) There are four traditionally recognized categories of RS, with a fifth type more recently identified. RS1 is starch that is contained within plant cells and is physically inaccessible to the salivary and pancreatic α-amylase, such as that in some vegetable tissues. RS2 is uncooked granular starch. For example, high-amylose corn (amylomaize) starch is marketed as a source of RS and dietary fiber because some of its granules remain ungelatinized even at typical cooking temperatures (100°C for products containing relatively high amounts of water and temperatures exceeding 100°C for low-moisture products). RS3 is retrograded starch (primarily retrograded amylose); examples of products containing RS3 are potatoes that have been boiled and cooled (for potato salad, for example) and breads and related products. RS4 is that portion of a chemically modified food starch that is resistant to digestion, while RS5 consists of lipid-complexed amylose. There is also the question of how to modify starch in such a way that glucose released from this most consumed component of the human diet is released at a rate that does not result in a large increase in the blood sugar level after a meal (known as a *hyperglycemic spike*).

Inulin and frutooligosaccharides (FOSs) derived from inulin are popular ingredients used to add dietary fiber to food products (see Section 3.3.16). These substances are also noteworthy prebiotics.

3.4.1 Summary

- Only monosaccharides can pass through the small intestinal wall into the blood stream, and only sucrose, lactose, starch, and starch-based oligosaccharides can be hydrolyzed to monosaccharides (D-glucose, D-fructose, and D-galactose) by human digestive enzymes.
- All other carbohydrates are components of dietary fiber, that is, are nondigestible food components. Dietary fiber contributes traditional health benefits, for example, increased fecal bulk, decreased stool transit time, and reduced serum cholesterol levels.
- Prebiotics are sources of dietary fiber that are fermented during transit through the gastrointestinal tract (most notably the colon), fostering the growth or bioactivity of select intestinal microflora that promote the health and well-being of the host. Even some forms of starch, referred to as *resistant starch*, are able to reach the colon in undigested form and serve as a prebiotic substrate for beneficial microflora.

CHAPTER PROBLEMS

1. Write the structures of D- and L-mannose in open-chain form.
2. Illustrate the conversion of the open-chain form of D-fructose to both its α-D-furanose and α-D-pyranose ring forms (write as Haworth projections).
3. Write the structures of α-D-galactopyranose and β-D-galactopyranose as (a) Haworth projections, and (b) conformational structures ($^4C_1$).
4. What is mutarotation? What is the consequence of mutarotation?
5. Show by equation how mannitol is made. What type of reaction does the sugar undergo in this process?
6. List (a) the reactants and (b) the reaction conditions required for nonenzymic browning (Maillard reaction).
7. Describe specific conditions that can be employed to minimize nonenzymic browning in foods.
8. Is maltose a glycoside? Why or why not?
9. Write the structures of lactose, maltose, and sucrose.
10. Explain why lactose and maltose can be direct reactants in the Maillard reaction while sucrose cannot be.
11. If you were to design the ideal pseudoplastic polysaccharide, what molecular features you build into it?
12. Draw a diagram of a typical polysaccharide gel, and the label the key components of the gel structure.
13. Describe the general molecular structures of amylose and amylopectin.
14. Why must starch first be heated in excess water to realize its functionality as a thickener and/or gelling agent?
15. Describe the events associated with starch gelatinization. Incorporate a diagram to enhance your explanation as needed.
16. What is starch retrogradation? Is it a desired or undesired phenomenon in food systems?
17. Explain the concept of cross-linking starch and why it is practiced.
18. Contrast the conditions required to bring about gel formation for HM and LM pectins. Describe the molecular feature that is primarily responsible for the differential gelling requirements of these two polysaccharides.
19. Identify an ideal hydrocolloid that could potentially be used to achieve each of the following characteristics: (a) high viscosity, (b) pseudoplastic behavior, (c) stability to high acid conditions, (d) emulsification, (e) fat replacement, (f) thermogelation, and (g) gelation in the presence of cations.
20. Define prebiotic. What are the potential benefits associated with a prebiotic?

REFERENCES

1. Aguilera, J.M., L. Cadoche, C. Lopez, and G. Guitierrez (2001). Microstructural changes of potato cells and starch granules heated in oil. *Food Research International 34:*939–947.
2. Becalski, A., B.P.-Y. Lau, D. Lewis, and S.W. Seaman (2003). Acrylamide in foods: Occurrence, sources, and modeling. *Journal of Agricultural and Food Chemistry 51:*802–808.
3. Belitz, H.-D., W. Grosch, and P. Schieberle, eds. (2004). *Food Chemistry*, Springer-Verlag, Berlin, Germany, Chapter 4.
4. BeMiller, J.N. (2007). *Carbohydrate Chemistry for Food Scientists*, 2nd edn., AACC International, St. Paul, MN.
5. BeMiller, J.N. and R.L. Whistler, eds. (2009). *Starch: Chemistry and Technology*, 3rd edn., Academic Press, New York.
6. Bertolini, A.C., ed. (2010). *Starches: Characterizations, Properties, and Application*, CRC Press, Boca Raton, FL.

7. Biliaderis, C.G. and M.S. Izydorczyk (2007). *Functional Food Carbohydrates*, CRC Press, Boca Raton, FL.
8. *British Journal of Nutrition* (2005). *93*(Suppl):1.
9. Campo, V.L., D.F. Kwano, D.B. da Silva, and I. Carvalho (2009). Carrageenans: Biological properties, chemical modifications and structural analysis—A review. *Carbohydrate Polymers 77:*167–180.
10. Charalampopoulos, D. and R.A. Rastall, eds. (2009). *Prebiotics and Probiotics Science and Technology*, Springer, New York.
11. Cho, S.S., ed. (2012). *Dietary Fiber and Health*, CRC Press, Boca Raton, FL.
12. Cho, S.S. and M.L. Dreher, eds. (2001). *Handbook of Dietary Fiber*, Marcel Dekker, New York.
13. Cho, S.S. and E.T. Finocchiaro, eds. (2009). *Handbook of Prebiotics and Probiotics Ingredients: Health Benefits and Food Applications*, CRC Press, Boca Raton, FL.
14. Cho, S.S. and P. Samuel, eds. (2009). *Fiber Ingredients: Food Applications and Health Benefits*, CRC Press, Boca Raton, FL.
15. Chronakis, I.S. (1998). On the molecular characteristics, compositional properties, and structural-functional mechanisms of maltodextrins: A review. *Critical Reviews in Food Science and Technology 38*:599–637.
16. Dickinson, E., ed. (1991). *Food Polymers, Gels, and Colloids*, The Royal Society of Chemistry, London, U.K.; 16a. Dill, W.L. (1993). Protein fructosylation: Fructose and the Maillard reaction. *American Journal of Clinical Nutrition 58*:779S–787S.
17. Dodzluk, H., ed. (2006). *Cyclodextrins and Their Complexes*, Wiley-VCH, New York.
18. Dumitriu, S., ed. (1998). *Polysaccharides*, Marcel Dekker, New York.
19. Eliasson, A.-C., ed. (2004). *Starch in Food: Structure, Function and Applications*, Woodhead Publishing, Cambridge, U.K.
20. Eliasson, A.-C., ed. (2006). *Carbohydrates in Foods*, 2nd edn., Taylor & Francis, Boca Raton, FL.
21. Embuscado, M.E., ed. (2014). *Functionalizing Carbohydrates for Food Applications*, DEStech Publications, Lancaster, PA.
22. Flamm, G., W. Glinsman, D. Kritchevsky, L. Prosky, and M. Roberfroid (2001). Inulin and oligofructose as dietary fiber: A review of the evidence. *Critical Reviews of Food Science and Nutrition 41*:353–362.
23. Flickinger, E.A., J.V. Loo, and G.C. Fahey, Jr. (2003). Nutritional responses to the presence of inulin and oligofructose in the diets of domesticated animals: A review. *Critical Reviews of Food Science and Nutrition 43*:19–60.
24. Freidman, M. (2003). Chemistry, biochemistry, and safety of acrylamide: A review. *Journal of Agricultural and Food Chemistry 51*:4504–4526.
25. Fuchs, A., ed. (1993). *Inulin and Inulin-Containing Crops*, Elsevier, Amsterdam, the Netherlands.
26. Gibson, G.R. and M.B. Roberfroid, eds. (2008). *Handbook of Prebiotics*, CRC Press, Boca Raton, FL.
27. Granvogl, M. and P. Schieberle (2006). Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. *Journal of Agricultural and Food Chemistry 54*:5933–5938.
28. Gray, J.A. and J.N. BeMiller (2003). Bread staling: Molecular basis and control. *Comprehensive Reviews of Food Science and Food Safety 2*:1–21.
29. Higley, J., J.-Y. Kim, K.C. Huber, and G. Smith (2012). Added versus accumulated sugars on color development and acrylamide formation in French-fried potato strips. *Journal of Agricultural and Food Chemistry 60*:8763–8771.
30. Hoefler, A.C. (2004). *Hydrocolloids*, American Association of Cereal Chemists, St. Paul, MN.
31. Holownia, P., B. Jaworska-Luczak, I. Wisniewska, P. Bilinski, and A. Wojtyla (2010). The benefits & potential health hazards posed by the prebiotic inulin—A review. *Polish Journal of Food and Nutrition Sciences 60*:201–211.
32. Hull, P. (2010). *Glucose Syrups: Technology and Applications*, Wiley-Blackwell, Chichester, U.K.; 32a. Imberty, A., A. Buléon, V. Tran, and S. Perez (1991). Recent advances in starch structure. *Starch/Stärke 43*:375–384.
33. Imeson, A., ed. (2010). *Food Stabilisers, Thickeners, and Gelling Agents*, Wiley-Blackwell, Oxford, U.K.
34. Izydorczyk, M.S. and J.E. Dexter (2008). Barley β-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products—A review. *Food Research International 41*:850–868.
35. Jane, J.-L., S.L. Kasemsuwan, S. Leas, H. Zobel, and J.F. Robyt (1994). Anthology of starch granule morphology by scanning electron microscopy. *Starch/Stärke 46*:121–129.
36. Jarvis, M.C. (1998). Intercellular separation forces generated by intracellular pressure. *Plant Cell and Environment 21*:1307–1310.

37. Jarvis, M.C., E. MacKenzie, and H.J. Duncan (1992). The textural analysis of cooked potato. 2. Swelling pressure of starch during gelatinization. *Potato Research 35*:93–102.
38. Kalyani Nair, K., S. Kharb, and D.K. Thompkinson (2010). Inulin dietary fiber with functional health attributes—A review. *Food Reviews International 26*:189–203.
39. Lapasin, R. and S. Pricl (1995). *Rheology of Industrial Polysaccharides*, Chapman & Hall, New York.
40. Labuza, T.P., G.A. Reineccius, V. Monnier, J. O'Brien, and J. Baynes, eds. (1995). *Maillard Reactions in Chemistry, Food, and Health*, CRC Press, Boca Raton, FL.
41. Madrigal, L. and E. Sangronis (2007). Inulin and derivatives as key ingredients in functional foods. *Archivos Latinoamericanos de Nutricion 57*:387–396.
42. Mathlouthi, M. and Reiser, P., eds. (1995). *Sucrose: Properties and Applications*, Blackie Academic & Professional, Glasgow, U.K.
43. Mathur, N.K. (2011). *Industrial Galactomannan Polysaccharides*, CRC Press, Boca Raton, FL.
44. McCleary, B.V. and L. Prosky, eds. (2001). *Advanced Dietary Fibre Technology*, Blackwell Science, London, U.K.
45. Miles, M.J., V.J. Morris, P.D. Orford, and S.G. Ring (1985). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research 135*:271–281.
46. Mitchell, H., ed. (2006). *Sweeteners and Sugar Alternatives in Food Technology*, Blackwell, Oxford, U.K.
47. Morris, V.J. (1994). Starch gelation and retrogradation. *Trends in Food Science and Technology 1*:2–6.
48. Nabors, L.O., ed. (2012). *Alternative Sweeteners*, 4th edn., CRC Press, Boca Raton, FL.
49. Nishinari, K. and E. Doi, eds. (1993). *Food Hydrocolloids*, Plenum Press, New York.
50. Nursten, H.E. (2005). *The Maillard Reaction: Chemistry, Biochemistry, and Implications*, The Royal Society of Chemistry, Cambridge, U.K.
51. Nussinovitch, A. (1997). *Hydrocolloid Applications: Gum Technology in Food and Other Applications*, Blackie Academic & Professional, London, U.K.
52. Ormerod, A., J. Ralfs, S. Jobling, and M. Gidley (2002). The influence of starch swelling on the material properties of cooked potatoes. *Journal of Materials Science 37*:1667–1673.
53. Parker, J.K., D.P. Balagiannis, J. Higley, G. Smith, B.L. Wedzicha, and D.S. Mottram (2012). Kinetic model for the formation of acrylamide during the finish-frying of commercial French-fries. *Journal of Agricultural and Food Chemistry 60*:9321–9331.
54. Pennington, N.L. and C.W. Baker, eds. (1990). *Sugar: A User's Guide to Sucrose*, Van Nostrand Reinhold, New York.
55. Phillips, G.O. and P.A. Williams, ed. (2009). *Handbook of Hydrocolloids*, 2nd edn., Woodhead Publishing, Cambridge, U.K.
56. Roberfroid, M. (2004). *Inulin-type Fructans: Functional Food Ingredients*, CRC Press, Boca Raton, FL.
57. Rydberg, P., S. Eriksson, E. Tareke, P. Karlson, L. Ehrenberg, and M. Tornqvist (2003). Investigations of factors that influence the acrylamide content of heated foodstuffs. *Journal of Agricultural and Food Chemistry 51*:7012–7018.
58. Schenck, F.W. and R.E. Hebeda, eds. (1992). *Starch Hydrolysis Products*, VCH Publishers, New York.
59. Seidermann, J. (1966). *Stärke-Atlas*, Paul Parey, Berlin, Germany.
60. Shi, Y.-C. and C.C. Maningat, eds. (2013). *Resistant Starch: Sources, Applications and Health Benefits*, Wiley-Blackwell, Chichester, UK.
61. Slade, L. and H. Levine (1989). A food polymer science approach to selected aspects of starch gelatinization and retrogradation, in *Frontiers in Carbohydrate Research—1* (R.P. Millane, J.N. BeMiller, and R. Chandrasekaran, eds.), Elsevier Applied Science, Amsterdam, the Netherlands, pp. 215–270.
62. Slavin, J. (2013). Fiber and prebiotics: Mechanisms and health benefits. *Nutrients 5*:1417–1435.
63. Stephen, A.M., G.O. Phillips, and P.A. Williams, eds. (2006). *Food Polysaccharides and Their Applications*, 2nd edn., CRC Press, Boca Raton, FL.
64. Swennen, K., C.M. Curtin, and J.A. Delcour (2006). Non-digestible oligosaccharides with prebiotic properties. *Critical Reviews in Food Science and Technology 46*:459–471.
65. Szente, L. and J. Szejtli (2004). Cyclodextrins as food ingredients. *Trends in Food Science and Technology 15*:137–142.
66. Tomasik, P. (2003). *Chemical and Functional Properties of Food Saccharides*, CRC Press, Boca Raton, FL.
67. Tomasik, P., M. Palasinski, and S. Wiejak (1989). The thermal decomposition of carbohydrates. Part 1. The decomposition of mono-, di-, and oligo-saccharides. *Advances in Carbohydrate Chemistry and Biochemistry 47*:203–278.

68. Tungland, B.C. and D. Meyer (2002). Nondigestible oligo- and polysaccharides (dietary fiber): Their physiology and role in human health and food. *Comprehensive Reviews in Food Science and Food Safety 1*:73–92.
69. Van Beynum, G.M.A. and J.A. Roels, eds. (1985). *Starch Conversion Technology*, Marcel Dekker, New York.
70. van der Kamp, J.W., N.-G. Asp, J.M. Jones, and G. Schaafsma, eds. (2004). *Dietary Fibre: Bio-Active Carbohydrates for Food and Feed*, Wageningen Academic Publishers, Wageningen, the Netherlands.
71. Varzakas, T., A. Labropoulos, and S. Anestis, eds. (2012). *Sweeteners: Nutritional Aspects, Applications, and Production Technology*, CRC Press, Boca Raton, FL.
72. Walter, R.H., ed. (1998). *Polysaccharide Association Structures in Food*, Marcel Dekker, New York.
73. Whistler, R.L. and J.N. BeMiller, eds. (1993). *Industrial Gums*, 3rd edn., Academic Press, San Diego, CA.
74. Whistler, R.L., J.N. BeMiller, and E.F. Paschall, eds. (1984). *Starch: Chemistry and Technology*, 2nd edn., Academic Press, New York.
75. Wilson, R., ed. (2007). *Sweetness*, 3rd edn., Wiley-Blackwell, Oxford, U.K.
76. Wurzburg, O.B., ed. (1986). *Modified Starches: Properties and Uses*, CRC Press, Boca Raton, FL.
77. Yaylayan, V.A. and A. Huyghues-Despointes (1994). Chemistry of Amadori rearrangement products: Analysis, synthesis, kinetics, reactions, and spectroscopic properties. *Critical Reviews in Food Science and Nutrition 34*:321–369.
78. Yaylayan, V.A., A. Wnorowski, and C. Perez Locas (2003). Why asparagine needs carbohydrates to generate acrylamide. *Journal of Agricultural and Food Chemistry 51*:1753–1757.
79. Young, R.A. and R.M. Rowell, eds. (1986). *Cellulose*, John Wiley, New York.
80. Zhang, G. and B.R. Hamaker (2010). Cereal carbohydrates and colon health. *Cereal Chemistry 87*:331–341.
81. Zyzak, D.V., R.A. Sanders, M. Stojanovic, D.H. Tallmadge, B.L. Eberhart, D.K. Ewald, D.C. Gruber et al. (2003). Acrylamide formation mechanisms in heated foods. *Journal of Agricultural and Food Chemistry 51*:4782–4787.

FURTHER READING

Belitz, H.-D., W. Grosch, and P. Schieberle, eds. (2004). *Food Chemistry*, Springer-Verlag, Berlin, Germany, Chapters 4 and 19.

BeMiller, J.N. (2007). *Carbohydrate Chemistry for Food Scientists*, 2nd edn., American Association of Cereal Chemists, St. Paul, MN.

BeMiller, J.N. (2010). Carbohydrate analysis, in *Food Analysis*, 4th edn. (S.S. Nielsen, ed.), Kluwer Academic/Plenum Publishers, New York, Chapter 10.

BeMiller, J.N. and K.C. Huber (2011). Starch, in *Ulmann's Encyclopedia of Industrial Chemistry*, 7th edn. (B. Elvers, ed.), Wiley-VCH, Weinheim, Germany.

Biliaderis, C.G. and M.S. Izydorczyk (2007). *Functional Food Carbohydrates*, CRC Press, Boca Raton, FL.

Cui, S.W., ed. (2005). *Food Carbohydrates: Chemistry, Physical Properties, and Applications*, CRC Press, Boca Raton, FL.

Eliasson, A.-C., ed. (2006). *Carbohydrates in Food*, 2nd edn., Marcel Dekker, New York.

Huber, K.C. and J.N. BeMiller (2010). Modified starch: Chemistry and properties, in *Starches: Characterization, Properties, and Applications* (A. Bertolini, ed.), Taylor & Francis Group, LLC, Boca Raton, FL.

Huber, K.C., J.N. BeMiller, and A. McDonald (2005). Carbohydrate chemistry, in *Handbook of Food Science, Technology, and Engineering*, Vol. 1 (Y. Hui, ed.), Taylor & Francis Group, LLC, Boca Raton, FL.

International Union of Pure and Applied Chemistry and International Union of Biochemistry and Molecular Biology. Nomenclature of Carbohydrates, *Pure and Applied Chemistry 68:*1919–2008 (1996), *Carbohydrate Research 297:*1–92 (1997), *Advances in Carbohydrate Chemistry and Biochemistry 52:*43–177 (1997), http://www.chem.qmul.ac.uk/iupac/2carb/ (1996). (*Note:* These published documents are identical.)

Imeson, A., ed. (2010). *Food Stabilisers, Thickeners, and Gelling Agents*, Wiley-Blackwell, Oxford, U.K.

Nursten, H.E. (2005). *The Maillard Reaction: Chemistry, Biochemistry, and Implications*, The Royal Society of Chemistry, Cambridge, U.K.

Stephen, A.M., G.O. Phillips, and P.A. Williams, eds. (2006). *Food Polysaccharides and Their Applications*, 2nd edn., CRC Press, Boca Raton, FL.

Tomasik, P., ed. (2003). *Chemical and Functional Properties of Food Saccharides*, CRC Press, Boca Raton, FL.

Wrolstad, R.E. (2012). *Food Carbohydrate Chemistry*, Wiley-Blackwell, London, U.K.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

4 Lipids

David Julian McClements and Eric Andrew Decker

CONTENTS

4.1 Introduction 173
4.2 Major Lipid Components 173
4.2.1 Fatty Acids 173
4.2.1.1 Nomenclature of Saturated Fatty Acids 173
4.2.1.2 Nomenclature of Unsaturated Fatty Acids 174
4.2.2 Acylglycerols 175
4.2.3 Phospholipids 176
4.2.4 Sphingolipids 177
4.2.5 Sterols 177
4.2.6 Waxes 178
4.2.7 Miscellaneous Lipids 178
4.2.8 Composition of Fats 178
4.2.9 Summary 180
4.3 Lipid Refining 180
4.3.1 Degumming 181
4.3.2 Neutralization 181
4.3.3 Bleaching 181
4.3.4 Deodorization 181
4.4 Molecular Interactions and Organization of Triacylglycerols 181
4.5 Physical Properties of Triacylglyerols 183
4.5.1 Rheological Properties 183
4.5.2 Density 186
4.5.3 Thermal Properties 186
4.5.4 Optical Properties 187
4.5.5 Electrical Properties 188
4.6 Solid Fat Content of Food Triacylglycerols 188
4.7 Crystallization of Triacylglycerols 189
4.7.1 Supercooling 190
4.7.2 Nucleation 191
4.7.3 Crystal Growth 194
4.7.4 Post-Crystallization Events 195
4.7.5 Crystal Morphology 195
4.7.6 Polymorphism 196
4.7.7 Crystallization of Edible Fats and Oils 197
4.7.8 Fat Crystallization in Emulsions 198
4.8 Altering the Solid-Fat Content of Food Lipids 199
4.8.1 Blending 199
4.8.2 Dietary Interventions 199
4.8.3 Genetic Manipulation 199
4.8.4 Fractionation 199
4.8.5 Hydrogenation 200

4.8.6 Interesterification 201
4.8.7 Summary 202
4.9 Functionality of Triacylglycerols in Foods 204
4.9.1 Texture 204
4.9.2 Appearance 204
4.9.3 Flavor 204
4.10 Chemical Deterioration of Lipids, Hydrolytic Reactions 205
4.11 Chemical Deterioration of Lipids, Oxidative Reactions 205
4.11.1 Chemical Pathway 205
4.11.1.1 Initiation 206
4.11.1.2 Propagation 208
4.11.1.3 Termination 208
4.11.2 Prooxidants 209
4.11.2.1 Prooxidant That Promote Formation of Lipid Hydroperoxides 210
4.11.2.2 Prooxidants That Promote Formation of Free Radicals 212
4.11.2.3 Prooxidants That Promote Decomposition of Hydroperoxides 212
4.11.3 Formation of Lipid Oxidation Decomposition Products 213
4.11.3.1 β-Scission Reaction 214
4.11.3.2 Additional Reactions of Fatty Acid Decomposition Products 216
4.11.3.3 Cholesterol Oxidation 216
4.11.4 Antioxidants 216
4.11.4.1 Control of Free Radicals 217
4.11.4.2 Tocopherols 217
4.11.4.3 Synthetic Phenolics 220
4.11.4.4 Plant Phenolics 220
4.11.4.5 Ascorbic Acid and Thiols 221
4.11.4.6 Control of Prooxidants 221
4.11.4.7 Control of Prooxidant Metals 221
4.11.4.8 Control of Singlet Oxygen 222
4.11.4.9 Control of Lipoxygenases 222
4.11.4.10 Control of Oxidation Intermediates 222
4.11.4.11 Superoxide Anion 222
4.11.4.12 Peroxides 223
4.11.4.13 Antioxidant Interactions 223
4.11.4.14 Physical Location of Antioxidants 224
4.11.5 Other Factors Influencing Lipid Oxidation Rates 224
4.11.5.1 Oxygen Concentration 224
4.11.5.2 Temperature 224
4.11.5.3 Surface Area 224
4.11.5.4 Water Activity 224
4.11.6 Measurement of Lipid Oxidation 225
4.11.6.1 Sensory Analysis 225
4.11.6.2 Primary Lipid Oxidation Products 225
4.11.6.3 Conjugated Double Bonds 225
4.11.6.4 Lipid Hydroperoxides 226
4.11.6.5 Secondary Lipid Oxidation Products 226
4.11.6.6 Analysis of Volatile Secondary Products 226
4.11.6.7 Carbonyls 226
4.11.6.8 Thiobarbituric Acid (TBA) 227
4.11.7 Summary 227

4.12 Food Lipids and Health 228
4.12.1 Bioactivity of Fatty Acids 228
4.12.2 *Trans* Fatty Acids 228
4.12.3 ω-3 Fatty Acids 228
4.12.4 Conjugated Linoleic Acid 229
4.12.5 Phytosterols 229
4.12.6 Carotenoids 229
4.12.7 Low-Calorie Lipids 229
4.12.8 Summary 230
References 230

4.1 INTRODUCTION

Lipids comprise a broad group of chemically diverse compounds that are linked together by the fact that they are all soluble in organic solvents. Food lipids are generally referred to as fats (solid) or oils (liquid), indicating their physical state at ambient temperatures. Food lipids are also classified as nonpolar (e.g., triacylglycerol and cholesterol) and polar lipids (e.g., phospholipids) to indicate differences in their solubility and functional properties. The total lipid content and the lipid composition of foods can vary tremendously. Since food lipids play an important role in food quality by contributing to attributes such as texture, flavor, nutrition, and caloric density, the manipulation of these important food components has been a major emphasis in food product development since food research began. This research has focused on the alteration of lipid composition to change the texture, alter the fatty acid and cholesterol composition, decrease total fat, alter bioavailability, and make lipids more oxidatively stable. In addition, the physical stability of lipids is important in food quality since many lipids exist as dispersions/emulsions that are thermodynamically unstable. In order to make changes in lipid composition while ensuring the production of high-quality foods, a fundamental understanding of the chemical and physical properties of lipids is critical. This chapter focuses on the chemical composition of lipids, their physical properties and crystallization/solid phase behavior, methods to modify the fatty acid and triacylglycerol composition and thus the physicochemical properties of lipids and their propensity to undergo oxidative deterioration, and the role of lipids in health and disease.

4.2 MAJOR LIPID COMPONENTS

The following section is a brief description of the nomenclature of the major classes of food lipids. For more information of lipid nomenclature, see O'Keefe [63] or the web page of the International Union of Pure and Applied Chemistry (IUPAC) (http://www.chem.qmul.ac.uk/iupac/lipid/).

4.2.1 Fatty Acids

The major components of lipids are the fatty acids that contain an aliphatic chain with a carboxylic acid group. Most natural fatty acids have an even number of carbons due to the biological process of fatty acid elongation where two carbons are added at a time. The majority of fatty acids in nature contain 14–24 carbons. While some fats contain small amounts of fatty acids with <14 carbons, significant levels of short-chain fatty acids are mainly found in tropical oils and dairy fats. Fatty acids are generally classified as either saturated or unsaturated, with the latter containing double bonds. Fatty acids can be described by systematic, common, and abbreviated names.

4.2.1.1 Nomenclature of Saturated Fatty Acids

The IUPAC has standardized systematic descriptions of fatty acids. The IUPAC system names the parent hydrocarbon of the fatty acid based on the number of carbons (e.g., 10 carbons would

TABLE 4.1
Systematic, Common, and Numerical Names for Fatty Acids Found in Foods

Systematic Name	Common Name	Numerical Abbreviation
Saturated fatty acids		
Hexanoic	Caproic	6:0
Octanoic	Caprylic	8:0
Decanoic	Capric	10:0
Dodecanoic	Lauric	12:0
Tetradecanoic	Myristic	14:0
Hexadecanoic	Palmitic	16:0
Octadecanoic	Stearic	18:0
Unsaturated fatty acids		
cis-9-Octadecenoic	Oleic	18:1 Δ9
cis-9, *cis*-12 Octadecadienoic	Linoleic	18:2 Δ9
cis-9, *cis*-12, *cis*-15 Octadecatrienoic	Linolenic	18:3 Δ9
cis-5, *cis*-8, *cis*-11, *cis*-14 Eicosatetraenoic	Arachidonic	20:4 Δ5
cis-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 Eicosapentaenoic	EPA	20:5 Δ5
cis-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 Docosahexaenoic	DHA	22:6 Δ4

be decane). Since fatty acids contain a carboxylic acid group, the terminal *e* in the name is replaced by *oic* (e.g., decanoic). Common names exist for most of the even number and many of the odd number fatty acids (Table 4.1). Many of the common names originate from the source that the fatty acid was commonly or traditionally isolated (e.g., palmitic acids and palm oil). A numerical system can be used for abbreviated names. The first number in this system designates the number of carbons in the fatty acids, while the second number designates the number of double bonds (e.g., hexadecanoic = palmitic = 16:0). Obviously, this second number will always be zero for the saturated fatty acids.

4.2.1.2 Nomenclature of Unsaturated Fatty Acids

Fatty acids that contain double bonds in their aliphatic chain are referred to as unsaturated fatty acids. In the IUPAC system, the *anoic* designation is changed to *enoic* to designate the presence of a double bond (Table 4.1). Based on the number of double bonds, the terms *di*, *tri*, *tetra*, and so on are added. Common names also exist for the unsaturated fatty acids (with the exception of some of the long-chain polyunsaturated fatty acids), and the numerical abbreviation system is similar to that of the saturated fatty acids with the second number indicating the number of double bonds (e.g., octadecadienoic = 18:2). The positions of the double bonds in the IUPAC system are numbered by the Δ method starting at the carboxylic acid carbon. For example, oleic acid which has 18 carbons and one double bond would be 9-octadecenoic acid, and linoleic acid which has 18 carbons and two double bonds would be 9,12-octadecadienoic acid. An alternative numbering system that indicates the position of the double bonds from the methyl end of the fatty acids is known as the omega (ω) system (sometimes given a shorthand notation of "n"). The omega system is sometimes useful because it can group fatty acids based on their biological activity and biosynthetic origin, since many enzymes recognize fatty acids from the free methyl end of the molecule when it is esterified to glycerol. For instance, the ω-3 fatty acids often have similar bioactivity in their ability to decrease blood triacylglycerol levels [11].

The natural configuration of double bonds in unsaturated fatty acids is the *cis* configuration. In this configuration, the carbons of the aliphatic chain extend on the same side of the double bond, while *trans* double bonds would have the carbons on opposite sides (Figure 4.1). Double bonds

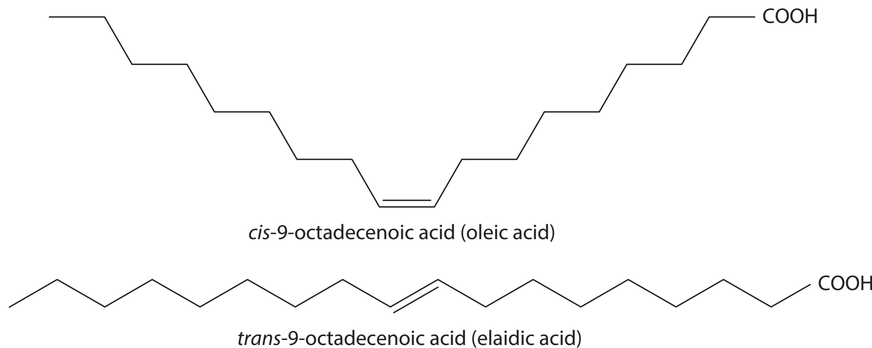


FIGURE 4.1 Differences between *cis* and *trans* double bonds in unsaturated fatty acids.

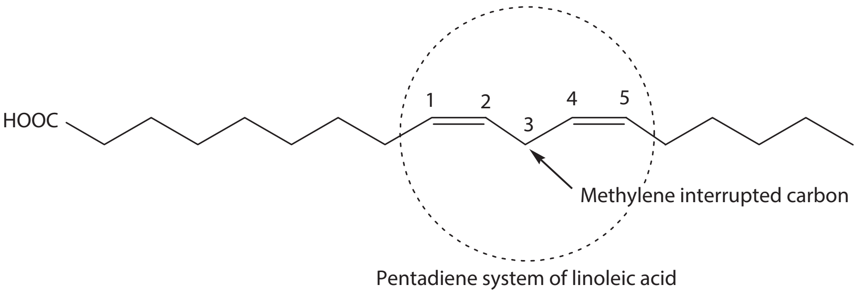


FIGURE 4.2 Pentadiene systems of the polyunsaturated fatty acid, linoleic acid.

in polyunsaturated fatty acids (≥2 double bonds) are most commonly in a methylene interrupted configuration, often termed the pentadiene system. In a pentadiene system, the two double bonds would be at carbons 1 and 4. In other words, the double bonds are not conjugated but, instead, are separated by a methylene carbon (Figure 4.2). This means that the double bonds of most unsaturated fatty acids are three carbons apart (e.g., 9, 12, 15 octadecatrienoic). It is therefore possible to predict the position of all the double bonds in most natural unsaturated fatty acids if the location of the first double bond is known. This is why the numerical abbreviation system will sometimes give only the number of double bonds and the position of the first double bond (e.g., 9, 12, 15 octadecatrienoic = 18:3Δ9 = 18:3ω3).

The presence of double bonds influences the melting point of the fatty acids. Double bonds in the *cis* configuration will cause the fatty acid to arrange in a bent configuration. Thus, unsaturated fatty acids are not linear, making it difficult for them to orient themselves into tight packing configurations. Since the unsaturated fatty acids do not pack tightly, van der Waals interactions between the molecules are relatively weak, and less energy is required to promote solid–liquid phase transitions, so that the melting point decreases. As more double bonds are added, the molecule becomes more bent, van der Waals interactions decrease further, and the melting point decreases. Fatty acids with double bonds in the *trans* configuration are more linear than unsaturated fatty acids in the *cis* configuration. This results in tighter packing of the molecules and higher melting points. For example, the approximate melting point of stearic acid (octadecanoic) is 70°C, oleic acid (*cis*-9-octadecenoic) is 5°C, and elaidic (*trans*-9-octadecenoic) is 44°C [66].

4.2.2 Acylglycerols

Over 99% of the fatty acids found in plants and animals are esterified to glycerol. Free fatty acids are not common in living tissues because they are cytotoxic due to their ability to disrupt cell membrane

organization. Once fatty acids are esterified onto glycerol, their surface activity decreases, as does their cytotoxicity.

Acylglycerols can exist as mono-, di-, and triesters, known as monoacylglycerols, diacylglycerols, and triacylglycerols, respectively. Triacylglycerols are the most common of the three in foods, although the mono- and diesters are sometimes used as food additives (e.g., emulsifiers). The central glycerol carbon of a triacylglycerol exhibits chirality if different fatty acids are present at the terminal carbons of the glycerol. Because of this, the three carbons on the glycerol portion of the triacylglycerol can be differentiated by stereospecific numbering (*sn*). If the triacylglycerol is shown in a planar Fischer projection, the carbons are numbered 1–3 from top to bottom.

Triacylglycerols can be named by several different systems. Triacylglycerols are often named using the common names of the fatty acids. If the triacylglycerol contains only one fatty acid (e.g., stearic acid, abbreviated as St) it could be named tristearin, tristearate, glycerol tristearate, tristearoyl glycerol, StStSt, or 18:0-18:0-18:0. Triacylglycerols that contain different fatty acids are named differently depending on whether the stereospecific location of each fatty acid is known. The nomenclature for these heterogeneous triacylglycerols replaces the *-ic* at the end of the fatty acid name with *-oyl*. If the stereospecific location is not known, a triacylglycerol containing palmitic acid, stearic acid, and oleic acid would be named palmitoyl-oleoyl-stearoyl-glycerol. Alternatively, this triacylglycerol could be named palmito-oleo-stearin or glycerol-palmito-oleo-stearate. If the stereospecific location of the fatty acids is known, *sn-* is added to the name such as in 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol, *sn*-1-palmito-2-oleo-3-stearin, or *sn*-glycerol-1-palmito-2-oleo-3-stearate. If two of the fatty acids are identical, the naming can be shortened as 1,2-dipalmitoyl-3-stearoyl-*sn*-glycerol, *sn*-1,2-dipalmito-3-stearin, or *sn*-glycerol-1,2-dipalmito-3-stearate. Heterogeneous triacylglycerols can also be named using fatty acid abbreviations such as in PStO or 16:0-18:0-18:1 (sterospecific location unknown) or *sn*-PStO, or *sn*-16:0-18:0-18:1 (sterospecific location known) for 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol.

4.2.3 Phospholipids

The phospholipids or phosphoglycerides are modifications of triacylglycerols where the phosphate groups are typically found in the *sn*-3 position (see Figure 4.3 for the structures of phospholipids). The simplest phospholipid is phosphatidic acid (PA), where the substitution group on the phosphate at *sn*-3 is an −OH. Other modifications of the substitution group on the phosphate at *sn*-3 result in phosphotidylcholine (PC), phosphotidylserine (PS), phosphotidylethanolamine (PE), and phosphotidylinositol (PI) (Figure 4.3). Nomenclature is similar to that of triacylglycerols, with the name and location of the phosphate group coming at the end of the name (e.g., 1-palmitoyl-2-stearoyl-*sn*-glycero-3-phosphoethanolamine). The term "lyso" signifies that a fatty acid has been removed from the phospholipid. In the food industry, lysophospholipids usually refer to a phospholipid where the fatty acid has been removed from the *sn*-2 position. Official nomenclature requires that the stereospecific location of the fatty acid removed should be named (e.g., 2-lysophospholipids, IUPAC). Phosphotidylcholine is commonly referred to as lecithin in the food industry; however, the lecithin sold as a food additive is not usually pure phosphotidylcholine.

The presence of the polar phosphate group on phospholipids makes these compounds surface active (see Chapter 7). This surface activity allows phospholipids to arrange in bilayers that are critical for the properties of biological cell membranes. Since cell membranes need to maintain fluidity, the fatty acids found in phospholipids are often unsaturated to prevent crystallization at environmental temperatures. The fatty acids at the *sn*-2 position are typically more unsaturated than those at the *sn*-1 position. The unsaturated fatty acids at the *sn*-1 and *sn*-2 positions can be released by phospholipases so they can be utilized as substrates for enzymes such as cyclooxygenase and lipoxygenase. The surface activity of phospholipids means that they can be used to modify the physical properties of lipids by acting as emulsifiers and by modifying lipid crystallization behavior (see Chapter 7).

FIGURE 4.3 Structures of phospholipids commonly found in foods.

4.2.4 Sphingolipids

Sphingolipids are lipids that commonly contain a sphingosine base. Common sphingolipids include sphingomyelin (a sphingophospholipid), ceramides, cerebrosides, and gangliosides. These lipids are most commonly found associated with cell membranes, especially in the nervous tissue. They are generally not major components of food lipids.

4.2.5 Sterols

Sterols are derivatives of steroids. These nonpolar lipids all have three six-carbon rings and a five-carbon ring that is attached to an aliphatic chain (Figure 4.4). Sterols have a hydroxyl group

FIGURE 4.4 Structures of sterols commonly found in foods.

attached to carbon 3 of the A ring. Sterol esters are sterols with a fatty acid esterified onto the hydroxyl group at carbon 3. Sterols are found in both plants (phytosterols) and animals. Cholesterol is the major sterol found in animal lipids. Plant lipids contain numerous sterols, with sitosterol and stigmasterol predominating. Cholesterol can be found in plant lipids as a minor sterol component. The hydroxyl group at carbon 3 of sterols makes these compounds surface active. Cholesterol therefore can orient itself into cell membranes where it is important in stabilizing membrane structure. Cholesterol is also important because it is the precursor for the synthesis of bile acids, and 7-dehydrocholesterol is the precursor for the ultraviolet irradiation production of vitamin D in the skin [68]. High blood cholesterol, in particular high low-density lipoprotein cholesterol, has been attributed to increased risk for cardiovascular disease. For this reason, many attempts have been made to alter dietary cholesterol levels by the removal of animal fats from the diet and of cholesterol in animal foods by the reduction in total fat. Phytosterols have been found to decrease cholesterol absorption and synthesis, and thus have been added to foods to reduce blood cholesterol levels (see Section 4.12).

4.2.6 Waxes

The strict chemical definition of a wax is an ester of a long-chain acid and a long-chain alcohol. In reality, industrial and food waxes are a combination of chemical classes including wax esters, sterol esters, ketones, aldehydes, alcohols, and sterols [68]. Waxes can be classified according to their origin as animal (beeswax), plant (carnauba wax), and mineral (petroleum waxes). Waxes are found on the surface of plant and animal tissues to inhibit water loss or to repel water. Waxes are commonly added to the surface of fruits to slow transpirational moisture losses during storage.

4.2.7 Miscellaneous Lipids

Other food lipids, including the fat-soluble vitamins (A, D, E, and K) and carotenoids, are covered in other chapters of this book.

4.2.8 Composition of Fats

Food lipids contain a wide variety of fatty acid compositions, as shown in Table 4.2. Several general trends can be seen among lipids. Most vegetable oils, especially those from oilseeds, are highly unsaturated and contain primarily fatty acids in the 18-carbon series. Oils high in oleic acid include olive and canola, oils high in linoleic include soybean and corn, and oils high in linolenic include linseed. Triacylglycerols from plant sources that contain high amounts of saturated fatty acids include cocoa butter and the tropical oils (e.g., coconut). The coconut and palm kernel are also unique in that they contain high amounts of the medium-chain fatty acids 8:0–14:0, with 12:0 predominating. The level of saturated fatty acids in fats and oils from animals is generally in the order of milk fat > sheep > beef > pig > chicken > turkey > marine fish, with palmitic and stearic being the major saturated fatty acids. The fatty acid composition of animal fats is dependent on the digestive system of the animal, with fat from nonruminants (e.g., poultry, pigs, and fish) being more dependent on the fatty acid compositions of their diets than ruminants. An example of this is pigs used to produce Iberian hams, where dietary regimes are manipulated to produce lard with a high oleic acid composition. Among the nonruminants, triacylglycerols from marine animals are unique because they contain high amounts of the ω-3 fatty acids, eicosapentaenoic and docosahexaenoic. In sheep and cows, dietary fatty acids are subject to biohydrogenation by microbial enzymes in the rumen. This results in the conversion of much of the dietary unsaturated fatty acids into saturated fatty acids and also the production of fatty acids with conjugated double bonds (including *trans* types) such as conjugated linoleic acid. Since ruminants consume primarily lipids of plant origin in which

TABLE 4.2
Fatty Acid Composition (%) of Common Foods

Food	4:0	6:0	8:0	10:0	12:0	14:0	16:0	16:1 Δ9	18:0	18:1 Δ9	18:2 Δ9	18:3 Δ9	20:5 Δ5	22:6 Δ4	Total Sat'd
Olive							13.7	1.2	2.5	71.1	10.0	0.6			16.2
Canola							3.9	0.2	1.9	64.1	18.7	9.2			5.5
Corn							12.2	0.1	2.2	27.5	57.0	0.9			14.4
Soybean						0.1	11.0	0.1	4.0	23.4	53.2	7.8			15.0
Linseed							4.8		4.7	19.9	15.9	52.7			9.5
Coconut		0.5	8.0	6.4	48.5	17.6	8.4		2.5	6.5	1.5				91.9
Cocoa						0.1	25.8	0.3	34.5	35.3	2.9				60.4
Butterfat	3.8	2.3	1.1	2.0	3.1	11.7	26.2	1.9	12.5	28.2	2.9	0.5			62.7
Beef fat				0.1	0.1	3.3	25.5	3.4	21.6	38.7	2.2	0.6			50.6
Pork fat				0.1	0.1	1.5	24.8	3.1	12.3	45.1	9.9	0.1			38.8
Chicken					0.2	1.3	23.2	6.5	6.4	41.6	18.9	1.3			31.1
Atlantic Salmon						5.0	15.9	6.3	2.5	21.4	1.1	0.6	1.9	11.9	23.4

Only the major fatty acids in these products are listed. All fatty acid compositions are adapted from White [93] with the exception of Atlantic Salmon, which is adapted from Ackman [1].

the fatty acids are primarily in the 18-carbon series, the end product of an exhaustive biohydrogenation pathway is stearic acid. Thus, butter as well as beef and sheep fats contain higher amounts of stearic acid than fats from nonruminants. Ruminal bacteria are also unique in that they can ferment carbohydrates to acetate and β-hydroxybutyrate. In the mammary gland, these substrates are converted to fatty acids to give butter fat a high concentration of saturated, short-chain (4:0 and 6:0) fatty acids that are not found in other food triacylglycerols. Ruminal bacteria also promote the formation of keto-, hydroxyl-, and branched-chain fatty acids. Because of the impact of ruminal bacteria on fatty acids, butter fat contains hundreds of different fatty acids.

The stereospecific location of fatty acids can also vary in food triacylglycerols. Triacylglycerols in some fats such as tallow, olive oil, and peanut oil have most of their fatty acids evenly distributed among all three positions of glycerol. However, some fats can have very specific trends for the stereospecific location of fatty acids. Many triacylglycerols from plants sources have the unsaturated fatty acids concentrated at the *sn*-2 position. The best example of this is cocoa butter, where over 85% of its oleic acid is *sn*-2, with palmitic and stearic acids being evenly distributed at *sn*-1 and *sn*-3. Triacylglycerols from animal fats tend to have saturated fatty acids concentrated at *sn*-2. For instance, palmitic acid is primarily at the *sn*-2 position in milkfat and lard. The stereospecific location of a fatty acid can be an important determinant on its impact in nutrition. When triacylglycerols are digested in the intestine, fatty acids from *sn*-1 and *sn*-3 are released by pancreatic lipase, resulting in two free fatty acids and an *sn*-2 monoacylglycerol. If long-chain saturated fatty acids are at *sn*-1 and *sn*-3, their bioavailability is lower because the free fatty acids can form insoluble calcium salts. Thus, placement of long-chain saturated fatty acids at *sn*-2 in milk fats may be a mechanism to ensure that these fatty acids are absorbed by infants. Since long-chain saturated fatty acids at *sn*-1 and *sn*-3 are absorbed inefficiently, they provide less calories [16] and have less impact on blood lipid profiles. For example, when lard has its fatty acids randomly distributed and thus has less palmitic acid at *sn*-2, it does not increase plasma palmitic acid or total lipid concentrations as high as unmodified lard where 65% of palmitic is at *sn*-2. This principle has been used to produce low-calorie triacylglycerols such as Salatrim (see Section 4.12).

4.2.9 Summary

- Fatty acids are the major building block of most food lipids.
- Fatty acids can be saturated or unsaturated, which affects their physical and biological properties.
- Food lipids vary widely in fatty acid composition as a function of the source of plant or animal from which the lipid is obtained.
- The position of fatty acids on triacylglycerols is also a function of the source of plant or animal from which the lipid is obtained.

4.3 LIPID REFINING

Triacylglycerols are extracted from both plant and animal sources. "Rendering" is a thermal processing operation that breaks down cellular structures to release the triacylglycerols from animal byproducts and oil-laden underutilized fish species. Plant triacylglycerols can be isolated by pressing (olives), solvent extraction (oilseeds), or a combination of the two (for a detailed discussion of fat and oil extraction see Reference 44). The resulting crude oils and fats from these processes will contain not only triacylglycerols but also lipids such as free fatty acids, phospholipids, lipid-soluble off-flavors and carotenoids, as well as nonlipid materials such as proteins and carbohydrates. These components must be removed to produce oils and fats with the desired color, flavor, and shelf-life. The major refining steps are described below.

4.3.1 Degumming

The presence of phospholipids will cause the formation of water-in-oil (W/O) emulsions in fats and oils. These emulsions will make the oil cloudy, and the water can present a hazard when the oils are heated to temperatures above 100°C (spattering and foaming). Phospholipids also contain amines that can interact with carbonyls to form browning products during thermal processing and storage. Degumming to remove phospholipids is accomplished by the addition of 1%–3% water at 60°C–80°C for 30–60 min. Small amounts of acid are often added to the water to increase the phospholipids' solubility. This occurs because the citric acid can bind calcium and magnesium, thereby decreasing phospholipid aggregation and making them more hydratable. Settling, filtering, or centrifugation is then used to remove the coalesced gums. With oils such as soybean, the phospholipids are recovered and sold as lecithin.

4.3.2 Neutralization

Free fatty acids must be removed from crude oils because they can cause off-flavors, decrease smoke point, accelerate lipid oxidation, cause foaming, and interfere with hydrogenation and interesterification operations. Neutralization is accomplished by reacting the oil with a solution of caustic soda and then removing the water containing the soaps of the free fatty acids. The amount of caustic soda used is dependent on the free fatty acid concentrations in the crude oil. The resulting soap stock can be used as animal feed or to produce surfactants and detergents.

4.3.3 Bleaching

Crude oils will contain pigments that produce undesirable colors (carotenoids, gossypol, etc.) and can promote lipid oxidation (chlorophyll). Pigments are removed by mixing the hot oil (80°C–110°C) with absorbents such as neutral clays, synthetic silicates, activated carbon, or activated earths. The absorbent is then removed by filtration. This process is usually done under vacuum since absorbents can accelerate lipid oxidation. An added benefit of bleaching is the removal of residual free fatty acids and phospholipids and the breakdown of lipid hydroperoxides.

4.3.4 Deodorization

Crude lipids contain undesirable aroma compounds such as aldehydes, ketones, and alcohols that occur naturally in the oil or are produced from lipid oxidation reactions that occur during extraction and refining. These volatile compounds are removed by subjecting the oil to steam distillation at high temperatures (180°C–270°C) at low pressures. Deodorization processes can also breakdown lipid hydroperoxides to increase the oxidative stability of the oil but can also result in the formation of *trans* fatty acids. The latter is the reason why most lipid-containing foods are not free of *trans* fatty acids. Oils can also be physically refined to remove both free fatty acids and off-flavors, thus skipping the neutralization step. This process requires higher temperatures, increases yield, but increases *trans* fatty acid formation [85]. After deodorization is complete, citric acid (0.005%–0.01%) is added to inactivate prooxidant metals. The deodorizer distillate will contain tocopherols and sterols, which can be recovered and used as antioxidants and functional food ingredients (phytosterols).

4.4 MOLECULAR INTERACTIONS AND ORGANIZATION OF TRIACYLGLYCEROLS

In the next few sections, we are primarily concerned with the molecular properties of lipids and their influence on food properties. In particular, we focus on how the structure, organization, and interactions of lipid molecules determine their functional properties (e.g., melting and crystallization,

surface activity, and interactions with other food components), which in turn determine the bulk physicochemical and sensory properties of food products (e.g., appearance, texture, stability, and flavor).

As discussed in previous sections, there are a number of different categories of lipids present in food systems, each with its own molecular characteristics. In this section, we will concentrate primarily on triacylglycerols because of their high natural abundance and major importance in food products. As mentioned earlier, triacylglycerols are esters of a glycerol molecule and three fatty acid molecules, and each fatty acid may have different numbers of carbon atoms and degrees of unsaturation in the hydrocarbon chain. The fact that there are many different types of fatty acid molecules, and that these fatty acids can be located at different positions on the glycerol backbone, means that there are a large number of possible triacylglycerol molecules present in foods. Indeed, edible fats and oils always contain a great many different types of triacylglycerol molecules, with the precise type and concentration depending on their origin [3,32,33].

Triacylglycerol molecules are portrayed as having a "tuning-fork" structure, with the two fatty acids at the ends of the glycerol molecule pointing in one direction, and the fatty acid in the middle pointing in the opposite direction (Figure 4.5). In the liquid state, there is considerable rotational freedom along the acyl chain where double bonds do not exist. They are predominantly nonpolar molecules, and so the most important types of molecular interaction that are responsible for their structural organization are van der Waals attraction and steric repulsion [43]. At a certain molecular separation (s^*), there is a minimum in the intermolecular pair potential $w(s^*)$ whose depth is a measure of the strength of the attractive interactions that hold the molecules together in the solid and liquid states (Figure 4.6). In the case of triacylglycerol molecules, s^* will be close to the distance between neighboring hydrocarbon chains. The structural organization of the molecules in triacylglycerols is primarily determined by its physical state, which depends on the balance between the attractive molecular interactions and the disorganizing influence of the thermal energy. Lipids tend to exist as liquids above their melting point and as solids at temperatures sufficiently below their melting point (Section 4.7).

Lipid molecules may adopt a variety of different structural organizations in both the solid and liquid states depending on their precise molecular characteristics, for example, chain length, degree of unsaturation, and polarity [37,38]. In the solid state, the organization of the lipid molecules may vary in a number of ways. Triacylglycerol molecules may stack together in crystals so that the height of the layers formed is approximately two (e.g., α and β-L2) or three (e.g., β-L3) fatty acid chains in dimensions (Figure 4.7). In addition, triacylglycerol molecules may pack together at different tilt angles with respect to the plane of the layers, for example, compare α and β-L2 (Figure 4.7). The crystals formed by triglyceride molecules can also be described in terms of the arrangement of the molecules within a "point lattice," for example, as hexagonal, triclinic, or orthorhombic (Figure 4.8). These differences mean that fat crystals can exist in a number of different polymorphic crystal forms, which have different physical properties and melting behavior (Section 4.7.5). The type of crystalline form adopted depends on the molecular structure and composition of the lipids, as well as on the environmental conditions during crystallization (cooling rate, holding

FIGURE 4.5 Chemical structure of a triacylglycerol molecule, which is assembled from three fatty acids and a glycerol molecule.

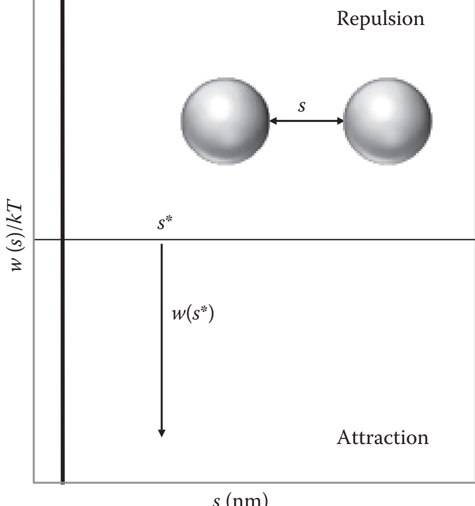


FIGURE 4.6 Strength of the attractive interactions between lipid molecules depends on the depth of the minimum in the overall molecular interaction potential.

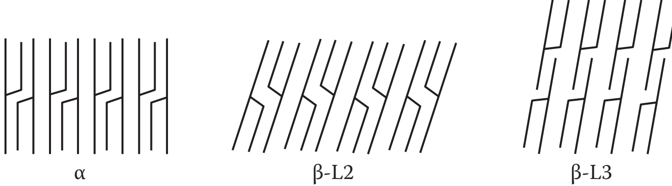


FIGURE 4.7 Common types of overall molecular organization of triacylglycerols within crystalline phases. (Adapted from Walstra, P., *Physical Chemistry of Foods*, Marcel Dekker, New York, 2003.)

temperature, shearing). Even in the liquid state, triacylglycerols are not completely randomly orientated but have some order due to self-organization of the lipid molecules into structural entities, for example, lamellar structures [37,38]. The size and number of these structural entities are believed to decrease as the temperature is increased.

It should be noted that the term *fat* is conventionally used to refer to a lipid that is predominantly solid-like at room temperature (around 25°C), whereas the term *oil* is used to refer to a lipid that is liquid, although these terms are often used interchangeably [91,92].

4.5 PHYSICAL PROPERTIES OF TRIACYLGLYEROLS

The physical properties of edible fats and oils depend primarily on the molecular structure, interactions, and organization of the triacylglycerol molecules that they contain [32,56,91]. In particular, the strength of the attractive interactions between the molecules and the effectiveness of their packing within a condensed phase largely determine their thermal behavior, density, and rheological properties (Table 4.3).

4.5.1 Rheological Properties

Most liquid triacylglycerol oils are Newtonian liquids with viscosities between ~30 and 60 mPa·s at room temperature (around 25°C), for example, corn oil, sunflower oil, canola oils, and

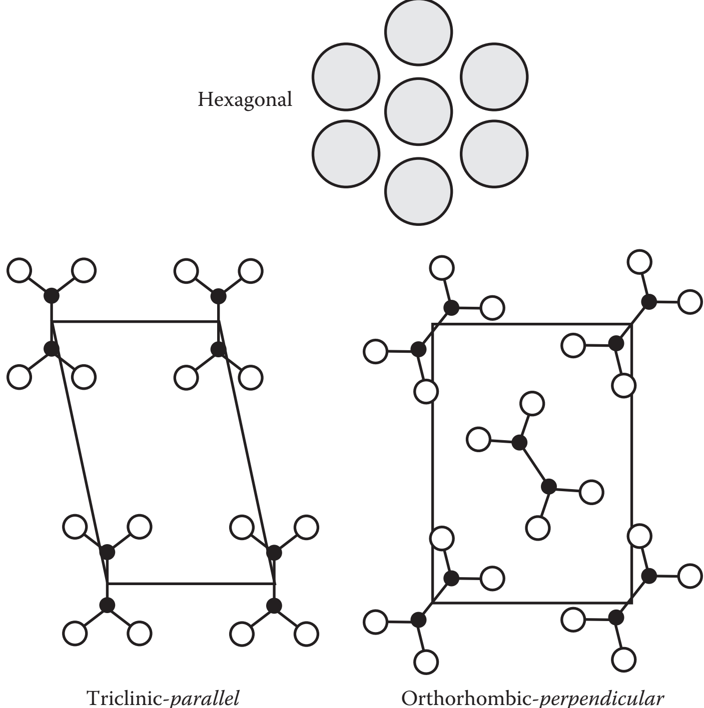


FIGURE 4.8 Three of the most common packing types of hydrocarbon chains: hexagonal, triclinic (*parallel*), and orthorhombic (*perpendicular*). For triclinic and orthorhombic packing, the black circles represent carbon atoms and the white circles represent hydrogen atoms. The hydrocarbon chains are viewed from the top. (Adapted from Walstra, P., *Physical Chemistry of Foods*, Marcel Dekker, New York, 2003.)

TABLE 4.3
Comparison of Some Bulk Physicochemical Properties of a Liquid Oil (Triolein) and Water at 20°C

	Oil	Water
Molecular weight	885	18
Melting point (°C)	5	0
Density (kg m^{-3})	910	998
Compressibility	5.03×10^{-10}	4.55×10^{-10}
Viscosity (mPa·s)	≈50	1.002
Thermal conductivity (W m^{-1} K^{-1})	0.170	0.598
Specific heat capacity (J kg^{-1} K^{-1})	1980	4182
Thermal expansion coefficient (°C^{-1})	7.1×10^{-4}	2.1×10^{-4}
Dielectric constant	3	80.2
Surface tension (mN m^{-1})	≈35	72.8
Refractive index	1.46	1.333

fish oil [15,21,78]. Nevertheless, castor oil tends to have a much higher viscosity than most other triacylglycerol oils because it contains an alcohol group along its hydrocarbon backbone, which is capable of forming hydrogen bonds with neighboring molecules. The viscosity of liquid oils tends to decrease steeply with increasing temperature and can be conveniently described by a logarithmic relationship.

Most "solid fats" actually consist of a mixture of fat crystals dispersed in a liquid oil matrix. The rheological properties of these solid fats are highly dependent on the concentration, morphology, interactions, and organization of the fat crystals present in the system [54,56,91]. Solid fats normally exhibit a type of rheological behavior known as "plasticity." A plastic material behaves like a solid below a critical applied stress, known as the yield stress (τ_0), but behaves like a liquid above this stress. The rheological behavior of an ideal plastic material, known as a *Bingham plastic,* is shown in Figure 4.9. For an applied *shear* stress, the rheological characteristics of this type of material can be described by the following equation:

$$\tau = G\gamma \quad (\text{for } \tau < \tau_0) \tag{4.1}$$

$$\tau - \tau_0 = \eta\dot{\gamma} \quad (\text{for } \tau \geq \tau_0) \tag{4.2}$$

where
- τ is the applied shear stress
- γ is the resultant shear strain
- $\dot{\gamma}$ is the rate of shear strain
- G is the shear modulus
- η is the Bingham shear viscosity
- τ_0 is the yield stress

In practice, solid fats tend to exhibit a nonideal plastic behavior. Above the yield stress, the fat may not flow like an ideal liquid and therefore exhibit non-Newtonian behavior, for example, shear thinning. Below the yield stress, the fat may also not behave like an ideal solid and exhibit some flow characteristics, for example, viscoelasticity. In addition, the yield stress may occur not at a well-defined value but over a range of stresses because there is a gradual breakdown of the fat crystal

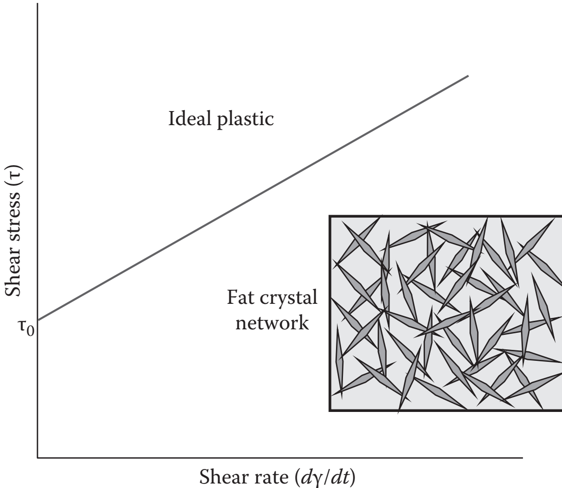


FIGURE 4.9 An ideal plastic material ("Bingham Plastic") behaves like a solid below a critical applied stress, known as the yield stress (τ_0), but behaves like a liquid above this stress.

network structure. The yield stress of a fat tends to increase with increasing solid fat content, and will be higher for crystal morphologies that are able to form three-dimensional networks that extend throughout the volume of the system more easily. Detailed discussions of the characteristics of plastic fats have recently been given elsewhere [54,56,91].

The structural origin of the plastic behavior of solid fats can be attributed to their ability to form a three-dimensional network of tiny fat crystals dispersed in a liquid oil matrix (Figure 4.9). Below a certain applied stress, there is a small deformation of the sample, but the weak bonds between the fat crystals are not disrupted. When the critical yield stress is exceeded, the weak bonds are broken and the fat crystals slide past one another, leading to flow of the sample. Once the force is removed, the flow stops, and the fat crystals begin to form new bonds with their neighbors. The rate at which this process occurs may have important implications for the functionality of the product, for example, the packing of margarines into containers after their production. In this case, it is important that the margarine flows into the container immediately after production and hardens later. The influence of the rheological characteristics of triacylglycerols on the physicochemical and sensory properties of foods is described later.

4.5.2 Density

The density of a lipid is the mass of material required to fill a given volume. This information is often important when designing food processing operations, since it determines the amount of material that can be stored in a tank or flow through a pipe of a given volume. The density of lipids is also important in certain food applications because it influences the overall properties of the system, for example, the creaming rate of oil droplets in oil-in-water (O/W) emulsions depends on the density difference between the oil and aqueous phases [59]. The densities of liquid oils tend to be ~910–930 kg m^{-3} at ambient temperature (around 25°C), and decrease with increasing temperature [15]. The densities of completely solidified fats tend to be ~1000–1060 kg m^{-3}, and also decrease with increasing temperature [78]. In many foods, the fat is partially crystalline and so the density depends on the solid fat content (SFC), that is, the fraction of the total fat phase that is solidified. The density of a partially crystalline fat tends to increase as the SFC increases, for example, after cooling below the crystallization temperature. Measurements of the density of a partially crystalline fat can therefore be used sometimes to determine its SFC.

The density of a particular lipid depends primarily on the efficiency of the packing of the triacylglycerol molecules within it: the more efficient the packing, the higher the density. Thus, triacylglycerols that contain linear saturated fatty acids are able to pack more efficiently that those that contain branched or unsaturated fatty acids, and so they tend to have higher densities [91,92]. The reason that solid fats tend to have higher densities than liquid oils is also because the molecules tend to be packed more efficiently. Nevertheless, this is not always the case in practice. For example, in lipid systems containing high concentrations of pure triacylglycerols that crystallize over a narrow temperature range, it has been shown that the density of the overall lipid system actually decreases upon crystallization because of void formation [39].

4.5.3 Thermal Properties

The most important thermal properties of lipids from a practical standpoint are the specific heat capacity (C_P), thermal conductivity (κ), melting point (T_{mp}), and enthalpy of fusion (ΔH_f) [21,32,78]. These thermal characteristics determine the total amount of heat that must be supplied (or removed) from a lipid system to change its temperature from one value to another, as well as the rate at which this process can be achieved. The specific heat capacities of most liquid oils and solid fats are ~2 J g^{-1}, and increase with increasing temperature [24]. Lipids are relatively poor conductors of heat and tend to have appreciably lower thermal conductivities (~0.165 W m^{-1} s^{-1}) than water

TABLE 4.4
Melting Points and Heats of Fusion of the Most Stable Polymorphic Forms of Selected Triacylglycerol Molecules

Triacylglycerol	Melting Point (°C)	ΔH_f (J g^{-1})
LLL	46	186
MMM	58	197
PPP	66	205
SSS	73	212
OOO	5	113
LiLiLi	−13	85
LnLnLn	−24	—
SOS	43	194
SOO	23	—

L = lauric acid (C12:0), M = myristic acid (C14:0), P = palmitic acid (C16:0), S = stearic acid (C16:0), O = oleic acid (C18:1), Li = linoleic (C18:2), Ln = linolenic (C18:3). The melting point also depends on polymorphic form, for example, for SSS it is 55°C, 63°C, and 73°C for the α, β′, and β forms, respectively. (Data from various sources.)

(~0.595 W m^{-1} s^{-1}). Detailed information about the thermal properties of different kinds of liquid and solid lipids has been provided elsewhere [15,24,34,78].

The melting point and heat of fusion of a lipid depend on the packing of the triacylglycerol molecules within the crystals formed: the more effective the packing, the higher the melting point and the enthalpy of fusion [43,91]. Thus, the melting points and heats of fusions of pure triacylglycerols tend to increase with increasing chain length, are higher for saturated than for unsaturated fatty acids, are higher for straight chained than for branched fatty acids, are higher for triacylglycerols with a more symmetrical distribution of fatty acids on the glycerol molecule, and are higher for more stable polymorphic forms (Table 4.4). The crystallization of lipids is one of the most important factors determining their influence on the bulk physicochemical and sensory properties of foods, and therefore it will be treated in some detail in Sections 4.7 and 4.9.

For some applications, knowledge of the temperature at which a lipid starts to breakdown due to thermal degradation is important, for example, frying or grilling. The thermal stability of lipids can be characterized by their smoke, flash, and fire points [64]. The *smoke point* is the temperature at which the sample begins to smoke when tested under specified conditions. The *flash point* is the temperature at which the volatile products generated by the lipid are produced at a rate where they can be temporarily ignited by the application of a flame but cannot sustain combustion. The *fire point* is the temperature at which the evolution of volatiles due to thermal decomposition occurs so quickly that continuous combustion can be sustained after the application of a flame. Measurements of these temperatures are particularly important when selecting lipids that are to be used at high temperatures, for example, during baking, grilling, or frying. The thermal stability of triacylglycerols is much better than that of free fatty acids; hence the propensity of lipids to breakdown during heating is largely determined by the amount of volatile organic material that they contain, such as free fatty acids.

4.5.4 Optical Properties

The optical properties of lipids are important practically because they influence the overall appearance of food materials (i.e., color and opacity), but also because they can be related to the molecular

characteristics of lipids so that their measurement can be used to assess oil quality [63]. The most important optical properties of lipids are their refractive index and absorption spectra. The refractive indices of liquid oils typically fall between 1.43 and 1.45 at room temperature (~25°C) [24]. The precise value of the refractive index of a particular liquid oil is mainly determined by the molecular structure of the triacylglycerols it contains. The refractive index tends to increase with the chain length, the number of double bonds, and the conjugation of double bonds. Empirical equations have been developed to relate the molecular structure of lipids to their refractive indices [24]. The refractive index of lipids is important in emulsified foods because the magnitude of the refractive index contrast determines the amount of light scattering and opacity [57].

The visible absorption spectra of an oil can have a pronounced influence on the final color of a food product. In addition, measurements of the absorption spectra may provide valuable information about the composition, quality, or properties of an oil, for example, the degree of unsaturation, extent of lipid oxidation, presence of impurities, and *cis–trans* isomerization [64]. Pure triacylglycerols tend to be colorless since they do not absorb any light in the visible region of the electromagnetic spectrum. Nevertheless, commercial oils tend to be colored because they contain appreciable amounts of pigmented impurities that absorb light, for example, carotenoids and chlorophyll. For this reason, oils often undergo a de-colorization step during their refinement.

4.5.5 Electrical Properties

Knowledge of the electrical properties of lipids is sometimes important because a number of analytical techniques used to analyze fatty foods are based on measurements of their electrical characteristics, for example, electrical conductivity measurements of fat concentration or electrical pulse counting of fat droplet size [59]. Lipids tend to have fairly low relative dielectric constants ($\varepsilon_R \approx 2–4$) because of the low polarity of triacylglycerol molecules (Table 4.3). The dielectric constant of pure triacylglycerols tends to increase with the chain length, polarity (e.g., due to the presence of −OH groups or to oxidation), and decreasing temperature [24]. Lipids also tend to be poor conductors of electricity, with relatively high electrical resistances.

4.6 SOLID FAT CONTENT OF FOOD TRIACYLGLYCEROLS

As mentioned earlier, edible triacylglycerols contain a variety of different fatty acids. If these fatty acids were randomly distributed on the glycerol backbone, the number of possible combinations of triacylglycerol molecules with different fatty acids at *sn*-1, *sn*-2, and *sn*-3 positions will depend on the number of different fatty acids in the lipid. The fatty acid combinations on triacylglycerols impact the liquid–solid phase transitions of the lipid since each triacylglycerol type has a different melting point. This means that food triacylglycerols do not typically have a sharp melting point, but, instead, they melt over a wide temperature range. This temperature range is often referred to as the "plastic range," since the existence of both liquid oil and solid fat usually gives the lipid rheological properties that are characterized as being plastic-like, that is, they act like a solid below a certain yield stress and as a liquid above this stress (Figure 4.9). While the term "plastic range" is commonly used, it is nevertheless possible that a fat can be partially crystalline and not have rheological properties that can be strictly classified as plastic. For example, a pourable lipid could contain nonaggregated fat crystals. The melting profile of triacylglycerols is commonly described in terms of the "solid fat content" (SFC), which specifies the fraction or percentage of lipid that is solid at a given temperature. Figure 4.10 shows the melting profile of a typical food triacylglycerol. At a sufficiently low temperature, the triacylglycerol is completely solid (SFC = 100%). As the temperature is increased, the fat enters the plastic range, with the shorter and more unsaturated triacylglycerols melting first, followed by the longer and more saturated ones until the lipid melts and is completely liquid (SFC = 0%). Due to the presence of different crystal types, the possibility of supercooling, and the solubility of high melting triacylglycerols in lower melting triacylglycerols, the melting

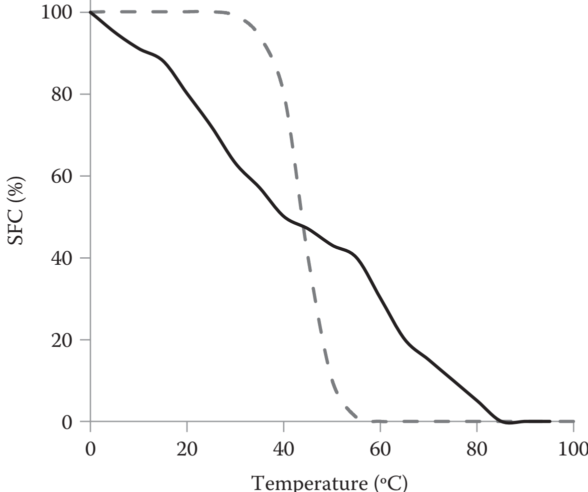


FIGURE 4.10 Comparison of the melting profile of a pure triacylglycerol and a typical edible fat. The edible fat melts over a much wider range of temperatures because it consists of a mixture of many different pure triacylglycerol molecules each with different melting points.

properties of lipids cannot be predicted directly from the triacylglycerol compositions [91]. The SFC of fatty foods is usually measured by calorimetry, changes in volume (dilatometry), or nuclear magnetic resonance (NMR) [64]. NMR is often the preferred method to measure SFC since it is rapid and non-destructive and requires little sample preparation.

Fatty acyl groups in natural fats are not usually randomly distributed. Some natural sources of fats have only a few combinations of different triacylglycerols, while others have numerous combinations of triacylglycerols. Fats that contain triacylglycerols with similar melting points tend to melt over a narrow temperature (plastic) range. These triacylglycerols will solidify into the most stable crystal states. On the other hand, fats that contain triacylglycerols and are more heterogeneous have a wide range of melting (plastic). Some lipids (butterfat) can have mixtures of high- and low-melting triacylglycerols, which produce a stepped rather than a smooth, continuous melting curve.

The solid fat content vs. temperature profile of an edible lipid is one of the most important factors determining its selection for a particular application since it affects many important functional attributes of food products. For example, it influences the appearance and stability of salad oils and dressings stored at refrigeration temperatures, the spreadability of margarines and butters under different conditions (e.g., refrigeration or ambient), the melting of chocolate in the mouth, and the texture of many baked products.

4.7 CRYSTALLIZATION OF TRIACYLGLYCEROLS

Solid–liquid phase transitions are an integral part of many processing operations used to produce food products, for example, margarine, butter, ice-cream, and whipped cream. The creation of food products with desirable properties therefore depends on an understanding of the major factors that influence the crystallization and melting of lipids in foods [37,38,56,91].

The contrasting arrangements of triacylglycerol molecules in the solid and liquid state are shown schematically in Figure 4.11. The physical state of a triacylglycerol at a particular temperature depends on its free energy, which is made up of contributions from both enthalpy and entropy terms: $\Delta G_{S\rightarrow L} = \Delta H_{S\rightarrow L} - T\Delta S_{S\rightarrow L}$ [5]. The enthalpy term ($\Delta H_{S\rightarrow L}$) represents the change in the overall strength of the molecular interactions between the triacylglycerols when they are converted from

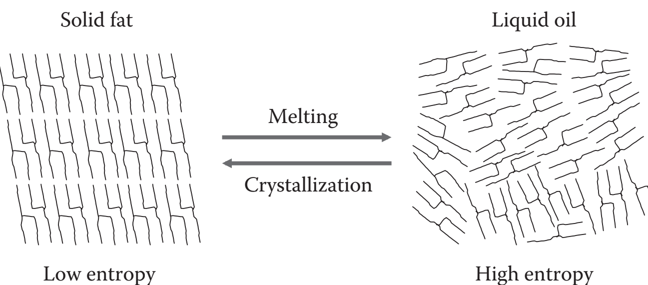


FIGURE 4.11 The arrangement of triacylglycerols in the solid and liquid states depends on a balance between the organizing influence of the attractive interactions between the molecules and the disorganizing influence of the thermal energy.

a solid to a liquid, whereas the entropy term ($\Delta S_{S \rightarrow L}$) represents the change in the randomness of the organization of the molecules brought about by the melting process. The strength of the bonds between the lipid molecules is greater in the solid state than in the liquid state because the molecules are able to pack more efficiently, and so $\Delta H_{S \rightarrow L}$ is positive (unfavorable), which favors the solid state. On the other hand, the entropy of the lipid molecules in the liquid state is greater than that in the solid state, and therefore $\Delta S_{S \rightarrow L}$ is positive (favorable), which favors the liquid state. At low temperatures, the enthalpy term dominates the entropy term ($\Delta H_{S \rightarrow L} > T\Delta S_{S \rightarrow L}$), and therefore the solid state has the lowest free energy [91]. As the temperature increases, the entropy contribution becomes increasingly important. Above a certain temperature, known as the *melting point*, the entropy term dominates the enthalpy term ($T\Delta S_{S \rightarrow L} > \Delta H_{S \rightarrow L}$) and so the liquid state has the lowest free energy. A material therefore changes from a solid to a liquid when its temperature is raised above the melting point. A solid-to-liquid transition (melting) is endothermic because energy must be supplied to the system to pull the molecules further apart. Conversely, a liquid-to-solid transition (crystallization) is exothermic because energy is released as the molecules come closer together. Even though the free energy of the solid state is lowest below the melting point, solid crystals may not appear until a liquid oil has been cooled well below the melting point because of a free energy penalty associated with nuclei formation.

Overall, the crystallization of fats can be conveniently divided into a number of stages: supercooling, nucleation, crystal growth, and post-crystallization events [37,38,55,91].

4.7.1 Supercooling

Although the solid form of a lipid is thermodynamically favorable at temperatures below its melting point, the lipid can persist in the liquid form below the melting point for a considerable time before any crystallization is observed. This is because of an activation energy (energy barrier) associated with nuclei formation that must be overcome before the liquid–solid phase transition can occur (Figure 4.12). If the magnitude of this activation energy is sufficiently high compared to the thermal energy, crystallization will not occur on an observable timescale, and the system will exist in a *metastable* state. The height of the activation energy barrier depends on the ability of crystal nuclei to be formed in the liquid oil that are stable enough to grow into crystals. The degree of supercooling of a liquid can be defined as $\Delta T = T_{mp} - T$, where T is the temperature and T_{mp} is the melting point. The value of ΔT at which crystallization is first observed depends on the chemical structure of the oil, the presence of any contaminating materials, the cooling rate, the microstructure of the lipid phase (e.g., bulk vs. emulsified oil), and the application of external forces [37,91]. Pure oils containing no impurities can often be supercooled by more than 10°C before any crystallization is observed.

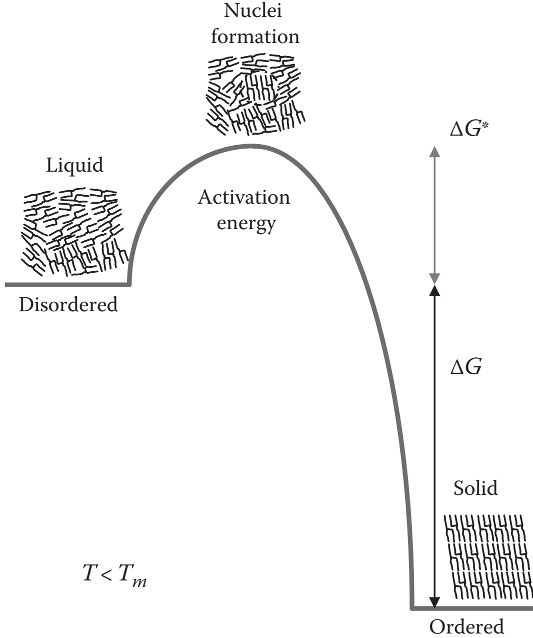


FIGURE 4.12 When the activation energy associated with nuclei formation is sufficiently high, a liquid oil can persist in a metastable state below the melting point of a fat.

4.7.2 Nucleation

Crystal growth can occur only after stable nuclei have been formed in a liquid. These nuclei are believed to be clusters of oil molecules that form small, ordered crystallites, and are formed when a number of oil molecules collide and become associated with each other [44,55]. There is a free energy change associated with the formation of one of these nuclei [37]. Below the melting point, the bulk crystalline state is thermodynamically favorable, and so there is a decrease in free energy when some of the oil molecules in the liquid cluster together to form a nucleus. This negative free energy (ΔG_V) change is proportional to the *volume* of the nucleus formed. On the other hand, the formation of a nucleus leads to the creation of a new interface between the solid and liquid phases, and this process involves an increase in free energy to overcome the interfacial tension. This positive free energy (ΔG_S) change is proportional to the *surface area* of the nucleus formed. The total free energy change associated with the formation of a nucleus is, therefore, a combination of a volume term and a surface term [91]:

$$\Delta G = \Delta G_V + \Delta G_S = \frac{4}{3}\pi r^3 \frac{\Delta H_{fus}\Delta T}{T_{mp}} + 4\pi r^2 \gamma_i \quad (4.3)$$

where
- r is the radius of the nuclei
- ΔH_{fus} is the enthalpy change per unit volume associated with the liquid-solid transition (which is negative)
- γ_i is the solid–liquid interfacial tension

The volume contribution becomes increasingly negative as the size of the nuclei increases, whereas the surface contribution becomes increasingly positive (Figure 4.13). Hence, the surface contribution tends to dominate for small nuclei, while the volume contribution tends to dominate for

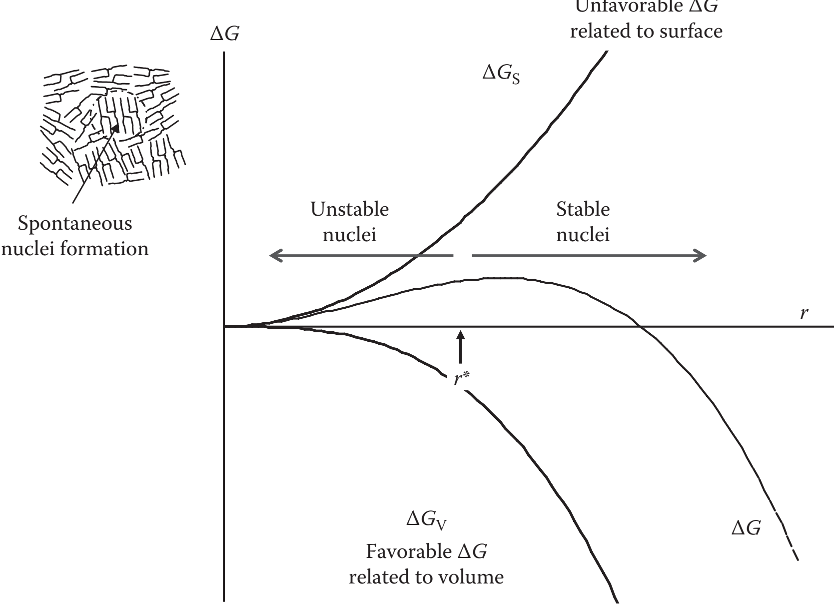


FIGURE 4.13 The critical size of a nucleus required for crystal growth depends on a balance between the volume and surface contributions to the free energy of nuclei formation. Nuclei that are spontaneously formed with radii below r^* grow, whereas those formed with radii below this value dissociate.

large nuclei. As a result, the overall free energy change associated with nuclei formation has a maximum value at a critical nucleus radius (r^*):

$$r^* = \frac{2\gamma_i T_{mp}}{\Delta H_{fus} \Delta T} \tag{4.4}$$

If a nucleus is spontaneously formed and has a radius that is below this critical size, then it will tend to dissociate so as to reduce the free energy of the system. On the other hand, if a nucleus is formed that has a radius above this critical value, then it will tend to grow into a crystal. This equation indicates that the critical size of nuclei required for crystal growth decreases as the degree of supercooling increases, which accounts for the increase in nucleation rate that is observed experimentally when the temperature is decreased.

The rate at which nucleation occurs can be mathematically related to the activation energy ΔG^* that must be overcome before stable nuclei are formed [37]:

$$J = A \exp\left(\frac{-\Delta G^*}{kT}\right) \tag{4.5}$$

where

J is the nucleation rate, which is equal to the number of stable nuclei formed per second per unit volume of material
A is a pre-exponential factor
k is Boltzmann's constant
T is the absolute temperature

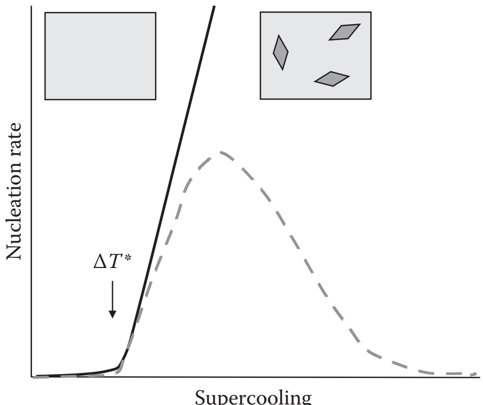


FIGURE 4.14 Theoretically, the rate of the formation of stable nuclei increases with supercooling (solid line), but in practice the nucleation rate decreases below a particular temperature because the diffusion of oil molecules is retarded by the increase in oil viscosity (broken line).

The value of ΔG^* is calculated by replacing r in Equation 4.3 with the critical radius given in Equation 4.4. The variation of the nucleation rate predicted by Equation 4.5 with the degree of supercooling (ΔT) is shown in Figure 4.14. The formation of stable nuclei is negligibly slow at temperatures just below the melting point, but increases dramatically when the liquid is cooled below a certain temperature T^*. In reality, the nucleation rate is observed to increase with the degree of cooling down to a certain temperature, after which it decreases upon further cooling. This is because the increase in viscosity of the oil that occurs as the temperature is decreased slows down the diffusion of oil molecules toward the liquid–nucleus interface [8,37]. Consequently, there is a maximum in the nucleation rate at a particular temperature (Figure 4.14).

The type of nucleation described above occurs when there are no impurities present in the oil, and is usually referred to as *homogeneous nucleation* [37,40]. If the liquid oil is in contact with foreign surfaces, such as the surfaces of dust particles, fat crystals, oil droplets, air bubbles, reverse micelles, or the vessel containing the oil, then nucleation can be induced at a higher temperature than expected for a pure system. Nucleation due to the presence of these foreign surfaces is referred to as *heterogeneous nucleation*, and can be divided into two types: primary and secondary [55]. Primary heterogeneous nucleation occurs when the foreign surfaces have a different chemical structure to that of the oil, whereas secondary heterogeneous nucleation occurs when the foreign surfaces are crystals with the same chemical structure as the liquid oil. Secondary heterogeneous nucleation is the basis for "seeding" nucleation in supercooled lipids. This process involves adding preformed triacylglycerol crystals to a supercooled liquid consisting of the same triacylglycerol so as to promote nucleation at a higher temperature than would otherwise be possible.

Heterogeneous nucleation occurs when the impurities provide a surface at which the formation of stable nuclei is more thermodynamically favorable than in the pure oil. As a result, the degree of supercooling required to initiate fat crystallization is reduced. On the other hand, certain types of impurities are capable of decreasing the nucleation rate of oils because they are incorporated into the surface of the growing nuclei and prevent any further oil molecules being incorporated [82]. Whether an impurity acts as a catalyst or an inhibitor of nucleation depends on its molecular structure and interactions with the nuclei. It should be noted that there is still considerable debate about the mathematical modeling of nucleation, since existing theories often give predictions of nucleation rates that are greatly different from experimental measurements [38,40,91]. Nevertheless, the general form of the dependence of nucleation rates on temperature is predicted fairly well by existing theories (see Figure 4.14).

4.7.3 Crystal Growth

Once stable nuclei have formed, they grow into crystals by incorporating molecules from the liquid oil at the solid–liquid interface [40]. Lipid crystals have a number of different faces, and each face may grow at an appreciably different rate. This partially accounts for the wide variety of different crystal morphologies that can be formed by lipids. The overall crystal growth rate depends on a number of factors, including mass transfer of the lipid molecules to the solid–liquid interface, mass transfer of noncrystallizing species away from the interface, incorporation of the liquid molecules into the crystal lattice, or removal of the heat generated by the crystallization process from the interface [37]. Any of these processes can be rate-limiting depending on molecular and physical properties of the lipids and the prevailing environmental conditions, for example, viscosity, thermal conductivity, crystal structure, temperature profile, and mechanical agitation. Consequently, a general theoretical model of crystal growth is difficult to construct. In crystallizing lipid systems, the incorporation of a molecule at the crystal surface is often rate-limiting at high temperatures, whereas the diffusion of a molecule to the solid–liquid interface is often rate-limiting at low temperatures. This is because the viscosity of the liquid oil increases as the temperature is lowered, and so the diffusion of a molecule is retarded. The crystal growth rate therefore tends to increase initially with increasing degree of supercooling until it reaches a maximum rate, after which it decreases [37]. The dependence of the growth rate on temperature therefore shows a similar trend to the nucleation rate; however, the temperature-dependence of the nucleation rate is usually different from that of the crystal growth rate (Figure 4.15). This difference accounts for the dependence of the number and size of crystals produced on the cooling rate and holding temperature. If a liquid oil is cooled to a temperature at which the nucleation rate is slower than the growth rate, then a small number of large crystals will be formed. On the other hand, if it is cooled to a temperature at which the growth rate is slower than the nucleation rate, then there will be a large number of small crystals formed. Experimentally, it has been observed

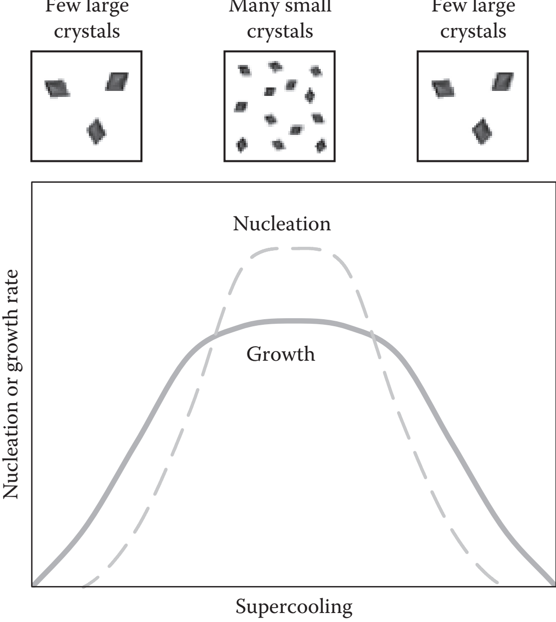


FIGURE 4.15 The nucleation and crystal grow rates have different temperature dependencies, which accounts for differences in the number and size of fat crystals produced under different cooling regimes.

that the rate of crystal growth is proportional to the degree of supercooling and inversely proportional to the viscosity of the melt [37,86].

A variety of mathematical theories have been developed to model the rate of crystal growth in crystallizing fats [37,38]. The most appropriate model for a specific situation depends on the rate-limiting step for that particular system under the prevailing environmental conditions, for example, mass transfer of the liquid molecules to the solid–liquid interface, mass transfer of the noncrystallizing species away from the interface, incorporation of the liquid molecules into the crystal lattice, or removal of the heat generated by the crystallization process from the interface. In practice, it is often difficult to develop fundamental models because of the complexity in mathematically describing the numerous physicochemical processes involved.

4.7.4 Post-Crystallization Events

Once the crystals have been formed in a lipid system, changes in their packing, size, composition, and interactions are likely to occur [37,91]. Post-crystallization events may involve a change from a less stable to a more stable polymorphic form due to rearrangement of the triacylglycerol molecules within the crystals. If a lipid forms mixed crystals (i.e., crystals that contain a mixture of different types of triacylglycerols), then there may be a change in the composition of the crystals during storage due to diffusion of triacylglycerol molecules between the crystals. There may also be a net growth in the average size of the crystals within a lipid with time due to Ostwald ripening, that is, growth of the large crystals at the expense of the smaller ones due to diffusion of oil molecules between the crystals. Finally, the bonds between fat crystals may strengthen over time during storage due to a sintering mechanism, that is, fusion of the crystals together. These post-crystallization changes can have pronounced influences on the bulk physicochemical and sensory properties of foods, and therefore it is important to understand and control them. For example, post-crystallization events often lead to an increase in the size of the crystals in a lipid, which is undesirable because it leads to a gritty perception during consumption.

4.7.5 Crystal Morphology

The morphology of the crystals depends on a number of internal (e.g., molecular structure, composition, packing, and interactions) and external factors (e.g., temperature–time profile, mechanical agitation, and impurities). In general, when a liquid oil is cooled rapidly to a temperature well below its melting point, a large number of small crystals are formed, but when it is cooled slowly to a temperature just below its melting point, a smaller number of larger crystals are formed [37,91]. This is because of the differences in the temperature dependences of the nucleation and crystallization rates (Figure 4.15). The nucleation rate tends to increase more rapidly with decreasing temperature than the crystallization rate up to a certain maximum value, and then it tends to decrease more rapidly with a further decrease in temperature. Thus, rapid cooling tends to produce many nuclei simultaneously, which subsequently grow into small crystals, whereas slow cooling tends to produce a smaller number of nuclei that have time to grow into larger crystals before more nuclei are formed (Figure 4.15). Crystal size has important implications for the rheology and organoleptic properties of many types of foods. When the crystals are too large, they are preceived as being "grainy" or "sandy" in the mouth. The efficiency of molecular packing in crystals also depends on the cooling rate. If a fat is cooled slowly, or the degree of supercooling is small, then the molecules have sufficient time to be efficiently incorporated into a crystal. At faster cooling rates, or higher degrees of the molecules have insufficient time to pack efficiently before another molecule is incorporated. Thus, rapid cooling tends to produce crystals that contain more dislocations, and in which the molecules are less densely packed [86]. The cooling rate therefore has an important impact on the morphology and functional properties of crystalline lipids in foods.

4.7.6 Polymorphism

Triacylglycerols exhibit a phenomenon known as *polymorphism*, which is the ability of a material to exist in a number of crystalline structures with different molecular packing [50,55,76]. Triacylglycerol molecules may pack into fat crystals in a number of different ways, leading to different polymorphic forms (Figures 4.7 and 4.8) having different physicochemical properties. The three most commonly occurring types of packing in triacylglycerols are hexagonal, orthorhombic, and triclinic, which are usually designated as α, β′, and β polymorphic forms, respectively [37,91]. The thermodynamic stability of the three forms decreases in the order β > β′ > α. Even though the β-form is the most thermodynamically stable, triacylglycerols often crystallize in the α-form initially because it has the lowest activation energy for nuclei formation (Figure 4.16). With time, the crystals transform to the most stable polymorphic form at a rate that depends on environmental condition such as temperature, pressure, and the presence of impurities. The conversion from one polymorphic form to another can be monitored using methods such as differential scanning calorimetry (DSC), which measures changes in the heat released (exothermic) or absorbed (endothermic) by a material when a phase transition occurs (Figure 4.17). In this example, when a fat initially in the α-form is heated, it undergoes a series of transitions: α–β′ transformation; β′ melting; β crystallization; and β melting. The time taken for these types of crystal transformation to occur is strongly influenced by the homogeneity of the triacylglycerol composition. The transition from the α-form tends to occur fairly rapidly for relatively homogeneous compositions where the triacylglycerols all have fairly similar molecular structures. On the other hand, the transition is relatively slow for multicomponent fats where the triacylglycerols have diverse molecular structures. The different types of polymorphic forms of lipids can be distinguished from each other using a variety of methods, including x-ray diffraction, DSC, as well as infrared (IR), NMR, and Raman spectroscopy [37,55,91]. These methods are largely based on the fact that different polymorphic forms have different unit cells, which can be categorized by their height, width, and angles of tilt (Figure 4.18). Knowledge of the polymorphic form of the crystals in lipids is often important because it can have a large impact on the thermal behavior and morphology of the crystals formed, and therefore on the physicochemical and sensory properties of foods. For example, the desirable textural characteristics

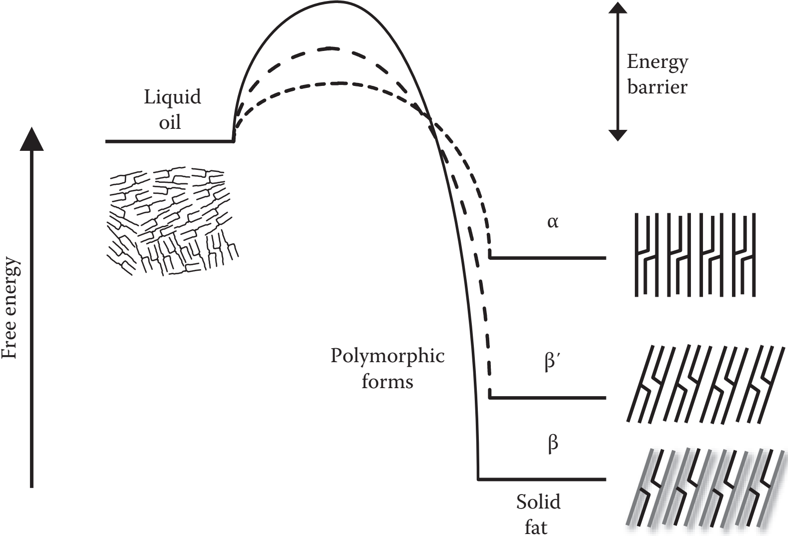


FIGURE 4.16 The polymorphic state that is initially formed when an oil crystallizes depends on the relative magnitude of the activation energies associated with nuclei formation.

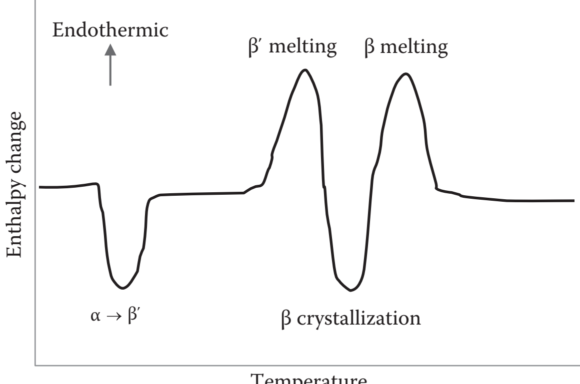


FIGURE 4.17 Polymorphic changes can be monitored using differential scanning calorimetry. This DSC thermogram shows transitions from one polymorph to another (α–β′), as well as melting and crystallization of different polymorphs (β or β′).

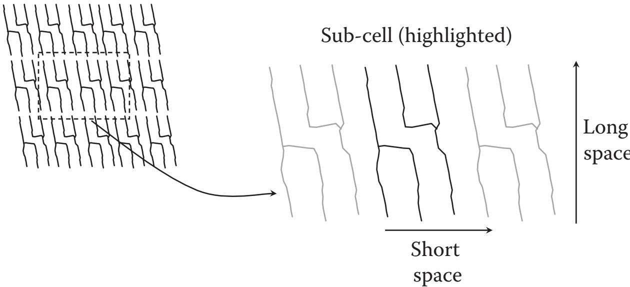


FIGURE 4.18 The unit cells in crystalline lipids can be characterized by their dimensions.

and appearance of chocolate depends on ensuring that the fat crystals are produced and maintained in the appropriate polymorphic form [2]. Edible lipids from different biological sources or that have been processed differently (e.g., fractionation, interesterification, or hydrogenation) often tend to adopt preferred polymorphic forms, typically β, or β′, since the α form is usually unstable [3]. Lipids that adopt a β′ form (such as palm oil and many hydrogenated oils) tend to give smaller crystals with a smoother texture, which is desirable in spreads and shortenings. On the other hand, lipids that adopt a β form often give larger crystals with a more gritty texture, which is undesirable for these applications.

4.7.7 Crystallization of Edible Fats and Oils

The melting point of a triacylglycerol depends on the chain length and the degree of unsaturation of its constituent fatty acids, as well as their relative positions along the glycerol molecule (Table 4.4). Edible fats and oils contain a complex mixture of numerous types of triacylglycerol molecules, each with a different melting point, and so they usually melt/solidify over a wide range of temperatures, rather than at a distinct temperature as would be the case for a pure triacylglycerol (Figure 4.10).

The melting profile of a fat is not simply the weighted sum of the melting profiles of its constituent triacylglycerols, because high-melting-point triacylglycerols are soluble in lower melting point ones [91]. For example, in a 50:50 mixture of tristearin and triolein it is possible to dissolve 10% of

solid tristearin in liquid triolein at 60°C. The solubility of a solid component in a liquid component can be predicted by assuming that they have widely differing melting points (>20°C):

$$\ln x = \frac{\Delta H_{fus}}{R}\left[\frac{1}{T_{mp}} - \frac{1}{T}\right] \tag{4.6}$$

where

x is the solubility, expressed as a mole fraction, of the higher melting point component in the lower melting point component

ΔH_{fus} is the molar heat of fusion

The structure and physical properties of crystals produced by cooling a complex mixture of triacylglycerols are strongly influenced by the cooling rate and temperature [37,55,91]. If an oil is cooled rapidly, all the triacylglycerols crystallize at nearly the same time and a *solid solution* is formed, which consists of homogeneous crystals in which the triacylglycerols are intimately mixed with each other. On the other hand, if the oil is cooled slowly, the higher melting point triacylglycerols crystallize first, while the low melting point triacylglycerols crystallize later, and so *mixed crystals* are formed. These crystals are heterogeneous and consist of some regions that are rich in high-melting-point triacylglycerols and other regions that are depleted in these triacylglycerols. Whether a crystalline fat forms mixed crystals or a solid solution influences many of its physicochemical properties, such as density, rheology, and melting profile, which could have an important influence on the properties of a food product. The type of crystal formed is influenced by the molecular compatibility of the various triacylglycerol molecules in the system, which depends on the chain length, unsaturation, and position of the fatty acids. A detailed review of the thermodynamic and kinetic aspects of fat crystallization and the type of crystal structures formed in mixed systems is given elsewhere [40]. Typically, a lipid may exhibit four different types of phase behavior depending on the nature of the triacylglycerol molecules present: (1) monotectic continuous solid solutions, (2) eutectic systems, (3) monotectic partial solid solutions, and (4) peritectic systems. A discussion of these different systems and the characteristics of lipid mixtures that typically lead to them is given in the article by Himawan et al. [40].

Once a fat has crystallized, the individual crystals may aggregate to form a three-dimensional network that traps liquid oil through capillary forces [55]. The interactions responsible for crystal aggregation in pure fats are primarily van der Waals interactions between the solid fat crystals, although "water bridges" may also play an important role in some products. Once aggregation has occurred, the fat crystals may partially fuse together, which strengthens the crystal network.

4.7.8 Fat Crystallization in Emulsions

The influence of fat crystallization on the bulk physicochemical properties of food emulsions depends on whether the fat forms the continuous phase or the dispersed phase. The characteristic stability and rheological properties of W/O emulsions, such as butter and margarine, are determined by the presence of a network of aggregated fat crystals within the continuous (oil) phase. The fat crystal network is responsible for preventing water droplets from sedimenting under the influence of gravity, as well as determining the textural attributes of the product. If there are too many fat crystals present, the product is firm and difficult to spread, but when there are too few crystals present, the product is soft and collapses under its own weight [91]. Selection of a fat with the appropriate melting characteristics is therefore one of the most important aspects of margarine and spread production. The melting profile of natural fats can be optimized for specific applications by various physical or chemical methods, including blending, interesterification, fractionation, and hydrogenation [32–34].

Fat crystallization also has a pronounced influence on the physicochemical properties of many O/W emulsions, such as milk or salad dressings. When the fat droplets are partially crystalline, a crystal from one droplet can penetrate into another droplet during a collision, which causes the two droplets to stick together. This phenomenon is known as *partial coalescence*, and leads to a dramatic increase in the viscosity of an emulsion, as well as a decrease in the stability to creaming [27]. Extensive partial coalescence can eventually lead to phase inversion, that is, conversion of an O/W emulsion to a W/O emulsion. This process is one of the most important steps in the production of butters, margarines, and spreads. Partial coalescence is also important in the production of ice cream and whipped creams, where an O/W emulsion is cooled to a temperature at which fat in the droplets partially crystallizes and is mechanically agitated to promote droplet collisions and aggregation [30]. The aggregated droplets form a two-dimensional network around the air bubbles and a three-dimensional network in the continuous phase, which contributes to the stability and texture of the product.

4.8 ALTERING THE SOLID-FAT CONTENT OF FOOD LIPIDS

Natural fats with desirable plastic ranges are not always available and are sometimes expensive. In addition, alteration of fatty acid profiles is often desirable to make the fat less susceptible to oxidation (decrease unsaturation) or more nutritionally desirable (increased unsaturation). Therefore, several technologies have been developed to alter the chemical structure and solid fat content of food lipids.

4.8.1 Blending

The simplest method to alter fatty acid composition and melting profile is by blending fats with different triacylglycerol compositions. This practice is performed in products such as frying oils and margarines.

4.8.2 Dietary Interventions

The fatty acid composition of animal fats can be altered by manipulation of the type of fats in the animal's diet. This practice is effective in nonruminants such as pigs, poultry, and fish. Increasing the levels of unsaturated fatty acids in fats from ruminants (cows and sheep) is not very efficient because bacteria in the rumen biohydrogenate the fatty acids before they reach the small intestine where they can be absorbed into the blood.

4.8.3 Genetic Manipulation

The fatty acid composition of fats can be manipulated genetically by altering the enzyme pathways that produce unsaturated fatty acids. Genetic manipulation has been done successfully by both traditional breeding programs and by genetic modification technologies. Several oils that have been obtained from genetically altered plants are commercially available. Most of these oils contain elevated levels of oleic acid.

4.8.4 Fractionation

The fatty acid and triacylglycerol composition of fats can also be altered by holding the fat at a temperature where the most saturated or long chain triacylglycerols will crystallize and then collecting either the solid (more saturated or long-chain) or liquid (more unsaturated or short-chain) phases. This is commonly done to vegetable oils in a process called *winterization*. Winterization is

necessary for oils used in products that are refrigerated to prevent the triacylglycerols from crystallizing and becoming cloudy. Winterization is also necessary for oils used in mayonnaise or salad dressings where crystallization would destabilize the emulsion. Palm, palm kernel (olein/stearin), butterfat, and fish oils are commonly fractionated to change their fatty acid composition.

4.8.5 Hydrogenation

Hydrogenation is a chemical process that adds hydrogen to double bonds. The process is used to alter lipids so they become more solid at room temperature, exhibit different crystallization behavior (by making them more compositionally homogeneous), and/or are more oxidatively stable. These goals are accomplished by the removal of double bonds to make the fatty acids more saturated. An additional use of hydrogenation is to bleach oils since the destruction of double bonds in compounds such as carotenoids will cause them to lose color. Products produced by hydrogenation include shortenings and partially hydrogenated oils that have improved oxidative stability.

The hydrogenation reaction requires a catalyst to speed up the reaction, hydrogen gas to provide the substrate, temperature control to initially heat up the oil to make it liquid and then cool the oil once the exothermic reaction is started, and agitation to mix the catalyst and substrates [45]. The oil used in hydrogenation must first be refined since contaminants will reduce the effectiveness of the catalysts. Hydrogenation is done as a batch or continuous process at temperatures ranging from 250°C to 300°C. Reduced nickel is the most common catalyst that is added at 0.01%–0.02%. The nickel is incorporated onto a porous support to provide a catalyst with high surface area that can be recovered by filtration. Mixing is a critical parameter since mass transfer of the reactants limits the reaction. The reaction takes 40–60 min, during which progress is monitored by change in the refractive index. Upon completion, catalysts are recovered by filtration so that they can be used in another reaction.

The mechanism of hydrogenation involves the unsaturated fatty acid associating with the catalyst, with metal–carbon complexes being formed at each end of the double bond (Figure 4.19, step 1). Hydrogen that is absorbed to the catalysts can then break one of the carbon–metal complexes to form a half-hydrogenated state, with the other carbon remaining linked to the catalysts (step 2). To complete hydrogenation, the half-hydrogenated state interacts with another hydrogen to break the remaining carbon–catalyst bond to produce a hydrogenated fatty acid (step 3). However, if hydrogen is not available, the reverse reaction can occur and the fatty acid is released from the catalyst and the double bond reforms (step 4). The double bond that reforms can be in the *cis* or *trans* configuration and can be at the same carbon number, or it can migrate to the adjacent carbon (e.g., a fatty acid with a double bond originally between carbons 9 and 10 can migrate to carbons 8 and 9 or 10 and 11). The propensity of the double bond to re-form is related to the concentration of hydrogen associated with the catalyst, with low hydrogen concentrations leading to the re-formation of the double bond and thus production of geometric and positional isomers. Thus, conditions such as low hydrogen pressure, low agitation, high temperature (reaction is faster than rate of hydrogen diffusion to the catalyst), and high catalyst concentrations (difficult to saturate catalyst with hydrogen) result in high levels of geometric and positional isomers. This can be problematic since *trans* fatty acids are negatively associated with cardiovascular disease.

The selectively of hydrogenation refers to the tendency of the hydrogenation process to hydrogenate one fatty acid faster than another (compared to random hydrogenation where all unsaturated fatty acids would be hydrogenated at similar rates). Hydrogenation of the most unsaturated fatty acids first is often desirable since this increases the oxidative stability of the oil with minimal formation of high-temperature-melting saturated triacylglycerols that cause problems with crystallization and texture. Selectivity occurs because the hydrogenation rate of polyunsaturated fatty acids is faster than monounsaturated fatty acids (partially due to the higher catalyst affinity

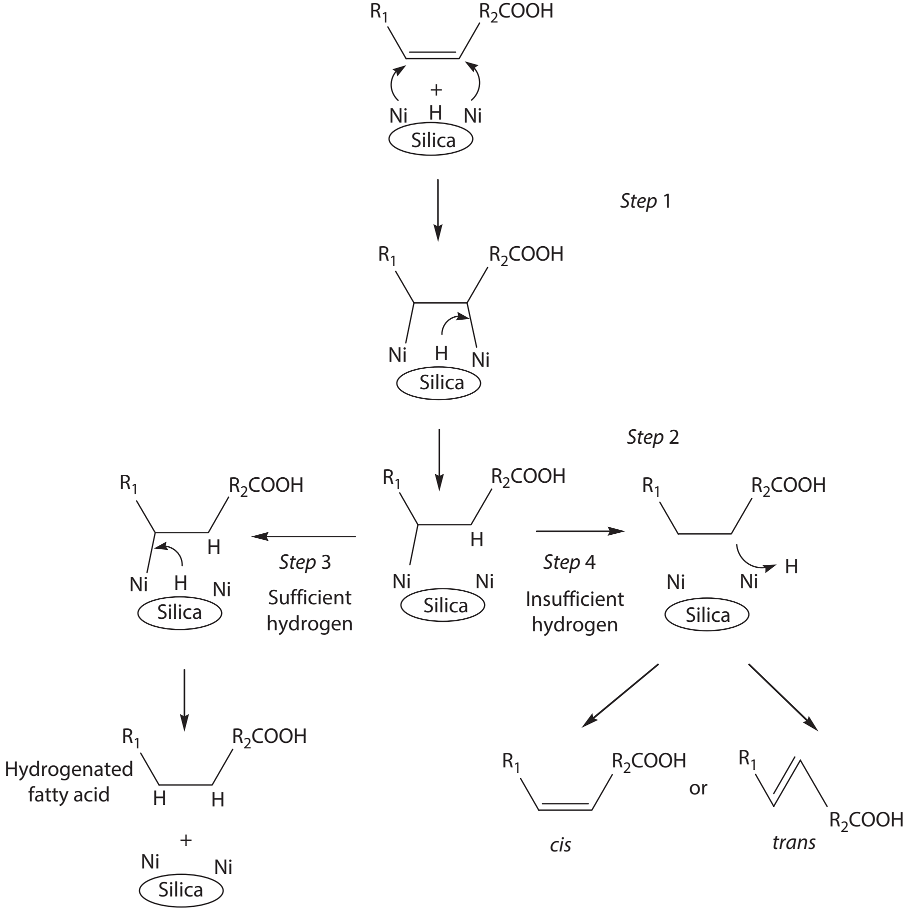


FIGURE 4.19 The pathways involved in hydrogenation that lead to formation of saturated fatty acids and *cis* and *trans* unsaturated fatty acids.

for pentadiene double bond systems than monounsaturated fatty acids). When hydrogen concentrations at the catalyst are low, hydrogenation is selective since polyunsaturated fatty acids are more rapidly hydrogenated than monounsaturated fatty acids. However, low hydrogen conditions also lead to high production of geometric and positional isomers, meaning that the lipid can contain high amounts of *trans* fatty acids.

4.8.6 Interesterification

Interesterification is a process that can change the melting profile of lipids without changing fatty acid composition [32,33]. Random interesterification works by rearranging the fatty acids to increase the number of different triacylglycerol types. Upon completion of random interesterification, all possible triacylglycerol combinations will be produced. This results in a change in the melting profile as new triacylglycerols are produced. In addition to alteration in melting profile, interesterification will also alter the crystallization behavior by making it more difficult for the lipid to form the most stable crystal type (e.g., β) since the triacylglycerol composition becomes more heterogeneous. Interesterification can be performed on mixtures of lipids such as a fat with a high-temperature melting range and an oil with a low-temperature melting range. If these two

lipid sources were simply blended, their melting profile could have a stair step appearance as the oil would melt first followed by the fat. Interesterification of these two lipids would create new triacylglycerols, with combinations of saturated and unsaturated fatty acids producing gradual melting throughout the plastic range. Another application would be to interesterify a lipid with a very homogeneous triacylglycerol composition to increase the heterogeneity of the triacylglycerols, a process that would widen the plastic range and make it more difficult for the lipid to form the most stable crystal types.

Interesterification does not always have to be random [32]. In directed interesterification, the reaction temperature is held low enough so that when highly saturated triacylglycerols are produced, they crystallize and are removed from participating in the reaction. This process would produce a liquid phase that is more unsaturated and a solid phase that is more saturated than the parent lipid. Interesterification can also be catalyzed by lipases. The advantage of lipases is that they can have specificity for different stereospecific locations on the triacylglycerol or for different fatty acids. This means that structured triacylglycerols can be produced with changes in fatty acid composition or triacylglycerol type (e.g., changes at *sn*-2 position). By altering fatty acid and/or triacylglycerol composition, these fats may have superior nutritional or physical properties. Interesterification can be performed by acidolysis, alcoholysis, glycerolysis, and transinteresterification [32]. Transinteresterification is the most common method used to alter the properties of food lipids. Alkylates of sodium (e.g., sodium ethylate) are commonly used to accelerate interesterification since they are inexpensive and active at low temperatures. The real catalyst for the reaction is thought to be a carbonyl anion of a diacylglycerol (Figure 4.20). The negative diacylglycerol can attack the slightly positive carbonyl group of a fatty acid on a triacylglycerol to form a transition-state complex. When interesterification takes place, the transition-state complex decomposes to transfer the fatty acid to the negative diacylglycerols plus the transfer of the anion to the site of the transferred fatty acid. This process can occur within the same (intraesterification) or a different (interesterification) triacylglycerol. To interesterify triacylglycerols, they must be very low in water, free fatty acids, and peroxides, which deactivate the catalyst. Random interesterification is performed at 100°C–150°C and is complete in 30–60 min. The reaction is stopped by the addition of water to inactivate the catalyst.

Interesterification was initially limited by its high cost and the low value of the resulting products such as shortenings and margarines. However, since the *trans* fatty acid labeling requirements prompted the removal of partially hydrogenated fats in foods, the utilization of interesterified oils has grown since their *trans* fatty acid composition is low because it is similar to their parent fats and oils.

4.8.7 Summary

Alteration of fatty acid profiles can alter the melting properties, oxidative stability, and nutritional quality of lipids.

- Alteration of fatty acids profiles can be accomplished by blending different sources or lipids or chemically modifying the fatty acid structure or triacylglycerol composition.
- Hydrogenation removes double bonds from fatty acids, which increases the melting ranges and oxidative stability.
- Hydrogenation can form nutritionally undesirable *trans* fatty acids.
- Interesterification rearranges fatty acids on triacylglycerol, which can alter the melting ranges.
- Interesterification can be used to produce solid fats with minimal *trans* fatty acid concentrations.

Diacylglycerol anion

FIGURE 4.20 Proposed mechanism of the interesterification reaction involving catalysis by the carbonyl anion of a diacylglycerol. (Adapted from Shahidi, F. and Wanasundara, J.P.K., *Crit. Rev. Food Sci. Nutr.*, 32, 67, 1992.)

4.9 FUNCTIONALITY OF TRIACYLGLYCEROLS IN FOODS

The ability of food scientists to improve the quality of food products depends on an improved understanding of the multiple roles that fats and oils play in determining their properties.

4.9.1 Texture

The influence of lipids on the texture of foods is largely determined by the nature of the lipid (e.g., solid vs. liquid) and the nature of the food matrix (e.g., bulk fat, emulsified fat, or structural fat). For liquid oils, such as cooking or salad oils, the texture is determined primarily by the viscosity of the oil over the temperature range of utilization. For partially crystalline fats, such as in chocolate, baked products, shortenings, butter, and margarine, the texture is mainly determined by the concentration, morphology, and interactions of the fat crystals [55]. In particular, the melting profile of the fat crystals plays a major role in determining properties such as texture, stability, spreadability, and mouthfeel. In O/W emulsions, the viscosity of the overall system is determined mainly by the concentration of oil droplets present rather than by the viscosity of the oil within the droplets [58]. The characteristic creamy texture of many food emulsions is determined by the presence of fat droplets, for example, creams, desserts, dressings, and mayonnaise. In W/O emulsions, the overall rheology of the system is largely determined by the rheology of the oil phase. In most food W/O emulsions, such as margarine, butter, and spreads, the oil phase is partially crystalline and has plastic-like properties. The rheology of these products is therefore determined by the solid fat content as well as the morphology and interactions of the fat crystals present, which in turn are governed by the crystallization and storage conditions [55]. For example, the "spreadability" of W/O emulsions such as margarines and butters is determined by the formation of a three-dimensional network of aggregated fat crystals in the continuous phase, which provides the product with mechanical rigidity. In many foods, the lipids form an integral part of a solid matrix that also contains various other components, for example, chocolate, cakes, cookies, crackers, biscuits, and cheese. The physical state of the lipids in these systems often plays an important role in determining their rheological properties, for example, firmness and snap.

4.9.2 Appearance

The characteristic appearance of many food products is strongly influenced by the presence of lipids. The appearance of pure lipids, such as cooking or salad oils, is mainly determined by the presence of pigmented impurities that absorb light, such as chlorophyll and carotenoids. Solid fats are often optically opaque because of the scattering of light by the fat crystals. The opacity of the fat depends on the concentration and size of the fat crystals present. The turbid, cloudy, or opaque appearance of food emulsions is a direct result of the immiscibility of oil and water, since this leads to a system where the droplets of one phase are dispersed in the other phase. Food emulsions usually appear optically opaque because the light passing through them is scattered by the droplets [57]. The intensity of the scattering depends on the concentration, size, and refractive index of the droplets present, so that both the color and opacity of food emulsions are strongly influenced by the presence of the lipid phase.

4.9.3 Flavor

The perceived flavor of a food is strongly influenced by the type and concentration of the lipids present. Triacylglycerols are relatively large molecules that have a low volatility and hence little inherent flavor. Nevertheless, different natural sources of edible fats and oils do have distinctive flavor profiles because of the characteristic volatile breakdown products and impurities that they contain. The flavor of many food products is also indirectly influenced by the presence of lipids because flavor

compounds can partition between oil, water, and gaseous phases according to their polarities and volatilities [57]. For this reason, the perceived aroma and taste of foods are often strongly influenced by the type and concentration of the lipids present.

Lipids also influence the mouthfeel of many food products [91,92]. Liquid oils may coat the tongue during mastication, which provides a characteristic oily mouthfeel. If a lipid phase contains fat crystals that are above a certain size, then an undesirable “gritty” mouthfeel is detected. The melting of fat crystals in the mouth causes a cooling sensation, which is an important sensory attribute of many fatty foods [88,89].

4.10 CHEMICAL DETERIORATION OF LIPIDS, HYDROLYTIC REACTIONS

Free fatty acids cause problems in foods because they can produce off-flavor, reduce oxidative stability, cause foaming, and reduce the smoke point (the temperature at which an oil begins to smoke). If the formation of free fatty acids results in the development of off-flavors (e.g., formation of short-chain free fatty acids in dairy products), this is known as *hydrolytic rancidity*. However, free fatty acids are sometimes desirable in products such as cheeses where they contribute to the flavor profiles.

Free fatty acids can be produced by enzymes called lipases. In living tissues, the activity of lipases is strictly controlled, since fatty acids can be cytotoxic by disrupting cellular membrane integrity. During the processing and storage of the biological tissues used as raw materials for foods, cellular structure and biochemical control mechanisms can be destroyed, and lipases can become active. A good example of this is seen in the production of olive oil, where the oil from the first pressing is low in free fatty acid concentrations. Oils from subsequent pressing and extraction of the pomace have higher free fatty acid concentrations as the cellular matrix is further disrupted and the activated lipases have time to hydrolyze the triacylglycerols. Triacylglycerol hydrolysis also occurs in frying oils due to the high processing temperatures and the introduction of water from the fried food. As the free fatty acid content of the frying oil increases, smoke point and oxidative stability decrease, and the tendency for foaming increases. Commercial frying oils are filtered on a regular basis with absorbents that are capable of binding free fatty acids to increase the useable life of the oil. Triacylglycerol hydrolysis will also occur at extreme pH values.

4.11 CHEMICAL DETERIORATION OF LIPIDS, OXIDATIVE REACTIONS

“Lipid oxidation” is a general term that is used to describe a complex sequence of chemical changes that result from the interaction of lipids with oxygen [25,61]. Triacylglycerols and phospholipids have low volatility and thus do not directly contribute to the aroma of foods. During lipid oxidation reactions, the fatty acids esterified to triacylglycerols and phospholipids will decompose to form small, volatile molecules that produce the off-aromas known as *oxidative rancidity*. In general, these volatile compounds are detrimental to food quality, although there are some food products such as fried foods, dried cereal, and cheeses where small amounts of lipid oxidation products are important positive components of their flavor profile.

4.11.1 Chemical Pathway

The centerpiece of these reactions is the molecular species known as *free radicals*. Free radicals are molecules or atoms that have unpaired electrons. Free-radical species can vary greatly in their energy. Radicals such as the hydroxyl radical (•OH) have very high energy and can oxidize virtually any molecule by causing hydrogen abstraction. Other molecules, such as the antioxidant α-tocopherol, can form free radicals with low energy. These antioxidants can slow down oxidation reactions by forming low-energy radicals that cannot attack molecules such as unsaturated fatty acids.

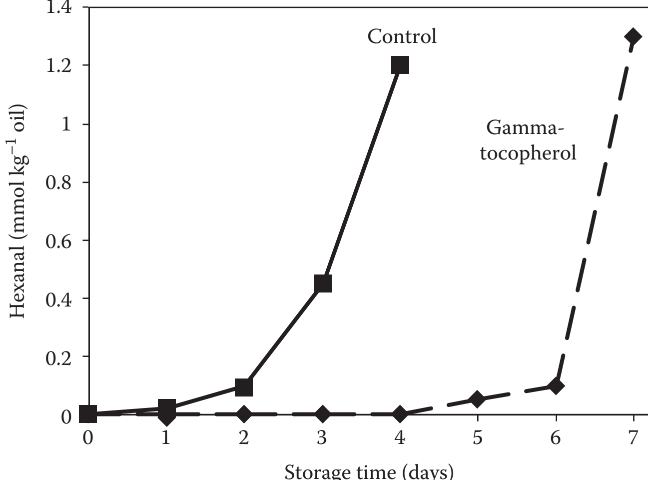


FIGURE 4.21 Impact of gamma tocopherol on the lag phase of the oxidation of corn oil-in-water emulsion. (Adapted from Huang, S.W. et al., *J. Agric. Food Chem.*, 42, 2108, 1994.)

The kinetics of lipid oxidation in foods often has a lag phase followed by an exponential increase in oxidation rate. The length of the lag phase is very important to food processors since this is the period where rancidity is not detected and the quality of the food is high. Once the exponential phase is reach, lipid oxidation proceeds rapidly and off-aroma development quickly follows. The length of the lag phase of oxidation will increase with decreasing temperature, oxygen concentrations, fatty acid unsaturation, activity of prooxidants, and increasing concentrations of antioxidants. Figure 4.21 shows how delta-tocopherol can increase the lag phase of the oxidation of a corn O/W emulsion [42].

The pathway of lipid oxidation has been simplified by dividing the reaction into three steps: initiation, propagation, and termination.

4.11.1.1 Initiation

This step describes the abstraction of a hydrogen from a fatty acid to form a fatty acid radical known as the alkyl radical ($L^{\bullet}$). Once the alkyl radical forms, the free radical is stabilized by its delocalization over the double bonds, resulting in double bond shifting and, in the case of polyunsaturated fatty acids, the formation of conjugated double bonds. This shift in location can produce double bonds in either the *cis* or *trans* configuration, with the latter predominating due to its greater stability. Figure 4.22 shows the initiation steps for hydrogen abstraction from the methylene-interrupted carbon of linoleic acid with double bond rearrangement, producing two isomers. When hydrogen is abstracted from oleic acid, the alkyl radical can exist at four different locations (Figure 4.23).

The ease of formation of fatty acid radicals increases with increasing unsaturation. The bond dissociation energy for the carbon–hydrogen covalent bond in an aliphatic chain is 98 kcal mol^{-1}. If a carbon atom is adjacent to an electron-rich double bond, the carbon–hydrogen covalent bond becomes weaker with the bond dissociation energy deceasing to 89 kcal mol^{-1}. In polyunsaturated fatty acids, the double bonds are in a pentadiene configuration with a methylene-interrupted carbon (Figure 4.24). Since the carbon–hydrogen covalent bond of the methylene-interrupted carbon is weakened by two double bonds, its bond dissociation energy is even lower at 80 cal mol^{-1}. As the bond dissociation energy of the carbon–hydrogen bond decreases, hydrogen abstraction becomes easier and lipid oxidation is faster. Linoleic acid (18:2) has been estimated to be 10–40 times more

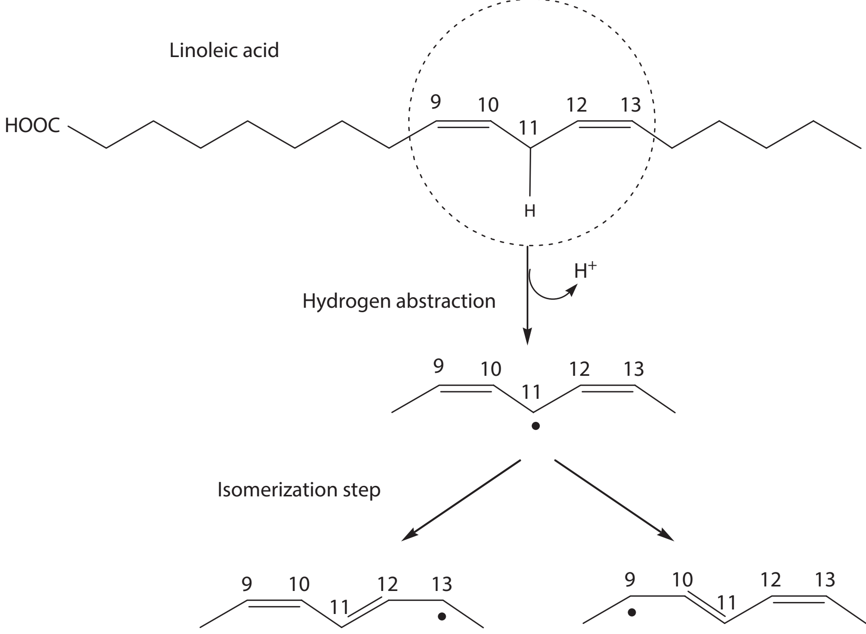


FIGURE 4.22 Initiation step of lipid oxidation for linoleic acid.

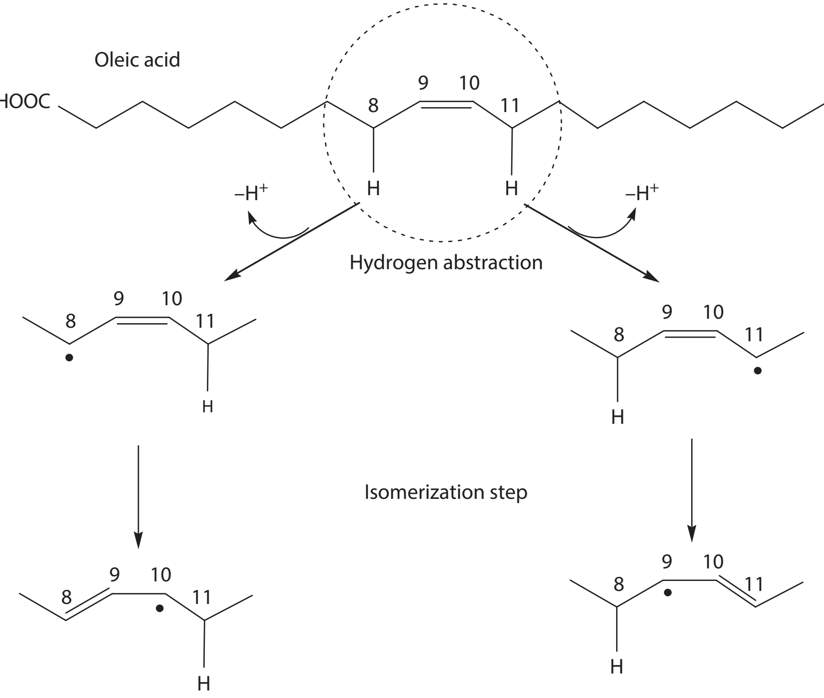


FIGURE 4.23 Initiation step of lipid oxidation for oleic acid.

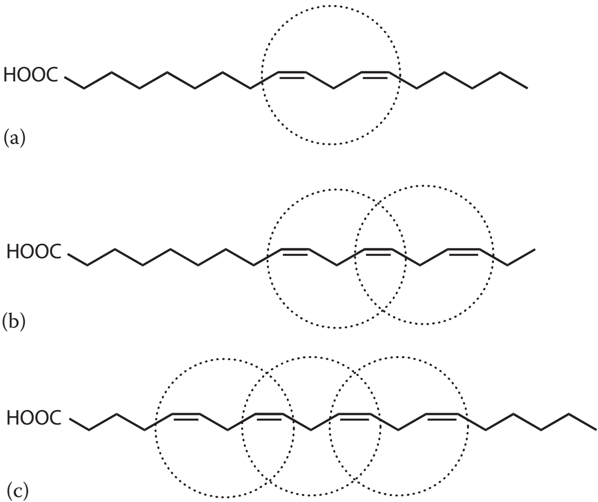


FIGURE 4.24 Pentadienes of (a) linoleic, (b) linolenic, and (c) arachadonic acids.

susceptible to oxidation than oleic acid (18:1). As additional double bonds are added onto polyunsaturated fatty acids, an additional methylene-interrupted carbon is added, resulting in the addition of another site for hydrogen abstraction. For example, linoleic (18:2) has one methylene-interrupted carbon, while linolenic (18:3) has two and arachadonic (20:4) has three (Figure 4.24). In most cases, oxidation rates double with the addition of a methylene-interrupted carbon. Thus, linolenic oxidizes twice as fast as linoleic and arachidonic oxidizes twice as fast as linolenic (four times faster than linoleic).

4.11.1.2 Propagation

The first step of propagation involves the addition of oxygen to the alkyl radical. Atmospheric or triplet oxygen is a biradical because it contains two electrons with the same spin direction that cannot exist in the same spin orbital. The free radicals on triplet oxygen are of low energy and rarely cause hydrogen abstraction. However, the free radicals on oxygen can react with the alkyl radical at a diffusion-limited rate. The combination of the alkyl radical with one of the radicals on triplet oxygen results in the formation of a covalent bond. The other radical on the oxygen remains free. The resulting radical is known as a *peroxyl radical* (LOO•). The high energy of peroxyl radicals allows them to promote the abstraction of a hydrogen from another molecule. Since the carbon–hydrogen covalent bond of unsaturated fatty acids is weak, they are susceptible to attack from peroxyl radicals. Hydrogen addition to the peroxyl radical results in the formation of a fatty acid hydroperoxide (LOOH) and the formation of a new alkyl radical on another fatty acid. Thus the reaction is propagated from one fatty acid to another. A schematic of this pathway for two linoleic molecules is shown in Figure 4.25. The location of the lipid hydroperoxide will correspond to the location of the original alkyl radicals, and thus linoleate will produce four hydroperoxides, and oleate will form two.

4.11.1.3 Termination

This reaction describes the combination of two radicals to form nonradical species. In the presence of oxygen, the predominant fatty acid is the peroxyl radical, since oxygen will be added onto alkyl radicals at diffusion-limited rates. Thus, under atmospheric conditions, termination reactions will occur between two peroxyl radicals. In low oxygen environments (e.g., frying oils), termination reactions can occur between alkyl radicals to form fatty acid dimers (Figure 4.26).

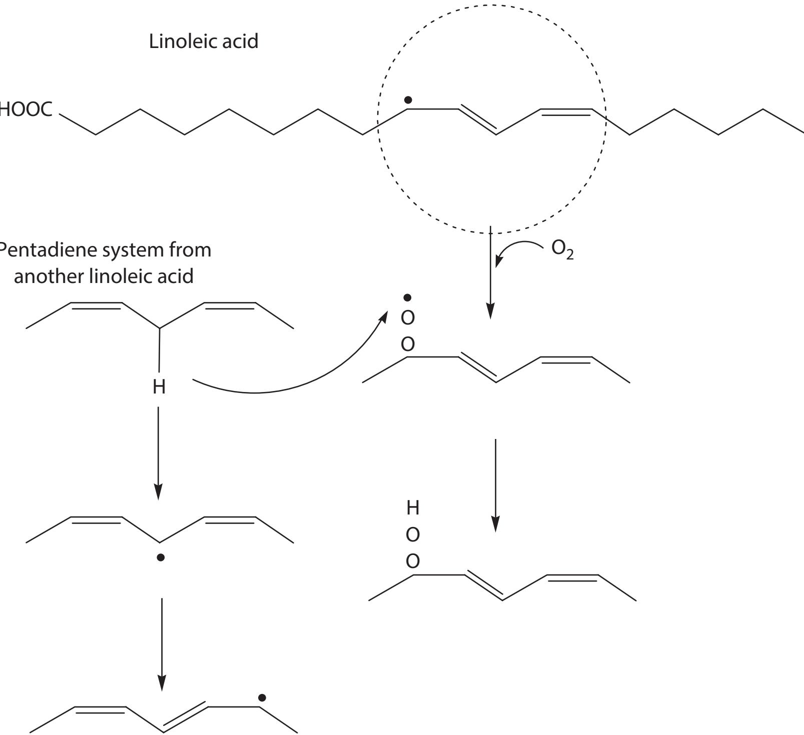


FIGURE 4.25 Propagation step of lipid oxidation for linoleic acid.

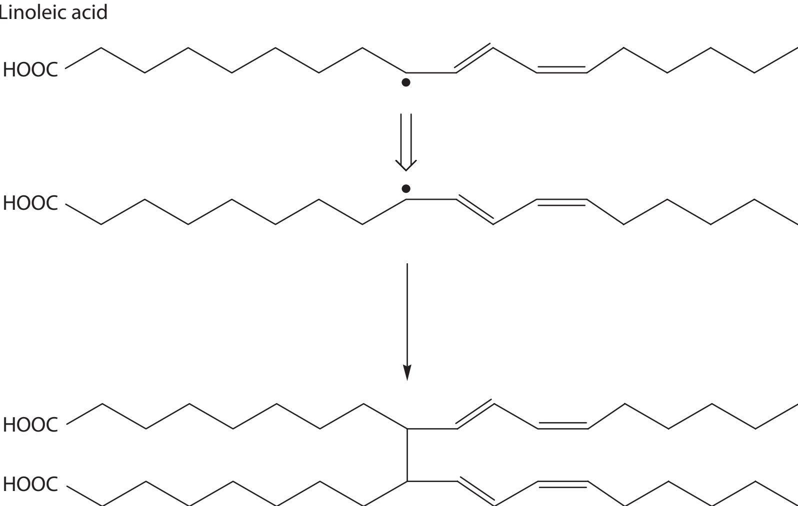


FIGURE 4.26 Example of a termination step of lipid oxidation under conditions of low oxygen concentrations.

4.11.2 Prooxidants

Lipid oxidation is often referred to as autoxidation. The prefix "auto" means "self-acting," and thus the term "autoxidation" has been used to describe the self-perpetuating generation of free radicals from unsaturated fatty acids in the presence of oxygen that occurs in lipid oxidation. In the initiation step, abstraction of hydrogen from unsaturated fatty acids results in the production of a single

free radical. The addition of oxygen to the alkyl radical to form a peroxyl radical and subsequent abstraction of hydrogen from another fatty acid or antioxidant to form a lipid hydroperoxide in the propagation step do not result in the formation of additional free radicals. Thus if "autoxidation" was the only reaction in lipid oxidation, the formation of oxidation products would increase linearly from time zero. However, in most foods the lag phase is followed by a rapid exponential increase in oxidation. This indicates that there are other reactions in lipid oxidation that produce additional free radicals.

Prooxidants, which are found in virtually all food systems, are compounds or factors that cause or accelerate lipid oxidation. Many prooxidants are not true catalysts, since they are altered during the reaction (e.g., singlet oxygen is converted to a hydroperoxide, and ferrous iron is converted to ferric). Prooxidants can accelerate lipid oxidation by direct interactions with unsaturated fatty acids to form lipid hydroperoxides (e.g., lipoxygenases and singlet oxygen) or by promoting the formation of free radicals (e.g., transition-metal- or ultraviolet-light-promoted hydroperoxide decomposition). It should be noted that lipid hydroperoxides do not contribute to off-aromas and thus do not directly cause rancidity. However, hydroperoxides are important substrates for rancidity since their decomposition results in the scission of the fatty acid to produce the low-molecular-weight, volatile compounds that produce off-aromas. The major prooxidants in foods are discussed.

4.11.2.1 Prooxidant That Promote Formation of Lipid Hydroperoxides

4.11.2.1.1 Singlet Oxygen

As mentioned earlier, triplet oxygen ($^3O_2$) is a biradical because its two electrons in the antibonding 2p orbital have the same (parallel or antiparallel) spin direction (Figure 4.27). The Pauli exclusion principle states that two electrons with the same spin direction cannot exist in the same electron orbital. Triplet oxygen cannot react directly with the electrons in the orbital of another molecule unless its electrons have matching parallel spin directions (two electrons in the orbital of a nonradical molecule would have opposite spin directions). If the electrons in the antibonding 2p orbital have opposite spin directions, oxygen is referred to as *singlet oxygen* ($^1O_2$). Singlet oxygen can exist in five different configurations, with the most common in foods being the $^1\Delta$ state where the electrons exist in the same orbital (for detailed description see Ref. [61]). Because singlet oxygen is more electrophilic than triplet oxygen, it can react with high-electron-density double bonds. Since the electrons in singlet oxygen match the spin direction of the electron in double bonds, it can react with an unsaturated fatty acid to directly form lipid hydroperoxides 1500 times faster than triplet oxygen. Singlet oxygen can react with either carbon at the end of a double bond, with the double bond then shifting to form a *trans* double bond. This means that oxidation of linoleate by singlet oxygen can produce four different hydroperoxides (Figure 4.27) compared to the typical two hydroperoxides produced in the propagation step of lipid oxidation (Figure 4.22). These different hydroperoxide locations will lead to the formation of several unique fatty acid decomposition products as will be discussed later.

Singlet oxygen is most commonly produced by photosensitization. Chlorophyll, riboflavin, and myoglobin are photosensitizers that can absorb energy from light to form an excited singlet state. The excited singlet state of the photosensitizer can then undergo intersystem crossing to produce an excited triplet state. The excited triplet state can react directly with substrates such as unsaturated fatty acids and abstract a hydrogen to cause initiation of lipid oxidation. This pathway is known as type 1 and will produce the same lipid hydroperoxides seen in the propagation step described previously for autoxidation. The excited triplet state of the photosensitizer can also react with triplet oxygen to form singlet oxygen and singlet state of the photosensitizer in the type 2 pathway. Type 1 and type 2 pathways are dependent on oxygen concentrations, with type 2 predominating in high oxygen environments. Singlet oxygen can also be formed chemically or enzymatically, or by the decomposition of hydroperoxides. However, production by photosensitization is believed to be the major pathway in foods.

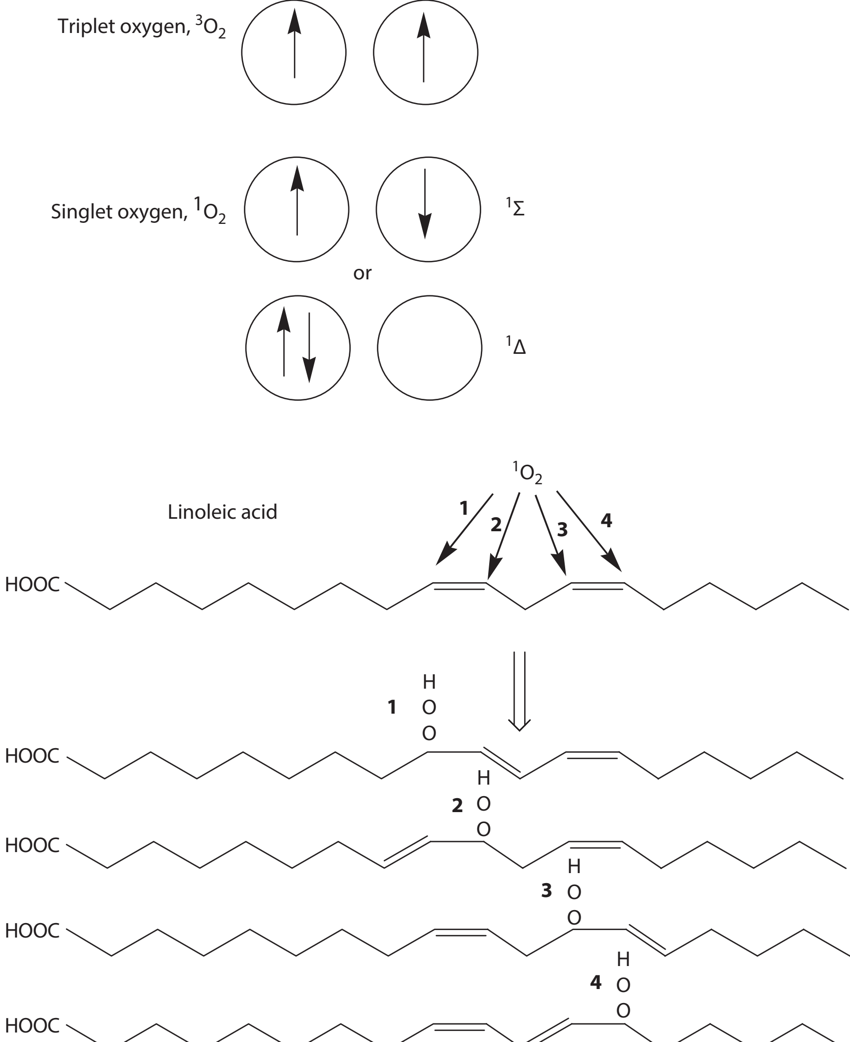


FIGURE 4.27 Singlet oxygen and singlet oxygen–promoted hydroperoxide formation on linoleic acid. (From Min, D.B. and Boff, J.M., Lipid oxidation in edible oil, in: *Food Lipid, Chemistry, Nutrition and Biotechnology*, eds. C.C. Akoh and D.B. Min, Marcel Dekker, New York, 2002, pp. 335–364.)

4.11.2.1.2 Lipoxygenase

Numerous plant tissues and selective animal tissues contain enzymes known as lipoxygenases, which produce lipid hydroperoxides. Lipoxygenases (LOX) from plant seed such as soybeans and peas exist as several isoforms (for review see Ref. [95]). In soybeans, isoform L-1 primarily reacts with free fatty acids and produces hydroperoxides at carbon 13 in both linoleic and linolenic acid. Isoform L-2 produces hydroperoxides at positions 9 and 13 and is active on both free and esterified linoleic and linolenic acid. Plant LOXs are cytoplasmic enzymes that contain a nonheme iron. The iron in inactive LOX is in the ferrous state. Activation occurs by the oxidation of the iron to the ferric state, a process that is usually promoted by a hydroperoxide. LOX catalyzes the abstraction of hydrogen from the methylene-interrupted carbon to form the alkyl radical and the conversion of the LOX iron back to the ferrous state. The enzyme can then control the stereospecific location where oxygen adds on to the alkyl radical to the peroxyl radical. An electron from the ferrous iron is then

donated to the peroxyl radical to form a peroxyl anion. When the peroxyl anion reacts with hydrogen to form the hydroperoxide, the fatty acid is released from the enzyme. Once oxygen is depleted from the system, the enzyme abstracts a hydrogen from a fatty acid and the iron is converted to ferrous. Since no oxygen is present, the alkyl radical is released, and LOX is returned to its inactive form. Lipoxygenases have also been reported in animal tissues especially those highly associated with the circulatory system (e.g., fish gills [28]).

4.11.2.2 Prooxidants That Promote Formation of Free Radicals

4.11.2.2.1 Ionizing Radiation

Foods are sometimes subjected to ionizing radiation to destroy pathogens and extend their shelf-life. However, ionizing radiation can convert molecules to excited states, which produce free radicals such as the production of hydroxyl radical ($^{\bullet}OH$) from water. The hydroxyl radical is the most reactive radical known, so it is capable of abstracting hydrogen from lipids as well as molecules such as proteins and DNA. Therefore, it is not surprising that irradiation of foods, especially muscle foods that are high in lipids and prooxidants, can increase oxidative rancidity.

4.11.2.3 Prooxidants That Promote Decomposition of Hydroperoxides

Lipid hydroperoxides are found in essentially all lipid-containing foods. Hydrogen peroxide is also found in food when it is utilized as a processing aid and when enzymes such as superoxide dismutase produce it in foods such as meats, poultry, and seafood. High-quality lipid-containing foods contain 1–100 nmole lipid hydroperoxide per gram of lipid. These are an estimated 40–1000 times greater than the estimated lipid hydroperoxide concentrations found *in vivo* (e.g., plasma lipids), suggesting that oxidation occurs during the extraction and refining of fats and oils [17]. Lipid hydroperoxides can be decomposed by high temperatures during thermal processing or by a variety of prooxidants. Upon decomposition, they produce additional radicals, a factor that could be responsible for the exponential increase in oxidation that is seen in many foods. The decomposition of lipid hydroperoxides also leads to the formation of alkoxyl radicals that can enter into β-scission reactions, which are responsible for decomposing fatty acids into low-molecular-weight compounds that are volatile enough to be perceived as rancidity.

4.11.2.3.1 Transition Metals

Transition metals are found in all foods since they are common constituents of raw food materials, water, ingredients, processing equipment, and packaging materials. Transition metals are one of the major food prooxidants that decrease the oxidative stability of foods and biological tissues through their ability to decompose hydroperoxides into free radicals [29,36]. These reactive metals decompose hydrogen and lipid peroxides through the following redox cycling pathway:

$$Mn^{n+} + LOOH \text{ or } HOOH \rightarrow Mn^{n+1} + LO^{\bullet} \text{ or } HO^{\bullet} + OH^{-}$$

$$Mn^{n+1} + LOOH \rightarrow Mn^{n+} + LOO^{\bullet} + H^{+}$$

where

Mn^{n+} and Mn^{n+1} are transition metals in their reduced and oxidized states
LOOH and HOOH are lipid and hydrogen peroxide
$LO^{\bullet}$, $HO^{\bullet}$, and $LOO^{\bullet}$ are alkoxyl, hydroxyl, and peroxyl radicals, respectively

Hydroxyl radical is produced from hydrogen peroxide, while alkoxyl radicals are produced from lipid hydroperoxides. When iron and hydrogen peroxide are involved in this pathway, it is known as the *Fenton reaction*. The concentration, chemical state, and the type of metal will influence the rate of hydroperoxide decomposition. Copper and iron are the most common transition metals in foods capable of participating in these reactions, with iron generally being found at greater concentrations than copper. Copper is more reactive with cuprous ions (Cu^{1+}), decomposing hydrogen peroxide over 50-fold faster

than ferrous ions (Fe^{2+}). Redox state is also important, with Fe^{2+} decomposing hydrogen peroxide over 10^5 times faster than Fe^{3+}. In addition, Fe^{2+} is more water soluble than Fe^{3+}, meaning that it is more available to promote hydroperoxide decomposition in water-based foods. The peroxide type is also important, with Fe^{2+} decomposing lipid hydroperoxides ~10 times faster than hydrogen peroxide [29,36].

Since the reduced state of transition metals are more efficient at decomposing hydroperoxides, reducing compounds capable of promoting the redox cycling of transition metals can promote lipid oxidation. Examples of prooxidative reductants include superoxide anion ($O_2^{-\bullet}$) and ascorbic acid. Superoxide anion is produced by the addition of an electron to triplet oxygen. The added electron in superoxide anion can then be transferred to a transition metal to cause its reduction. Superoxide anion is produced by enzymes, by the release of oxygen from oxymyoglobin to produce metmyoglobin, or by cells such as phagocytes. The redox cycling of iron by superoxide anion to promote lipid oxidation is shown in the following pathways. This pathway is known as the Haber–Weiss reaction.

$$Fe^{3+} + O_2^{-\bullet} \rightarrow Fe^{+2} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{+3} + {}^{\bullet}OH + OH^-$$

$$\text{Net: } O_2^{-\bullet} + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + OH^-$$

Ascorbic acid can also participate in Haber–Weiss-like reactions; however, unlike superoxide anion, ascorbic acid can also act as an antioxidant. Thus, at high ascorbate concentration, its antioxidant activity may outweigh its ability to accelerate metal-promoted oxidation, resulting in a net antioxidant effect.

Transition metals associated with proteins can also promote hydroperoxide decomposition. The heme proteins are the best studied of this group, with the iron in myoglobin, hemoglobin, peroxidases, and catalase being able to promote both hydrogen and lipid hydroperoxide decomposition. In some cases, heme proteins have been suggested to cause homolytic scission of lipid hydroperoxides, meaning that the breakdown of the hydroperoxide will produce two free radicals (hydroxyl and alkoxyl). Thermal denaturation of these proteins can increase their prooxidant activity, presumably due to increased exposure of the heme iron that is able to more effectively interact with hydroperoxides. Thermal denaturation of myoglobin is believed to be one of the factors that cause the acceleration of lipid oxidation in cooked meats, a problem known as *warmed-over flavor*.

4.11.2.3.2 Light and Elevated Temperatures

UV and visible light can promote the decomposition of hydroperoxides to produce free radicals. Thus, packaging to decrease light exposure can reduce lipid oxidation rates. Increasing the temperature increases the lipid oxidation rate in essentially all foods, so temperature control is an important way to control rancidity. Elevated temperatures will also decompose lipid hydroperoxides. In fact, lipid hydroperoxide accumulation is often not seen in frying oils since the hydroperoxides breakdown very rapidly after formation.

4.11.3 Formation of Lipid Oxidation Decomposition Products

Once lipid hydroperoxides are decomposed into alkoxyl radicals, a number of different reaction schemes can occur. The products of these reaction schemes will depend on the fatty acid type as well as the location of the hydroperoxide on the fatty acid. In addition, decomposition products can be unsaturated and even have intact pentadiene structures, meaning that the oxidation products can be further oxidized. This results in literally hundreds of different fatty acid decomposition products. Since the type of fatty acid decomposition products will depend on the fatty acid composition of the food, lipid oxidation can have different effects on sensory properties. For example, oxidation of vegetable oils that have predominately ω-6 fatty acids will produce “grassy” and “beany” odors, while oxidation of the long chain ω-3 fatty acids in marine oils will produce “fishy” aromas.

One of the reasons why lipid hydroperoxide decomposition will cause decomposition of the fatty acid is that these pathways produce the alkoxyl radical (LO•). The alkoxyl radical is more energetic than both the alkyl and peroxyl radicals, meaning that it can attack other unsaturated fatty acids, other pentadiene groups within the same fatty acid, or the covalent bonds adjacent to the alkoxyl radical. This last reaction, known as the β-*scission reaction*, is important to food quality since it causes fatty acids to decompose into low-molecular-weight compounds, which are perceived as rancidity.

4.11.3.1 β-Scission Reaction

The highly energetic alkoxyl radical has the ability to abstract a hydrogen from the carbon–carbon bond on either side of the oxygen radical. Unesterified linoleic acid will be used to demonstrate more details of this reaction. One should remember that the decomposition product on the carboxylic acid end of the fatty acid would usually be esterified to the glycerol of a triacylglycerol or phospholipid. Thus, this decomposition product would not be volatile and thus would not contribute to rancidity unless it undergoes further decomposition reactions to form low-molecular-weight compounds. Figure 4.28 shows the oxidation of linoleic acid to produce a hydroperoxides at carbon 9. In step 1, the hydroperoxide decomposes into the alkoxyl radical. Step 2 shows the β-scission reaction that occurs because the high-energy alkoxyl radical can abstract an electron from adjacent carbon–carbon bonds to cleave the fatty acid chain. β-Scission breaks the fatty acid on either side of the alkoxyl radical. If cleavage of the fatty acid is on the

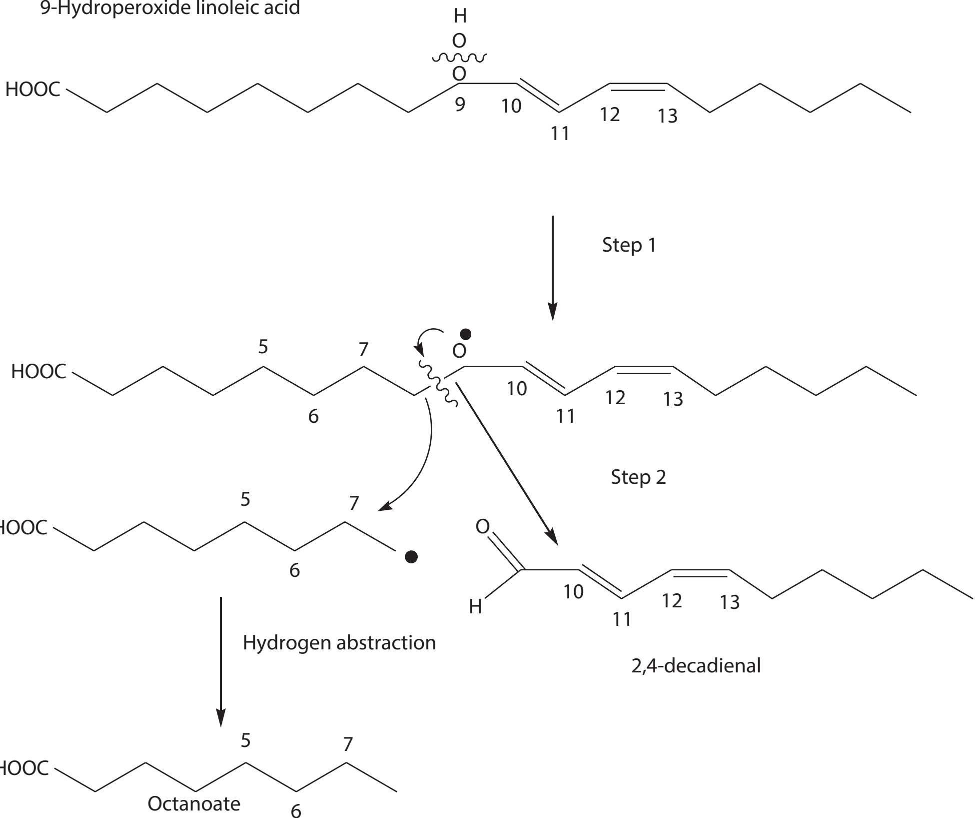


FIGURE 4.28 β-Scission decomposition products produced from 9-linoleic acid hydroperoxide when fatty acid cleavage occurs on the carboxylic acid side of the hydroperoxide. (From Frankel, E.N., *Lipid Oxidation,* 2nd edn., Oily Press, Scotland, 2005.)

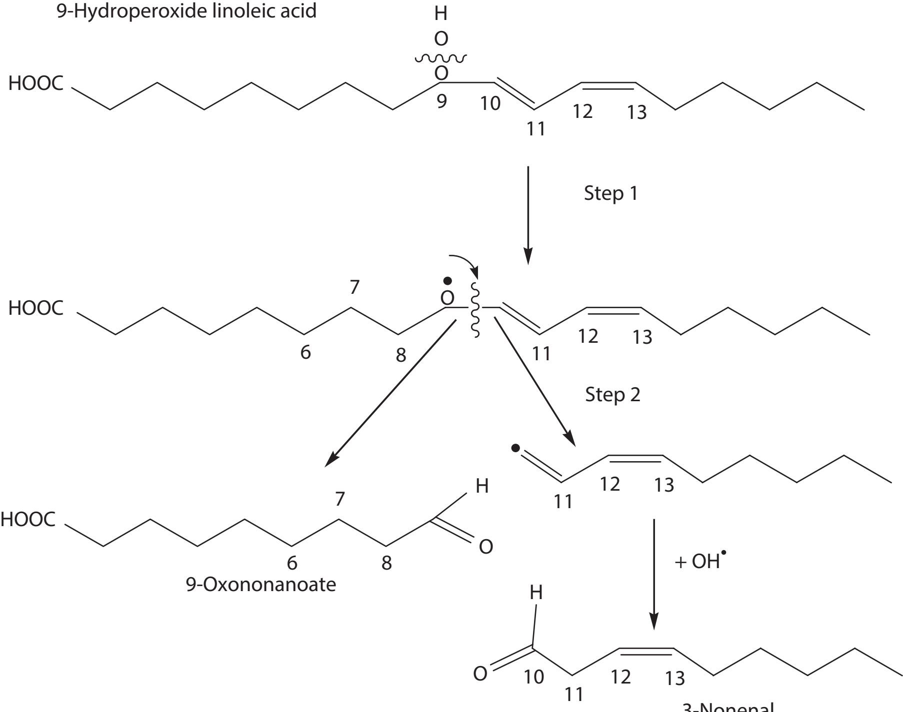


FIGURE 4.29 β-Scission decomposition products produced from 9-linoleic acid hydroperoxide when fatty acid cleavage occurs on the methyl side of the hydroperoxide. (From Frankel, E.N., *Lipid Oxidation,* 2nd edn., Oily Press, Scotland, 2005.)

carboxylic acid end of the fatty acid, the decomposition products will be octanoate and 2, 4-decadienal (Figure 4.28). Cleavage on the opposite side of the alkoxyl radical (Figure 4.29, methyl end of the fatty acid), 9-oxononanoate and a nine-carbon vinyl radical will be produced. Vinyl radicals can interact with hydroxyl radicals to form aldehydes, thus producing 3-nonenal. Similar pathways will occur if the hydroperoxide is on carbon 13. Cleavage on the carboxylic acid end will produce 12-oxo-9-dodecenoate and hexanal. Cleavage on the methyl end of the fatty acid will produce 13-oxo-9,11-tridecadienoate and pentane.

When singlet oxygen attacks linoleic acid, it will form hydroperoxides at all of the carbons associated with double bonds (Figure 4.27). This means that it will form hydroperoxides at carbons 9 and 13, as in free-radical-initiated oxidation, plus hydroperoxides at carbons 10 and 12. Typical products from the β-scission reaction from an alkoxyl radical at carbon 10 will produce 9-oxononanoate and 3-nonenal from cleavage on the carboxylic acid end and 10-oxo-8-decenoate and 2-octene from cleavage at the methyl end of the fatty acid. Typical products from the β-scission reaction from an alkoxyl radical at carbon 12 will produce 9-undecenoate and 2-heptenal from cleavage on the carboxylic acid end and 12-oxo-9-dodecenoate and hexanal from cleavage at the methyl end of the fatty acid.

As one can see from the above discussion on the β-scission products and other free-radical reactions of linoleic acid, numerous products can be formed. For a detailed discussion on β-scission decomposition products, see Frankel [25]. Pathways similar to this will occur with other unsaturated fatty acids producing additional unique compounds. In addition, the decomposition products often contain double bonds and, in some cases, intact pentadiene systems. These double bond systems can undergo hydrogen abstraction or singlet oxygen attack, which will result in the formation of additional decomposition products. While the above discussion shows the theoretical decomposition

products of linoleic acid, in reality not all of these products have been detected. This is likely due to the ability of these compounds to undergo additional decomposition reactions.

4.11.3.2 Additional Reactions of Fatty Acid Decomposition Products

In addition to the fatty acid hydroperoxide products described previously, fatty acids radicals can undergo a series of other reactions to form compounds such as olefins, alcohols, carboxylic acids, ketones, epoxides, and cyclic products (for review see Ref. [25]). Alkyl radicals will react with hydrogen and hydroxyl radicals to produce olefins and alcohols. As mentioned earlier, alkoxyl radicals are high-energy radicals. Thus, they can abstract hydrogen from other molecules such as unsaturated fatty acids or antioxidants to produce fatty acid alcohols. Alkoxyl radicals can also lose an electron and be converted to a ketone or link to an adjacent carbon to form an epoxide. Peroxyl radicals can react with double bonds within the same fatty acid to produce cyclic products such as bicyclic endoperoxides.

Aldehydes produced from the oxidative decomposition of fatty acids are important because of their impact on off-flavor development. The aldehydes can also react with nucleophilic food components. In particular, they interact with sulfhydryls and amines in proteins, which may alter the functionality of the protein. One example is the ability of unsaturated aldehydes to react with histidine in myoglobin via a Michael addition–type reaction [22]. This reaction is thought to contribute to the conversion of myoglobin to metmyoglobin to produce meat discoloration.

Fatty acid decomposition products can also form dimers and polymers [25]. This can occur via radical–radical termination reactions. In the presence of oxygen (peroxyl and alkoxyl radicals), polymerization involves the formation of peroxide or ether linkages. In the absence of oxygen (alkyl radicals), polymerization occurs through carbon–carbon crosslinks. These carbon–carbon crosslinks often occur when oils are subjected to high temperatures where oxygen solubility is low. Methyl esters of fatty acids will crosslink much more readily than the fatty acids in triacylglycerols. Crosslinking of fatty acids in triacylglycerols is generally significant only in frying oils.

4.11.3.3 Cholesterol Oxidation

Cholesterol contains a double bond between carbons 5 and 6 (Figure 4.4). As with fatty acids, this double bond is susceptible to free-radical attack and can undergo decomposition reactions to produce alcohols, ketones, and epoxides [81]. The most notable of the cholesterol oxidation pathway begins with the formation of a hydroperoxide at carbon 7. This hydroperoxide can decompose into an alkoxyl radical, which in turn can undergo rearrangements to 5,6 epoxides, 7-hydroxylcholesterol, and 7-ketocholesterol. These cholesterol oxidation products are potentially cytotoxic and have been linked to the development of atherosclerosis. Cholesterol oxidation products have primarily been found in animal food products that have undergone thermal processing, such as cooked meats, tallow, lard, and butter, as well as dried dairy and egg products.

4.11.4 Antioxidants

Oxidative stress occurs in all organisms in an oxygenated environment. Thus biological systems have developed a wide variety of antioxidant systems to protect against oxidation. There is no uniform definition of an antioxidant because there are numerous chemical mechanisms that can be used to inhibit oxidation. The biological tissues from which foods are obtained generally contain several endogenous antioxidant systems. Unfortunately, food processing operations can remove antioxidants or cause additional oxidative stress that can overcome the endogenous antioxidants systems in the food. Therefore it is common to incorporate additional antioxidant protection into processed foods. Antioxidant mechanisms of compounds that are used to increase the oxidative stability of foods include the control of free radicals, prooxidants, and oxidation intermediates.

4.11.4.1 Control of Free Radicals

Many antioxidants slow lipid oxidation by inactivating or scavenging free radicals, thereby inhibiting initiation, propagation, and β-scission reactions. Free-radical scavengers (FRSs) or chain-breaking antioxidants can interact with peroxyl ($LOO^\bullet$) and alkoxyl ($LO^\bullet$) radicals by the following reactions.

$$LOO^\bullet \text{ or } LO^\bullet + FRS \rightarrow LOOH \text{ or } LOH + FRS^\bullet$$

FRSs inhibit lipid oxidation by reacting faster with free radicals than unsaturated fatty acids. FRSs are thought to interact mainly with peroxyl radicals because propagation is the slowest step of lipid oxidation, meaning that peroxyl radicals are often found in the greatest concentration of all radicals in the systems; peroxyl radicals have lower energies than radicals such as alkoxyl radicals [10] and therefore preferentially react with the low-energy hydrogens of FRSs than polyunsaturated fatty acids; and FRSs are generally found at low concentrations and therefore do not compete effectively with initiating radicals (e.g., $^\bullet OH$) that can oxidize the first compound they come in contact with [52].

Antioxidant efficiency is dependent on the ability of the FRSs to donate hydrogen to a free radical. As the energy of a hydrogen bound to an FRS decreases, the transfer of the hydrogen to the free radical is more energetically favorable and therefore more rapid. The ability of an FRS to donate its hydrogen to a free radical can be predicted with the help of standard one-electron reduction potentials [10]. Any compound that has a reduction potential lower than the reduction potential of a free radical (or oxidized species) is capable of donating its hydrogen to that free radical unless the reaction is kinetically unfeasible. For example, FRSs including α-tocopherol ($E^{\circ\prime}$ = 500 mV), catechol ($E^{\circ\prime}$ = 530 mV), and ascorbate ($E^{\circ\prime}$ = 282 mV) all have reduction potentials below that of peroxyl radicals ($E^{\circ\prime}$ = 1000 mV) and are therefore capable of donating their hydrogen to the peroxyl radical to form a hydroperoxide.

The efficiency of the FRS is also dependent on the energy of the resulting free radical scavenger radical ($FRS^\bullet$). If the FRS· is a low-energy radical, then the likelihood of the $FRS^\bullet$ catalyzing the oxidation of unsaturated fatty acids decreases. Effective FRSs form low-energy radicals due to resonance delocalization, as shown in Figure 4.30 [77]. Effective FRSs also produce radicals that do not react rapidly with oxygen to form hydroperoxides. When radical scavengers form hydroperoxides, they can undergo decomposition reactions that produce additional radicals which could cause oxidation of unsaturated fatty acids. FRS radicals may participate in termination reactions with other $FRS^\bullet$ or lipid radicals to form nonradical species. This means that each FRS is capable of inactivating at least two free radicals, the first being inactivated when the FRS interacts with peroxyl or alkoxyl radicals, and the second when the $FRS^\bullet$ enters a termination reactions with another $FRS^\bullet$ or lipid radical (Figure 4.31).

Phenolic compounds possess many of the properties of an efficient FRS. These compounds donate a hydrogen from their hydroxyl groups, and the subsequent phenolic radical can have low energy as the radical is delocalized throughout the phenolic ring structure. The effectiveness of a phenolic FRS is often increased by substitution groups on the phenolic ring, which increase the ability of the FRS to donate hydrogen to lipid radicals and/or increase the stability of the $FRS^\bullet$ [77]. In foods, the efficiency of phenolic FRSs is also dependent on their volatility, pH sensitivity, and polarity. In the following, we give examples of the most common FRSs in foods.

4.11.4.2 Tocopherols

Tocopherols are a group of compounds that have a hydroxylated ring system (chromanol ring) with a phytol chain (Figure 4.32). Differences in tocopherol homologs are due to differences in methylation on the chromanol ring, with α being trimethylated, β (positions 5 and 8) and γ (positions 7 and 8) being dimethylated, and δ being monomethylated (position 8). Tocotrienols differ from tocopherols in that they have three double bonds in their phytyl chain. Tocopherols have three asymmetric

OH

ROO•

ROOH

O•

O

O

O

FIGURE 4.30 Resonance delocalization of phenol radical. (Adapted from Shahidi, F. and Wanasundara, J.P.K., *Crit. Rev. Food Sci. Nutr.,* 32, 67, 1992.)

carbons, and thus each homolog can have eight possible steroisomers. Natural tocopherols are found in the all *rac* or *RRR* configuration. Synthetic tocopherols have steroisomers with combinations of *R* and *S* configurations. The steroisomer configuration of α-tocopherol is important because only the *RRR* and 2*R*-steroisomers (*RSR*, *RRS*, and *RSS*) have significant vitamin E activity and can be used for the establishment of the recommended daily allowance (RDA) of vitamin E in the United States [23]. α-Tocopherol is commonly sold as an acetate ester when used as a nutritional supplement or a food ingredient. The acetate ester is hydrolyzed in the gastrointestinal tract by lipase to regenerate α-tocopherol. The acetate ester form of tocopherols blocks the hydroxyl group and decreases

FIGURE 4.31 Termination reaction between and antioxidant radical and a lipid peroxyl radical (ROO•).

FIGURE 4.32 Structure of α-tocopherol.

the molecule's susceptibility to oxidative degradation. It should be noted that the blocking of the hydroxyl group by the acetate ester removes the antioxidant activity of tocopherol. The esterification of α-tocopherols also increases its stability, thereby maintaining vitamin E activity during storage.

Reactions between tocopherols and lipid peroxyl radicals lead to the formation of a lipid hydroperoxide and several resonance structures of tocopheroxyl radicals. These radicals can interact with other lipid radicals or with each other to form a variety of termination products. The types and amounts of these products are dependent on oxidation rates, radical species, physical location

(e.g., bulk vs. membrane lipids), and tocopherol concentration (see Ref. [52] for more details). Tocopherols are generally insoluble in water. However, they do vary in polarity, with α-tocopherol (trimethylated) being the most nonpolar and δ-tocopherol (monomethylated) being the most polar. These differences in polarity alter the surface activity of the tocopherols, a factor that may impact their antioxidant activity (see the section on physical location of antioxidants).

4.11.4.3 Synthetic Phenolics

Phenol is not a good antioxidant, but addition of substitution groups onto the phenolic ring can enhance its antioxidant activity. Thus the majority of synthetic antioxidants are substituted monophenolic compounds. The most common synthetic FRSs used in foods include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), and propyl gallate (Figure 4.33). These synthetic FRSs vary in polarity in the order: BHT (most nonpolar) > BHA > TBHQ > propyl gallate. As with other FRSs, interactions between the FRS and lipid radicals result in the formation of a low-energy, resonance-stabilized phenolic radical, which neither rapidly catalyzes the oxidation of unsaturated fatty acids nor reacts with oxygen to form unstable antioxidant hydroperoxides that decompose into high-energy free radicals that can promote oxidation. Synthetic phenolics are effective in numerous food systems; however, their use in the food industry is declining due to the consumer demand for all natural products.

4.11.4.4 Plant Phenolics

Plants contain a diverse group of phenolic compounds, including simple phenolics, phenolic acids, anthocyanins, hydrocinnamic acid derivatives, and flavonoids. These phenolics are widely distributed in fruits, spices, tea, coffee, seeds, and grains. All phenolic classes have the structural requirements of FRSs, although their activity varies greatly. Factors influencing the FRS activity of plant phenolics include the position and degree of hydroxylation, polarity, solubility, reducing potential, stability of the phenolic to food processing operations, and stability of the phenolic radical.

Rosemary extracts are the most commercially important source of natural phenolics used as a foods additive to inhibit lipid oxidation by FRSs. Carnosic acid, carnosol, and rosmarinic acid are the major FRSs in rosemary extracts (Figure 4.34). Rosemary extracts can inhibit lipid oxidation in a wide variety of food products including meats, bulk oils, and lipid emulsions [4,26,60]. Utilization of phenolic antioxidants from crude herb extracts such as rosemary is often limited by the presence of flavor compounds such as monoterpenes. Phenolics found naturally in foods are important to the endogenous

OH, $C(CH_3)_3$, OCH_3 — 2-BHA; OH, $C(CH_3)_3$, OCH_3 — 3-BHA
Butylated hydroxyanisole

OH, $(H_3C)_3C$, $C(CH_3)_3$, CH_3
Butylated hydroxytoluene

OH, $C(CH_3)_3$, OH
Tertiary butylhydroxyquionone

OH, HO, OH, $COOC_3H_7$
Propyl gallate

FIGURE 4.33 Structures of synthetic antioxidants used in foods.

FIGURE 4.34 Structures of phenolic antioxidants found in rosemary extracts.

oxidative stability of foods. Natural phenolic levels in foods can vary as a function of plant maturity, variety, tissue type, growing conditions and postharvest age, and storage conditions [9,41,84].

4.11.4.5 Ascorbic Acid and Thiols

Free radicals are generated in the water phase of foods by processes such as the Fenton reaction, which produces hydroxyl radicals from hydrogen peroxide. In addition, free radicals can be surface-active, meaning that they would migrate from the lipid phase toward the water phase in lipid dispersions. Since free radicals are found in the aqueous phase, biological systems containing water-soluble compounds are capable of free-radical scavenging. Ascorbic acid and thiols scavenge free radicals, resulting in the formation of low-energy radicals [18]. Thiols such as cysteine and glutathione may contribute to the oxidative stability of plant and muscle foods, but they are rarely added to foods as antioxidants. One exception to this is the thiols found in proteins that can inhibit lipid oxidation in food products [20,87]. Ascorbate and its isomer erythorbic acid can both scavenge free radicals. Both have similar activity, but erythorbic acid is more cost effective. Ascorbic acid is also available as a conjugate with palmitic acid. The conjugate is lipid-soluble and surface-active, meaning that it can be effective in bulk oils and emulsions. In the gastrointestinal tract, ascorbyl palmitate is hydrolyzed to ascorbic and palmitic acids, and thus there are no restrictions on its usage levels.

4.11.4.6 Control of Prooxidants

The rate at which lipids oxidize in foods is very much dependent on prooxidant concentrations and activity (e.g., transition metals, singlet oxygen, and enzymes). Control of prooxidants is therefore a very effective strategy to increase the oxidative stability of foods. Both endogenous and exogenous antioxidants will impact the activity of transition metals and singlet oxygen.

4.11.4.7 Control of Prooxidant Metals

Iron and copper are examples of important prooxidant transition metals that accelerate lipid oxidation by promoting hydroperoxide decomposition. The prooxidative activity of metals is altered by chelators or sequestering agents. Chelators inhibit the activity of prooxidant metals by one or more of the following properties: prevention of metal redox cycling; occupation of all metal coordination sites; formation of insoluble metal complexes; and/or steric hinderance of interactions between metals and lipids or oxidation intermediates (e.g., hydroperoxides) [18]. Some metal chelators can increase oxidative reactions by increasing the metal solubility and/or altering the redox potential.

The tendency of a chelator to accelerate or inhibit prooxidant activity depends on the metal and chelator concentrations. For instance, ethylenediaminetetraacetic acid (EDTA) is ineffective or prooxidative when the EDTA/iron ratios are ≤1 and antioxidative when the ratio is >1 [53].

The main metal chelators found in foods contain multiple carboxylic acid (e.g., EDTA and citric acid) or phosphate groups (e.g., polyphosphates and phytate). Most chelators act in the aqueous phase of foods, but some will also partition into the lipid phase (e.g., citric acid), thus allowing them to inactivate lipid-soluble metals. Chelators must be ionized to be active, and therefore their activity decreases at pH values below the pK_a of the ionizable groups. The most common chelators used as food additives are citric acid, EDTA, and polyphosphates. The effectiveness of phosphates increases with increasing number of phosphate groups. Thus tripolyphosphate and hexametaphosphate are more effective than phosphoric acid [83]. Prooxidant metals can also be controlled by binding to proteins such as transferrin, phosvitin, lactoferrin, ferritin, and casein (for review see Ref. [18]).

4.11.4.8 Control of Singlet Oxygen

As mentioned previously, singlet oxygen is an excited state of oxygen that can promote the formation of lipid hydroperoxides. Carotenoids are a diverse group (>600 different compounds) of yellow to red colored polyenes. The activity of singlet oxygen can be controlled by carotenoids by both chemical and physical quenching mechanisms [51,67]. Carotenoids chemically quench singlet oxygen when singlet oxygen attacks the double bonds of the carotenoid. This reaction will lead to the formation of carotenoid breakdown products containing aldehydes, ketones, and endoperoxide. These reactions cause carotenoid decomposition, leading to loss of antioxidant activity and color. The more effective mechanism of singlet oxygen inactivation by carotenoids is physical quenching. Carotenoids physically quench singlet oxygen by a transfer of energy from singlet oxygen to the carotenoid to produce an excited state of the carotenoid and ground-state triplet oxygen. Energy is dissipated from the excited carotenoid by vibrational and rotational interactions with the surrounding solvent to return the carotenoid to the ground state. The presence of nine or more conjugated double bonds in a carotenoid is necessary for physical quenching. Carotenoids that have six carbon oxygenated ring structures at the end their polyenes are often more effective at physically quenching singlet oxygen. Carotenoids can also physically absorb the energy of photoactivated sensitizers, preventing them from promoting the formation of singlet oxygen.

4.11.4.9 Control of Lipoxygenases

Lipoxygenases are active lipid oxidation catalysts found in plants and some animal tissues. Lipoxygenase activity can be controlled by heat inactivation and plant-breeding programs that decrease the concentration of these enzymes in edible tissues.

4.11.4.10 Control of Oxidation Intermediates

Compounds are found in foods that indirectly influence lipid oxidation rates by interacting with prooxidant metals or oxygen to form reactive species. Examples of such compounds include superoxide anion and hydroperoxides.

4.11.4.11 Superoxide Anion

Superoxide participates in oxidative reactions by reducing transition metals to a more active state or by promoting the release of iron bound to protein. In addition, at low pH values, superoxide will form its conjugated acid, the perhydroxyl radical, which can directly catalyze lipid oxidation [46]. Due to the prooxidant nature of superoxide anion in oxidative reactions, biological systems contain superoxide dismutase (SOD). SOD catalyzes the conversion of superoxide anion to hydrogen peroxide by the following reaction:

$$2O_2^{-\bullet} + 2H^+ \rightarrow O_2 + H_2O_2$$

4.11.4.12 Peroxides

Peroxides are important intermediates of oxidative reactions since they decompose via transition metals, irradiation, and elevated temperatures to form free radicals. Hydrogen peroxide exists in foods as a result of direct addition (e.g., aseptic processing operations) and formation in biological tissues by mechanisms including the dismutation of superoxide by SOD and the activity of peroxisomes and leukocytes. The inactivation of hydrogen peroxide is catalyzed by catalase, a heme-containing enzyme, by the following reaction [46]:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Glutathione peroxidase is a selenium-containing enzyme that can decompose both lipid hydroperoxides and hydrogen peroxide using reduced glutathione (GSH) as a cofactor [46]:

$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$$

or

$$LOOH + 2GSH \rightarrow LOH + H_2O + GSSG$$

where
GSSG is oxidized glutathione
LOH is a fatty acid alcohol

4.11.4.13 Antioxidant Interactions

Food systems usually contain endogenous, multicomponent antioxidant systems. In addition, exogenous antioxidants can be added to processed foods. The presence of multiple antioxidants will enhance the oxidative stability of the product due to interactions between antioxidants. Synergism is often used to describe antioxidant interactions. For an antioxidant interaction to be synergistic, the effect of the antioxidant combination must be greater than the sum of the effects of the two individual antioxidants. The effectiveness of many antioxidant combinations often is equal to or less than their additive effect. Thus caution should be used when claiming synergistic activity.

Enhanced antioxidant activity can be observed in the presence of two or more different FRSs. In the presence of two FRSs, it is possible that one FRS (the primary FRS) will react more rapidly with lipid free radicals than the other due to lower bond disassociation energies or due to the fact that its physical location is closer to the site where free radicals are being generated. In the presence of multiple FRSs, the primary FRS that is rapidly oxidized can be regenerated by a secondary FRS, with the free radical being transferred from the primary to the secondary FRS. This process is seen with α-tocopherol and ascorbic acid. In this system, α-tocopherol is the primary FRS due to its presence in the lipid phase. Ascorbic acid then regenerates the tocopheroxyl radical or possibly the tocopherylquinone back to α-tocopherol, resulting in the formation of the dehydroascorbate [10]. The net result is that the primary FRS (α-tocopherol) is maintained in an active state where it can continue to scavenge free radicals in the lipid phase of the food.

Chelators and FRS combinations can result in synergistic inhibition of lipid oxidation [25]. These enhanced interactions occur by a "sparing" effect provided by the chelator. Since the chelator will decrease the amounts of free radicals formed in the food by inhibiting metal-catalyzed oxidation, the eventual inactivation of the FRS through reactions such as termination or autoxidation will be slower. Thus, by decreasing free-radical generation and thus decreasing FRS inactivation, concentrations of the FRSs will be higher.

Since multicomponent antioxidant systems can inhibit oxidation by many different mechanisms (e.g., FRS, metal chelation, and singlet oxygen quenching), the use of multiple antioxidants

can greatly enhance the oxidative stability of foods. Thus when designing antioxidant systems, the antioxidants used should have different mechanisms of action and/or physical properties. Determining which antioxidants would be the most effective depends on factors such as type of oxidation catalysts, physical state of the food, and factors that influence the activity of the antioxidants themselves (e.g., pH, temperature, and ability to interact with other compounds/antioxidants in the foods).

4.11.4.14 Physical Location of Antioxidants

Antioxidants can show a wide range of effectiveness depending on the physical nature of the lipid [25,71]. For example, hydrophilic antioxidants are often less effective in O/W emulsions than lipophilic antioxidants, whereas lipophilic antioxidants are less effective in bulk oils than hydrophilic antioxidants [25,71]. This observation has been coined the "antioxidant paradox." Differences in the effectiveness of the antioxidants in bulk oils and emulsions are due to their physical location in the two systems. Polar antioxidants are more effective in bulk oils presumably because they can accumulate in reverse micelles within the oil [12], the locations where lipid oxidation reactions would be greatest due to the coexistence of surface active hydroperoxides and prooxidants such as metals [90]. In contrast, predominantly nonpolar antioxidants are more effective in O/W emulsions because they are retained in the oil droplets and/or accumulate at the oil–water interface, the location where, again, lipid oxidation reactions are prevalent. Conversely, in O/W emulsions, polar antioxidants would tend to partition into the aqueous phase where they would not be able to protect the lipid.

4.11.5 Other Factors Influencing Lipid Oxidation Rates

4.11.5.1 Oxygen Concentration

Reduction of oxygen concentrations is a common method used to inhibit lipid oxidation. However, the addition of oxygen to the alkyl radical is a diffusion-limited reaction, so it has been suggested that to effectively inhibit lipid oxidation, most of the oxygen must be removed from the system. Since oxygen solubility is higher in oil than water, removal of oxygen to stop lipid oxidation can be difficult unless vacuum conditions are used. Unfortunately, very little research has been conducted on lipid oxidation at intermediate oxygen concentrations.

4.11.5.2 Temperature

Increasing temperature generally increases lipid oxidation rates. However, increasing temperatures also decrease oxygen solubility, so in some cases high temperatures can slow oxidation. This can happen in heated bulk oil. However, if food is fried in heated oil, aeration of the oil occurs, leading to acceleration of oxidation. Elevated temperatures can also cause antioxidants to degrade, volatilize, and, in the cases of antioxidant enzymes, become inactivated through denaturation.

4.11.5.3 Surface Area

Increasing the surface area of lipids can increase lipid oxidation rates since this can lead to increased exposure to oxygen and prooxidants. This has recently been observed in bulk oils that contain nanostructures formed by naturally occurring surfactants (e.g., phospholipids) and water [12].

4.11.5.4 Water Activity

As water is removed from a food system, lipid oxidation rates generally decrease. This is likely due to a decrease in the mobility of reactants such as transition metals and oxygen. In some foods, continued removal of water will result in an acceleration of lipid oxidation. This acceleration of lipid oxidation at very low water activity is thought to be due to the loss of a protective water solvation layer surrounding lipid hydroperoxides [12,13].

4.11.6 Measurement of Lipid Oxidation

As one can see from the foregoing discussion on lipid oxidation pathways, numerous oxidation products can be formed from a single fatty acid. In addition, these decomposition products often contain double bonds and in some cases intact pentadiene systems. These double bond systems can undergo hydrogen abstraction or singlet oxygen attack, which will result in the formation of additional decomposition products. Since food lipids can contain many different unsaturated fatty acids and can be exposed to several different prooxidants, hundreds of decomposition products can be formed. In addition, many oxidation products are unstable (hydroperoxides) and can react with other food components (aldehydes). Thus the complexity of these pathways and factors make analysis of lipid oxidation very challenging. In the following, we give a summary of the most common analytical techniques used to monitor the oxidation products in food lipids.

4.11.6.1 Sensory Analysis

The gold standard of lipid oxidation measurements is sensory analysis, since this is the only technique that directly monitors the off-aromas and off-flavors generated by oxidative reactions. In addition, sensory analysis can be highly sensitive because humans can detect certain aroma compounds at levels below or close to detection levels that can be achieved by chemical and instrumental techniques. Sensory analysis of oxidized lipids must be done with a panel that is trained in the identification of oxidation products. This training is usually product-specific, since the oxidation products from different fatty acids can produce different sensory profiles. Due to the necessity for extensive training, sensory analysis is often time consuming and cost prohibitive and obviously is not suitable for the rapid and extensive analysis required for quality control operations. Thus, many chemical and instrumental techniques have been developed. In the best case scenario, these chemical and instrumental techniques are most useful when correlated with sensory analysis. Numerous tests exist for the measurement of oxidative deterioration of foods. The most common methods and their advantages and disadvantages are discussed in the following.

4.11.6.2 Primary Lipid Oxidation Products

Primary lipid oxidation products are compounds that are produced by the initiation and propagation steps of lipid oxidation. Since these are the first oxidation products produced, they can appear early in the oxidative deterioration of lipids. However, during the latter stages of oxidation, the concentrations of these compounds decrease, as their formation rates become slower than their decomposition rates. A disadvantage of using primary products to measure oxidation is that primary products are not volatile and thus do not directly contribute to off-flavors and aromas. In addition, under certain conditions such as high temperatures (frying oils) or high amounts of reactive transition metals, the concentration primary products may not increase since their decomposition rates are high. This would produce misleading results since a very rancid oil could have very low concentrations of primary lipid oxidation products.

4.11.6.3 Conjugated Double Bonds

Conjugated double bonds are rapidly formed in polyunsaturated fatty acids upon the abstraction of hydrogen in the initiation step. Conjugated dienes have an absorption maximum at 234 nm with a molar extinction coefficient of $2.5 \times 10^4\ M^{-1}\ cm^{-1}$ [7]. This extinction coefficient gives an intermediate level of sensitivity compared to other techniques. Conjugated dienes can be useful for simple oil systems, but it is often ineffective in complex foods where many compounds exist that also absorb at similar wavelengths and thus cause interference. Sometimes conjugated diene values are used interchangeably with lipid hydroperoxides since many lipid hydroperoxides will contain a conjugated diene system. However, this equivalence should be discouraged because fatty acid breakdown products can also contain conjugated double bonds and also because monounsaturated fatty acids (e.g., oleic) will form hydroperoxides that do not have a conjugated diene system. Conjugated trienes

can also be measured in foods at 270 nm. This technique is useful only with lipids that have more than or equal to three double bonds and thus is limited to highly unsaturated oils such as those from linseed and fish.

4.11.6.4 Lipid Hydroperoxides

A very common method to measure the oxidative quality of lipids is to measure fatty acid hydroperoxides. Most methods that measure lipid hydroperoxides rely on the ability of the hydroperoxides to oxidize an indicator compound. Peroxide values are expressed as milliequivalents (meq) of oxygen per kg of oil, with 1 meq equal to 2 mmol of hydroperoxide. The most common titration method uses the hydroperoxide-promoted conversion of iodide to iodine. Iodine is then titrated with sodium thiosufite to produce iodide, which is measured with a starch indicator [70]. This method is relatively insensitive (0.5 meq/kg oil) and can require up to 5 g of lipid; thus it is practical only for isolated fats and oils. Lipid hydroperoxide–promoted oxidation of ferrous to ferric ions can also be used, with ferric ions being detected with ferric ion–specific chromophores such as thiocyanate or xylenol orange [79]. These methods are much more sensitive than the titration methods, with the thiocyanate method having an extinction coefficient of 4.0×10^4 M^{-1} cm^{-1} allowing analysis to be performed with milligram quantities of lipids [79].

4.11.6.5 Secondary Lipid Oxidation Products

Secondary lipid oxidation products are compounds that arise from the decomposition of fatty acid hydroperoxides via reactions such as β-scission. As described previously, these reactions can generate hundreds of different compounds, both volatile and nonvolatile, from the oxidation of food lipids. Since it is virtually impossible to measure all of these compounds simultaneously, these methods generally focus on the analysis of a single compound or a class of compounds. A drawback of these methods is that the formation of secondary products relies on the decomposition of lipid hydroperoxides. Thus in certain cases (e.g., presence of antioxidants), the concentrations of secondary products can be low while the primary product concentrations are high. In addition, compounds in foods containing amine and sulfhydryl groups (e.g., proteins) can interact with secondary products that contain functional groups such as aldehydes, thus making them difficult to measure. An advantage of these measurements is that they measure many of the products from fatty acid decomposition which are responsible for the off-flavors and aromas in rancid oils and thus have higher correlation with sensory analysis.

4.11.6.6 Analysis of Volatile Secondary Products

Volatile lipid oxidation products are typically measured by gas chromatography using direct injection, static or dynamic headspace, or solid-phase microextraction (SPME) [48]. Using these systems, lipid oxidation can be measured using specific products (e.g., hexanal for lipids high in ω-6 fatty acids and propanal for lipids high in ω-3 fatty acids), product classes (e.g., hydrocarbons or aldehydes), or by total volatiles. Each method can give different profiles of volatiles due to the differences in their ability to drive the volatiles out of the sample. The advantage of measuring volatile lipid oxidation products is a stronger correlation with sensory analysis compared to primary oxidation products. The disadvantage is the expense of instrumentation and the difficulty in analyzing large amounts of samples especially in lipids that oxidize rapidly (these techniques are often time consuming). In addition, these methods often use heating steps to drive the volatiles into the headspace. In some foods such as meats, these heating steps may increase lipid oxidation rates by cooking the food. In general, lipids should be sampled at the lowest temperature possible. An additional problem is the loss of volatiles by processes such as steam distillation in frying oils.

4.11.6.7 Carbonyls

Carbonyls arising from lipid oxidation can be determined by reacting lipids with 2,4-dinitrophenylhydrazine to form a complex that absorbs light at 430–460 nm. This method is

limited by the presence of other carbonyls in foods that can cause interference [70]. HPLC techniques have been developed to separate carbonyls arising from lipid oxidation from interfering compounds. However, these techniques are expensive and time consuming, and are therefore not routinely used in food lipids.

Carbonyls can also be measured by conjugation with anisidine to form products that absorb at 350 nm. This method is useful because it can measure nonvolatile, high-molecular-weight carbonyls. Thus, anisidine is used to measure oxidation in products such as fish oils since these oils commonly undergo steam distillation during refining. Anisidine is therefore useful in fish oils because it can give an indication of the quality of the oil prior to steam distillation, since nonvolatile, high-molecular-weight compounds are retained in the oil.

4.11.6.8 Thiobarbituric Acid (TBA)

The TBA assay is based on the reaction between TBA and carbonyls to form red fluorescent adducts under acidic conditions [94]. The assay can be conducted on whole samples, sample extracts, or distillates, and adduct formation can be conducted under a number of varying temperature (25°C–100°C) and time (15 min–20 h) protocols. The compound often attributed to be the primary lipid oxidation product detected by TBA is malonaldehyde (MDA) whose TBA adduct absorbs strongly at 532 nm. MDA is a dialdehyde produced by a two-step oxidative degradation of fatty acids with three or more double bonds. This means that MDA yield during the oxidation of lipids is dependent on the fatty acid composition, with highly unsaturated fatty acids producing high amounts of MDA. TBA can also react with aldehydic lipid oxidation products other than malonaldehyde, especially unsaturated aldehydes.

The TBA assay suffers from nonspecificity due to its ability to react with nonlipid carbonyls such as ascorbic acid, sugars, and nonenzymic browning products. These compounds can form TBA adducts, which absorb over the range 450–540 nm. Often, it is more appropriate to refer to TBA reactive substances (TBARSs) to acknowledge that compounds in addition to MDA can generate pink chromophores. To decrease problems with interfering compounds, the TBA–MDA complex can be measured directly by fluorescence or HPLC techniques.

The TBA assay can be a useful method for analysis of lipid oxidation in foods since it is simple and inexpensive. However, the nonspecificity of the method requires an understanding of the test's limitations, so improper comparisons and conclusions are not made. To minimize potential misinterpretation of TBA analysis, it is suggested that analysis of fresh, nonoxidized samples be conducted to obtain baseline data on TBA reactive substances in product that do not arise from lipid oxidation. However, the TBA method should be avoided in foods where concentrations of interfering compounds are high. In addition, attempts to use TBA to compare oxidation between products with different fatty acid compositions are inappropriate since the MDA yield varies with the fatty acid composition.

4.11.7 Summary

Hydrolysis of triacylglycerol can impact food quality by releasing fatty acids, which negatively impact flavor, physical properties, and oxidative stability of fats and oils.

- Oxidation rancidity occurs via autocatalytic free-radical reactions.
- Oxidative rancidity occurs when fatty acids are decomposed into low-molecular-weight aldehydes and ketones.
- Prooxidant such as transition metals, singlet oxygen, and enzymes are often the major cause of lipid oxidation in foods.
- Antioxidants slow oxidation by scavenging free radicals and/or decreasing the activity of prooxidants.
- Lipid oxidation is also influenced by factors such as oxygen concentrations, fatty acid unsaturation, temperature, and water activity.

- Sensory analysis is the gold standard for measuring oxidative rancidity.
- Lipid oxidation can be monitored by measuring the primary oxidation products, but these tend to not be strongly correlated with rancidity.
- Secondary lipid oxidation products originate from fatty acid decomposition and can be more strongly correlated with rancidity.

4.12 FOOD LIPIDS AND HEALTH

4.12.1 Bioactivity of Fatty Acids

Dietary lipids have often been negatively associated with health. Since obesity is highly correlated with numerous diseases such as heart disease and diabetes, the negative role of lipids in health is often attributed to their high caloric density of 9 kcal g^{-1}. Specific dietary lipids have also been associated with the risk of heart disease due to their ability to modulate low-density lipoprotein (LDL)-cholesterol levels in the blood. Since LDL-cholesterol levels are often associated with the development of heart disease, several dietary strategies have been proposed to decrease LDL-cholesterol, including consumption of dietary saturated fatty acids at <10% of calories, limiting dietary cholesterol to <300 mg day^{-1}, and keeping dietary *trans* as low as possible [19]. Recently, the role of dietary saturated fatty acids in heart disease has been questioned, since saturated fatty acids raise "good" HDL-cholesterol. In addition, the biological effect of saturated fatty acids varies with fatty acid type [14].

4.12.2 *Trans* Fatty Acids

Trans fatty acids have gained attention because of their unique role in heart disease through their ability to both increase LDL-cholesterol and decrease HDL-cholesterol [47]. This behavior is partially due to the geometric configuration of *trans* fatty acids, which is more similar to that of saturated fatty acids than of unsaturated fatty acids. Because of the potential harm of *trans* fatty acids, foods are required to disclose *trans* fatty acid concentrations on their nutritional labels in many countries. In the United States, foods with less than 0.5 g of fat/serving do not have to label *trans* fatty acids as long as no claims are made about fat, fatty acids, or cholesterol content. This is because refining causes the formation of *trans* fatty acids, so most commercial oils are not *trans*-free. Because of the labeling requirement, *trans* fatty acid concentrations in processed foods have decreased dramatically [75].

While a large amount of research has been devoted to the negative aspects of dietary lipids on health, more recently evidence is growing that some dietary lipids can reduce the risk of several diseases. These bioactive lipids include ω-3 fatty acids, phytosterols, carotenoids, and conjugated linoleic acid.

4.12.3 ω-3 Fatty Acids

As agricultural practices have advanced, the profile of dietary lipids in Western societies has changed dramatically. Our ancestors are thought to have consumed diets with approximately equal amounts of ω-6 and ω-3 fatty acids. The development of modern agriculture increased the availability of refined fats, especially vegetable oils, changing our dietary ω-6 to ω-3 ratio to >7:1. This is an extremely rapid change on an evolutionary timescale, and is problematic since humans interconvert ω-6 and ω-3 fatty acids at slow rates. A lower amount of ω-3 fatty acids in the diet is important because these bioactive lipids play a vital role in membrane fluidity, cellular signaling, gene expression, and eicosanoid metabolism. Therefore, consumption of dietary ω-3 fatty acids (ω-3s) is essential for the promotion and maintenance of good health, especially for pregnant and lactating women and individuals with coronary heart disease, immune-response disorders, and compromised

mental health. There is strong evidence that the level of ω-3s currently consumed by the general population is inadequate [19]. Numerous food companies are attempting to increase the levels of these bioactive lipids in their products by direct incorporation of ω-3 fatty acids into foods or by feeding ω-3 fatty acids to livestock. These approaches are commonly impeded by the oxidative deterioration of ω-3 fatty acids during the processing and storage of fortified food products.

4.12.4 Conjugated Linoleic Acid

The two double bonds of linoleic acid are normally in a methylene-interrupted system, where two single bonds separate the double bonds. However, the double bond system is sometimes altered, resulting in isomerization of the double bonds to a conjugated configuration. This isomerization can occur during processes such as hydrogenation, and is common during the biohydrogenation process promoted by bacteria in ruminants. These isomers, known as *conjugated linoleic acid* (CLA), have gained widespread interest due to their ability to inhibit cancer [35], lower blood cholesterol [49], inhibit the onset of diabetes, and influence weight gain [69]. The different isomers have different biological effects, with 9-*cis*, 11-*trans* linoleic acid showing anticarcinogenic activity and 10-*trans*, 12-*cis* linoleic acid having the ability to influence body fat accumulation. The 9-*cis*, 11-*trans* isomer of CLA is the predominant isomer found in dairy and beef products [80]. Molecular mechanisms of CLA bioactivity have been attributed to their ability to modulate of eicosanoid formation and gene expression. A meta-analysis of dietary CLA consumption by humans indicated that CLA had little impact on body composition [65].

4.12.5 Phytosterols

The major phytosterols are sitosterol, campesterol, and stigmasterol. Dietary phytosterols have little to no absorption into the blood. Their bioactivity lies in the fact that they can inhibit the bioavailability of both dietary and biliary (produced by intestinal cells) cholesterol [72,73]. Intake of 1.5–2 g day^{-1} of phytosterols can reduce LDL-cholesterol by 8%–15%. Since phytosterols primarily inhibit cholesterol absorption, they are most effective when consumed with a cholesterol-containing meal. Phytosterols have very high melting points, and therefore exist as lipid crystals at the temperatures common to many foods. To minimize crystallization, phytosterols are commonly esterified to unsaturated fatty acids to increase their lipid solubility.

4.12.6 Carotenoids

Carotenoids are a diverse group (>600 different compounds) of yellow to red colored polyenes that are lipid-soluble. Vitamin A is an essential nutrient obtained from carotenoids such as β-carotene. The bioactivity of other carotenoids has been a research area of great interest. This interest was initially focused on the antioxidant activity of carotenoids. However, when clinical trials were conducted to evaluate dietary β-carotene in subjects at risk to free-radicals stress (smokers), β-carotene was found to increase lung cancer rates [6]. Other carotenoids have been found to have health benefits. Lutein and zeaxanthin can enhance visual activity [31]. The health benefits of tomatoes have been attributed to the carotenoid lycopene [62]. Interestingly, cooked tomatoes have greater lycopene bioavailability, presumably due to the thermally induced conversion of *trans*-lycopene to *cis*-lycopene.

4.12.7 Low-Calorie Lipids

One of the other health concerns of dietary triacylglycerols is their high caloric density. Many attempts have been made to produce low-fat foods that have the same sensory attributes as their full-fat counterparts by using fat mimetics. Fat mimetics are nonlipid compounds such as proteins

or carbohydrates that can produce fat-like properties at lower caloric values. A similar approach has been attempted to produce lipid components with no calories or with lower caloric contents (fat substitutes). The first commercial non-caloric lipid was sucrose fatty acid esters (Proctor & Gamble's Olestra). This compound is non-caloric because the presence of more than or equal to six fatty acids esterified to sucrose sterically prevents lipase from hydrolyzing the ester bond to release free fatty acids that can be absorbed into the blood. The nondigestibility of sucrose fatty acid esters means that they pass through the gastrointestinal tract and are excreted in the feces. This property can cause gastrointestinal problems such as diarrhea.

Structured lipids with lower caloric density have also been used in the food industry (e.g., Nabisco's Salatrim). These products are based on the principle that fatty acids at *sn*-1 and *sn*-3 of triacylglycerol are released as free fatty acids upon hydrolysis by pancreatic lipase. If *sn*-1 and *sn*-3 have long-chain saturated fatty acids (≥16 carbons), their release can lead to interactions with divalent cations to form insoluble soaps that are not readily bioavailable. Structured low-calorie fats also use short-chain fatty acids (≤6 carbons) at the *sn*-2 position. After hydrolysis by pancreatic lipase, *sn*-2 monoacylglycerol is absorbed into the intestinal endothelial cells. The short-chain fatty acids at *sn*-2 eventually are metabolized in the liver where they yield fewer calories than long-chain fatty acids. The combination of both long-chain saturated fatty acids at *sn*-1 and *sn*-3 and short-chain fatty acids at *sn*-2 produces a triacylglycerol with 5–7 cal g^{-1}.

4.12.8 Summary

Lipids can be both deleterious and beneficial to health.

- *Trans* fatty acid have been negatively associated with health because they increase LDL- and decrease HDL-cholesterol.
- Omega-3 fatty acids, conjugated linoleic acid, phytosterols, and carotenoids are examples of lipids that positively influence health.
- The caloric content of lipids can be decreased by altering their digestion and metabolism.

REFERENCES

1. Ackman, R. G. 2000. Fatty acids in fish and shellfish. In *Fatty Acids in Foods and Their Health Implications*, 2nd edn, ed. C.K. Chow, Marcel Dekker Inc., New York, pp. 155–186.
2. Afoakwa, E. O., Paterson, A., and Fowler, M. 2007. Factors influencing rheological and textural qualities in chocolate—A review. *Trends Food Sci. Technol.* 18(6):290–298.
3. Akoh, C. C. and Min, D. B. 2008. *Food Lipids: Chemistry, Nutrition, and Biotechnology* (3rd edn.). Boca Raton, FL: CRC Press.
4. Aruoma, O. I., Halliwell, B., Aeschbach, R., and Löligers, J. 1992. Antioxidant and pro-oxidant properties of active rosemary constituents: Carnosol and carnosol and carnosic acid. *Xenobiotica* 22:257–268.
5. Atkins, P. and de Paula, J. 2014. *Physical Chemistry: Thermodynamics, Structure, and Change* (10th edn.). Oxford, U.K.: Oxford University Press.
6. Bendich, A. 2004. From 1989 to 2001: What have we learned about the "biological actions of beta-carotene"? *J. Nutr.* 134:225S–230S.
7. Beuge, J. A. and Aust, S. D. 1978. Microsomal lipid peroxidation. *Meth. Enzymol.* 52:302–310.
8. Boistelle, R. 1988. Fundamentals of nucleation and crystal growth. In: *Crystallization and Polymorphism of Fats and Fatty Acids*, eds. N. Garti, and K. Sato, Marcel Dekker, New York, Chap 5.
9. Britz, S. J. and Kremer, D. F. 2002. Warm temperatures or drought during seed maturation increase free α-tocopherol in seeds of soybean (glycine max). *J. Agric. Food Chem.* 50:6058–6063.
10. Buettner, G. R. 1993. The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol, and ascorbate. *Arch. Biochem. Biophys.* 300:535–543.
11. Calder, P. C. 2014. Very long chain omega-3 (n-3) fatty acids and human health. *Eur. J. Lipid Sci. Technol.* 116:1280–1300.

12. Chen, B., McClements, D. J., and Decker, E. A. 2011. Minor components in food oils: A critical review of their roles on lipid oxidation chemistry in bulk oils and emulsions. *Crit. Rev. Food Sci. Nutr.* 51:901–916.
13. Chen, H., Lee, D. J., and Schanus, E. G. 1992. The inhibitory effect of water on the Co^{2+} and Cu^{2+} catalyzed decomposition of methyl linoleate hydroperoxides. *Lipids* 27:234–239.
14. Chowdhury, R., Warnakula, S., Kunutsor, S., Crowe, F., Ward, H. A., and Johnson, L. 2014. Association of dietary, circulating, and supplement fatty acids with coronary risk a systematic review and meta-analysis. *Ann. Intern. Med.* 160:658–658.
15. Coupland, J. N., and McClements, D. J. 1997. Physical properties of liquid edible oils. *J. Am. Oil Chem. Soc.* 74(12):1559–1564.
16. Decker, E. A. 1996. The role of stereospecific saturated fatty acid position on lipid nutrition. *Nutr. Rev.* 54:108–110.
17. Decker, E. A. and McClements, D. J. 2001. Transition metal and hydroperoxide interactions: An important determinant in the oxidative stability of lipid dispersions. *Inform* 12:251–255.
18. Decker, E. A. 2002. Nomenclature and classification of lipids. In: *Food Lipids, Chemistry, Nutrition and Biotechnology*, eds. C. C. Akoh and D. B. Min, Marcel Dekker, New York, pp. 517–542.
19. Dietary Guidelines for Americans. 2010. U.S. Department of Agriculture and U.S. Department of Health and Human Services, (7th edn.). Washington, DC: U.S. Government Printing Office.
20. Elias, R. J., Kellerby, S. S., and Decker, E. A. 2008. Antioxidant activity of proteins and peptides in foods. *Crit. Rev. Food Sci. Nutr.* 48:430–441.
21. Fasina, O. O. and Colley, Z. 2008. Viscosity and specific heat of vegetable oils as a function of temperature: 35C to 180C. *Int. J. Food Prop.* 11(4):738–746.
22. Faustman, C., Liebler, D. C., McClure, T. D., and Sun, Q. 1999. Alpha, beta-unsaturated aldehydes accelerate oxymyoglobin oxidation. *J. Agric. Food Chem.* 47:3140–3144.
23. Food and Nutrition Board, Institute of Medicine. 2001. *Vitamin E, in Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids.* Washington, DC: National Academy Press, pp. 186–283.
24. Formo, M. W. 1979. Physical properties of fats and fatty acids. In: *Bailey's Industrial Oil and Fat Products* (5th edn.), ed. D. Swern, Vol. 1, New York: John Wiley & Sons.
25. Frankel, E. N. 2005. *Lipid Oxidation* (2nd edn.). Scotland: Oily Press.
26. Frankel, E. N., Huang, S-W., Aeschbach, R., and Prior, E. 1996. Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. *J. Agric. Food Chem.* 44:131–135.
27. Fredrick, E., Walstra, P., and Dewettinck, K. 2010. Factors governing partial coalescence in oil-in-water emulsions. *Adv. Colloid Interface Sci.* 153(1–2):30–42.
28. German, J. B. and Creveling, R. K. 1990. Identification and characterization of a 15-lipoxygenase from fish gills. *J. Agric. Food Chem.* 38:2144–2147.
29. Girotti, A. W. 1998. Lipid hydroperoxide generation, turnover and effector action in biological systems. *J. Lipid Res.* 39:1529–1542.
30. Goff, H. D. and Hartel, R. W. 2013. *Ice Cream*. New York: Springer.
31. Granado, F., Olmedilla, B., and Blanco, I. 2003. Nutritional and clinical relevance of lutein in human health. *Br. J. Nutr.* 90:487–502.
32. Gunstone, F. D., Harwood, J. L., and Dijkstra, A. J. 2007. *The Lipid Handbook* (3rd edn.). Boca Raton, FL: CRC Press.
33. Gunstone, F. D. 2008. *Oils and Fats in the Food Industry*. Chichester, U.K.: Blackwell Publishing.
34. Gunstone, F. D. 2013. Composition and properties of edible oils. In: *Edible Oil Processing* (2nd edn.), eds. W. Hamm, R. J. Hamilton, and G. Calliauw. Hoboken, NJ: Wiley Blackwell, pp. 1–39.
35. Ha, Y. L., Grimm, N. K., and Pariza, M. W. 1987. Anticarcinogens from fried ground beef: Heat-altered derivatives of linoleic acid. *Carcinogenesis* 8:1881–1887.
36. Halliwell, B. and Gutteridge, J. M. 1990. Role of free radicals and catalylic metal ions in human disease: An overview. *Meth. Enzymol.* 186:1–88.
37. Hartel, R. W. 2001. *Crystallization in Foods*. Gaithersburg, MD: Aspen Publishers.
38. Hartel, R. W. 2013. Advances in food crystallization. *Annu. Rev. Food Sci. Technol.* 4:277–292.
39. Hernqvist, L. 1984. On the structure of triglycerides in the liquid-state and fat crystallization. *Fette Seifen Anstrichmittel* 86(8):297–300.
40. Himawan, C., Starov, V. M., and Stapley, A. G. F. 2006. Thermodynamic and kinetic aspects of fat crystallization. *Adv. Colloid Interface Sci.* 122(1–3):3–33.

41. Howard, L. R., Pandjaitan, N., Morelock, T., and Gil, M. I. 2002. Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season. *J. Agric. Food Chem.* 50:5891–5896.
42. Huang, S. W., Frankel, E. N., and German J. B. 1994. Antioxidant activity of alpha-tocopherols and gamma-tocopherols in bulk oils and in oil-in-water emulsions. *J. Agric. Food Chem.* 42:2108–2114.
43. Israelachvili, J. 2011. *Intermolecular and Surface Forces* (3rd edn.). London, U.K.: Academic Press.
44. Iwahashi, M. and Kasahara, Y. 2011. Dynamic molecular movements and aggregation structures of lipids in a liquid state. *Curr. Opin. Colloid Interface Sci.* 16(5):359–366.
45. Johnson, L. A. 2002. Recovery, refining, converting and stabilizing edible oils. In: *Food Lipids, Chemistry, Nutrition and Biotechnology*, eds. C. C. Akoh and D. B. Min. New York: Marcel Dekker, pp. 223–274.
46. Kanner, J., German, J. B., and Kinsella, J. E. 1987. Initiation of lipid peroxidation in biological systems. *Crit. Rev. Food Sci. Nutr.* 25:317–364.
47. Khosla, P. and Hayes, K. C. 1996. Dietary trans-monounsaturated fatty acids negatively impact plasma lipids in humans: Critical review of the evidence. *J. Am. Coll. Nutr.* 15:325–339.
48. Larick, D. K. and Parker, J. D. 2002. Chromatographic analysis of secondary lipid oxidation products. In: *Current Protocols in Food Analytical Chemistry*, ed. R. Wrolstad. New York: John Wiley & Sons, pp. D2.2.1–D2.4.9.
49. Lee, K. N., Kritchevsky, D., and Pariza, M. W. 1994. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* 108:19–25.
50. Lee, A. Y., Erdemir, D., and Myerson, A. S. 2011. Crystal polymorphism in chemical process development. In *Annual Review of Chemical and Biomolecular Engineering*, ed. J. M Prausnitz, Vol. 2, pp. 259–280.
51. Liebler, D. C. 1992. Antioxidant reactions of carotenoids. *Ann. NY Acad. Sci.* 691:20–31.
52. Liebler, D. C. 1993. The role of metabolism in the antioxidant function of vitamin E. *Crit. Rev. Toxicol.* 23:147–169.
53. Mahoney, J. R. and Graf, E. 1986. Role of α tocoperol, ascorbic acid, citric acid and EDTA as oxidants in a model system. *J. Food Sci.* 51:1293–1296.
54. Marangoni, A. G. and Tang, D. 2008. Modeling the rheological properties of fats: A perspective and recent advances. *Food Biophys.* 3(2):113–119.
55. Marangoni, A. G. and Wesdorp, L. H. 2012. *Structure and Properties of Fat Crystal Networks* (2nd edn.). Boca Raton, FL: CRC Press.
56. Marangoni, A. G., Acevedo, N., Maleky, F., Co, E., Peyronel, F., Mazzanti, G., and Pink, D. 2012. Structure and functionality of edible fats. *Soft Matter* 8(5):1275–1300.
57. McClements, D. J. 2002. Theoretical prediction of emulsion color. *Adv. Colloid Interface Sci.* 97(1–3):63–89.
58. McClements, D. J. 2005. *Food Emulsions: Principles, Practice, and Techniques* (2nd edn.). Boca Raton, FL: CRC Press.
59. McClements, D. J. 2007. Critical review of techniques and methodologies for characterization of emulsion stability. *Crit. Rev. Food Sci. Nutr.* 47(7):611–649.
60. Mielche, M. M. and Bertelsen, G. 1994. Approaches to the prevention of warmed oven flavour. *Trends Food Sci. Technol.* 5:322–327.
61. Min, D. B. and Boff, J. M. 2002. Lipid oxidation in edible oil. In: *Food Lipid, Chemistry, Nutrition and Biotechnology*, eds. C. C. Akoh and D. B. Min. New York: Marcel Dekker, pp. 335–364.
62. Nguyen, M. L. and Schwartz, S. J. 1999. Lycopene: Chemical and biological properties. *Food Technol.* 53:38–45.
63. O'Keefe, S. F. 2002. Nomenclature and classification of lipids. In: *Food Lipids, Chemistry, Nutrition and Biotechnology*, eds. C. C. Akoh and D. B. Min. New York: Marcel Dekker, pp. 1–40.
64. O'Keefe, S. F. and Pike, O. A. 2014. Fat characterization. In: *Food Analysis*, ed. S. S. Nielsen. New York: Springer, pp. 239–260.
65. Onakpoya, I. J., Posadzki, P. P., Watson, L. K., Davies, L. A., and Ernst, E. 2012. The efficacy of long-term conjugated linoleic acid (CLA) supplementation on body composition in overweight and obese individuals: A systematic review and meta-analysis of randomized clinical trials. *Eur. J. Nutr.* 51:127–134.
66. O'Neil, M. J. 2006. *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals* (14th edn.). Whitehouse Station, NJ: Merck.
67. Palozza, P. and Krinksky, N. I. 1992. Antioxidant effect of carotenoids in vivo and in vitro—An overview. *Meth. Enzymol.* 213:403–420.

68. Parish, E. J., Boos, T. L., and Li, S. 2002. The chemistry of waxes and sterols. In: *Food Lipids, Chemistry, Nutrition and Biotechnology*, eds. C. C. Akoh and D. B. Min. New York: Marcel Dekker, pp. 103–132.
69. Park, Y., Storkson, J. M., Albright, K. J., Liu, W., and Pariza, M. W. 1999. Evidence that the trans-10, cis-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* 34:235–241.
70. Pegg, R. B. 2002. Spectrophotometric measurement of secondary lipid oxidation products. In: *Current Protocols in Food Analytical Chemistry*, ed. R. Wrolstad. New York: John Wiley & Sons, pp. D2.4.1–D2.4.18.
71. Porter, W. L. 1993. Paradoxical behavior of antioxidants in food and biological systems. *Tox. Indus. Health* 9:93–122.
72. Quilez, J., Garcia-Lorda, P., and Salas-Salvado, J. 2003. Potential uses and benefits of phytosterols in diet: Present situation and future directions. *Clin. Nutr.* 22:343–351.
73. Russell, J. C., Eqart, H. S., Kelly, S. E., Kralovec, J., Wright, J. L. C., and Dolphin, P. J. 2002. Improvement of vascular disfunction and blood lipids of insulin resistant rats by a marine oil-based phytosterol compound. *Lipids* 37:147–152.
74. Rousseau, D. and Marangoni, A. G. 2002. Chemical interesterification of food lipids: Theory and practice. In: *Food Lipids, Chemistry, Nutrition and Biotechnology*, eds. C. C. Akoh and D. B. Min. New York: Marcel Dekker, pp. 301–334.
75. Ratnayake, W. M. N., L'Abbe, M. R., Farnworth, S., Dumais, L., Gagnon, C., Lampi, B. et al. 2009. Trans fatty acids: Current contents in Canadian foods and estimated intake levels for the Canadian population. *J. AOAC Int.* 92:1258–1276.
76. Sato, K., Bayes-Garcia, L., Calvet, T., Cuevas-Diarte, M. A., and Ueno, S. 2013. External factors affecting polymorphic crystallization of lipids. *Eur. J. Lipid Sci. Technol.* 115(11):1224–1238.
77. Shahidi, F. and Wanasundara, J. P. K. 1992. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* 32:67–103.
78. Shahidi, F. 2005. *Bailey's Industrial Oil and Fat Products, Edible Oil and Fat Products: Chemistry, Properties, and Health Effects*, Vol. 1. New York: Wiley-Interscience.
79. Shantha, N. C. and Decker, E. A. 1994. Rapid sensitive iron-based spectrophotometric methods for the determination of peroxide values in food lipids. *J. AOAC Int.* 77:421–424.
80. Shantha, N. C., Crum, A. D., and Decker, E. A. 1994. Conjugated linoleic acid concentrations in cooked beef containing antioxidants and hydrogen donors. *J. Food Lipids* 2:57–64.
81. Smith, L. L. and Johnson, B. H. 1989. Biological activities of oxysterols. *Free Rad. Biol. Med.* 7:285–332.
82. Smith, K. W., Bhaggan, K., Talbot, G., and van Malssen, K. F. 2011. Crystallization of fats: Influence of minor components and additives. *J. Am. Oil Chem. Soc.* 88(8):1085–1101.
83. Sofos, J. N. 1986. Use of phosphates in low sodium meat products. *Food Technol.* 40:52–57.
84. Talcott, S. T., Howard, L. R., and Brenes, C. H. 2000. Antioxidant changes and sensory properties of carrot puree processed with and without periderm tissue. *J. Agric. Food Chem.* 48:1315–1321.
85. Tasan, M. and Demirci, M. 2003. Trans FA in sunflower oil at different steps of refining. *J. Am. Oil Chem. Soc.* 80:825–828.
86. Timms, R. E. 1991. Crystallization of fats. *Chem. Ind.* May:342.
87. Tong, L. M., Sasaki, S., McClements, D. J., and Decker, E. A. 2000. Mechanisms of antioxidant activity of a high molecular weight fraction of whey. *J. Agric. Food Chem.* 48:1473–1478.
88. van Aken, G. A., Vingerhoeds, M. H., and de Wijk, R. A. 2011. Textural perception of liquid emulsions: Role of oil content, oil viscosity and emulsion viscosity. *Food Hydrocoll.* 25(4):789–796.
89. van Vliet, T., van Aken, G. A., de Jongh, H. H. J., and Hamer, R. J. 2009. Colloidal aspects of texture perception. *Adv. Colloid Interface Sci.* 150(1):27–40.
90. Waraho, T., McClements, D. J., and Decker, E. A. 2011. Mechanisms of lipid oxidation in food dispersions. *Trends Food Sci. Technol.* 22:3–13.
91. Walstra, P. 2003. *Physical Chemistry of Foods*. New York: Marcel Dekker.
92. Walstra, P. 1987. Fat crystallization. In: *Food Structure and Behaviour*, eds. J. M. V. Blanshard and P. Lillford. London, U.K.: Academic Press, Chap 5.
93. White, P. J. 2000. Fatty acids in oilseeds. In *Fatty Acids in Foods and Their Health Implications*, 2nd edn, ed. C.K. Chow, Marcel Dekker Inc., New York, pp. 227–263.
94. Yu, T. C. and Sinnhuber, R. O. 1967. An improved 2-thiobarbituric acid (TBA) procedure for measurement of autoxidation in fish oils. *J. Am. Oil Chem. Soc.* 44:256–261.
95. Zhuang, H., Barth, M. M., and Hildebrand, D. 2002. Fatty acid oxidation in plant lipids. In: *Food Lipids, Chemistry, Nutrition and Biotechnology*, eds. C. C. Akoh and D. B. Min. New York: Marcel Dekker, New York, pp. 413–464.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

5 Amino Acids, Peptides, and Proteins

Srinivasan Damodaran

CONTENTS

5.1 Introduction 237
5.2 Physicochemical Properties of Amino Acids 238
5.2.1 General Properties 238
5.2.1.1 Structure and Classification 238
5.2.1.2 Stereochemistry of Amino Acids 240
5.2.1.3 Acid–Base Properties and Relative Polarity of Amino Acids 241
5.2.1.4 Hydrophobicity of Amino Acids 244
5.2.1.5 Optical Properties of Amino Acids 246
5.2.2 Chemical Reactivity of Amino Acids 247
5.2.3 Summary 251
5.3 Protein Structure 251
5.3.1 Structural Hierarchy in Proteins 251
5.3.1.1 Primary Structure 251
5.3.1.2 Secondary Structure 253
5.3.1.3 Tertiary Structure 257
5.3.1.4 Quaternary Structure 260
5.3.2 Forces Involved in the Stability of Protein Structure 261
5.3.2.1 Steric Strains 261
5.3.2.2 van der Waals Interactions 261
5.3.2.3 Hydrogen Bonds 262
5.3.2.4 Electrostatic Interactions 263
5.3.2.5 Hydrophobic Interactions 264
5.3.2.6 Disulfide Bonds 265
5.3.3 Conformational Stability and Adaptability of Proteins 267
5.3.4 Summary 268
5.4 Protein Denaturation 269
5.4.1 Thermodynamics of Denaturation 270
5.4.2 Denaturing Agents 272
5.4.2.1 Physical Agents 272
5.4.2.2 Chemical Agents 279
5.4.3 Summary 284
5.5 Functional Properties of Proteins 284
5.5.1 Protein Hydration 286
5.5.2 Solubility 290
5.5.2.1 pH and Solubility 292
5.5.2.2 Ionic Strength and Solubility 293
5.5.2.3 Temperature and Solubility 295
5.5.2.4 Organic Solvents and Solubility 295

5.5.3 Interfacial Properties of Proteins ... 295
5.5.3.1 Emulsifying Properties ... 299
5.5.3.2 Foaming Properties ... 304
5.5.4 Flavor Binding ... 308
5.5.4.1 Thermodynamics of Protein–Flavor Interactions ... 309
5.5.4.2 Factors Influencing Flavor Binding ... 310
5.5.5 Viscosity ... 311
5.5.6 Gelation ... 313
5.5.7 Texturization ... 316
5.5.7.1 Spun-Fiber Texturization ... 317
5.5.7.2 Extrusion Texturization ... 317
5.5.8 Dough Formation ... 318
5.6 Protein Hydrolysates ... 321
5.6.1 Functional Properties ... 322
5.6.2 Allergenicity ... 324
5.6.3 Bitter Peptides ... 324
5.7 Nutritional Properties of Proteins ... 324
5.7.1 Protein Quality ... 325
5.7.2 Digestibility ... 325
5.7.2.1 Protein Conformation ... 325
5.7.2.2 Antinutritional Factors ... 327
5.7.2.3 Processing ... 328
5.7.3 Evaluation of Protein Nutritive Value ... 328
5.7.3.1 Biological Methods ... 328
5.7.3.2 Chemical Methods ... 329
5.7.3.3 Enzymatic and Microbial Methods ... 330
5.8 Processing-Induced Physical, Chemical, and Nutritional Changes in Proteins ... 330
5.8.1 Changes in Nutritional Quality and Formation of Toxic Compounds ... 330
5.8.1.1 Effect of Moderate Heat Treatments ... 330
5.8.1.2 Compositional Changes during Extraction and Fractionation ... 332
5.8.1.3 Chemical Alteration of Amino Acids ... 332
5.8.1.4 Effects of Oxidizing Agents ... 337
5.8.1.5 Carbonyl–Amine Reactions ... 340
5.8.1.6 Other Reactions of Proteins in Foods ... 343
5.8.2 Changes in the Functional Properties of Proteins ... 345
5.9 Chemical and Enzymatic Modification of Proteins ... 346
5.9.1 Chemical Modifications ... 346
5.9.1.1 Alkylation ... 347
5.9.1.2 Acylation ... 348
5.9.1.3 Phosphorylation ... 349
5.9.1.4 Sulfitolysis ... 349
5.9.1.5 Esterification ... 350
5.9.2 Enzymatic Modification ... 350
5.9.2.1 Enzymatic Hydrolysis ... 350
5.9.2.2 Plastein Reaction ... 351
5.9.2.3 Protein Cross-Linking ... 351
References ... 351

5.1 INTRODUCTION

Proteins play a central role in biological systems. Although DNA carries the basic information—mostly the codes for proteins sequences—the biochemical reactions and processes, including decoding of the information on DNA, that sustain the life of a cell/organism are exclusively performed by enzymes, which are proteins. Thousands of enzymes have been discovered. Each one of them catalyzes a highly specific biological reaction in cells. In addition to functioning as enzymes, proteins (such as collagen, keratin, and elastin) also function as structural components of cells, bones, nails, hair, tendons, etc., in complex organisms. The functional diversity of proteins essentially arises from their chemical makeup.

Proteins are highly complex polymers, made up of 20 different amino acids. The amino acid constituents are linked to each other in a linear sequence via substituted amide bonds. Unlike the glycosidic bonds in polysaccharides and phosphodiester bonds in nucleic acids, which are single bonds, the substituted amide linkage in proteins is a partial double bond, which further underscores the unique structural property of protein polymers. The functional diversity of proteins fundamentally lies in the multitude of three-dimensional conformations that can be generated by rearranging the amino acid sequence in proteins. For instance, a small protein of 200 amino acid residues can be arranged in 20^{200} different sequences, and each one of these sequences would have different three-dimensional structures and biological functions. To signify their biological importance, these macromolecules were named proteins, derived from the Greek word *proteois*, which means of the first kind.

At the elemental level, proteins contain 50%–55% carbon, 6%–7% hydrogen, 20%–23% oxygen, 12%–19% nitrogen, and 0.2%–3.0% sulfur on w/w basis. Protein synthesis occurs in ribosomes. After synthesis, cytoplasmic enzymes modify some of the amino acid constituents. This changes the elemental composition of some proteins. Proteins that are not enzymatically modified in cells are called "homoproteins" and those that are covalently modified or complexed with nonprotein components are called "conjugated proteins" or "heteroproteins." The nonprotein components are often referred to as "prosthetic groups." Examples of conjugated proteins include *nucleoproteins* (e.g., ribosomes), *glycoproteins* (e.g., ovalbumin, κ-casein), *phosphoproteins* (e.g., α- and β-caseins, kinases, phosphorylases), *lipoproteins* (e.g., proteins of egg yolk, several plasma proteins), and *metalloproteins* (e.g., hemoglobin, myoglobin, cytochromes, several enzymes). Glyco- and phosphoproteins contain covalently linked carbohydrate and phosphate groups, respectively, whereas the other conjugated proteins are noncovalent complexes containing nucleic acids, lipids, or metal ions. These noncovalent complexes can be dissociated under appropriate conditions.

Proteins also can be classified according to their three-dimensional structural organization. *Globular proteins* are those that exist in spherical or ellipsoidal shapes, resulting from folding or collapsing of the polypeptide chain(s) on itself. On the other hand, *fibrous proteins* are rod-shaped molecules containing twisted linear polypeptide chains (e.g., tropomyosin, collagen, keratin, and elastin). Fibrous proteins also can be formed by linear aggregation of small globular proteins (e.g., actin and fibrin). While a majority of enzymes are globular proteins, fibrous proteins invariably function as *structural proteins* in bones, nails, tendons, skin, and muscles.

The diverse biological functions of proteins can be categorized as *enzyme catalysts*, *structural proteins*, *contractile proteins* (myosin, actin, tubulin), *electron transporters* (cytochromes), ion pumps, *hormones* (insulin, growth hormone), *transfer proteins* (serum albumin, transferrin, hemoglobin), *antibodies* (immunoglobulins [Ig's]), *storage proteins* (egg albumen, seed proteins), and *toxins*. Storage proteins are found mainly in eggs and plant seeds. These proteins act as sources of nitrogen and amino acids for germinating seeds and embryos. Toxins are a part of the defense mechanism in certain microorganisms, animals, and plants for survival against predators.

All proteins are essentially made up of the same primary 20 amino acids. Some proteins however do not contain all 20 amino acids. The structural and functional differences among thousands of proteins arise from the sequence in which the amino acids are linked together via amide bonds. Literally, billions of trillions of proteins with unique properties can be synthesized by changing the amino acid sequence, the type and ratio of amino acids, and the length of the polypeptide chain.

All biologically produced proteins can be used as *food proteins*. However, for practical purposes, *food proteins* may be defined as those that are easily digestible, nontoxic, nutritionally adequate, functionally usable in food products, available in abundance, and agriculturally sustainable. Traditionally, milk, meats (including fish and poultry), eggs, cereals, legumes, and oilseeds have been the major sources of food proteins. Many of these are mainly storage proteins in animal and plant tissues, which act as the nitrogen source for the growing embryo or infants. Because of the burgeoning world population, which is expected to reach nine billion by the year 2050, there is a critical need to develop nontraditional sources of proteins for human nutrition to meet the future demand. The suitability of such new protein sources for use in foods, however, depends on their cost and their ability to fulfill the functional role of protein ingredients in processed and domestically prepared foods.

The functional properties of proteins in foods are related to their structural and other physicochemical characteristics. A fundamental understanding of the physical, chemical, nutritional, and functional properties of proteins and the changes these properties undergo during processing and storage are essential if the performance of proteins in foods were to be improved and if new or less costly sources of proteins were to compete with traditional food proteins.

5.2 PHYSICOCHEMICAL PROPERTIES OF AMINO ACIDS

5.2.1 General Properties

5.2.1.1 Structure and Classification

α-Amino acids are the basic structural units of proteins. These amino acids consist of an α-carbon atom covalently attached to a hydrogen atom, an amino group, a carboxyl group, and an

$$NH_2\text{—}\overset{\displaystyle H}{\underset{\displaystyle R}{\overset{|}{\underset{|}{C^{\alpha}}}}}\text{—}COOH \quad (5.1)$$

R group, which is commonly referred to as the side chain. The structures of amino acids (shown in Figure 5.1) differ only in the chemical nature of the side chain R group. The physicochemical properties, such as net charge, solubility, chemical reactivity, and hydrogen bonding potential, of the amino acids are dependent on the chemical nature of the R group.

A majority of natural proteins usually contain up to 20 different amino acids linked together via amide bonds. Of these, 19 amino acids contain the primary amine group and 1 (proline) contains a secondary imine group. Some enzymes (e.g., glutathione peroxidase and formate dehydrogenase) contain selenocysteine, which has been recognized as a new 21st natural amino acid in proteins [1]. A special selenocysteine-specific tRNA incorporates selenocysteine in a limited number of proteins using the stop codon UGA during translation using a mechanism known as translational recoding [2]. Bioinformatics analysis indicates that there are at least 25 genes coding for selenocysteine proteins in the human genome [3].

The amino acids listed in Figure 5.1 have genetic codes, including selenocysteine. That is, each one of these amino acids has a specific *t*-RNA that translates the genetic information on *m*-RNA

Aliphatic amino acids

Glycine (Gly, G) — GG(N)

Alanine (Ala, A) — GC(N)

Valine (Val, V) — GU(N)

Leucine (Leu, L) — UUA, UUG, CU(N)

Isoleucine (Ile, I) — AUU, AUC, AUA

Proline (Pro, P) — CC(N)

Aromatic amino acids

Phenylalanine (Phe, F) — UUU, UUC

Tyrosine (Tyr, Y) — AUA, UAC

Tryptophan (Trp, W) — UGG

Basic amino acids

Lysine (Lys, K) — AAA, AAG

Arginine (Arg, R) — AGA, AGG, CG(N)

Histidine (His, H) — CAU, CAC

Acidic amino acids

Aspartic acid (Asp, D) — GAU, GAC

Glutamic acid (Glu, E) — GAA, GAG

Amide amino acids

Asparagine (Asn, N) — AAU, AAC

Glutamine (Gln, Q) — CAA, CAG

Hydroxy amino acid

Serine (Ser, S) — AGU, AGC

Threonine (Thr, T) — AC(N)

Sulfur amino acids

Cysteine (Cys, C) — UGU, UGC

Methionine (Met, M) — AUG

Selenocysteine (SeCys) — UGA

FIGURE 5.1 Primary α-amino acids that occur in proteins.

into an amino acid sequence during protein synthesis. After proteins are synthesized and released from ribosomes, the side chains of some of the amino acid residues in select

COO^- / $^+H_3N-C-H$ / CH_2 / CH_2 / CH–OH / CH_2 / NH_3^+

Hydroxylysine

COO^- / $^+H_2N-C-H$ / H_2C CH_2 / CH / OH

Hydroxyproline

COO^- / $^+H_3N-C-H$ / CH_2 / CH / ^-OOC COO^-

γ-Carboxyglutamate

COO^- / $^+H_3N-C-H$ / CH_2 / $PO_4^{=}$

Phosphoserine

Derived amino acids (5.2)

proteins go through posttranslational enzymatic modification. These *derived amino acids* are either cross-linked amino acids or simple derivatives of single amino acids. Proteins that contain derived amino acid residues are called *conjugated* proteins. Cystine, which is S–S cross-linked cysteine residues found in most proteins, is a good example of a cross-linked amino acid. Other cross-linked amino acids, such as desmosine, isodesmosine, and di- and tri-tyrosine, are found in structural proteins such as elastin and resilin. Several simple derivatives of amino acids are found in several proteins. For example, 4-hydroxyproline and 5-hydroxylysine are found in collagen. These are the result of posttranslational modification during maturation of collagen fiber. Phosphoserine and phosphothreonine are found in several proteins, including caseins. *N*-methyllysine is found in myosin, and γ-carboxy-glutamate is found in several blood clotting factors and calcium binding proteins.

5.2.1.2 Stereochemistry of Amino Acids

With the exception of Gly, the α-carbon atom of all amino acids is chiral because of four different chemical groups attached to it. As a result, 19 of the 21 amino acids exhibit optical activity, that is, they rotate the plane of linearly polarized light. In addition to the α-carbon atom, the β-carbon atoms of Ile and Thr are also asymmetric, and therefore both Ile and Thr can exist in four enantiomeric forms. Among the derived amino acids, hydroxyproline and hydroxylysine also contain two asymmetric carbon centers. All proteins found in nature contain only L-amino acids. Conventionally, the L- and D-enantiomers are represented as this nomenclature is based on D- and L-glyceraldehyde configurations, and not on the actual direction of rotation of linearly polarized light. That is, the L-configuration does not refer to levorotation as in the case of L-glyceraldehyde. In fact most of the L-amino acids are dextrorotatory, not levorotatory.

COOH / $H-C^{\alpha}-NH_2$ / R

D-Amino acid

COOH / $H_2N-C^{\alpha}-H$ / R

L-Amino acid

(5.3)

5.2.1.3 Acid–Base Properties and Relative Polarity of Amino Acids

Since amino acids contain a carboxyl group (acidic) and an amino group (basic), they behave both as acids and bases; that is, they are *ampholytes*. For example, Gly, the simplest of all amino acids, can exist in three different ionized states, depending on the pH of the solution.

$$NH_3^+\text{—}\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle R}{|}}{C^\alpha}}\text{—}COOH \underset{}{\overset{K_1}{\rightleftharpoons}} NH_3^+\text{—}\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle R}{|}}{C^\alpha}}\text{—}COO^- \overset{K_2}{\rightleftharpoons} NH_2\text{—}\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle R}{|}}{C^\alpha}}\text{—}COO^- \quad (5.4)$$

At around neutral pH, both α-amino and α-carboxyl groups are ionized and the molecule becomes *dipolar* or a *zwitterion*. The pH at which the dipolar ion becomes electrically neutral is called the "isoelectric point" (pI). When the zwitterion is titrated with an acid, the COO^- group is protonated. The pH at which the concentrations of COO^- and COOH are equal is known as pK_{a1} (which is a negative logarithm of the acid dissociation constant K_{a1}). Similarly, when the zwitterion is titrated with a base, the NH_3^+ group is deprotonated. As before, the pH at which $[NH_3^+] = [NH_2]$ is known as pK_{a2}. A typical electrometric titration curve for a dipolar amino acid is shown in Figure 5.2. In addition to the α-amino and α-carboxyl groups, the side chains of Lys, Arg, His, Asp, Glu, Cys, and Tyr also contain ionizable groups. The pK_{a3} values of all the ionizable groups in amino acids are given in Table 5.1. The isoelectric points of amino acids can be estimated from their pK_{a1}, pK_{a2}, and pK_{a3} values, using the following expressions:

For amino acids with no charged side chain, $pI = (pK_{a1} + pK_{a2})/2$.
For acidic amino acids, $pI = (pK_{a1} + pK_{a3})/2$.
For basic amino acids, $pI = (pK_{a2} + pK_{a3})/2$.

The subscripts 1, 2, and 3 refer to α-carboxyl, α-amino, and side chain ionizable groups, respectively.

In proteins, the α-COOH of one amino acid is covalently coupled to the α-NH_2 of the next amino acid in the protein sequence through an amide bond. As a result, the only ionizable groups in proteins are the N-terminus amino group, the C-terminus carboxyl group, and ionizable groups on side chains. The pK_a values of the ionizable groups in proteins are different from those of free amino acids (Table 5.2). The significant shift in the pK_a values in

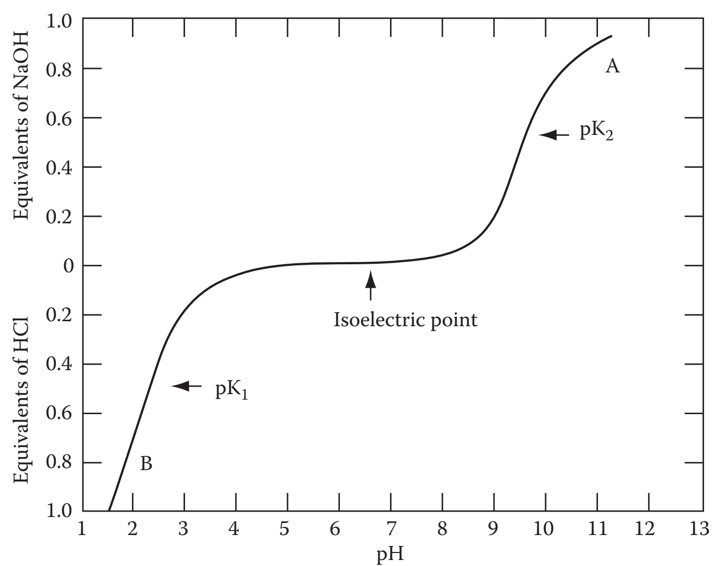


FIGURE 5.2 Titration curve of a typical amino acid. (From Tanford, C., *J. Am. Chem. Soc.*, 79, 5333, 1957.)

TABLE 5.1
Properties of Ionizable Groups in Free Amino Acids at 25°C

Amino Acid	pK_{a1} (–COOH)	pK_{a2} (NH_3^+)	pK_{a3} (Side Chain)	pI
Alanine	2.34	9.69	—	6.00
Arginine	2.17	9.04	12.48	10.76
Asparagine	2.02	8.80	—	5.41
Aspartic acid	1.88	9.60	3.65	2.77
Cysteine	1.96	10.28	8.18	5.07
Glutamine	2.17	9.13	—	5.65
Glutamic acid	2.19	9.67	4.25	3.22
Glycine	2.34	9.60		5.98
Histidine	1.82	9.17	6.00	7.59
Isoleucine	2.36	9.68	—	6.02
Leucine	2.30	9.60	—	5.98
Lysine	2.18	8.95	10.53	9.74
Methionine	2.28	9.21	—	5.74
Phenylalanine	1.83	9.13	—	5.48
Proline	1.94	10.60	—	6.30
Serine	2.20	9.15	—	5.68
Threonine	2.21	9.15	—	5.68
Tryptophan	2.38	9.39	—	5.89
Tyrosine	2.20	9.11	10.07	5.66
Valine	2.32	9.62	—	5.96

proteins, compared to those in free amino acids, is related to altered electronic and dielectric environments of these groups in the three-dimensional structure of proteins. (This property is important in enzymes.)

The degree of ionization of an ionizable group in proteins as well as in amino acids at any given solution pH can be determined using the *Henderson–Hasselbalch equation*:

$$\text{pH} = \text{pK}_\text{a} + \log\frac{[\text{Conjugated base}]}{[\text{Conjugated acid}]} \tag{5.5}$$

Using the Henderson–Hasselbalch equation, the net (fractional) charge carried by an ionizable group can be determined using the following equations: For groups that carry a charge in the dissociated state and uncharged in the protonated state (e.g., carboxyl, sulfhydryl, and phenolic groups), the fractional negative charge at any given solution pH is given by

$$\text{Negative charge} = \frac{-1}{1+10^{(\text{pK}_\text{a}-\text{pH})}} \tag{5.6}$$

For groups that carry a (positive) charge in the protonated state and neutral in the deprotonated state (e.g., amine and guanidinium groups), the fractional positive charge at any solution pH is given by

$$\text{Positive charge} = \frac{1}{1+10^{(\text{pH}-\text{pK}_\text{a})}} \tag{5.7}$$

TABLE 5.2
Average pK_a Values of Ionizable Groups in Proteins

Ionizable Group	pK_a	Acid Form ↔ Base Form
Terminal COOH	3.75	$-COOH \leftrightarrow -COO^-$
Terminal NH_2	7.8	$-NH_3^+ \leftrightarrow -NH_2$
Side chain COOH (Glu, Asp)	4.6	$-COOH \leftrightarrow -COO^-$
Side chain NH_2	10.2	$-NH_3^+ \leftrightarrow -NH_2$
Imidazole	7.0	CH_2-imidazole (NH^+, HN) ↔ CH_2-imidazole (N, HN)
Sulfhydryl	8.8	$-SH \leftrightarrow -S^-$
Phenolic	9.6	CH_2-phenyl-OH ↔ CH_2-phenyl-O^-
Guanidyl[a]	13.8[a]	$-NH-C(=^+NH_2)-NH_2$ ↔ $-NH-C(=NH)-NH_2$

[a] From Reference 117.

The net charge of a protein or a peptide at a given pH can be then estimated by summing up all the positive and negative charges at the given pH.

Amino acids can be classified into several categories based on the nature of interaction of the side chains with water. Amino acids with aliphatic (Ala, Ile, Leu, Met, Pro, and Val) and aromatic side chains (Phe, Trp, and Tyr) are hydrophobic, and hence they exhibit limited solubility in water (Table 5.3). Polar (hydrophilic) amino acids are quite soluble in water and they are either charged (Arg, Asp, Glu, His, and Lys) or uncharged (Ser, Thr, Asn, Gln, and Cys). The side chains of Arg and Lys contain guanidyl and amino groups, respectively, and thus are positively charged (basic) at neutral pH. The imidazole group of His is basic in nature. However, at neutral pH its net charge is only slightly positive. The side chains of Asp and Glu acids contain a carboxyl group and they carry a net negative charge at neutral pH. Both the basic and acidic amino acids are strongly hydrophilic. The net charge of a protein at physiological conditions is dependent on the relative numbers of basic and acidic amino acids residues in the protein.

The polarities of uncharged neutral amino acids fall between those of hydrophobic and charged amino acids. Ser and Thr are polar because of the ability of their OH group to hydrogen bond with water. Since Tyr also contains an ionizable phenolic group, which ionizes at alkaline pH, it is also considered to be a polar amino acid. However, based on its solubility characteristics at neutral pH, it should be regarded as a hydrophobic amino acid. The amide group of Asn and Gln is able to interact with water through hydrogen bonding. Upon acid or alkaline hydrolysis, the amide group of Asn and Gln is converted to carboxyl group with release of ammonia. A majority of Cys residues in proteins exists as cystine, which is a disulfide cross-linked dimer of Cys created by oxidation of the thiol groups.

Proline is a unique amino acid because it is the only *imino acid* in proteins. In proline, the propyl side chain is covalently linked to both the α-carbon atom and the α-amino group, forming a pyrrolidine ring structure.

TABLE 5.3
Properties of Amino Acids at 25°C

Amino Acid	Molecular Weight	Residue Volume, Δ^3	Residue Area,[a] Δ^2	Solubility (g L^{-1})	Hydrophobicity (kcal mol^{-1})[b,c] (ΔG^0_{tr})
Ala	89.1	89	115	167.2	0.4
Arg	174.2	173	225	855.6	−1.4
Asn	132.1	111	160	28.5	−0.8
Asp	133.1	114	150	5.0	−1.1
Cys	121.1	109	135	—	2.1
Gln	146.1	144	180	7.2 (37°C)	−0.3
Glu	147.1	138	190	8.5	−0.9
Gly	75.1	60	75	249.9	0
His	155.2	153	195	—	0.2
Ile	131.2	167	175	34.5	2.5
Leu	131.2	167	170	21.7	2.3
Lys	146.2	169	200	739.0	−1.4
Met	149.2	163	185	56.2	1.7
Phe	165.2	190	210	27.6	2.4
Pro	115.1	113	145	620.0	1.0
Ser	105.1	89	115	422.0	−0.1
Thr	119.1	116	140	13.2	0.4
Trp	204.2	228	255	13.6	3.1
Tyr	181.2	194	230	0.4	1.3
Val	117.1	140	155	58.1	1.7

[a] From Reference 118.
[b] From Reference 119.
[c] The ΔG values are relative to glycine based on the side chain distribution coefficients (K_{eq}) between 1-octanol and water.

5.2.1.4 Hydrophobicity of Amino Acids

One of the major factors affecting physicochemical properties, such as structure, solubility, and fat-binding properties, of proteins and peptides is the hydrophobicity of the constituent amino acid residues [4]. Hydrophobicity can be defined as the excess free energy of a solute dissolved in water compared to that in an organic solvent under similar conditions. The most direct and simplest way to estimate hydrophobicities of amino acid side chains is experimental determination of free energy changes for dissolution of amino acid side chains in water and in an organic solvent, such as octanol or ethanol. The chemical potential of an amino acid dissolved in water can be expressed by

$$\mu_{AA,w} = \mu^o_{AA,w} + RT\ln\left(\gamma_{AA,w} X_{AA,w}\right) \quad (5.8)$$

where

$\mu^o_{AA,w}$ is the standard chemical potential of the amino acid
$\gamma_{AA,w}$ is the activity coefficient
$X_{AA,w}$ is the concentration
T is the absolute temperature
R is the gas constant

Similarly, the chemical potential of an amino acid dissolved in an organic solvent, for example, octanol, can be expressed as

$$\mu_{AA,oct} = \mu^o_{AA,oct} + RT\ln\left(\gamma_{AA,oct} X_{AA,oct}\right) \quad (5.9)$$

In saturated solutions, in which $X_{AA,w}$ and $X_{AA,oct}$ represent solubility of the amino acid in water and octanol, respectively, the chemical potentials of the amino acid in water and in octanol are the same, that is,

$$\mu_{AA,w} = \mu_{AA,oct} \tag{5.10}$$

Therefore

$$\mu^{o}_{AA,oct} + RT\ln\left(\gamma_{AA,oct}X_{AA,oct}\right) = \mu^{o}_{AA,w} + RT\ln\left(\gamma_{AA,w}X_{AA,w}\right) \tag{5.11}$$

The quantity $\mu^{o}_{AA,w} - \mu^{o}_{AA,oct}$, which represents the difference between the standard chemical potentials of the amino acid arising from the interaction of the amino acid with water and with octanol, can be defined as the standard free energy change $\Delta G^{0}_{tr,(oct\rightarrow w)}$ for transfer of the amino acid from octanol to water. Thus, assuming that the ratio of activity coefficients is one, the previous equation can be expressed as

$$\Delta G^{0}_{tr,(oct\rightarrow w)} = -RT\ \ln\left(\frac{S_{AA,w}}{S_{AA,oct}}\right) \tag{5.12}$$

where $S_{AA,oct}$ and $S_{AA,w}$ represent solubilities in mole fraction units of the amino acid in octanol and water, respectively.

As is true of all other thermodynamic parameters, ΔG^{o}_{tr} is an additive function. That is, if a molecule has two chemical groups, A and B, covalently attached, the ΔG^{o}_{tr} for transfer from one solvent to another solvent is the sum of the free energy changes for transfer of group A and group B. That is,

$$\Delta G^{o}_{tr,AB} = \Delta G^{o}_{tr,A} + \Delta G^{o}_{tr,B} \tag{5.13}$$

The same logic can be applied to transfer of an amino acid from octanol to water. For example, Val can be considered as a derivative of Gly with an isopropyl side chain at the α-carbon atom.

COO^-

^+H_3N—C—H   Glycyl group

CH

H_3C CH_3 Propyl group

The free energy change of transfer of valine from octanol to water can then be written as

$$\Delta G^{o}_{tr,Val} = \Delta G^{o}_{tr,Gly} + \Delta G^{o}_{tr,side\ chain} \tag{5.14}$$

or

$$\Delta G^{o}_{tr,side\ chain} = \Delta G^{o}_{tr,Val} - \Delta G^{o}_{tr,Gly} \tag{5.15}$$

In other words, the hydrophobicities of amino acid side chains can be determined by subtracting $\Delta G^{0}_{tr,Gly}$ from $\Delta G^{0}_{tr,AA}$.

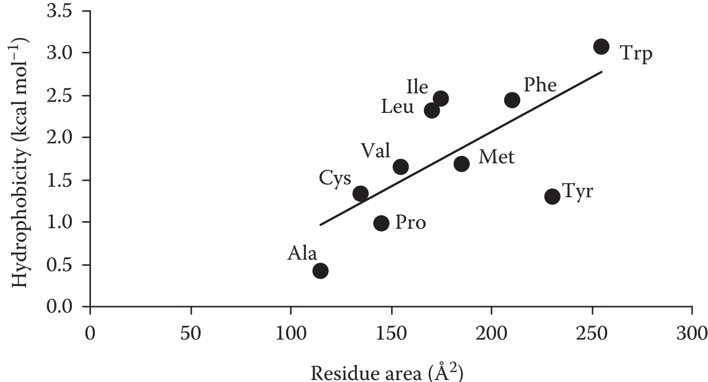


FIGURE 5.3 Correlation between surface area and hydrophobicity of nonpolar amino acid residues.

The hydrophobicity values of amino acid side chains, that is, the free energy change for transfer of an amino acid side chain from the octanol phase to water phase, obtained in this manner are given in Table 5.3. Amino acid side chains with large positive ΔG^0_{tr} values are hydrophobic; they would prefer to be in an organic phase rather than in an aqueous phase. In proteins, these amino acid residues would tend to locate themselves in the protein interior and away from water, where the polarity of the environment is similar to that of an organic phase. Amino acid residues with negative ΔG^o_{tr} values are hydrophilic, and these residues would tend to locate themselves on the surface of protein molecules in contact with the aqueous phase.

The hydrophobicity of a nonpolar side chain is a linear function of the surface area of contact between the nonpolar side chain and the surrounding aqueous phase. This is shown in Figure 5.3.

5.2.1.5 Optical Properties of Amino Acids

The aromatic amino acids Trp, Tyr, and Phe absorb light in the near-ultraviolet (UV) region (250–300 nm). In addition, Trp and Tyr also exhibit fluorescence in the UV region. The maximum wavelengths of absorption and fluorescence emission of the aromatic amino acids are given in Table 5.4. These amino acid residues are responsible for UV absorption properties of proteins in the 250–300 nm range, with maximum absorption at about 280 nm for most proteins. Since both absorption and fluorescence properties of these amino acids are influenced by the polarity of their environment, changes in the optical properties of proteins are often used as a means to monitor conformational changes in proteins.

TABLE 5.4
Ultraviolet Absorbance and Fluorescence of Aromatic Amino Acids

Amino Acid	λ_{max} of Absorbance (nm)	Molar Extinction Coefficient (L mol^{-1} cm^{-1})	λ_{max} of Fluorescence (nm)
Phenylalanine	260	190	282[a]
Tryptophan	278	5500	348[b]
Tyrosine	275	1340	304[b]

[a] Excitation at 260 nm.
[b] Excitation at 280 nm.

5.2.2 Chemical Reactivity of Amino Acids

The reactive groups, such as amino, carboxyl, sulfhydryl, phenolic, hydroxyl, thioether (Met), imidazole, and guanyl groups in proteins, can participate in chemical reactions in a manner similar to small organic molecules containing these groups. Typical reactions for various side chain groups are presented in Table 5.5. Several of these reactions can be used to alter the hydrophilic and hydrophobic properties and the functional properties of proteins and peptides. Some of these reactions also can be used to quantify amino acids and specific amino acid residues in proteins. For example, reaction of amino acids with ninhydrin, *O*-phthaldialdehyde, or fluorescamine is regularly used in the quantification of amino acids.

Reaction with ninhydrin: The ninhydrin reaction is often used to quantify free amino acids. When an amino acid is reacted with an excess amount of ninhydrin, one mole each of ammonia, aldehyde, CO_2, and hydrindantin are formed for every mole of amino acid consumed (Equation 5.16). The liberated ammonia subsequently reacts with one mole of ninhydrin and one mole of hydrindantin, forming a purple color product known as Ruhemann's purple, which has maximum absorbance at 570 nm. Proline and hydroxyproline give a yellow color product, which has maximum absorbance at 440 nm. These color reactions provide the basis for colorimetric determination of amino acids.

$$\text{Ninhydrin} + R{-}CH(NH_2){-}COOH \longrightarrow \text{Ruhemann's purple} \; +R{-}CHO \; +CO_2 \; +3H_2O \quad (5.16)$$

The ninhydrin reaction is usually used to determine the amino acid composition of proteins. In this case, the protein is first acid hydrolyzed to the amino acid level. The freed amino acids are then separated and identified using ion exchange/hydrophobic chromatography. The column eluates are reacted with ninhydrin and quantified by measuring absorbance at 570 and 440 nm.

Reaction with O-phthaldialdehyde: Reaction of amino acids with *O*-phthaldialdehyde (1,2-benzene dicarbonal) in the presence of 2-mercaptoethanol yields a highly fluorescent derivative that has an excitation maximum at 380 nm and a fluorescence emission maximum at 450 nm.

$$\text{O-phthaldialdehyde} + R{-}CH(NH_2){-}COOH \xrightarrow[\text{(mercaptoethanol)}]{HS{-}CH_2{-}CH_2{-}OH} \text{isoindole}(S{-}CH_2{-}CH_2{-}OH)\,N{-}CH(R){-}COOH \quad (5.17)$$

Reaction with fluorescamine: Reaction of amino acids, peptides, and proteins containing primary amines with fluorescamine yields a highly fluorescent derivative with fluorescence

TABLE 5.5
Chemical Reactions of Functional Groups in Amino Acids and Proteins

Type of Reaction	Reagent and Conditions	Product	Remarks
A. Amino groups			
1. Reductive alkylation	HCHO, $NaBH_4$ (formaldehyde)	Ⓡ$-\overset{+}{N}H(CH_3)_2$	Useful for radiolabeling proteins
2. Guanidation	$NH{=}C(O{-}CH_3){-}NH_2$ (*O*-methylisourea) pH 10.6, 4°C for 4 days	Ⓡ$-NH-C(=NH_2^+)-NH_2$	Converts lysyl side chain to homoarginine
3. Acetylation	Acetic anhydride	Ⓡ$-NH-C(=O)-CH_3$	Eliminates the positive charge
4. Succinylation	Succinic anhydride	Ⓡ$-NH-C(=O)-(CH_2)_2-COOH$	Introduces a negative charge at lysyl residues
5. Thiolation	(Thioparaconic acid)	Ⓡ$-NH-C(=O)-CH_2-CH(COOH)-CH_2-SH$	Eliminates positive charge and initiates thiol group at lysyl residues
6. Arylation	1-Fluoro-2,4-dinitrobenzene (FDNB)	Ⓡ$-NH-C_6H_2(NO_2)_3$	Used for the determination of amino groups
	2,4,6-Trinitrobenzene sulfonic acid (TNBS)	Ⓡ$-NH-C_6H_2(NO_2)_3$	The extinction coefficient is $1.1 \times 10^4\ M^{-1}\ cm^{-1}$ at 367 nm; used to determine reactive lysyl residues in proteins
7. Deamination	1.5 M $NaNO_2$ in acetic acid, 0°C	$R-OH + N_2 + H_2O$	

(*Continued*)

TABLE 5.5 (*Continued*)
Chemical Reactions of Functional Groups in Amino Acids and Proteins

Type of Reaction	Reagent and Conditions	Product	Remarks
B. Carboxyl groups			
1. Esterification	Acidic methanol	Ⓡ—$COOCH_3 + H_2O$	Hydrolysis of the ester occurs at pH > 6.0
2. Reduction	Borohydride in tetrahydrofuran, trifluoracetic acid	Ⓡ—CH_2OH	
3. Decarboxylation	Acid, alkali, heat treatment	R—CH_2—NH_2	Occurs only with amino acid, not with proteins
C. Sulfhydryl group			
1. Oxidation	Performic acid	Ⓡ—CH_2—SO_3H	
2. Blocking	CH_2—CH_2 (ring with NH) (Ethyleneimine)	Ⓡ—CH_2—S—$(CH_2)_2$—NH_3^+	Introduces amino group
	Iodoacetic acid	Ⓡ—CH_2—S—CH_2—COOH	Introduces one amino group
	CH—CO / ‖ O / CH—CO (Maleic anhydride)	Ⓡ—CH_2—S—CH_2—COOH, with CH_2—COOH on the CH_2	Introduces two negative charges for each SH group blocked
	p-Mercuribenzoate	Ⓡ—CH_2—S—Hg—C_6H_4—COO^-	The extinction coefficient of this derivative at 250 nm (pH 7) is 7500 M^{-1} cm^{-1}; this reaction is used to determine SH content of proteins
	N-Ethylmaleimide	Ⓡ—CH_2—S—CH—CO—NH—CO—CH_2 (ring, CH bonded to CH_2)	Used for blocking SH groups

(*Continued*)

TABLE 5.5 (*Continued*)
Chemical Reactions of Functional Groups in Amino Acids and Proteins

Type of Reaction	Reagent and Conditions	Product	Remarks
	5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB)	Ⓡ—S—S—(C₆H₃)(COO⁻)—NO₂ + ⁻S—(C₆H₃)(COO⁻)—NO₂ (Thionitrobenzoate)	One mole of thionitrobenzoate is released; the ϵ_{412} of thionitrobenzoate is 13,600 M^{-1} cm^{-1}; this reaction is used to determine SH groups in proteins
D. Serine and threonine			
1. Esterification	CH_3—COCl	Ⓡ—O—C(=O)—CH_3	
E. Methionine			
1. Alkyl halides	CH_3I	Ⓡ—CH_2—$S^{\oplus}$(CH_3)—CH_3	
2. β-Propiolactone	CH_2—CH_2—CO (ring closed through —O—)	Ⓡ—CH_2—$S^{\oplus}$(CH—CH_2—COOH)—CH_3	

emission maximum at 475 nm when excited at 390 nm. This method can be used to quantify amino acids as well as proteins and peptides.

$$\text{Fluorescamine} + R\text{—CH(NH}_2\text{)—COOH} \longrightarrow \text{product} + H_2O \qquad (5.18)$$

Fluorescamine

5.2.3 Summary

- Proteins are made up of 21 naturally occurring amino acid. Selenocysteine has been recognized as the 21st amino acid.
- The acid–base properties of amino acid residues in a protein determine the net charge of a protein at a given solution pH.
- Hydrophobicity of amino acid residues is defined as the free energy change for the transfer of the side chain of a residue from an organic phase to an aqueous phase. Octanol is used as the reference solvent since its dielectric constant is similar to that of a protein's interior.
- The aromatic amino acid residues in proteins are responsible for the near-UV absorption spectrum of proteins.

5.3 PROTEIN STRUCTURE

5.3.1 Structural Hierarchy in Proteins

Four levels of protein structure exist: *primary, secondary, tertiary,* and *quaternary.*

5.3.1.1 Primary Structure

The primary structure of a protein refers to the linear sequence in which the constituent amino acids are covalently linked through amide bonds, also known as peptide bonds. The amide linkage results from condensation of the α-carboxyl group of ith amino acid and the α-amino group of i + 1th amino acid with removal of a water molecule. In this linear sequence, all the amino acid residues are in the L-configuration. A protein with n amino acid residues contains n − 1 peptide linkages.

$$\text{—NH—CH(R}_1\text{)—COOH} + \text{NH}_2\text{—CH(R}_2\text{)—COOH} \xrightarrow{-H_2O} \text{—NH—CH(R}_1\text{)—}\underbrace{\text{C(=O)—N(H)}}_{\text{Peptide bond}}\text{—CH(R}_2\text{)—COOH} \qquad (5.19)$$

Peptide bond

The terminus with the free α-amino group is known as the N-terminal, and that with the free α-COOH group is known as the C-terminal. By convention, the N-terminal represents the beginning and the C-terminal the end of the polypeptide chain when primary sequence information is indicated.

The chain length (n) and the sequence in which the n residues are linked act as the code for formation of secondary and tertiary structures and the ultimate physicochemical, structural, and biological functionality of a protein. The molecular mass of proteins ranges from a few thousand Daltons (Da) to over a million Da. For example, titin, which is a single-chain protein found in muscle has a molecular weight of over one million Da, whereas secretin has a molecular weight of about 2300 Da. The molecular weight of most proteins is in the range of 10,000–100,000 Da.

The backbone of polypeptides can be depicted as repeating units of $-N-C-C^{\alpha}-$ or $-{}^{\alpha}C-C-N$. The expression $-NH-{}^{\alpha}CHR-CO-$ relates to an amino acid residue, whereas $-{}^{\alpha}CHR-CO-NH-$

Amino acid residue

Peptide unit

$$-NH-{}^{\alpha}CH(R_i)-C(=O)-N(H)-{}^{\alpha}CH(R_{i+1})-COOH \quad (5.20)$$

represents a *peptide unit*. Although the CO−NH bond is depicted as a single covalent bond, in reality it has a partial double bond character because of the resonance structure caused by delocalization of electrons (Equation 5.21).

$$-C(=O)-\ddot{N}(H)- \rightleftharpoons -C(O^-)=N^+(H)-$$

$$-C-C(O^{-(-0.42)})\cdots N(H^{+(+0.2)})-C \quad \text{Dipole length} = 0.88\ \text{Å} \quad (5.21)$$

This has several important structural implications in proteins.

- First, the resonance structure precludes protonation of the peptide N−H group.
- Second, the partial double bond restricts rotation of the CO−NH bond to a maximum of 6°, known as ω-angle. Because of this restriction, each six-atom segment ($-C^{\alpha}-CO-NH-C^{\alpha}-$) of the peptide backbone lies in a single plane. The polypeptide backbone, in essence, can be depicted as a series of $-C^{\alpha}-CO-NH-C^{\alpha}$-planes connected at the C^{α} atoms as shown in the following scheme. Since peptide bonds constitute about one-third of the total covalent bonds of the backbone, their restricted rotational freedom drastically reduces backbone flexibility. Only the $N-C^{\alpha}$ and $C^{\alpha}-C$ bonds have rotational freedoms, and these are termed ϕ (*phi*) and ψ (*psi*) dihedral angles, respectively. These are also known as main-chain torsion angles.

$$-{}^{\alpha}C(R_1)-C(O)\cdots N(H)-{}^{\alpha}C(R_2)-C(O)\cdots N(H)-{}^{\alpha}C(R_3)-C(O)\cdots N(H)-{}^{\alpha}C(R_4)- \quad (5.22)$$

- Third, delocalization of electrons also imparts a partial negative charge to the carbonyl oxygen atom and a partial positive charge to the hydrogen atom of the N−H group. Because of this, hydrogen bonding (*dipole–dipole interaction*) between the C=O and N−H groups of peptide backbone is possible under appropriate conditions.
- Another consequence of the partial double-bond nature of the peptide bond is that the four atoms attached to the peptide bond can exist either in *cis* or *trans* configuration. However, almost all protein peptide bonds exist in the *trans* configuration.

$O^{\delta-}$ $^{\alpha}C_{i+1}$ C N $^{\alpha}C_i$ $H^{\delta+}$ $O^{\delta-}$ $H^{\delta+}$ C N $^{\alpha}C_i$ $^{\alpha}C_{i+1}$ (5.23)

trans *cis*

This is due to the fact that the *trans* configuration is thermodynamically more stable than the *cis* configuration. Since *tran* → *cis* transformation increases the free energy of the peptide bond by 8.3 kcal mol^{-1}, isomerization of peptide bonds does not occur in proteins. One exception to this is peptide bonds involving proline residues. Since the free energy change for *trans* → *cis* transformation of peptide bonds involving proline residues is only about 1.86 kcal mol^{-1}, at high temperatures these peptide bonds sometimes do undergo *trans* → *cis* isomerization.

Although the $N-C^{\alpha}$ and $C^{\alpha}-C$ bonds are truly single bonds, and thus the ϕ (*phi*) and ψ (*psi*) dihedral angles can theoretically have 360° rotational freedom, in reality their rotational freedoms are restricted by steric hindrances from side chain atoms. These restrictions further decrease the flexibility of the polypeptide chain.

5.3.1.2 Secondary Structure

Secondary structure refers to the periodic spatial arrangement of amino acid residues at certain segments of the polypeptide chain. The periodic structures arise when consecutive amino acid residues in a segment assume the same set of ϕ and ψ torsion angles. The twist of the ϕ and ψ angles is driven by near-neighbor or short-range noncovalent interactions between amino acid side chains, which lead to a decrease in local free energy. The *aperiodic* or *random* structure refers to those regions of the polypeptide chain where successive amino acid residues have different sets of ϕ and ψ torsion angles.

In general, two forms of periodic (regular) secondary structures are found in proteins. These are helical structures and extended sheetlike structures. The geometric characteristics of various regular structures found in proteins are given in Table 5.6.

5.3.1.2.1 Helical Structures

Protein helical structures are formed when the ϕ and ψ angles of consecutive amino acid residues are twisted to a same set of values. By selecting different combinations of ϕ and ψ angles, it is theoretically possible to create several types of helical structures with different geometries. However, α-helix is the predominant helical structure found in proteins, as it is the most stable of all the helical structures. Short segments of the 3_{10}*-helix* also have been located in several globular proteins.

The geometry of the α-helix is shown in Figure 5.4. The pitch of this helix, that is, the increase in axial length per rotation, is 5.4 Å. Each helical rotation involves 3.6 amino acid residues, with each residue extending the axial length by 1.5 Å. The angle of rotation on the axis per residue is 100° (i.e., 360°/3.6). In this configuration, the amino acid side chains are oriented perpendicular to the axis of the helix.

The α-helices are stabilized by hydrogen bonding. In this structure, each backbone N−H group is hydrogen bonded to the C=O group of the fourth preceding residue. Thirteen backbone atoms are

TABLE 5.6
Geometric Characteristics of Regular Polypeptide Conformations

Structure	ϕ	ψ	n	r	h (Å)	t
Right-handed α-helix	−58°	−47°	3.6	13	1.5	100°
π-Helix	−57°	−70°	4.4	16	1.15	81.8°
3_{10}-Helix	−49°	−26°	3	10	2	120°
Parallel β-sheet	−119°	+113°	2	—	3.2	—
Antiparallel β-sheet	−139°	+135°	2	—	3.4	—
Polyproline I (*cis*)	−83°	+158°	3.33		1.9	
Polyproline II (*trans*)	−78°	+149°	3.00		3.12	

ϕ and ψ represent dihedral angles of the N−C_α and C_α−C bonds, respectively; n is number of residues per turn; r is the number of backbone atoms within a hydrogen-bonded loop of helix; h is the rise of helix per amino acid residue; t = 360°/n, twist of helix per residue.

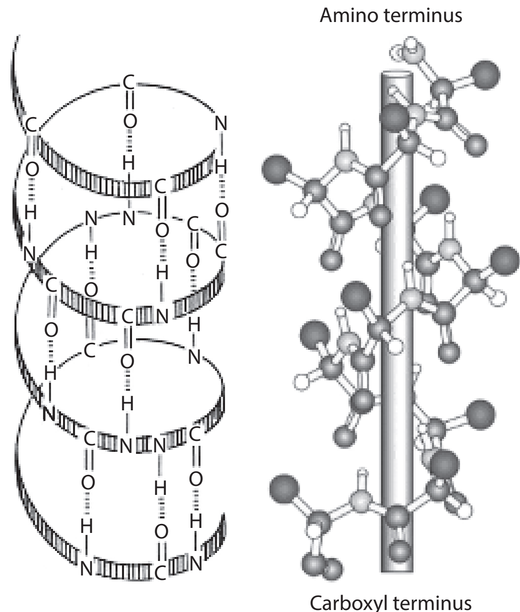


FIGURE 5.4 Spatial arrangement of polypeptides in α-helix. (From https://www.google.com/search?q=alpha+helix.)

in this hydrogen-bonded loop; thus the α-helix is sometimes called the 3.6_{13} helix (Figure 5.4). The hydrogen bonds are oriented parallel to the helix axis, and the N, H, and O atoms of the hydrogen bond lie almost in a straight line, that is, the hydrogen bond angle is almost zero. The hydrogen bond length, that is, the N−H···O distance, is about 2.9 Å, and the strength of this bond is about 4.5 kcal mol^{-1}. The α-helix can exist in either a right- or left-handed orientation. These are mirror images of each other. However, the right-handed orientation is the common one in naturally occurring proteins.

The details for α-helix formation are embedded as a binary code in the amino acid sequence. The binary code is related to the arrangement of polar and nonpolar residues in the sequence. Polypeptide segments with repeating seven amino acid (heptet) sequences of the kind −P−N−P−P−N−N−P−, where P and N are polar and nonpolar residues, respectively, readily form α-helices

in aqueous solutions [5]. It is the binary code, and not the precise identities of the polar and nonpolar residues in the heptet sequence, that dictates α-helix formation. Slight variations in the binary code of the heptet are tolerated, provided other inter- or intramolecular interactions are favorable for α-helix formation. For example, tropomyosin, a muscle protein, exists entirely in a coiled-coil α-helical rod form. The repeating heptet sequence in this protein is −N−P−P−N−P−P−P−, which is slightly different from the previous sequence. Despite this variation, tropomyosin exists entirely in the α-helix form because of other stabilizing interactions in the coiled-coil rod [6].

Most of the α-helical structures found in proteins are amphiphilic in nature, that is, one-half of the helix's surface is occupied by hydrophobic residues and the other half by hydrophilic residues. This is schematically shown in the form of an α-helical wheel in Figure 5.5. In most proteins, the nonpolar surface of the helix faces the protein interior and is generally engaged in hydrophobic interactions with other nonpolar surfaces.

In proline residues, because of the ring structure formed by covalent attachment of the propyl side chain to the amino group, rotation of the $N-C^{\alpha}$ bond is not possible, and therefore the ϕ angle has a fixed value of 70°. In addition, since there is no hydrogen at the nitrogen atom, it cannot form hydrogen bonds. Because of these two attributes, segments containing proline residues cannot form α-helices. In fact, proline is considered to be an α-helix breaker. Proteins containing high levels of proline residues tend to assume a random or aperiodic structure. For example, proline residues constitute about 17% of the total amino acid residues in β-casein, and 8.5% in α_{s1}-casein, and because of the uniform distribution of these residues in their primary structures, the formation of α-helices and other ordered secondary structures is precluded in these proteins. However, polyproline is able to form two types of helical structures, termed "polyproline I" and "polyproline II." In polyproline I, the peptide bonds are in the *cis* configuration, and in polyproline II they are in *trans*. Other geometric characteristics of these helices are given in Table 5.6. Collagen, which is the most abundant animal protein, exists as polyproline II–type helix. In collagen, on an average, every third residue is

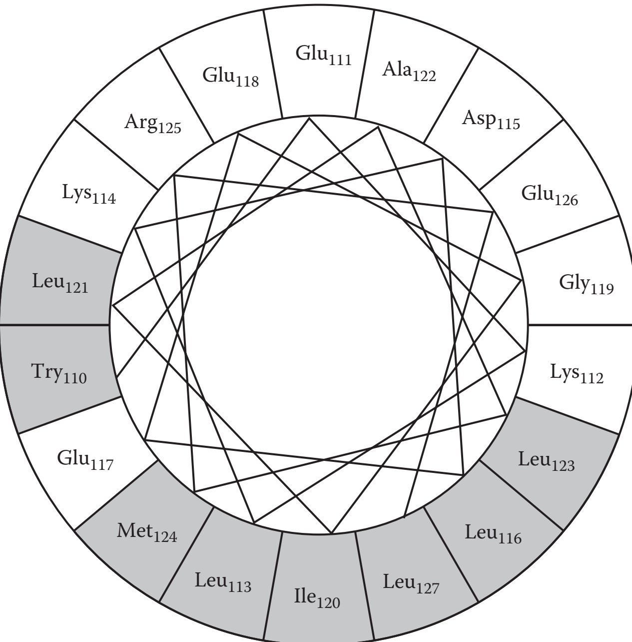


FIGURE 5.5 Cross-sectional view of the helical structure of residues 110–127 of bovine growth hormone. The top of the helical wheel (unfilled) represents the hydrophilic surface, and the bottom (filled) represents the hydrophobic surface of the amphiphilic helix. (From He, X.M. and Carter, D.C., Atomic structure and chemistry of human serum albumin, *Nature*, 358, 209–214, 1992. Reprinted with permission of AAAS.)

a glycine, which is followed usually by a proline residue. Three polypeptide chains are entwined to form a triple helix, and the stability of the triple helix is maintained by interchain hydrogen bonds. This unique triple helix structure is responsible for the high tensile strength of collagen.

5.3.1.2.2 β-Sheet Structure

The β-*sheet* is an extended structure with specific geometries given in Table 5.6. In this extended form, the C=O and N−H groups are oriented perpendicular to the direction of the chain, and therefore hydrogen bonding is possible only between segments (i.e., intersegment), and not within a segment (i.e., intrasegment). The β-strands are usually about 5–15 amino acid residues long. In proteins, two β-strands of the same molecule interact via hydrogen bonding, forming a sheetlike structure known as β-pleated sheet. In the sheetlike structure, the side chains are oriented perpendicular (above and below) to the plane of the sheet. Depending on the N → C directional orientations of the strands, two types of β-pleated sheet structures, namely, *parallel* β-*sheet* and *antiparallel* β-*sheet*, can form (Figure 5.6). In parallel β-sheet, the N → C directions of the β-strands run parallel to each other, whereas in the other sheet, they run opposite to each other. These differences in chain directions affect the geometry of hydrogen bonds. In antiparallel β-sheets, the N−H···O atoms lie in a straight line (zero H-bond angle), which enhances the stability of the hydrogen bond, whereas in parallel β-sheets, they lie at an angle, which reduces the stability of the hydrogen bonds. Antiparallel β-sheets are, therefore, more stable than parallel β-sheets.

The binary code that specifies formation of β-sheet structures in proteins is −N−P−N−P−N−P−N−P−. Clearly, polypeptide segments containing alternating polar and nonpolar residues have a high propensity to form β-sheet structures. Segments rich in bulky hydrophobic side chains, such

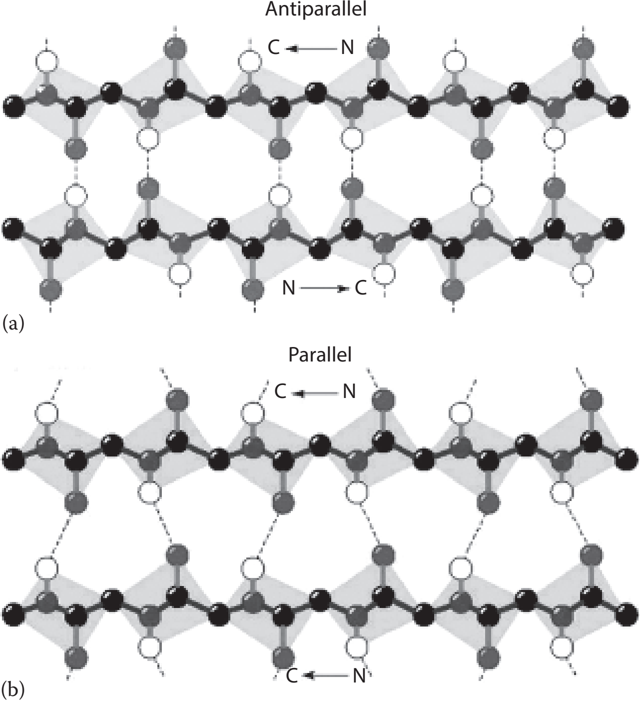


FIGURE 5.6 Parallel (a) and antiparallel (b) β-sheets. The dotted lines represent hydrogen bonds between peptide groups. The side chains at C_α atoms are oriented perpendicular (up or down) to the direction of the backbone. (From Brutlag, D.L., Advanced molecular biology course, http://cmgm.stanford.edu/biochem201/slides/protein structure, 2000.)

TABLE 5.7
Secondary Structure Content of Selected Globular Proteins[a]

Protein	%α-Helix	%β-Sheet	%β-Turns	%Aperiodic
Deoxyhemoglobin	85.7	0	8.8	5.5
Bovine serum albumin	67.0	0	0	33.0
α_{s1}-Casein	15.0	12.0	19.0	54.0
β-Casein	12.0	14.0	17.0	57.0
κ-Casein	23.0	31.0	14.0	32.0
Chymotrypsinogen	11.0	49.4	21.2	18.4
Immunoglobulin G	2.5	67.2	17.8	12.5
Insulin (dimer)	60.8	14.7	10.8	15.7
Bovine trypsin inhibitor	25.9	44.8	8.8	20.5
Ribonuclease A	22.6	46.0	18.5	12.9
Egg lysozyme	45.7	19.4	22.5	12.4
Ovomucoid	26.0	46.0	10.0	18.0
Ovalbumin	49.0	13.0	14.0	24.0
Papain	27.8	29.2	24.5	18.5
α-Lactalbumin	26.0	14.0	0	60.0
β-Lactoglobulin	6.8	51.2	10.5	31.5
Soy 11S	8.5	64.5	0	27.0
Soy 7S	6.0	62.5	2.0	29.5
Phaseolin	10.5	50.5	11.5	27.5
Myoglobin	79.0	0	5.0	16.0

[a] Compiled from various sources. The values represent % of total number of amino acid residues.

as Val and Ile, also have a tendency to form a β-sheet structure. As expected, some variation in the code is tolerated.

The β-sheet structure is generally more stable than the α-helix. Proteins that contain large fractions of β-sheet structure usually exhibit high denaturation temperatures. Examples are β-lactoglobulin (51% β-sheet) and soy 11S globulin (64% β-sheet), which have thermal denaturation temperatures of 75.6°C and 84.5°C, respectively. On the other hand, the denaturation temperature of bovine serum albumin, which has about 64% α-helix structure, is only about 64°C [7]. When solutions of α-helix-type proteins are heated and cooled, the α-helix is usually converted to β-sheet [7]. Conversion of α-helix to β-sheet structure occurs spontaneously in prion-like proteins under certain solution conditions [8]. However, heat-induced conversion from β-sheet to α-helix has not been observed in proteins.

Another common structural feature found in proteins is the β-bend or β-turn. This arises as a result of 180° reversal of the polypeptide chain involved in β-sheet formation. The hairpin-type bend is the result of antiparallel β-sheet formation, and the crossover bend is the result of parallel β-sheet formation. Usually, a β-bend involves a four-residue segment folding back on itself and the bend is stabilized by a hydrogen bond. The amino acid residues Asp, Cys, Asn, Gly, Tyr, and Pro are common in β-bends.

The α-helix and β-sheet contents of several proteins are given in Table 5.7.

5.3.1.3 Tertiary Structure

Tertiary structure refers to the equilibrium spatial arrangement attained when a linear protein chain with secondary structure segments folds further into a compact three-dimensional form.

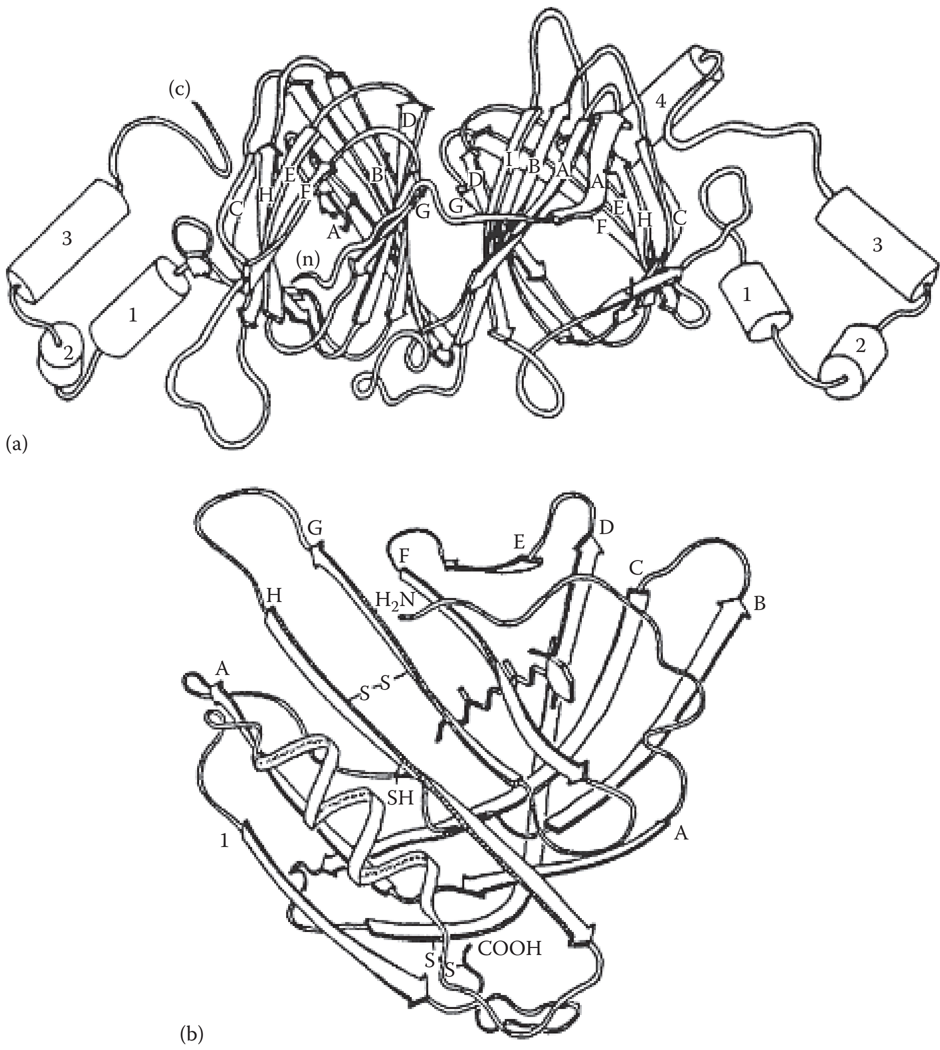


FIGURE 5.7 Tertiary structures of (a) phaseolin subunit and (b) β-lactoglobulin. The arrows indicate β-sheet strands, and the cylinders indicate α-helix. ([a]: From Lawrence, M.C. et al., *EMBO J.*, 9, 9, 1990; [b]: Papiz, M.Z. et al., *Nature*, 324, 383, 1986.)

The tertiary structures of β-lactoglobulin and phaseolin (the storage protein in kidney beans) are shown in Figure 5.7. Transformation of a protein from a linear configuration (primary structure) into a folded tertiary structure is a complex process. At the molecular level, the details for formation of a protein tertiary structure are present in its amino acid sequence, that is, when a native protein is denatured, it folds back to the original tertiary folded structure upon removal of the denaturant. From a thermodynamic viewpoint, formation of tertiary structure involves optimization of various favorable noncovalent interactions (hydrophobic, electrostatic, van der Waals, and hydrogen bonding) within a protein molecule so that these forces overcome the destabilizing effect of the conformational entropy of the polypeptide chain and the net free energy of the molecule is reduced to the minimum possible value [9]. The most important rearrangement that accompanies the reduction in free energy during tertiary structure formation is the relocation of most of the hydrophobic residues to the interior of the protein structure and away from the aqueous environment, and relocation of most of the hydrophilic residues, especially charged residues, to the protein–water interface.

Although there is a strong general tendency for hydrophobic residues to be buried in the protein interior, in most proteins this is accomplished only partly because of steric constraints. In fact, in most globular proteins, nonpolar residues occupy about 40%–50% of the water-accessible surface [10]. Also, some polar groups are inevitably buried in the interior of proteins; however, these buried polar groups are invariably hydrogen bonded to other polar groups, such that their free energies are minimized in the apolar environment of the protein interior. The ratio of apolar and polar surfaces on a protein's surface enormously influences several of its physicochemical properties.

The folding of a protein from a random structure to an ordered folded tertiary structure is accompanied by a reduction in protein–water interfacial area. One of the theories that has been put forward to explain protein folding is the *excluded volume effect*: According to this theory, the energy cost to create a cavity in water against its cohesive energy to house a protein molecule is larger for the unfolded state than the folded of the protein that has a smaller water-accessible surface area [11,12]. The difference between the energy costs of small cavity formation in the folded state versus large cavity formation in unfolded state acts as the driving force (solvophobic force) for protein folding. To rephrase, the excluded volume effect is fundamentally related to the tension at the protein–water interface, and protein folding occurs in order to minimize the protein–water interfacial area.

The "accessible interfacial area" of a protein is defined as the total interfacial area of a three-dimensional space, occupied by the protein, as determined by figuratively rolling a spherical water molecule of radius 1.4 Å over the entire surface of the protein molecule. Examination of the water-accessible surface area of a large number of native globular proteins had shown that the water-accessible interfacial area (in Å^2) is a simple function of the molecular weight, M, of a protein and it follows Equation 5.10:

$$A_s = 6.3M^{0.73} \tag{5.24}$$

In contrast, the total water-accessible interfacial area of a nascent (unfolded) polypeptide in its extended state (i.e., fully stretched molecule with no secondary, tertiary or quaternary structure) is also a function of the molecular weight and follows Equation 5.10:

$$A_t = 1.48M + 21 \tag{5.25}$$

The net surface area of a protein that has been buried during formation of a globular tertiary structure is

$$A_b = A_t - A_s = (1.48M + 21) - 6.3M^{0.73} \tag{5.26}$$

The fraction and distribution of hydrophilic and hydrophobic residues in the primary structure affects several physicochemical properties of the protein. For instance, the shape of a folded protein molecule is dictated by its amino acid sequence. If a protein contained a large number of hydrophilic residues distributed uniformly in the sequence, it would assume an elongated or rodlike shape. This is because, for a given mass, an elongated shape has a large surface-area-to-volume ratio, and therefore more hydrophilic residues can be placed on the surface in contact with water. On the other hand, if a protein contained a large number of hydrophobic residues, it would assume a globular (roughly spherical) shape, which has the least surface-area-to-volume ratio, enabling burial of a large number of hydrophobic residues in the protein interior. Among globular proteins, it is generally found that the larger the size of a protein, the larger is the ratio of hydrophobic to hydrophilic amino acid residues.

The tertiary structures of several single polypeptide proteins are made up of domains. "Domains" are defined as those regions of the polypeptide sequence that independently fold up into a tertiary structure. These are, in essence, mini-proteins within a single protein. The structural stability of each

domain is largely independent of the others. In most single-chain proteins, the domains fold independently and then interact with each other to form the unique tertiary structure of the protein. In some proteins, as in the case of phaseolin (Figure 5.7), the tertiary structure may contain two or more distinct domains (structural entities) connected by a segment of the polypeptide chain. The number of domains in a protein usually depends on its molecular weight. Small proteins (e.g., lysozyme, β-lactoglobulin, and α-lactalbumin) with 100–150 amino acid residues usually form a single domain tertiary structure. Large proteins, such as Ig, contain multiple domains. The light chain of IgG contains two domains, and the heavy chain contains four domains. The size of each of these domains is about 120 amino acid residues. Similarly, human serum albumin, which is made up of 585 amino acid residues, has three homologous domains, and each domain contains two subdomains [13].

5.3.1.4 Quaternary Structure

Quaternary structure refers to the spatial arrangement of a protein when it contains more than one polypeptide chain. Several biologically important proteins exist as dimers, trimers, tetramers, etc. These quaternary complexes (also referred to as oligomers) are made up of subunits (monomers) of the same polypeptides (homogeneous) or of different polypeptides (heterogeneous). For example, β-lactoglobulin exists as a dimer in the pH range 5–8, as an octomer in the pH range 3–5, and as a monomer above pH 8, and the monomeric units of these complexes are identical. On the other hand, hemoglobin is a tetramer made up of two different polypeptide chains, that is, α and β chains.

Formation of oligomeric structures is the result of specific protein–protein interactions. These are primarily driven by noncovalent interactions such as hydrogen bonding and hydrophobic and electrostatic interactions. The fraction of hydrophobic amino acid residues in a protein influences the tendency to form oligomeric structures. Proteins that contain more than 30% hydrophobic amino acid residues exhibit a greater tendency to form oligomeric structures than those that contain fewer hydrophobic amino acid residues.

Formation of quaternary structure is primarily driven by the thermodynamic requirement to bury exposed hydrophobic surfaces of subunits. When the hydrophobic amino acid content of a protein is greater than 30%, it becomes physically impossible in some proteins to form a tertiary structure with all of the nonpolar residues buried in the interior. Consequently, there is a greater likelihood of having large hydrophobic patches on the surface, and hydrophobic interaction between those patches leads to formation of dimers, trimers, etc. (Figure 5.8).

Many food proteins, especially cereal and legume proteins, exist as oligomers of different polypeptides. As would be expected, these proteins typically contain more than 35% hydrophobic amino acid residues (Ile, Leu, Trp, Tyr, Val, Phe, and Pro). In addition, they also contain 6%–12% proline. As a consequence, cereal proteins exist in complex oligomeric structures. The major storage proteins

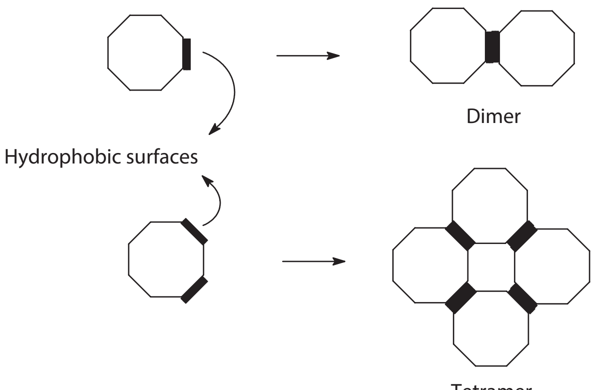


FIGURE 5.8 Schematic representation of formation of dimers and oligomers in proteins.

of soybean, namely, β-conglycinin and glycinin, contain about 41% and 39% hydrophobic amino acid residues, respectively. β-Conglycinin is a trimeric protein made up of three different subunits, and it exhibits complex association–dissociation phenomenon as a function of ionic strength and pH [14]. Glycinin is made up of 12 subunits, 6 of the subunits being acidic and the others basic. Each basic subunit is cross-linked to an acidic subunit via a disulfide bond. The six acidic–basic pairs are held together in an oligomeric state by noncovalent interactions. Glycinin also exhibits complex association–dissociation behavior as a function of ionic strength [14].

In oligomeric proteins, the accessible surface area, A_S, is a function of the molecular weight of the oligomer [10], which is represented as follows:

$$A_S = 5.3M^{0.76} \quad (5.27)$$

This relationship is different from that which applies to monomeric proteins (Equation 5.24). The surface area buried when the native oligomeric structure is formed from its constituent polypeptide subunits can be estimated from the following equation:

$$A_b = A_t - A_S = (1.48M + 21) - 5.3M^{0.76} \quad (5.28)$$

where

A_t is the total accessible area of the nascent polypeptide subunits in their fully extended state
M is the molecular weight of the oligomeric protein

5.3.2 Forces Involved in the Stability of Protein Structure

The process of folding of a random polypeptide chain into a unique three-dimensional structure is quite complex. As mentioned earlier, the information for attaining the biologically native conformation is encoded in the amino acid sequence of the protein. In the 1960s, Anfinsen and coworkers showed that when denatured ribonuclease was added to a physiological buffer solution, it refolded to its native conformation and regained almost 100% of its biological activity. Several enzymes have been subsequently shown to exhibit similar propensity. The slow but spontaneous transformation of an unfolded state to a folded state is facilitated by several intramolecular noncovalent interactions. The native conformation of a protein is a thermodynamic state in which various favorable interactions are maximized and the unfavorable ones are minimized such that the overall free energy of the protein molecule is at the lowest possible value. The forces that contribute to protein folding may be grouped into two categories: (1) intramolecular interactions emanating from forces intrinsic to the protein molecule and (2) intramolecular interactions affected by the surrounding solvent. van der Waals and steric interactions belong to the former, and hydrogen bonding, electrostatic, and hydrophobic interactions belong to the latter.

5.3.2.1 Steric Strains

Although the ϕ and ψ angles of the peptide backbone can theoretically have 360° rotational freedom, their values are very much restricted in polypeptides because of steric hindrance from side chain atoms. As a result, segments of a polypeptide chain can assume only a limited number of configurations. Distortions in the planar geometry of the peptide unit or stretching and bending of bonds will cause an increase in the free energy of the molecule. Therefore, folding of the polypeptide chain can occur only in such a way that deformation of bond lengths and bond angles are avoided.

5.3.2.2 van der Waals Interactions

These are dipole-induced dipole and induced dipole-induced dipole interactions between neutral atoms in protein molecules. When two atoms approach each other, each atom induces a dipole in the other via polarization of the electron cloud. The interaction between these induced dipoles has an attractive as well as a repulsive component. The magnitudes of these forces are dependent on the interatomic distance. The attractive energy is inversely proportional to the sixth power of the

interatomic distance, and the repulsive interaction is inversely proportional to the 12th power of this distance. Therefore, at a distance r, the net interaction energy between two atoms is given by the potential energy function

$$E_{vdW} = E_a + E_r = \frac{A}{r^6} + \frac{B}{r^{12}} \tag{5.29}$$

where

A and B are constants for a given pair of atoms
E_a and E_r are the attractive and repulsive interaction energies, respectively

van der Waals interactions are very weak, decrease rapidly with distance, and become negligible beyond 6 Å. The van der Waals interaction energy for various pairs of atoms ranges from −0.04 to −0.19 kcal mol^{-1}. In proteins, however, since numerous pairs of atoms are involved in van der Waals interactions, the sum of its contribution to protein folding and stability could be significant.

5.3.2.3 Hydrogen Bonds

The hydrogen bond involves the interaction of a hydrogen atom that is covalently attached to an electronegative atom (such as N, O, or S) with another electronegative atom. Schematically, a hydrogen bond may be represented as D–H···A, where D and A, respectively, are the donor and acceptor electronegative atoms. The strength of a hydrogen bond ranges between 2 and 7.9 kcal mol^{-1}, depending on the pair of electronegative atoms involved and the bond angle.

Proteins contain several groups capable of forming hydrogen bonds. Some of the possible candidates are shown in Figure 5.9. Among these groups, the greatest number of hydrogen bonds are formed between the N−H and C=O groups of the peptide bonds in α-helix and β-sheet structures.

Hydrogen bond between peptide groups

Hydrogen bond between unionized carboxyl groups

Hydrogen bond between phenolic or hydroxyl group and ionized carboxyl groups

Hydrogen bond between phenolic or hydroxyl group and peptide carbonyl group

Hydrogen bond between side chain amide groups

Hydrogen bond between side chain carboxyl group and histidine side chain

FIGURE 5.9 H-bonding groups in proteins. (From Scheraga, H.A., Intramolecular bonds in proteins. II. Noncovalent bonds, in: *The Proteins*, Neurath, H. (ed.), 2nd edn., Vol. 1, Academic Press, New York, 1963, pp. 478–594.)

The peptide hydrogen bond can be considered as a strong permanent dipole–dipole interaction between the $N^{\delta-}{-}H^{\delta+}$ and $C^{\delta+}{=}O^{\delta-}$ dipoles:

μ_1
$\delta+$ H
θ
$\delta-$ N - - - - - - $O^{\delta-}$ ═══ $C^{\delta+}$ - - - - → μ_2 (5.30)

The strength of the hydrogen bond is given by the potential energy function

$$E_{H-bond} = \frac{\mu_1 \mu_2}{4\pi\varepsilon_0 \varepsilon r^3} \cos\theta \tag{5.31}$$

where
μ_1 and μ_2 are the dipole moments
ε_0 is the permittivity of the vacuum
ε is the dielectric constant of the medium
r is the distance between the electronegative atoms
θ is the hydrogen bond angle

The hydrogen bond energy is directly proportional to the product of the dipole moments and to the cosine of the bond angle and is inversely proportional to the third power of the N···O distance and to the dielectric permittivity of the medium. The strength of the hydrogen bond reaches a maximum when θ is zero, and it is zero when θ is 90°. The hydrogen bonds in α-helix and antiparallel β-sheet structures have a θ value very close to zero, whereas those in parallel β-sheets have larger θ values. The optimum N···O distance for maximum hydrogen bond energy is 2.9 Å. At shorter distances the electrostatic repulsive interaction between the $N^{\delta-}$ and $O^{\delta-}$ atoms causes a significant decrease in the strength of the hydrogen bond. At longer distances weak dipole–dipole interaction between the N−H and C=O groups decreases the strength of the hydrogen bond. The strength of N−H···O=C hydrogen bonds in the interior of proteins, where the dielectric constant ε of the environment is close to 1, is typically about 4.5 kcal mol^{-1}. The “strength” refers to the amount of energy needed to break the bond.

The existence of hydrogen bonds in proteins is well established. Since formation of each hydrogen bond decreases the free energy of the protein by about −4.5 kcal mol^{-1}, it is commonly assumed that they may act not only as a stabilizing force of the folded structure but also as a driving force for protein folding. This assumption, however, is questionable, because, being a strong hydrogen bond former, water can compete for hydrogen bonding with N−H and C=O groups of proteins and prevent formation of N−H···O=C hydrogen bonds, and therefore the N−H···O=C hydrogen bond formation cannot act a driving force for formation of α-helix and β-pleated sheets in proteins. The hydrogen bond is primarily an ionic interaction. Like any other ionic interactions, its stability depends upon the dielectric permittivity of the environment. The stability of hydrogen bonds in α-helix and β-pleated sheets is mainly due to a low dielectric environment created by hydrophobic interactions between nonpolar side chains. These bulky side chains prevent access of water to the N−H···O=C hydrogen bond sites within the secondary structure. Thus, they are stable only as long as the local nonpolar environment is maintained.

5.3.2.4 Electrostatic Interactions

As noted earlier, proteins contain several amino acid residues with ionizable groups. At neutral pH, Asp and Glu residues are negatively charged, and Lys, Arg, and His are positively charged. At high alkaline pH, Cys and Tyr residues also assume a negative charge.

Depending on the relative number of negatively and positively charged residues, proteins assume either a net negative or a net positive charge at neutral pH. The pH at which the net charge is zero is called the "isoelectric pH (pI)." The isoelectric pH is different from the "isoionic point." Isoionic point is the pH of a protein solution in the absence of electrolytes. The isoelectric pH of a protein can be estimated from its amino acid composition and the pK_a values of the ionizable groups using the Henderson–Hasselbalch equation (Equations 5.6 and 5.7).

With few exceptions, almost all charged groups in proteins are located on the surface of the protein molecule and in contact with the surrounding aqueous solvent. Since proteins assume either a net positive or a net negative charge at neutral pH, one might expect that the net repulsive interaction between like charges within a protein would destabilize protein structure. It is also reasonable to assume that attractive interactions between oppositely charged groups at certain critical locations might contribute to the stability of the protein structure. In reality, however, the strengths of these repulsive and attractive forces are minimized in aqueous solutions because of the high dielectric permittivity of water. The electrostatic interaction energy between two fixed charges q_1 and q_2 separated by distance r is given by

$$E_{ele} = \pm \frac{q_1 q_2}{4\pi\varepsilon_0 \varepsilon r} \quad (5.32)$$

In vacuum or air ($\varepsilon = 1$), the electrostatic interaction energy between two charges at a distance of 3–5 Å is about ±110 to ±66 kcal mol^{-1}. In water ($\varepsilon = 80$), however, this interaction energy is reduced to ±1.4 to ±0.84 kcal mol^{-1}, which is of the order of thermal energy (RT) of the protein molecule at 37°C. Furthermore, since the distance between charges in a protein molecule is typically much farther than 5 Å, the attractive and repulsive electrostatic interactions between charges on protein surface do not contribute significantly to protein stability. In any case, the electrostatic interactions within a protein are already taken into account before arriving at the final folded structure of a protein.

Although electrostatic interactions may not act as the primary driving force for protein folding, their penchant to remain exposed to the aqueous environment certainly would influence the folding pathway.

5.3.2.5 Hydrophobic Interactions

It should be obvious from the foregoing discussions that, in aqueous solutions, intramolecular hydrogen bonding and electrostatic interactions in a polypeptide chain do not possess sufficient energy to act as driving forces for protein folding. These polar interactions in proteins are not very stable in an aqueous environment, and their stabilities depend on maintenance of an apolar environment. The major force driving protein folding comes from hydrophobic interactions among nonpolar groups.

In aqueous solutions, the hydrophobic interaction between nonpolar groups is the result of thermodynamically unfavorable interaction between water and nonpolar groups. When a hydrocarbon is dissolved in water, the standard free energy change (ΔG) is positive and the volume (ΔV) and enthalpy change (ΔH) are negative. Even though ΔH is negative, meaning that there is favorable interaction between water and the hydrocarbon, ΔG is positive. Since $\Delta G = \Delta H - T\Delta S$ (where T is the temperature and ΔS is the entropy change), the positive change in ΔG must result from a large negative change in entropy, which offsets the favorable change in ΔH. The decrease in entropy is caused by formation of a clathrate or cage-like water structure around the hydrocarbon (see Chapter 2). Because of the net positive change in ΔG, interaction between water and nonpolar groups is highly unfavorable. As a consequence, in aqueous solutions, nonpolar groups tend to aggregate, so that the area of direct contact with water is minimized. This water structure–induced interaction between nonpolar groups in aqueous solutions is known as hydrophobic interaction.

Since the hydrophobic interaction is the antithesis of dissolution of nonpolar groups in water, ΔG for hydrophobic interaction is negative, and ΔV, ΔH, and ΔS are positive. Unlike other noncovalent interactions, hydrophobic interactions are endothermic; that is, they are stronger at high temperatures than at low temperatures. In contrast, both hydrogen bonding and electrostatic interactions are exothermic in nature, and therefore they are weaker at high temperatures than at low temperatures. The variation of hydrophobic free energy with temperature usually follows a quadratic function, that is

$$\Delta G_{H\phi} = a + bT + cT^2 \quad (5.33)$$

where
a, b, and c are constants
T is the absolute temperature

The distance dependence of hydrophobic interaction energy between two spherical nonpolar molecules follows the expression [15]

$$E_{H\phi} = -20 \frac{R_1 R_2}{R_1 + R_2} e^{-D/D_0} \text{ kcal mol}^{-1} \quad (5.34)$$

where
R_1 and R_2 are the radii of the nonpolar molecules
D is the distance in nm between the molecules
D_0 is the decay length (1 nm)

Unlike electrostatic, hydrogen bonding, and van der Waals interactions, which follow an inverse power law relationship with distance between interacting groups, the hydrophobic interaction follows an exponential relationship with distance between interacting groups. Thus, it is effective over relatively long distances, for example, 10 nm. While Equation 5.34 is useful for estimating hydrophobic interaction energy between ideal nonpolar spherical particles, it is not useful in the case of proteins because of structural complexities involved and the irregular distribution of hydrophobic patches on protein surface.

The hydrophobic free energy of a protein can be estimated using other empirical correlations. The hydrophobic free energy of aliphatic hydrocarbons as well as amino acid side chains is directly proportional to the nonpolar surface area accessible to water. This is shown in Figure 5.10 [16]. The proportionality constant, that is, the slope, varies between 22 cal mol^{-1} $Å^{-2}$ for Ala, Val, Leu, and Phe, and 26 cal mol^{-1} $Å^{-2}$ for Ser, Thr, Trp, and Met side chains. On average, the hydrophobicity of amino acid side chains is about 24 cal mol^{-1} $Å^{-2}$. This is close to the 25 cal mol^{-1} $Å^{-2}$ value for alkanes (the slope for hydrocarbons in Figure 5.10). This means that for the removal of every $Å^2$ area of nonpolar surface from the water environment, a protein will lose its hydrophobic free energy by about 24 cal mol^{-1}. Thus, the total hydrophobic free energy reduction during folding of a protein from the unfolded to folded state can be estimated by multiplying the total buried surface area A_b (see Equation 5.28) by 24 cal mol^{-1} $Å^{-2}$.

The buried surface area in several globular proteins and the estimated hydrophobic free energies are shown in Table 5.8. It is evident that hydrophobic free energy contributes significantly to the stability of protein structure.

5.3.2.6 Disulfide Bonds

Disulfide bonds are the only covalent side chain cross-links found in proteins. They can occur both intramolecularly and intermolecularly. In monomeric proteins, disulfide bonds are formed as a result of protein folding. When two Cys residues are brought into proximity with proper orientation,

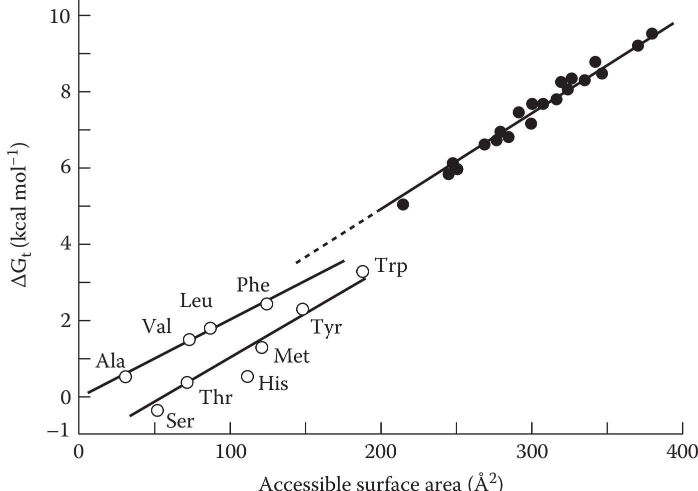


FIGURE 5.10 The relationship between hydrophobicity and accessible surface area of amino acid side chains (open circles) and hydrocarbons (filled circles). (From Richards, F.M., *Annu. Rev. Biophys. Bioeng.*, 6, 151, 1977; Courtesy of Annual Reviews, Palo Alto, CA.)

TABLE 5.8
Accessible Surface Area (A_s), Buried Surface Area (A_b), and Estimated Change in Hydrophobic Free Energy of Protein Unfolding

Protein	M.W. (Da)	A_s (Å^2)	A_b (Å^2)	$\Delta G_h\phi$ (kcal mol^{-1})
Parvalbumin	11,450	5,930	11,037	269
Cytochrome C	11,930	5,570	12,107	294
Ribonuclease A	13,690	6,790	13,492	329
Lysozyme	14,700	6,620	15,157	369
Myoglobin	17,300	7,600	18,025	439
Retinol binding protein	20,050	9,160	20,535	500
Papain	23,270	9,140	25,320	617
Chymotrypsin	25,030	10,440	26,625	648
Subtilisin	27,540	10,390	30,390	739
Carbonic anhydrase B	28,370	11,020	30,988	755
Carboxypeptidase A	34,450	12,110	38,897	947
Thermolysin	34,500	12,650	38,431	935

A_s values are from Reference 10. A_b was calculated from Equations 5.25 and 5.26.

oxidation of the sulfhydryl groups by molecular oxygen results in disulfide bond (S−S) formation. Once formed, S−S bonds help stabilize the folded structure of proteins.

Protein mixtures containing S−S and Cys residues can undergo sulfhydryl–disulfide interchange reactions, shown as follows:

S—S HS → HS S—S (5.35)

This interchange reaction also can occur when a solution containing only one protein species is heated above its denaturation temperature if it contained at least a free sulfhydryl group and a disulfide bond. This interchange reaction, if it occurs at room temperature, can destabilize a protein molecule.

In summary, the formation of a unique three-dimensional protein structure is the net result of various repulsive and attractive noncovalent interactions; formation of disulfide bond in the folded state further stabilizes the folded conformation.

5.3.3 Conformational Stability and Adaptability of Proteins

The stability of the native protein structure is defined as the difference in free energy between the native and denatured (or unfolded) states of the protein molecule. This is usually denoted as ΔG_D. This refers to the amount of energy needed to unfold a protein from the native state to the denatured state.

All of the noncovalent interactions discussed earlier contribute to the stability of the native protein structure. If we considered only the noncovalent interactions, the ΔG_D of the native state would amount to hundreds of kcal mol^{-1} (e.g., see Table 5.8 for the contribution from hydrophobic interactions). However, several experimental studies have shown that the net ΔG_D of proteins is only in the range of 5–20 kcal mol^{-1}. This would mean that there is another force within the protein chain that tries to destabilize the native structure. This counter force is the conformational entropy of the polypeptide chain. When a protein is transformed from a disordered state to a folded state, the loss of translational, rotational, and vibrational motions that exist in a disordered state is lost or restricted in the folded state, and this causes a large decrease in the conformational entropy of the protein chain. The increase in free energy resulting from this loss of conformational entropy partly offsets the decrease in free energy caused by favorable noncovalent interactions in the folded state. As a result, the net free energy change favoring the folded state is reduced to a level of about 5–20 kcal mol^{-1}. Thus, the various interaction energies contributing to the net free energy change for the process D (denatured) ⇄ N (native) can be expressed as

$$\Delta G_{D\rightarrow N} = \Delta G_{H\text{-bond}} + \Delta G_{ele} + \Delta G_{H\phi} + \Delta G_{vdW} - T\Delta S_{conf} \tag{5.36}$$

where

$\Delta G_{H\text{-bond}}$, ΔG_{ele}, $\Delta G_{H\phi}$, and ΔG_{vdW}, respectively, are free energy changes for hydrogen bonding, electrostatic, hydrophobic, and van der Waals interactions

ΔS_{conf} is the conformational entropy change of the polypeptide chain

The ΔS_{conf} of a protein in the unfolded state is in the range of 1.9–10 cal mol^{-1} K^{-1} per residue. Usually, an average value of 4.7 cal mol^{-1} K^{-1} per residue is assumed, which corresponds to an ~10-fold increase in the number of conformations available to an average amino acid residue in the unfolded state than in the folded state [17]. In the unfolded state, a protein with 100 amino acid residues at 310 K will have conformational entropy of $-T\Delta S_{conf}$, which will be about $-4.7 \times 100 \times 310 = -145.7$ kcal mol^{-1}. In the folded state, the loss of this conformational entropy, that is, $-T(-\Delta S_{conf}) = T\Delta S_{conf}$, acts as a destabilizing force.

The ΔG_D values, that is, energy required to unfold, of various proteins are presented in Table 5.9. It should be noted that in spite of numerous intramolecular interactions, proteins are only marginally stable. The ΔG_D values of most proteins correspond to energy equivalent to one to three hydrogen bonds or about two to five hydrophobic interactions, suggesting that breakage of a few noncovalent interactions in a protein would cause destabilization of the native structure of most proteins.

Conversely, it appears that proteins are not designed to be rigid molecules. They are in a metastable state and their structure can easily adapt to any change in their environment. This conformational

TABLE 5.9
ΔG_D Values for Selected Proteins

Protein	pH	T (°C)	ΔG_D (kcal mol^{-1})
α-Lactalbumin	7	25	4.4
Bovine β-lactoglobulin A + B	7.2	25	7.6
Bovine β-lactoglobulin A	3.15	25	10.2
Bovine β-lactoglobulin B	3.15	25	11.9
T4 lysozyme	3.0	37	4.6
Hen egg-white lysozyme	7.0	37	12.2
Gactin	7.5	25	6.5
Lipase (from *Aspergillus*)	7.0	—	11.2
Troponin	7.0	37	4.7
Ovalbumin	7.0	25	6.0
Cytochrome C	5.0	37	7.9
Ribonuclease	7.0	37	8.1
α-Chymotrypsin	4.0	37	8.1
Trypsin	—	37	13.2
Pepsin	6.5	25	10.9
Growth hormone	8.0	25	14.2
Insulin	3.0	20	6.5
Alkaline phosphatase	7.5	30	20.3

ΔG_D represents $G_U - G_N$, where G_U and G_N are free energies of the denatured and native states, respectively, of a protein molecule. Compiled from several sources.

adaptability might be necessary to enable proteins to carry out their biological functions. For example, efficient binding of substrates or prosthetic ligands to enzymes might require reorganization of polypeptide segments at the binding sites. On the other hand, proteins that require high structural stability to perform their physiological functions usually are stabilized by intramolecular disulfide bonds, which effectively counter the conformational entropy (i.e., the tendency of the polypeptide chain to unfold).

5.3.4 Summary

- The primary structure of a protein refers to the amino acid sequence of the protein.
- The peptide bond has a partial double bond character. This imposes four important structural implications on the protein backbone.
- Alpha-helix and beta-sheet structures are the major ordered secondary structures in proteins. The information for the formation of these structures is embedded in the form of a binary code in the amino acid sequence.
- Alpha-helix and beta-sheet structures are amphiphilic in nature, that is, they possess distinct hydrophobic and hydrophilic surfaces.
- Because proline residue has a fixed 70°ϕ angle, it cannot be included in α-helix and β-sheet structures.
- Tertiary structure refers to the final spatial structure of a protein in which the ordered secondary structures and periodic regions are collapsed into a globular form in which a majority of nonpolar groups are buried in the interior and the hydrophilic groups are exposed to water.

- The major noncovalent interactions that drive protein folding are van der Waals, hydrogen bonding, and electrostatic and hydrophobic interactions.
- The net free energy change for the transformation of a protein from an unfolded state to a folded state is typically in the range of 5–20 kcal mol^{-1}. Thus, protein structure is only marginally stable.

5.4 PROTEIN DENATURATION

The native structure of a protein is the net result of various attractive and repulsive interactions stemming from assorted intramolecular forces as well as interaction of various protein groups with surrounding aqueous medium; it is largely the product of the protein's environment. The native state is thermodynamically the most stable state with the lowest possible free energy. Any change in its environment, such as pH, ionic strength, temperature, and solvent composition, will affect the electrostatic and hydrophobic forces within the molecule, and, as a result, the molecule will assume a new equilibrium structure. Subtle changes in structure that do not drastically alter the molecular architecture of the protein are usually regarded as "conformational adaptability," whereas major changes in the secondary, tertiary, and quaternary structures without cleavage of backbone peptide bonds are regarded as "denaturation." From a structural standpoint, while the native structure of a protein is a well-defined entity with structural coordinates for each and every atom in the molecule obtainable from its crystallographic structure, it is not the case for the denatured state. Denaturation is a phenomenon wherein a well-defined initial state of a protein formed under physiological conditions is transformed into an ill-defined final state under nonphysiological conditions using a denaturing agent. It does not involve any chemical changes in the protein. Because of a greater degree of rotational freedom of the dihedral angles of the polypeptide chain, a denatured protein can assume several conformational states differing only marginally in free energy. This is shown schematically in Figure 5.11. Some denatured states possess more residual folded (secondary) structures than others. It should be noted that even in the fully denatured state, typical globular proteins, with the exception of gelatin, do not behave like a true random coil. This is so because the partial double bond character of the peptide bond and local steric restrictions imposed by bulky side chains do not permit 360° rotational freedom for the covalent bonds in the polypeptide backbone.

The intrinsic viscosity ([η]) of a fully denatured protein is a function of the number of amino acid residues, and is expressed by the empirical equation (Equation 5.18):

$$[\eta] = 0.716\, n^{0.66} \tag{5.37}$$

where n is the number of amino acid residues in the protein.

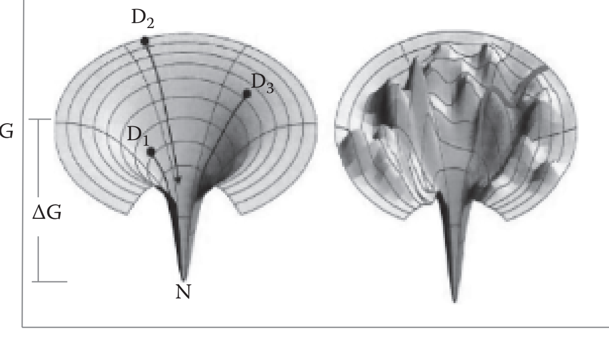


FIGURE 5.11 Schematic representation of the energy of a protein molecule as a function of its conformation. The conformation with the lowest energy is usually the native state. (From Sadi-Carnot, Energy landscape, *Encyclopedia of Human Thermodynamics, Human Chemistry, and Human Physics*, 2015. www.eoht.info/page/Energy+landscape.)

Often, denaturation has a negative connotation, because it indicates loss of some properties. Enzymes lose their activity upon denaturation. In the case of food proteins, although denaturation usually causes loss of solubility and some functional properties, in some cases protein denaturation is highly desirable. For instance, partial denaturation of proteins at the air–water and oil–water interfaces improves their foaming and emulsifying properties, whereas excessive thermal denaturation of soy proteins diminishes their foaming and emulsifying properties. On the other hand, in general, denatured proteins are more digestible than native proteins. In protein beverages, where high solubility and dispersibility of proteins is required, partial protein denaturation during processing may cause flocculation and precipitation during storage of the product and thus may adversely affect its sensory attributes. Thermal denaturation is also a prerequisite for heat-induced gelation of food proteins. Thus, to develop appropriate processing strategies, it is imperative to have a basic understanding of the environmental and other factors that affect structural stability of proteins in food systems.

5.4.1 Thermodynamics of Denaturation

Denaturation is a phenomenon that involves transformation of a well-defined, folded structure of a protein, formed under physiological conditions, to an unfolded state under nonphysiological conditions. Since structure is not an easily quantifiable parameter, direct measurement of the mole fraction of a protein in the native and denatured states in a solution is not possible. However, denaturation invariably affects a protein's chemical and physical properties, such as UV absorbance, fluorescence, viscosity, sedimentation coefficient, optical rotation, circular dichroism, reactivity of sulfhydryl groups, and enzyme activity. Thus, protein denaturation can be studied by monitoring changes in these physical and chemical properties.

When changes in a physical or chemical property, y, is monitored as a function of denaturant concentration or temperature, many monomeric globular proteins exhibit denaturation profiles as shown in Figure 5.12, where y_N and y_D are y values for the native and denatured states, respectively, of the protein.

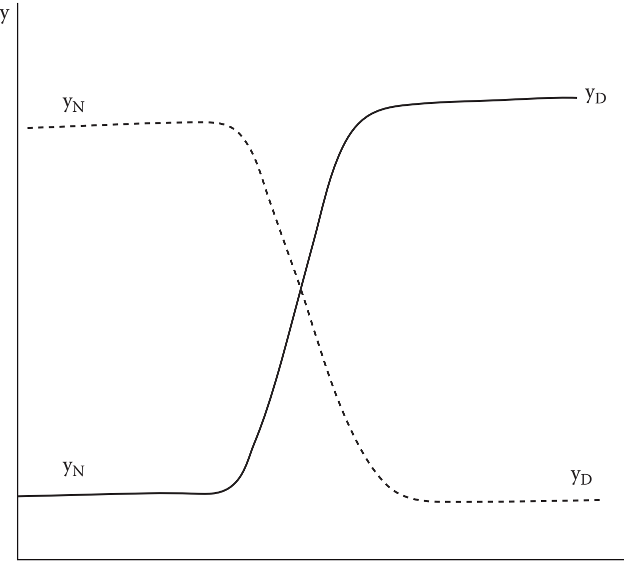


FIGURE 5.12 Typical protein denaturation curves. y represents any measurable physical or chemical property of the protein molecule that varies with protein conformation. y_N and y_D are the values of y for the native and denatured states, respectively.

For most proteins, as denaturant concentration (or temperature) is increased, the value of y remains mostly unchanged initially, and above a critical denaturant concentration (or temperature), its value changes abruptly from y_N to y_D within a narrow range of denaturant concentration or temperature. For a majority of globular proteins, this transition is very steep, indicating that protein denaturation is a cooperative process. That is, once a protein molecule begins to unfold, or once a few interactions in the protein are broken, the whole molecule completely unfolds when denaturant concentration (or temperature) is increased slightly above the threshold. This cooperative nature of unfolding suggests that globular proteins can exist either in the native or denatured state, and intermediate states are not possible. This is known as a "two-state transition" model. For this two-state model, the equilibrium between the native and the denatured state in the cooperative transition region can be expressed as

$$N \xleftrightarrow{K_D} D$$

$$K_D = \frac{[D]}{[N]} \tag{5.38}$$

where K_D is the equilibrium constant. Since the concentration of denatured protein molecules in the absence of a denaturant is extremely low (about 1 in 10^9), experimental determination of [D] is not possible. However, in the transition region, that is, at sufficiently high denaturant concentration (or sufficiently high temperature), an increase in the population of the denatured protein molecule would permit experimental determination [D] and therefore the apparent equilibrium constant, $K_{D,app}$. In the transition region, where both native and denatured protein molecules are present, the value of y can be expressed as

$$y = f_N y_N + f_D y_D \tag{5.39}$$

where
- f_N and f_D are the fractions of the protein in the native and denatured states
- y_N and y_D are y values for the native and denatured states, respectively

From Figure 5.12,

$$f_N = \frac{y_D - y}{y_D - y_N} \tag{5.40}$$

$$f_D = \frac{y - y_N}{y_D - y_N} \tag{5.41}$$

and the apparent equilibrium constant is given by

$$K_{D,app} = \frac{f_D}{f_N} = \frac{y - y_N}{y_D - y} \tag{5.42}$$

and the free energy of denaturation is given by

$$\Delta G_{D,app} = -RT \ln K_{D,app} \tag{5.43}$$

A plot of $\Delta G_{D,app}$ versus denaturant concentration is usually linear, and thus the K_D and ΔG_D of the protein in pure water (i.e., in the absence of denaturant) can be obtained from the y-intercept.

By determining K_D at various temperatures, the enthalpy of denaturation, ΔH_D, can be determined using the van't Hoff equation:

$$\Delta H_D = -R\frac{d(\ln K_D)}{d(1/T)} \tag{5.44}$$

Monomeric proteins containing two or more domains with different structural stabilities usually exhibit multiple transition steps in the denaturation profile. If the transition steps are well separated, the stabilities of each domain can be obtained from the transition profile by using the two-state model mentioned earlier. Denaturation of oligomeric proteins usually proceeds via dissociation of subunits, followed by denaturation of the subunits.

Protein denaturation is reversible, especially for small monomeric proteins. When the denaturant is removed from the protein solution (or the sample is cooled), in the absence of aggregation, most monomeric proteins would refold to their native conformation under appropriate solution conditions, such as pH, ionic strength, redox potential, and protein concentration. Many proteins refold when the protein concentration is below 1 μM. Above 1 μM protein concentration, refolding is partially inhibited because of greater intermolecular interaction at the cost of intramolecular interactions. A redox potential comparable to that of biological fluid facilitates formation of the correct pairs of disulfide bonds during refolding.

5.4.2 Denaturing Agents

5.4.2.1 Physical Agents

5.4.2.1.1 Temperature and Denaturation

Heat is the most commonly used denaturing agent in foods. Proteins undergo varying degree of denaturation during processing, depending on time and temperature employed. This can affect their functional properties in foods, and it is, therefore, important to understand solution conditions affecting protein denaturation.

When a protein solution is gradually heated above a critical temperature, it undergoes a sharp transition from the native state to the denatured state. The temperature at the transition midpoint, where the concentration ratio of native and denatured states is one, is known either as the melting temperature T_m, or the denaturation temperature T_d. The temperature-induced denaturation of proteins is primarily due to the effect of temperature on the stability of noncovalent interactions. Hydrogen bonding and electrostatic interactions, which are exothermic in nature, are destabilized, and hydrophobic interactions, which are endothermic, are stabilized as the temperature is increased. The strength of hydrophobic interactions reaches a maximum at about 70°C–80°C and decreases thereafter. In addition to noncovalent interactions, the temperature effect on conformational entropy, $T\Delta S_{conf}$, also plays a destabilizing major role in the stability of proteins. The conformational entropy of the chain increases as the temperature is increased, which favors the unfolded state. The net stability of a protein at a given temperature is then the sum of these stabilizing and destabilizing forces. However, a careful analysis of the temperature effect on various interactions in proteins reveals the following: In globular proteins, the majority of charged groups exist on the surface of the protein molecule, fully exposed to the high dielectric aqueous medium. Because of the dielectric screening effect of water, attractive and repulsive electrostatic interactions between charged residues are greatly reduced. In addition, at physiological ionic strength, that is, at 0.15 M, screening of charged groups in proteins by counter ions further reduces electrostatic interactions in proteins. Because of these facts, the role of electrostatic interactions in protein stability is not significant. Similarly, hydrogen bonds are unstable in an aqueous environment, and therefore their stability in proteins is dependent on hydrophobic interactions that create local low dielectric environments. This implies that so long as a nonpolar environment is maintained, the hydrogen bonds in proteins would remain

intact when the temperature is increased. These facts suggest that although polar interactions are affected by temperature, they generally do not play a major role in heat-induced denaturation of proteins. Based on these considerations, the stability of the native state of a protein can be simply regarded as the net free energy difference arising from hydrophobic interactions that tend to favor the folded state and the conformational entropy of the chain that favor the unfolded state. That is,

$$\Delta G_{N\to D} = \Delta G_{H\phi} + \Delta G_{conf} \tag{5.45}$$

Since the enthalpy change (ΔH) for hydrophobic interactions is very small, Equation 5.45 can be expressed as

$$\Delta G_{N\to D} = -T(\Delta S_{water}) - T\Delta S_{conf} \tag{5.46}$$

The dependence of protein stability on temperature at constant pressure can be expressed as

$$\frac{\partial \Delta G_{N\to D}}{\partial T} = \frac{\partial \Delta G_{H\phi}}{\partial T} + \frac{\partial \Delta G_{conf}}{\partial T} \tag{5.47}$$

Hydrophobic interactions are strengthened at higher temperatures and therefore $(\partial \Delta G_{H\phi}/\partial T) > 0$ at higher temperatures. Conformational entropy of protein chain increases upon unfolding of the protein and therefore $(\partial \Delta G_{conf}/\partial T) < 0$. As the temperature is increased, the interplay between these opposing forces reaches a point at which $\partial \Delta G_{N\to D}/\partial T = 0$. The temperature at which this occurs signifies the denaturation temperature (T_d) of the protein. The relative contributions of the major forces to stability of a protein molecule as a function of temperature are depicted in Figure 5.13.

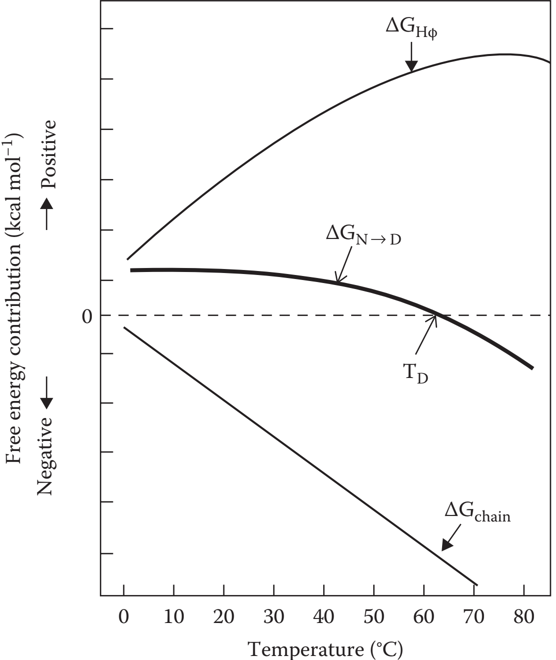


FIGURE 5.13 Relative changes in free energy contributions by hydrogen bonding, hydrophobic interactions, and conformational entropy to the stability of proteins as a function of temperature.

TABLE 5.10
Thermal Denaturation Temperatures (T_d) and Mean Hydrophobicities of Proteins

Protein	T_d	Mean Hydrophobicity (kcal mol^{-1} Residue^{-1})
Trypsinogen	55	0.89
Chymotrypsinogen	57	0.90
Elastase	57	
Pepsinogen	60	0.97
Ribonuclease	62	0.78
Carboxypeptidase	63	
Alcohol dehydrogenase	64	
Bovine serum albumin	65	1.02
Hemoglobin	67	0.96
Lysozyme	72	0.90
Insulin	76	1.00
Egg albumin	76	0.97
Trypsin inhibitor	77	
Myoglobin	79	1.05
α-Lactalbumin	83	1.03
Cytochrome C	83	1.06
β-Lactoglobulin	83	1.09
Avidin	85	0.92
Soy glycinin	92	
Broadbean 11S protein	94	
Sunflower 11S protein	95	
Oat globulin	108	

Source: Data were compiled from Bull, H.B. and Breese, K., *Arch. Biochem. Biophys.*, 158, 681, 1973.

Note that the temperature does not significantly affect the stability of hydrogen bonds in proteins. The T_d values of some proteins are listed in Table 5.10.

It is often assumed that the lower the temperature, the greater will be the stability of a protein. This is not always true. Some proteins are denatured at cold temperatures [19]. For example (Figure 5.14), the stability of lysozyme increases with lowering of temperature, whereas those of myoglobin and a mutant phage T4 lysozyme show maximum stability at about 30°C and 12.5°C, respectively. Below and above these temperatures, myoglobin and phage T4 lysozyme are less stable. These two proteins undergo cold-induced denaturation when stored below 0°C. Cold denaturation is mainly due to weakening of hydrophobic interactions within a protein, which allows the destabilizing effect of conformational entropy to dominate, resulting in unfolding. The temperature of maximum stability (minimum free energy) depends on the relative impact of temperature on the stabilizing and destabilizing forces in proteins. Proteins that are primarily stabilized by hydrophobic interactions are more stable at about ambient temperature than they are at refrigeration temperature. Intramolecular disulfide bonds in proteins tend to stabilize proteins at low as well as high temperatures because they counter conformational entropy of the protein chain.

Several food proteins undergo reversible dissociation and denaturation at low temperature. Glycinin, one of the storage proteins of soybean, aggregates and precipitates when stored at 2°C and then becomes soluble when returned to ambient temperature. When skim milk is stored

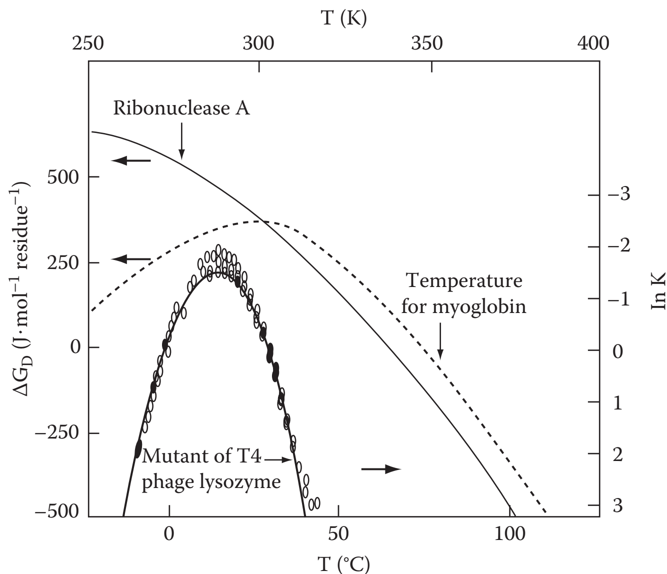


FIGURE 5.14 Variation of protein stability (ΔG_D) with temperature for myoglobin (----), ribonuclease A (—), and a mutant of T4 phage lysozyme (O–O). K is the equilibrium constant. (Compiled from Chen, B. and Schellman, J.A., *Biochemistry*, 28, 685, 1989; Lapanje, S., *Physicochemical Aspects of Protein Denaturation*, Wiley-Interscience, New York, 1978.)

at 4°C, β-casein dissociates from casein micelles, and this alters the physicochemical and renneting properties of casein micelles. Several oligomeric enzymes, such as lactate dehydrogenase and glyceraldehyde-phosphate dehydrogenase, lose most of their enzyme activity when stored at 4°C; this has been attributed to dissociation of the subunits. However, when warmed to and held at ambient temperature for a few hours, they reassociate and completely regain their activity [20].

The amino acid composition affects thermal stability of proteins. Proteins that contain a greater proportion of hydrophobic amino acid residues, especially Val, Ile, Leu, and Phe, tend to be more stable than the more hydrophilic proteins. A strong positive correlation also exists between thermostability and the number percent of certain amino acid residues. For example, statistical analysis of 15 different proteins has shown that thermal denaturation temperatures of these proteins are positively correlated ($r = 0.98$) to the sum of number percent of Asp, Cys, Glu, Lys, Leu, Arg, Trp, and Tyr residues. On the other hand, thermal denaturation temperatures of the same set of proteins are negatively correlated ($r = -0.975$) to the sum of number percent of Ala, Asp, Gly, Gln, Ser, Thr, Val, and Tyr (Figure 5.15) [21]. Other amino acid residues have little influence on T_d.

Thermal stability of proteins from thermophilic and hyperthermophilic organisms, which can withstand extremely high temperatures, is also attributed to their unique amino acid composition [22]. These proteins contain lower levels of Asn and Gln residues than those from mesophilic organisms. The implication here is that because Asn and Gln are susceptible to deamidation at high temperatures, higher levels of these residues in mesophilic proteins may partly contribute to instability. The Cys, Met, and Trp contents, which can be oxidized easily at high temperatures, are also low in hyperthermostable proteins. On the other hand, thermostable proteins have high levels of Ile and Pro [23,24]. The high Ile content is believed to help in better packing of the interior core of the protein [25], which reduces buried cavities or void spaces. The absence of void spaces can reduce mobility of the polypeptide chain at high temperatures, and this minimizes the increase in the configurational entropy of the polypeptide chain at high temperatures.

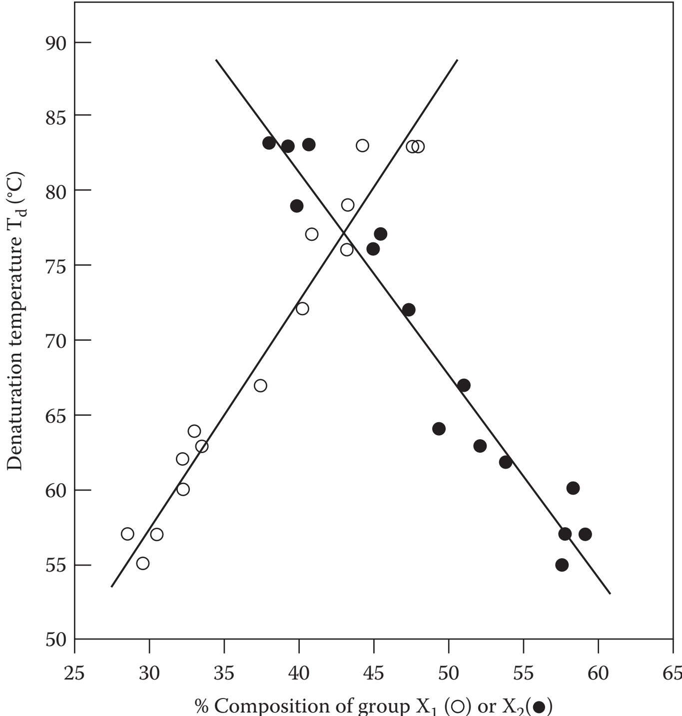


FIGURE 5.15 Group correlations of amino acid residues to thermal stability of globular proteins. Group X_1 represents Asp, Cys, Glu, Lys, Leu, Arg, Trp, and Tyr. Group X_2 represents Ala, Asp, Gly, Gln, Ser, Thr, Val, and Tyr. (Adapted from Ponnuswamy, P.K. et al., *Int. J. Biol. Macromol.*, 4, 186, 1982.)

A high content of Pro, especially in the loop regions of the protein chain, is believed to provide rigidity to the structure [26,27]. Examination of crystallographic structures of several proteins/enzymes from thermophilic organisms show that they also contain a significantly higher number of ion pairs in crevices of proteins and a substantially higher amount of buried water molecules engaged in hydrogen bonding bridge between segments than in their mesophilic counterparts [28,29]. Taken together, it appears that polar interactions (both salt bridges and hydrogen bonding between segments) in the nonpolar protein interior are responsible for thermostability of proteins from thermophilic and hyperthermophilic organisms, and such an environment is facilitated by a high content of Ile. As discussed earlier, it is conceivable that each salt bridge in the protein interior, where the dielectric constant is about 4, could increase the stability of protein structure by about 20 kcal mol^{-1}. In general, thermostable enzymes are characterized by a more highly hydrophobic core, tighter packing, deleted or shortened loops, greater rigidity through increased Pro content in loops, fewer and/or smaller voids, smaller surface-area-to-volume ratio, fewer thermolabile residues, increased hydrogen bonding, and more salt bridges/ion pairs and networks of salt bridges [24].

Thermal denaturation of monomeric globular proteins is mostly reversible at very low protein concentration, for example, less than one μM. However, thermal denaturation can become irreversible when the protein is heated at 90°C–100°C for a prolonged period even at neutral pH. This irreversibility occurs because of several chemical changes in the protein, such as deamidation of Asn and Gln residues, cleavage of peptide bonds at Asp residues, and destruction of Cys and cystine residues [30,31]. Furthermore, at high protein concentration (i.e., >1 μM), intermolecular protein–protein interaction between denatured protein molecules leads to aggregation/coagulation, which prevents the possibility of protein renaturation/refolding to its native structure. The energy-state

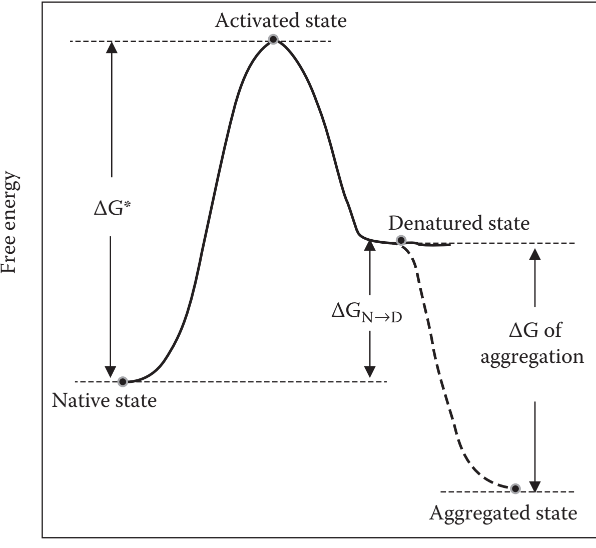


FIGURE 5.16 Schematic representation of the free energy differences between native, activated, denatured, and aggregated state of a protein.

diagram of such a system is schematically shown in Figure 5.16. Note that the free energy of the protein in the aggregated state is lower than that of the native state.

Water greatly facilitates thermal denaturation of proteins [32]. Dry protein powders are extremely stable to thermal denaturation. T_d decreases sharply as the water content is increased from 0 to 0.35 g water (g protein)$^{-1}$ (Figure 5.17). An increase in water content from 0.35 to 0.75 g water (g protein)$^{-1}$

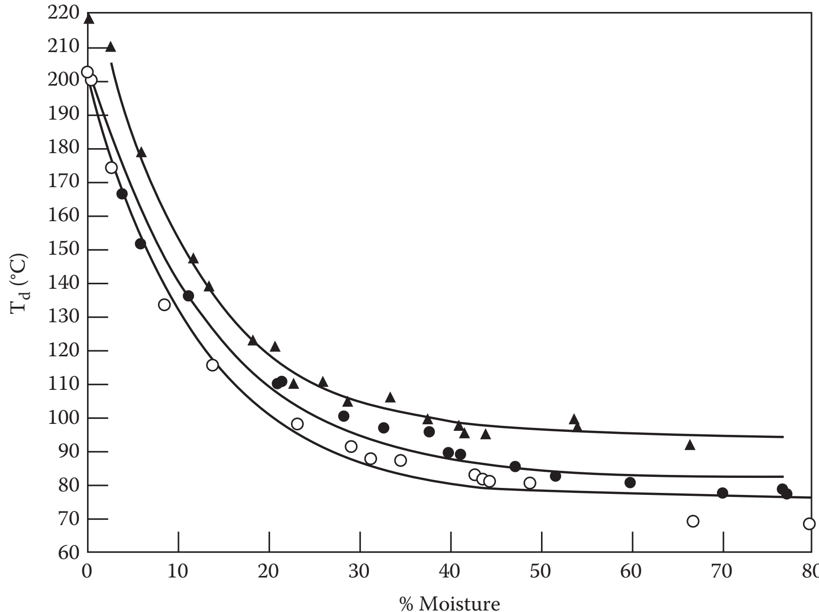


FIGURE 5.17 Influence of water content on the thermal denaturation temperature (T_d) of soy protein. (From Tsukada, H. et al., *Biosci. Biotechnol. Biochem.*, 70, 2096, 2006.)

causes only a marginal decrease in T_d. Above 0.75 g water (g protein)$^{-1}$, the T_d of the protein is the same as in a dilute protein solution. The effect of hydration on thermostability is related to protein dynamics. In the dry state, proteins have a static structure, that is, the polypeptide segments have restricted mobility. As the water content is increased, hydration and partial penetration of water into surface cavities causes swelling of the protein. This swollen state, which represents conversion of protein from an amorphous to a rubbery state, reaches a maximum value at water content of 0.3–0.4 g (g protein)$^{-1}$ at room temperature. The swelling of the protein increases chain mobility and flexibility, and the protein molecule assumes a more dynamic molten structure. When heated, this dynamic flexible structure provides greater access of water to salt bridges and peptide hydrogen bonds than is possible in the dry state, resulting in lowering of T_d.

Additives such as salts and sugars affect thermostability of proteins in aqueous solutions. Sucrose, lactose, glucose, and glycerol stabilize proteins against thermal denaturation [33]. Addition of 0.5 M NaCl to proteins such as β-lactoglobulin, soy proteins, serum albumin, and oat globulin significantly increases their T_d [7,34,35].

5.4.2.1.2 Hydrostatic Pressure and Denaturation

One of the thermodynamic variables that affect conformation of proteins is hydrostatic pressure. Unlike temperature-induced denaturation, which usually occurs in the range of 40°C–80°C at one atmospheric pressure, pressure-induced denaturation can occur at 25°C if the pressure is sufficiently great. Most proteins undergo pressure-induced denaturation in the range of 1–12 kbar as evidenced from changes in their spectral properties. The midpoint of pressure-induced transition occurs at 4–8 kbar [36,37].

Pressure-induced denaturation occurs mainly because proteins are flexible and compressible. Although amino acid residues are densely packed in the interior of globular proteins, some void spaces invariably exist and this leads to compressibility. The average partial specific volume of globular proteins in the hydrated state, υ^o, is about 0.74 mL g^{-1}. The partial specific volume can be considered as the sum of three components:

$$\upsilon^o = V_C + V_{Cav} + \Delta V_{Sol} \tag{5.48}$$

where

V_C is the sum of the atomic volumes
V_{Cav} is the sum of the volumes of the void spaces in the interior of the protein
ΔV_{Sol} is the volume change due to hydration [38]

The larger the V_{cav}, the larger is the contribution of void spaces to partial specific volume and the more unstable the protein will be when pressurized. Fibrous proteins are mostly devoid of void spaces, and hence they are more stable to hydrostatic pressure than globular proteins.

Pressure-induced denaturation of globular proteins is usually accompanied by a reduction in volume of about 30–100 mL mol^{-1}. This decrease in volume is caused by two factors: elimination of void spaces as the protein unfolds and hydration of the nonpolar amino acid residues that become exposed during unfolding. The latter event results in a decrease in volume (see Section 5.3.2). The volume change is related to the free energy change by the expression

$$\Delta V = \frac{d(\Delta G)}{dp} \tag{5.49}$$

where p is the hydrostatic pressure.

If a typical globular protein completely unfolds during pressurization, the volume change should be about 2%. However, 30–100 mL mol^{-1} volume change observed in pressure-denatured proteins

corresponds to only about 0.5% change in volume. This indicates that proteins are only partially unfolded even at hydrostatic pressure as high as 10 kbar.

Pressure-induced protein denaturation is highly reversible. Most enzymes, in dilute solutions, regain their activity once the pressure is decreased to atmospheric pressure [39]. However, regeneration of native structure is a slow process. In the case of oligomeric proteins and enzymes, subunits first dissociate at 0.001–2 kbar and then subunits denature at higher pressures [40]; when the pressure is removed, the subunits reassociate and almost complete restoration of enzyme activity occurs after several hours.

High hydrostatic pressures are being investigated as a food-processing tool, for example, for microbial inactivation or gelation. Since high hydrostatic pressure (2–10 kbar) irreversibly damages cell membranes and causes dissociation of organelles in microorganisms, it will inactivate vegetative microorganisms [41]. Pressure gelation of egg white, 16% soy protein solution, or 3% actomyosin solution can be achieved by application of 1–7 kbar hydrostatic pressure for 30 min at 25°C. These pressure-induced gels are softer than thermally induced gels [42]. Also, exposure of beef muscle to 1–3 kbar hydrostatic pressure causes partial fragmentation of myofibrils, which may be useful as a means of tenderizing meat and gelation of myofibrillar proteins [43]. Pressure processing, unlike thermal processing, does not harm essential amino acids, natural color, and flavor, nor does it cause toxic compounds to develop. Thus, processing of foods with high hydrostatic pressure, though may be costly, may prove to be advantageous for certain food products.

5.4.2.1.3 Shear and Denaturation

High mechanical shear generated by shaking, kneading, whipping, etc., can cause denaturation of proteins. Many proteins denature and precipitate when they are vigorously agitated. In this case, denaturation occurs because of incorporation of air bubbles and adsorption of protein molecules to the air–liquid interface. Since the air–liquid interface has an excess free energy compared to the bulk phase, proteins undergo conformational change at the interface. The extent of conformational change depends on the flexibility of the protein. Highly flexible proteins denature more readily at an air–liquid interface than do rigid proteins. Upon interfacial denaturation, the nonpolar residues of denatured protein orient toward the gas phase and the polar residues orient toward the aqueous phase.

Several food-processing operations involve high pressure, shear, and high temperature, for example, extrusion, high speed blending, homogenization. When a rotating blade produces a high shear rate, subsonic pulses are created, and cavitation also occurs at the trailing edges of the blade. Both these events contribute to protein denaturation. The greater the shear rate, the greater the degree of denaturation. The combination of high temperature and high shear force causes irreversible denaturation of proteins. For example, when a 10%–20% whey protein solution at pH 3.5–4.5 and at 80°C–120°C is subjected to a shear rate of 7,500–10,000 s^{-1}, it forms insoluble spherical macrocolloidal particles of about 1 μm diameter. A hydrated material produced under these conditions, "Simplesse," has a smooth, emulsion-like organoleptic character.

5.4.2.2 Chemical Agents

5.4.2.2.1 pH and Denaturation

Proteins are more stable against denaturation at their isoelectric point than at any other pH. At neutral pH, most proteins are negatively charged, and a few are positively charged. Since the net electrostatic repulsive energy is small in an aqueous medium, and since this electrostatic energy is already accounted for during the formation of the native protein structure at neutral physiological pH, most proteins are stable at around neutral pH. However, when the pH is shifted to very low or very high values, the net charge of the protein changes accordingly and strong intramolecular electrostatic repulsion causes swelling and unfolding of the protein molecule. The degree of unfolding is greater at extreme alkaline pH values than it is at extreme acid pH values. The former behavior

is attributed to ionization of partially buried carboxyl, phenolic, and sulfhydryl groups that cause unraveling of the polypeptide chain as they attempt to migrate to the aqueous environment. The pH-induced denaturation is mostly reversible. However, in some cases, partial hydrolysis of peptide bonds, deamidation of Asn and Gln, destruction of sulfhydryl groups at alkaline pH, or aggregation can result in irreversible denaturation of proteins.

5.4.2.2.2 Organic Solvents and Denaturation

Organic solvents affect the stability of hydrophobic interactions, hydrogen bonding, and electrostatic interactions in proteins in different ways [44]. Since nonpolar side chains are more soluble in organic solvents than in water, organic solvents weaken hydrophobic interactions in proteins. In contrast, since the stability of hydrogen bonds in proteins is dependent on a low dielectric permittivity environment, certain organic solvents may actually strengthen or promote formation of peptide hydrogen bonds. For example, 2-chloroethanol causes an increase in α-helix content in globular proteins. The action of organic solvents on electrostatic interactions is twofold. By decreasing dielectric permittivity, organic solvents enhance electrostatic interactions between oppositely charged groups and also enhance repulsion between groups with like charge. The net effect of an organic solvent on protein structure, therefore, usually depends on the magnitude of its effect on various polar and nonpolar interactions. At low concentration, some organic solvents can stabilize enzymes against denaturation. At high concentrations, however, all organic solvents cause denaturation of proteins because of their solubilizing effect on nonpolar side chains.

5.4.2.2.3 Denaturation by Small-Molecular-Weight Additives

Because protein folding is driven by solvent properties, a change in solvent properties is expected to cause a corresponding change in protein stability. Several water miscible/soluble cosolvents, such as sugars, polyhydric alcohols, urea, poly(ethylene glycol), and certain amino acids alter protein stability in aqueous solutions [45,46]. While some of these cosolvents (e.g., urea and guanidinium hydrochloride) destabilize protein structure, other cosolvents, especially sugars, polyols, and some amino acids (osmolytes), increase protein stability [45,46]. Sugars tend to stabilize the native structure. In the case of neutral salts, while certain salts, such as sulfate, phosphate, and fluoride salts of sodium, termed as "kosmotropes," stabilize protein structure, other salts, such as bromide, iodide, perchlorate, and thiocyanate, termed as "chaotropes," destabilize protein structure.

According to prevailing theories, a combination of two mechanisms, namely, preferential interaction of water and cosolvent molecules with the protein surface (i.e., the solvent exchange model) and the excluded volume effect, governs the stability of globular proteins in cosolvent solutions [45,47–50]. According to the preferential interaction model, if the affinity of the protein surface is greater for cosolvent than for water, the cosolvent binds to protein loci with release of water from the protein loci to the bulk phase, and, if the protein loci has greater affinity for water than for cosolvent, water preferentially binds to protein loci and the cosolvent is excluded from the protein domain (Figure 5.18). The thermodynamics of binding to and exclusion of the solvent components from the protein surface are regarded as symmetrical phenomena [47]. In the case of denaturing cosolvents, binding of cosolvent molecules to the protein loci shifts the folded $\rightleftharpoons$ unfolded equilibrium in favor of the unfolded state, as more binding loci are available for the cosolvent in the unfolded state than in the folded state and the opposite process occurs in the case of osmolytes. In the case of stabilizing cosolvents, the opposite occurs, that is, water preferentially binds to the protein surface and this binding increases the stability of the protein.

While the preferential interaction model seems to apparently explain the mode of action of denaturants on protein stability, there is no clear evidence in the literature that the stabilizing effect of osmolytes is indeed due to preferential hydration of the protein surface. If the model were valid for osmolytes, then one would expect a positive correlation between the preferential hydration parameter (i.e., $\partial g_1/\partial g_2$ at constant temperature, pressure, and other solution condition, where g_1 is grams of water and g_2 is gram of protein) and the thermal stability of proteins in osmolyte solutions.

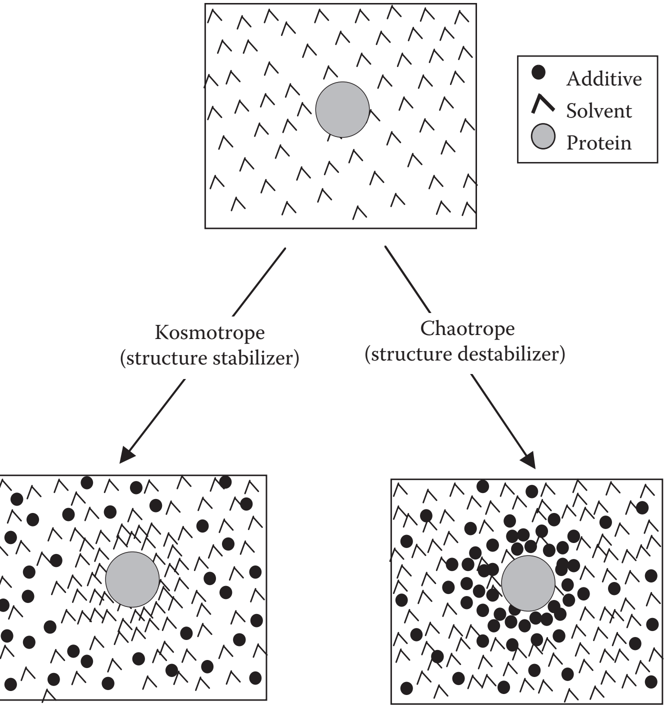


FIGURE 5.18 Schematic representation of preferential binding and preferential hydration of protein in the presence of additives. (Adapted from Creighton, T.E., *Proteins: Structures and Molecular Properties*, W.H. Freeman & Co., New York, 1993, pp. 158–159.)

However, a critical examination of literature data reveals no correlation between the preferential hydration parameter and thermal transition temperature (T_m) of several proteins. As an example, the relationship between the preferential hydration parameter and thermal transition temperature of α-chymotrypsin in sucrose solutions is presented in Figure 5.19. The lack of correlation casts a doubt on whether preferential hydration is the mechanism by which osmolytes influence protein stability. It should be noted, however, that in contrast to the preferential hydration parameter, preferential exclusion of osmolytes (i.e., $\partial g_3/\partial g_2$, where the subscript 3 refers to osmolyte) from the vicinity of the protein domain exhibits a linear correlation with T_m. In contrast, however, it should be noted that the preferential exclusion of sucrose (i.e., $\partial g_3/\partial g_2$, where the subscript 3 refers to sucrose) from the vicinity of the protein domain exhibits a linear correlation with T_m. Thus, logically, it can be inferred that the effect of an osmolyte on protein stability might be directly linked to the force(s) responsible for exclusion of the osmolyte away from the protein's domain, and water accumulation at the protein surface might be only a consequence of this exclusion rather than its cause.

It is apparent that the exact mechanism by which osmolytes (and denaturants) alter protein stability remains unresolved. Philosophically, for a variety of osmolytes that differ in chemical and physical properties to have similar effects on protein stability, the fundamental mechanism involved ought to be a universal one. Its origin might not be rooted merely in interactions of water and cosolvent with groups on the protein surface, but in a three-body quantum electrodynamic interaction between the protein phase and osmolyte across the water medium. In a recent study it has been shown that the thermal stability of several protein in various cosolvents was linearly related to the electrodynamic pressure arising from protein-cosolvent interaction (Figure 5.20) [33,51]. The electrodynamic pressure was positive (repulsive) for osmolytes that increased the stability of proteins, whereas it was negative (attractive) denaturants, suggesting that Lifshift–van der Waals three-body electrodynamic interaction might be the universal mechanism by which cosolvents exert their effect on protein stability.

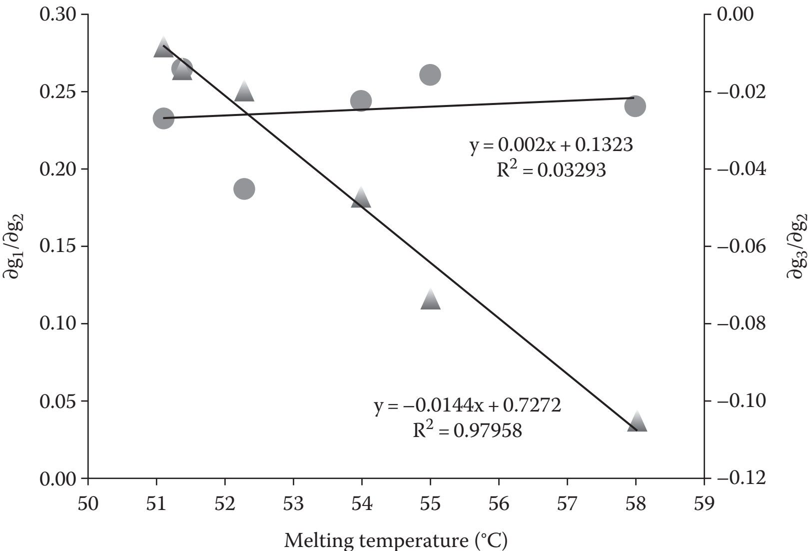


FIGURE 5.19 Relationship between melting temperature and preferential hydration parameter ($\partial g_1/\partial g_2$) (●) and preferential binding parameter of sucrose ($\partial g_3/\partial g_2$) (▲) to α-chymotrypsin. (Drawn using data from Lee, J.C. and Timasheff, S.N., *J. Biol. Chem.*, 256, 7193, 1981.)

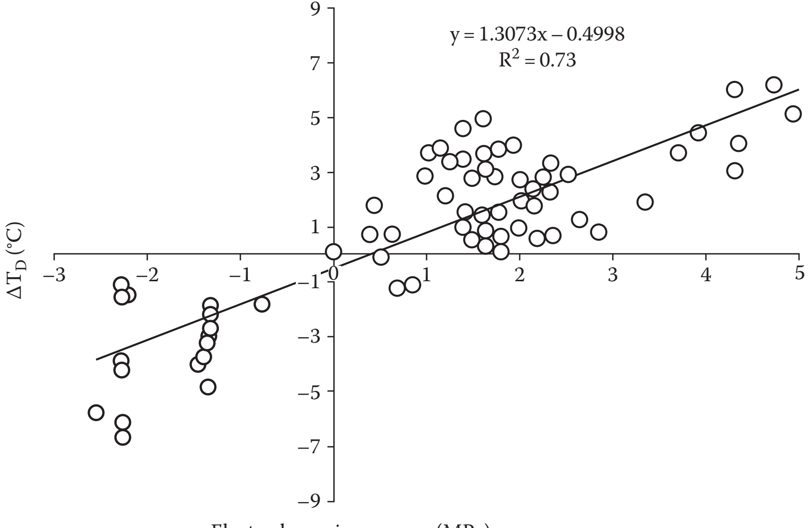


FIGURE 5.20 Relationship between electrodynamic pressure and net change in thermal denaturation temperature, T_d, of various proteins. (From Damodaran, S., *Biochemistry*, 52, 8363, 2013.)

When a protein is exposed to a mixture of stabilizing and destabilizing cosolvents, the net effect on protein stability generally follows an additivity rule. For example, sucrose and polyols are considered to be protein structure stabilizers, whereas guanidine hydrochloride (GuHCl) is a structure destabilizer. When sucrose is mixed with GuHCl, the concentration of GuHCl required for unfolding proteins increases with an increase in sucrose concentration [52].

5.4.2.2.4 Organic Solutes and Denaturation

Organic solutes, notably urea and GuHCl, cause denaturation of proteins. For many globular proteins the midpoint of transition from the native to denatured state occurs at 4–6 M urea and at 3–4 M GuHCl at room temperature. Complete denaturation often occurs in 8 M urea and in about 6 M GuHCl. GuHCl is a more powerful denaturant than urea because of its ionic character. Many globular proteins do not undergo complete denaturation even in 8 M urea, whereas in 8 M GuHCl they usually exist in a random coil state.

Denaturation of proteins by urea and GuHCl is thought to involve two mechanisms. The first mechanism involves preferential binding of urea and GuHCl to the denatured protein. Removal of denatured protein as a protein–denaturant complex shifts the N ↔ D equilibrium to the right. As the denaturant concentration is increased, continuous conversion of the protein to protein–denaturant complex eventually results in complete denaturation of the protein. Since binding of denaturants to denatured protein is very weak, a high concentration of denaturant is needed to shift N ↔ D equilibrium to the right. The second mechanism involves solubilization of hydrophobic amino acid residues in urea and GuHCl solutions. Since urea and GuHCl have the potential to form hydrogen bonds, at high concentration these solutes break down the hydrogen-bonded structure of water. This destructuring of solvent water makes it a better solvent for nonpolar residues. This results in unfolding and solubilization of apolar residues from the interior of the protein molecule.

Urea- or GuHCl-induced denaturation is reversible. However, complete reversibility of urea-induced protein denaturation is sometimes difficult. This is because some urea converts to cyanate and ammonia. Cyanate reacts with amine groups and alters the charge of the protein.

5.4.2.2.5 Detergents and Denaturation

Detergents, such as sodium dodecyl sulfate (SDS), are powerful protein denaturing agents. SDS at 3–8 mM concentration denatures most globular proteins. The mechanism involves preferential binding of detergent to the denatured state. This causes a shift in equilibrium between the native and denatured states. Unlike urea and GuHCl, detergents bind strongly to denatured proteins, and this is the reason for complete denaturation at a relatively low detergent concentration of 3–8 mM. Because of this strong binding, detergent-induced denaturation is irreversible. Globular proteins denatured by SDS do not exist in a random coil state; instead, they assume a helical rod shape in SDS solutions. This rod shape is properly regarded as denatured.

5.4.2.2.6 Chaotropic Salts and Denaturation

Salts affect protein stability in two different ways. At low concentrations, ions interact with proteins via nonspecific electrostatic interactions. This electrostatic neutralization of protein charges usually stabilizes protein structure. Complete charge neutralization by ions occurs at or below 0.2 M ionic strength and it is independent of the nature of the salt. However, at higher concentrations (>1 M), salts have ion specific effects that influence the structural stability of proteins. Salts such as Na_2SO_4 and NaF enhance, whereas NaSCN and $NaClO_4$ weaken, protein stability. Protein structure is influenced more by anions than by cations. For example, the effect of various sodium salts on the thermal denaturation temperature of β-lactoglobulin is shown in Figure 5.21. At equal ionic strength, Na_2SO_4 and NaCl increase T_D, whereas NaSCN and $NaClO_4$ decrease it. Regardless of their chemical makeup and conformational differences, the structural stability of macromolecules is affected by high concentrations of salts [53,54]. NaSCN and $NaClO_4$ are strong denaturants. The relative ability of various anions at isoionic strength to influence the structural stability of protein (and DNA) in general follows the series $F^- < SO_4^{=} < Cl^- < Br^- < I^- < ClO_4^- < SCN^- < Cl_3CCOO^-$. This ranking is known as the Hofmeister series or chaotropic series. Floride, chloride, and sulfate salts are structure stabilizers, whereas the salts of other anions are structure destabilizers.

The mechanism of salts effects on the structural stability of proteins is still unknown, but is believed to be related to their relative ability to bind to and alter hydration properties of proteins.

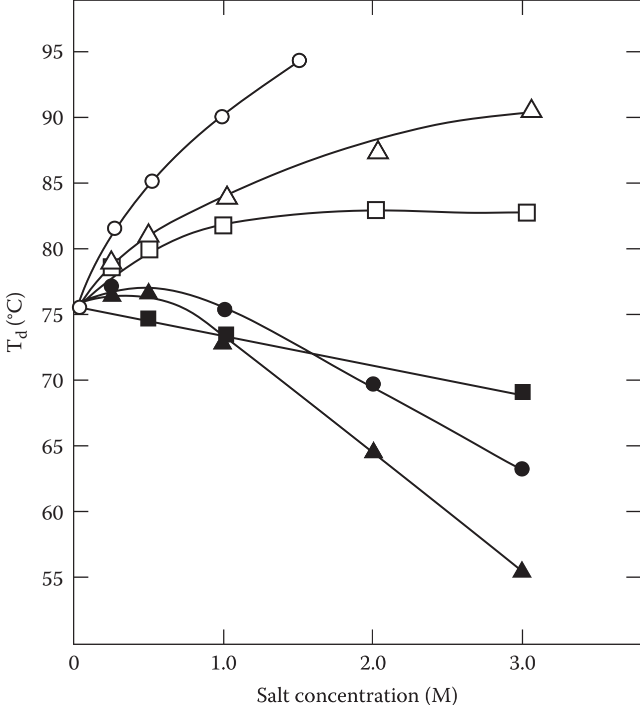


FIGURE 5.21 Effects of various sodium salts on the temperature of denaturation, T_d, of β-lactoglobulin at pH 7.0. ○, Na_2SO_4; Δ, NaCl; □, NaBr; ●, $NaClO_4$; ▲, NaSCN; ■, urea. (From Damodaran, S., *Int. J. Biol. Macromol.*, 11, 2, 1989.)

Salts that stabilize proteins enhance hydration of proteins and bind weakly, whereas salts that destabilize proteins decrease protein hydration and bind strongly [53]. However, whether or not these effects are mediated via changes in bulk water structure is not well understood [54]. As discussed in Section 5.4.2.2.3, the mechanism of Hofmeister salt effect on protein stability might be due to three-body electrodynamic interaction between protein and ions across the water medium [33,51].

5.4.3 Summary

- Protein denaturation involves transformation of a protein from a native folded state to an unfolded state.
- Protein denaturation can be monitored by measuring change in physical properties, such as UV absorption, fluorescence, sedimentation coefficient, and viscosity, as a function of denaturant concentration.
- Typical protein denaturants are temperature, extremes of pH, pressure, organic solvents, organic solutes, and chaotropic salts.

5.5 FUNCTIONAL PROPERTIES OF PROTEINS

Food preferences by human beings are based primarily on sensory attributes such as texture, flavor, color, and appearance. The sensory attributes of a food are the net effect of complex interactions among various minor and major components of food. Proteins greatly influence on the sensory attributes of foods. For example, the sensory properties of bakery products are related to the viscoelastic and dough-forming properties of wheat gluten; the textural and succulence characteristics of meat

TABLE 5.11
Functional Roles of Food Proteins in Food Systems

Function	Mechanism	Food	Protein Type
Solubility	Hydrophilicity	Beverages	Whey proteins
Viscosity	Water binding, hydrodynamic size and shape	Soups, gravies, and salad dressings, deserts	Gelatin
Water binding	Hydrogen bonding, ionic hydration	Meat sausages, cakes, and breads	Muscle proteins, egg proteins
Gelation	Water entrapment and immobilization, network formation	Meats, gels, cakes, bakeries, cheese	Muscle proteins, egg and milk proteins
Cohesion–adhesion	Hydrophobic, ionic, and hydrogen bonding	Meats, sausages, pasta, baked goods	Muscle proteins, egg proteins, whey proteins
Elasticity	Hydrophobic bonding, disulfide cross-links	Meats, bakery	Muscle proteins, cereal proteins
Emulsification	Adsorption and film formation at interfaces	Sausages, bologna, soup, cakes, dressings	Muscle proteins, egg proteins, milk proteins
Foaming	Interfacial adsorption and film formation	Whipped toppings, ice cream, cakes, desserts	Egg proteins, milk proteins
Fat and flavor binding	Hydrophobic bonding, entrapment	Low-fat bakery products, doughnuts	Milk proteins, egg proteins, cereal proteins

Source: Kinsella, J.E. et al., Physicochemical and functional properties of oilseed proteins with emphasis on soy proteins, in: *New Protein Foods: Seed Storage Proteins*, Altshul, A.M. and Wilcke, H.L., eds., Academic Press, London, U.K., 1985, pp. 107–179.

products are largely dependent on muscle proteins (actin, myosin, actomyosin, and several soluble meat proteins); the textural and curd-forming properties of dairy products are due to the unique colloidal structure of casein micelles; and the structure of some cakes and the whipping properties of some desert products depend on the properties of egg-white proteins. The functional roles of various proteins in different food products are listed in Table 5.11. Functionality of food proteins refers to the physical and chemical properties that influence the performance of proteins in food systems during processing, storage, preparation, and consumption.

The sensory attributes of foods are achieved by complex interactions among various ingredients. For example, the sensory attributes of a cake emanate from gelling/heat-setting, foaming, and emulsifying properties of the ingredients used. Therefore, for a protein to be useful as an ingredient in cakes and other such products, it must possess multiple functionalities. Proteins of animal origin, for example, milk (caseins), egg, and meat proteins, are widely used in fabricated foods. These proteins are mixtures of several proteins with wide ranging physicochemical properties and they are capable of performing multiple functions. For example, egg white possesses multiple functionalities such as gelation, emulsification, foaming, water binding, and heat coagulation, which make it a highly desirable protein in many foods. The multiple functionalities of egg white arise from complex interactions among its protein constituents, namely, ovalbumin, conalbumin, lysozyme, ovomucin, and other albumin-type proteins. Plant proteins (e.g., soy and other legume and oilseed proteins) and other proteins, such as whey proteins, are used to a limited extent in conventional foods. Though these proteins are also mixtures of several proteins, they do not perform well as animal proteins in most food products. The exact molecular properties of proteins that are responsible for the various desirable functionalities in food are poorly understood.

The physical and chemical properties that govern protein functionality include size; shape; amino acid composition and sequence; net charge and distribution of charges; hydrophobicity/hydrophilicity

TABLE 5.12
Linkage between the Physicochemical Aspects of Proteins and Their Impact on Functionalities in Foods

General Property	Functions Affected
1. Hydration	Solubility, dispersibility, wettability, swelling, thickening, water absorption, water-holding capacity
2. Surface activity	Emulsification, foaming, flavor binding, pigment binding
3. Hydrodynamic/rheological	Elasticity, viscosity, cohesiveness, chewiness, adhesion, stickiness, gelation, dough formation, texturization

ratio; secondary, tertiary, and quaternary structures; molecular flexibility/rigidity; and ability to interact/react with other components. Since proteins possess a multitude of physical and chemical properties, it is difficult to delineate the impact of each of these molecular properties on any given functional property.

On an empirical level, the various functional properties of proteins can be viewed as manifestations of three molecular aspects of proteins: (1) hydration properties, (2) protein surface-related properties, and (3) size- and shape-dependent hydrodynamic/rheological properties (Table 5.12). Although much is known about the physicochemical properties of several food proteins, prediction of functional properties from their molecular properties has not been successful. A few empirical correlations between molecular properties and certain functional properties in model protein systems have been established. However, behavior in model systems often is not the same as behavior in real food products. This disconnection is attributable, in part, to denaturation of proteins during food fabrication. The extent of denaturation depends on pH, temperature, other processing conditions, and product characteristics. In addition, in real foods, proteins interact with other food components, such as lipids, sugars, polysaccharides, salts, and other minor components, and this modifies their functional behavior. Despite these inherent difficulties, considerable progress has been made toward understanding the relationship between various physicochemical properties of protein molecules and their functional properties.

5.5.1 Protein Hydration [55]

Water is an essential constituent of foods. The rheological and textural properties of foods depend on the interaction of water with other food constituents, especially with proteins and polysaccharides. Water modifies the physicochemical properties of proteins. For example, the plasticizing effect of water on amorphous and semicrystalline food proteins changes their glass transition temperature (see Chapter 2) and T_D. The glass transition temperature refers to the conversion of a brittle amorphous solid (glass) to a flexible rubbery state, whereas the melting temperature refers to transition of a crystalline solid to a disordered structure.

Many functional properties of proteins, such as dispersibility, wettability, swelling, solubility, thickening/viscosity, water-holding capacity, gelation, coagulation, emulsification, and foaming, depend on water–protein interaction. In low- and intermediate-moisture foods, such as bakery and comminuted meat products, the ability of proteins to bind water is critical to the acceptability of these foods. The ability of a protein to exhibit a proper balance of protein–protein and protein–water interactions is critical to their thermal gelation properties.

Water molecules bind to several groups in proteins. These include charged groups (ion–dipole interactions); backbone peptide groups; the amide groups of Asn and Gln; hydroxyl groups of Ser, Thr, and Tyr residues (all dipole–dipole interactions); and nonpolar residues (dipole-induced dipole interaction, hydrophobic hydration).

TABLE 5.13
Hydration Capacities of Amino Acid Residues[a]

Amino Acid Residue	Hydration (Moles H_2O (Mol Residue)$^{-1}$)
Polar	
Asn	2
Gln	2
Pro	3
Ser, The	2
Trp	2
Asp (unionized)	2
Glu (unionized)	2
Tyr	3
Arg (unionized)	3
Lys (unionized)	4
Ionic	
Asp^-	6
Glu^-	7
Tyr^-	7
Arg^+	3
His^+	4
Lys^+	4
Nonpolar	
Ala	1
Gly	1
Phe	0
Val, Ile, Leu, Met	1

Source: Kuntz, I.D., *J. Am.Chem. Soc.*, 93, 514, 1971.

[a] Represents unfrozen water associated with amino acid residues based on nuclear magnetic resonance studies of polypeptide.

The *water binding capacity* of proteins is defined as grams of water bound per gram of protein when a dry protein powder is equilibrated with water vapor at 90%–95% relative humidity. The water binding capacities (also sometimes called hydration capacity) of various polar and nonpolar groups of proteins are given in Table 5.13. Amino acid residues with charged groups bind about 6 moles of water per residue, the uncharged polar residues bind about 2 mol residue^{-1}, and the nonpolar groups bind about 1 mol residue^{-1}. The water binding capacity of a protein therefore is related, in part, to its amino acid composition—the greater the number of charged residues, the greater is the water binding capacity.

The water binding capacity of a protein can be calculated from its amino acid composition using the empirical equation:

$$a = f_C + 0.4f_P + 0.2f_N \tag{5.50}$$

where

a is g water (g protein)$^{-1}$

f_C, f_P, and f_N are the fractions of the charged, polar, and nonpolar residues, respectively, in the protein

The experimental hydration capacities of several monomeric globular proteins agree very well with those calculated from Equation 5.50. This, however, is not true for oligomeric proteins. Since oligomeric structures involve partial burial of the protein surface at the subunit–subunit interface, calculated values are usually greater than experimental values. On the other hand, the experimental hydration capacity of casein micelles (~4 g water (g protein)$^{-1}$) is much larger than that predicted by Equation 5.50. This is because of the enormous amount of void space within the casein micelle structure, which imbibes water through capillary action and physical entrapment.

On a macroscopic level, water binding to proteins occurs in a stepwise process. The high affinity ionic groups are solvated first at low water activity, followed by polar and nonpolar groups. The sequence of steps involved at increasing water activity is presented in Figure 5.22 (see also Chapter 2). Sorption isotherms of proteins, that is, the amount of water bound per gram of protein as a function of relative humidity is invariably a sigmoidal curve (see Chapter 2). For most proteins, saturated monolayer coverage of water occurs at a water activity (a_W) of about 0.7–0.8, and multilayers of water are formed at $a_W > 0.8$. The saturated monolayer coverage corresponds to about 0.3–0.5 g water (g protein)$^{-1}$. The saturated monolayer water is primarily associated with ionic,

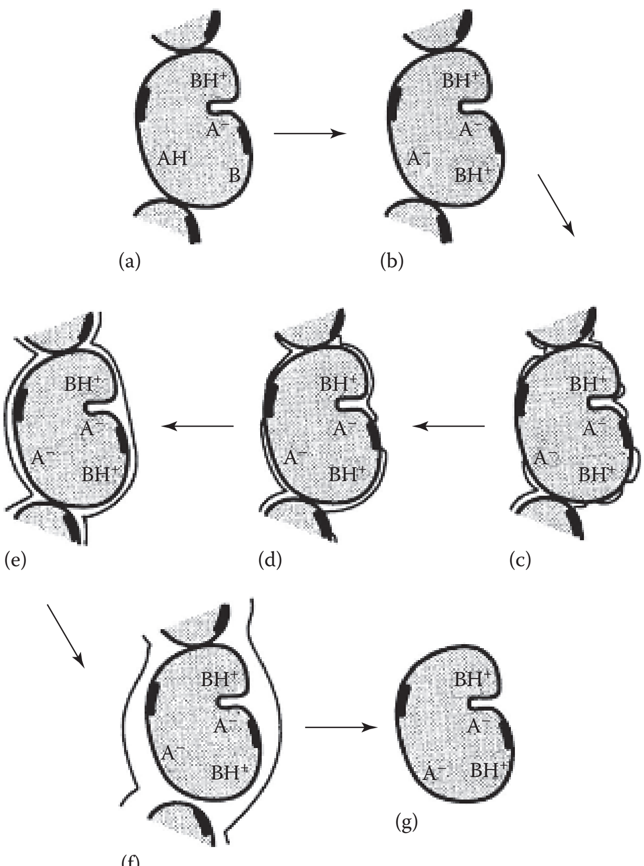


FIGURE 5.22 Sequence of steps involved in hydration of a protein. (a) Unhydrated protein. (b) Initial hydration of charged groups. (c) Water cluster formation near polar and charged sites. (d) Completion of hydration at the polar surfaces. (e) Hydrophobic hydration of nonpolar patches; completion of monolayer coverage. (f) Bridging between protein-associated water and bulk water. (g) Completion of hydrodynamic hydration. (From Rupley, J.A. et al., Thermodynamic and related studies of water interacting with proteins, in: *Water in Polymers*, Rowland, S.P. (ed.), ACS Symposium Series 127, American Chemical Society, Washington, DC, 1980, pp. 91–139.)

TABLE 5.14
Hydration Capacities of Various Proteins

Protein	g Water (g Protein)$^{-1}$
Pure proteins[a]	
Ribonuclease	0.53
Lysozyme	0.34
Myoglobin	0.44
β-Lactoglobulin	0.54
Chymotrypsinogen	0.23
Serum albumin	0.33
Hemoglobin	0.62
Collagen	0.45
Casein	0.40
Ovalbumin	0.30
Commercial protein preparations[b]	
Whey protein concentrates	0.45–0.52
Sodium caseinate	0.38–0.92
Soy protein	0.33

Note: Compiled from various sources.
[a] At 90% relative humidity.
[b] At 95% relative humidity.

polar, and apolar groups on the surface of the protein. This water is unfreezable, does not take part as a solvent in chemical reactions, and is often referred to as "bound" water, which should be understood to mean water with "hindered" mobility. In the hydration range of 0.07–0.27 g g^{-1}, the energy required for desorption of water from the protein surface is only about 0.18 kcal mol^{-1} at 25°C. Since the thermal kinetic energy of water at 25°C is about ~0.6 kcal mol^{-1}, which is greater than the free energy of desorption, water molecules in the monolayer are reasonably mobile.

At $a_W = 0.9$, proteins bind about 0.3–0.5 g water (g protein)$^{-1}$ (Table 5.14). At $a_W > 0.9$, liquid (bulk) water condenses into the clefts and crevices of protein molecules, or in the capillaries of insoluble protein systems, such as myofibrils. The properties of this water are similar to those of bulk water. This water is known as hydrodynamic water, which moves with the protein molecule.

Several environmental factors, such as pH, ionic strength, temperature, type of salts, and protein conformation influence the water binding capacity of proteins. Proteins are least hydrated at their isoelectric pH, where enhanced protein–protein interactions results in minimal interaction with water. Above and below the isoelectric pH, because of the increase in the net charge and repulsive forces, proteins swell and bind more water. The water binding capacity of most proteins is greater at pH 9–10 than at any other pH. This is due to ionization of sulfhydryl and tyrosine residues. Above pH 10, the loss of positively charged ε-amino groups of lysyl residues results in reduced water binding.

At low concentrations (<0.2 M), salts increase the water binding capacity of proteins. This is because hydrated salt ions, especially the anions, bind (weakly) to charged groups on proteins. At this low concentration, binding of ions to proteins does not affect the hydration shell of the charged groups on the protein, and the increase in water binding essentially comes from water associated with the bound ions. However, at high salt concentrations much of the existing water is bound by salt ions, resulting in dehydration of the protein.

The water binding capacity of proteins generally decreases as the temperature is raised, because of decreased hydrogen bonding and decreased hydration of ionic groups. The water binding

capacity of a denatured protein is generally about 10% greater than that of the native protein. This is due to an increase in surface-area-to-mass (or volume) ratio with exposure of some previously buried polar and hydrophobic groups. If denaturation leads to aggregation of the protein, then its water binding capacity may actually decrease because of displacement of water by enhanced protein–protein interactions. Denatured food proteins generally exhibit low solubility in water. Their water binding capacities, however, are not drastically different from those in the native state. Thus, the water binding capacity cannot be used to predict the solubility characteristics of proteins. The solubility of a protein is dependent not only on water binding capacity but also on other thermodynamic factors.

In food applications, the water-holding capacity of a protein is more important than the water binding capacity. Water-holding capacity refers to the ability of the protein to imbibe water and retain it against gravitational force within a protein matrix, such as protein gels or beef and fish muscle. This water refers to the sum of the bound water, hydrodynamic water, and the physically entrapped (capillary) water. The physically entrapped water contributes more to water-holding capacity than do the bound and hydrodynamic water. However, studies have shown that the water-holding capacity of proteins is positively correlated with water binding capacity. The ability of proteins to entrap water is associated with juiciness and tenderness of comminuted meat products and desirable textural properties of bakery and other gel-type products.

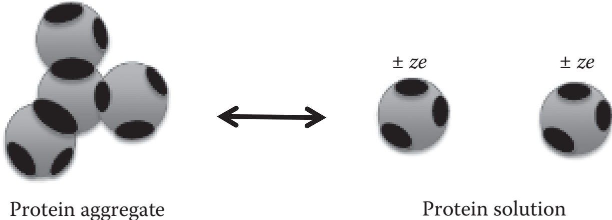
5.5.2 Solubility

The functional properties of proteins are often affected by protein solubility, and those most affected are thickening, foaming, emulsifying, and gelling. Insoluble proteins have very limited uses in food.

The solubility of a protein is the thermodynamic manifestation of the equilibrium between protein–protein and protein–solvent interactions:

$$\text{Protein} - \text{Protein} + \text{Water} \rightleftarrows \text{Protein} - \text{Water} \tag{5.51}$$

Aggregation of proteins, which eventually leads to protein insolubility, in water involves a balance between repulsive electrostatic interaction, which favors solubilization, and attractive van der Waals and hydrophobic interaction, which favors precipitation, between protein molecules, as shown in the following figure. The dark patches represent hydrophobic patches on the protein surface, and z and e are the net number of charge and charge of an electron (1.6×10^{-19} C), respectively.



The net free energy change for dissolution of a protein can be expressed as

$$E_{net} = E_{elec} + E_{vdW} + E_{H\phi}$$

If the repulsive electrostatic potential energy is greater (more negative) than the sum of the attractive (positive) van der Waals and hydrophobic potential energies, that is, if E_{net} were negative, then the protein would dissolve into solution. Conversely, if E_{net} of the process were positive, the protein would aggregate and precipitate. The van der Waals and hydrophobic interactions are always

attractive and therefore the electrostatic term (and consequently the hydrophilicity of protein) determines whether or not a protein would dissolve at a given pH.

Since proteins are colloidal particles with several positively and negatively charged groups on the surface, Equation 5.32, which is appropriate for interaction between two fixed point charges, cannot adequately describe electrostatic interaction between two protein molecules of colloidal dimension. The net interaction energy between two colloidal particles can be described using the DLVO theory as

$$E_{net} \approx \left(\frac{2\pi\sigma^2 R}{\varepsilon_o \varepsilon \kappa^2} e^{-\kappa D}\right) + \left(\frac{AR}{12D}\right) + \left(E_{H\phi}\right) \tag{5.52}$$

where

- σ is the surface charge density of the protein molecule
- R is its radius
- ε_o is the dielectric permittivity of vacuum
- ε is the dielectric constant of the medium (water)
- κ is the Debye length
- D is surface-to-surface distance between protein molecules
- A is the Hamaker constant

The Debye length is dependent on the ionic strength of the medium according to the following equation:

$$\kappa^{-1} = \left(\frac{\varepsilon_o \varepsilon kT}{2N_A e^2 I}\right)^{1/2} \tag{5.53}$$

where

- k is the Boltzmann constant
- T is the temperature
- N_A is the Avogadro's number
- e is the charge of an electron
- I is the ionic strength of the medium (in mol m^{-3})

For a 1:1 electrolyte, such as NaCl, Equation 5.53 simplifies to $\kappa^{-1} = 0.304/[NaCl]^{1/2}$ nm; and for a 2:1 salt, such as $CaCl_2$, $\kappa^{-1} = 0.176/[CaCl_2]^{1/2}$ nm. Since $\sigma = q/4\pi R^2$ (where q = ze is the net charge), Equation 5.52 can be simplified as

$$E_{net} \approx \left(\frac{z^2 kT}{4\pi R^3 N_A I} e^{-\kappa D}\right) + \left(\frac{AR}{12D}\right) + \left(E_{H\phi}\right) \tag{5.54}$$

The electrostatic interaction potential is inversely proportional to the radius of the particle and the ionic strength I and directly proportional to net number of charges (z) of the particle. Since z is dependent on the pH of the medium, the electrostatic interaction potential will be zero at the isoelectric pH of the protein, and therefore, the attractive van der Waals and hydrophobic interaction potentials would dominate and cause precipitation of the protein. The Hamaker constant A for proteins in water is typically about 10^{-21} J, and therefore the van der Waals attraction potential is dependent on the radius of the protein. On the other hand, since the composition of protein surface is inhomogeneous and nonpolar groups are distributed as patches on the surface, Equation 5.34 cannot be used to determine the hydrophobic interaction potential $E_{H\phi}$. However, if the interacting hydrophobic patches are large, and if the surface area of the patches are known, then one could estimate the hydrophobic potential at contact distance (D = 0) between hydrophobic patches using the buried surface area concept (see Figure 5.10).

Bigelow [56] proposed that the solubility of a protein is fundamentally related to the average hydrophobicity of the amino acid residues and the charge frequency. The average hydrophobicity is defined as

$$\Delta G = \sum \frac{\Delta g_{residue}}{n} \tag{5.55}$$

where

$\Delta g_{residue}$ is the hydrophobicity of each amino acid side chain obtained from the free energy change for transfer from octanol to water (see Section 5.2.1.4)

n is the total number of residues in the protein

The charge frequency is defined as

$$f = \left(\frac{n^+ + n^-}{n} \right) \tag{5.56}$$

where

n^+ and n^- are the total number of positively and negatively charged residues, respectively

n is the total number of residues

According to Bigelow [56], the smaller the average hydrophobicity and the larger the charge frequency, the greater will be the solubility of the protein. Although this empirical correlation is true for most proteins, it is not an absolute one. Solubility of a protein is dictated by the hydrophilicity and hydrophobicity of the protein surface that contacts with the surrounding water, rather than the average hydrophobicity and charge frequency of the molecule as a whole. Since a majority of hydrophobic residues are buried in the interior of the protein, only those nonpolar groups that are on the surface would affect the solubility. The fewer the number of surface hydrophobic patches, the greater would be the solubility.

Based on solubility characteristics, proteins are classified into four categories: *albumins* are those that are soluble in water at pH 6.6 (e.g., serum albumin, ovalbumin, and α-lactalbumin), *globulins* are those that are soluble in dilute salt solutions at pH 7.0 (e.g., glycinin, phaseolin, and β-lactoglobulin), *glutelins* are those that are soluble only in acid (pH 2) and alkaline (pH 12) solutions (e.g., wheat glutelins), and *prolamines* are those soluble in 70% ethanol (e.g., zein and gliadins). Both prolamines and glutelins are highly hydrophobic proteins.

In addition to these intrinsic physicochemical properties, solubility is influenced by several solution conditions, such as pH, ionic strength, temperature, and the presence of organic solvents.

5.5.2.1 pH and Solubility

At pH values below and above the isoelectric pH, proteins carry a net positive or a net negative charge, respectively. Electrostatic repulsion and hydration of charged residues promote solubilization of the protein. When solubility is plotted against pH, most food proteins exhibit a U-shaped curve. Minimum solubility occurs at about the isoelectric pH of proteins. A majority of food proteins are acidic proteins; that is, the sum of Asp and Glu residues is greater than the sum of Lys, Arg, and His residues. Therefore, they exhibit minimum solubility at pH 4–5 (isoelectric pH) and maximum solubility at alkaline pH. The occurrence of minimum solubility near the isoelectric pH is due primarily to the lack of electrostatic repulsion, which promotes aggregation and precipitation via hydrophobic interactions. Some food proteins are highly soluble at their isoelectric pH, for example, β-lactoglobulin (pI 5.2) and bovine serum albumin (pI 5.3). This is because these proteins contain a large ratio of surface hydrophilic residues to surface nonpolar groups. It should be

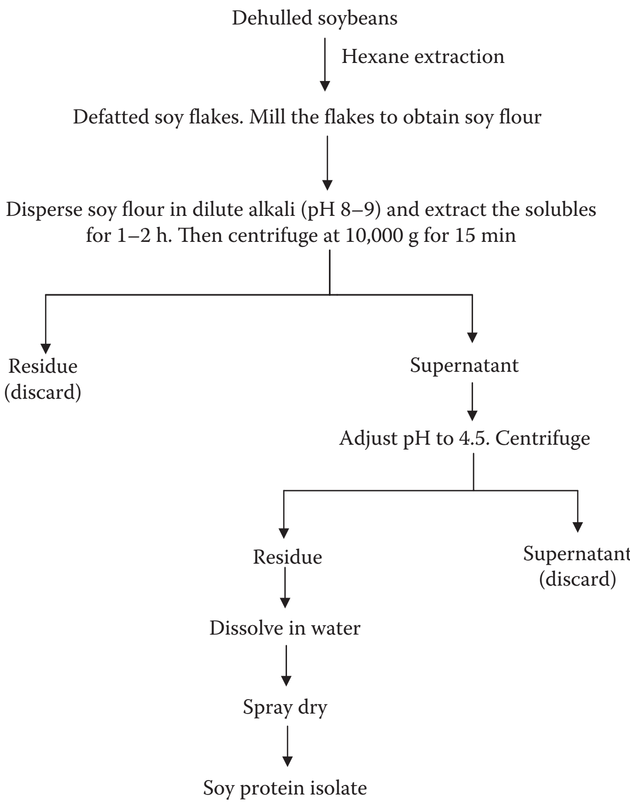


FIGURE 5.23 A typical industrial process for isolation of soy protein from defatted soy flour.

remembered that even though a protein is electrically neutral at its pI, it still has an equal number of positive and negative charges on the surface, contributing to hydrophilicity of the protein. If the hydrophilicity and the hydration repulsion forces arising from these charged residues are greater than the protein–protein hydrophobic interactions, then the protein will still be soluble at the pI.

Since most proteins are highly soluble at alkaline pH 8–9, protein extraction from plant sources, such as soybean flour, is carried out at this pH. Shown in Figure 5.23 is a typical industrial process for the isolation of soy protein based on its pH-solubility behavior.

Heat denaturation changes the pH-solubility profile of proteins (Figure 5.24). Native whey protein isolate (WPI) is completely soluble in the pH range 2–9, but when heated at 70°C for 1–10 min, a typical U-shaped solubility profile develops with a solubility minimum at pH 4.5. The change in the solubility profile upon heat denaturation is due to an increase in the hydrophobicity of the protein surface as a consequence of unfolding. Unfolding alters the balance between protein–protein and protein–solvent interactions in favor of the former.

5.5.2.2 Ionic Strength and Solubility

The ionic strength of a salt solution is given by

$$\mu = 0.5 \Sigma C_i Z_i^2 \tag{5.57}$$

where

C_i is the concentration of an ion
Z_i is its valence

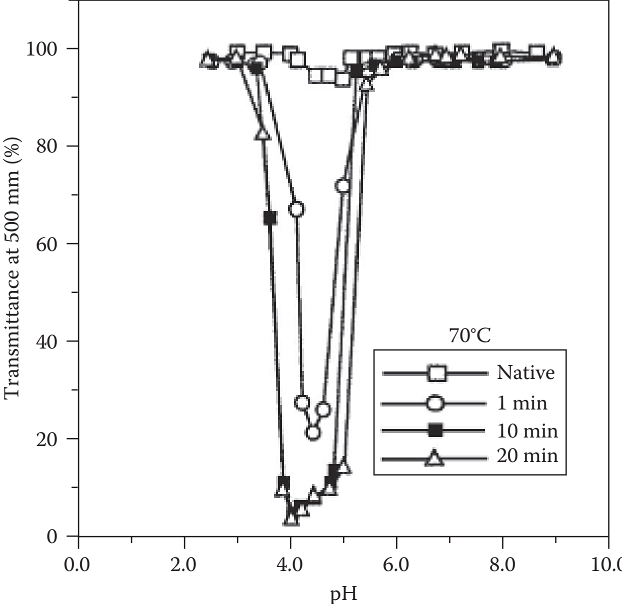


FIGURE 5.24 pH-solubility profile of whey protein isolate solutions heated at 70°C for various times. (From Zhu, H. and Damodaran, S., *J. Agric. Food Chem.*, 42, 846, 1994.)

At low ionic strength (<0.5 M), ions neutralize charges at the surface of proteins. This charge screening affects solubility in two different ways, depending on the characteristics of the protein surface. Solubility decreases for those proteins that contain a high incidence of nonpolar patches, and it increases for those that do not. The former behavior is typical for soy proteins, and the latter behavior is exhibited by β-lactoglobulin. While the decrease in solubility is caused by enhanced hydrophobic interactions, the increase in solubility is caused by a decrease in the ionic activity of the protein macro-ion. At ionic strength >1.0 M, salts have ion specific effects on protein solubility. As salt concentration is increased, sulfate and fluoride salts progressively decrease solubility (salting-out), whereas bromide, iodide, thiocyanate, and perchlorate salts increase solubility (salting-in). At constant ionic strength, relative effectiveness of various ions on solubility follows the Hofmeister series with anions promoting solubility in the order $SO_4^{=} < F^- < Cl^- < Br^- < I^- < ClO_4^- < SCN^-$, and cations decreasing solubility in the order $NH_4^+ < K^+ < Na^+ < Li^+ < Mg^{2+} < Ca^{2+}$. This behavior is analogous to the effects of salts on the thermal denaturation temperature of proteins (see Section 5.4).

Generally, solubility of proteins in salt solutions follows the relation

$$\log\left(\frac{S}{S_0}\right) = \beta - K_S C_S \tag{5.58}$$

where

S and S_0 are solubilities of the protein in the salt solution and in water, respectively
K_S is the salting-out constant
C_S is the molar concentration of salt
β is a constant characteristic of only protein

K_S is positive for salting-out-type salts and negative for salting-in-type salts.

5.5.2.3 Temperature and Solubility

At constant pH and ionic strength, the solubility of most proteins generally increases with temperature between 0°C and 40°C. Exceptions occur with highly hydrophobic proteins, such as β-casein and some cereal proteins, which exhibit a negative relationship with temperature. Above 40°C, the increase in thermal kinetic energy causes protein unfolding (denaturation), exposure of nonpolar groups, aggregation, and precipitation, that is, decreased solubility.

5.5.2.4 Organic Solvents and Solubility

Addition of organic solvents, such as ethanol or acetone, lowers the permittivity of an aqueous medium. This increases intra- and intermolecular electrostatic forces, both repulsive as well as attractive. The repulsive intramolecular electrostatic interactions cause unfolding of the protein molecule. In the unfolded state, the low permittivity of the medium promotes intermolecular hydrogen bonding between the exposed peptide groups and attractive intermolecular electrostatic interactions between oppositely charged groups. These intermolecular polar interactions lead to precipitation of the protein in organic solvents or reduced solubility in an aqueous medium. The role of hydrophobic interactions in causing precipitation in organic solvents is minimal because of the solubilizing effect of organic solvents on nonpolar residues. One exception is prolamine-type proteins. These proteins are so hydrophobic that they are soluble only in 70% ethanol.

Since solubility of proteins is intimately related to their structural states, it is often used as a measure of the extent of denaturation during extraction, isolation, and purification processes. It is also used as an index of the potential applications of proteins. Commercially prepared protein concentrates and isolates show a wide range of solubility. The solubility characteristics of these protein preparations are expressed as *protein solubility index* (PSI) or *protein dispersibility index* (PDI). Both of these terms express the % of soluble protein present in a protein sample. The PSI of commercial protein isolates varies from 25% to 80%.

5.5.3 Interfacial Properties of Proteins

Several natural and processed foods are either foam or emulsion-type products. These types of dispersed systems are unstable unless a suitable amphiphilic substance is present at the interface between the two phases (see Chapter 7). Proteins are amphiphilic molecules and they migrate spontaneously to the air–water interface or oil–water interface. This spontaneous migration of proteins from a bulk liquid to an interface indicates that the free energy of proteins is lower at the interface than it is in the bulk aqueous phase. Thus, when equilibrium is established, the concentration of protein in the interfacial region is always much greater than it is in the bulk aqueous phase. Unlike small molecule surfactants, proteins form a highly viscoelastic film at an interface, which has the ability to withstand mechanical shocks during storage and handling. Thus, protein-stabilized foams and emulsions are more stable than those prepared with small molecule surfactants, and because of this, proteins are extensively used for these purposes.

Although all proteins are amphiphilic, they differ significantly in their surface-active properties. Differences in surface-active properties among proteins cannot be attributed to differences in the ratio of hydrophobic to hydrophilic residues alone. If a large hydrophobicity/hydrophilicity ratio were the primary determinant of surface activity of proteins, then plant proteins, which contain more than 40% hydrophobic amino acid residues, should be better surfactants than albumin-type proteins, such as ovalbumin and bovine serum albumin, which contain less than 30% hydrophobic amino acid residues. On the contrary, ovalbumin and serum albumin are better emulsifying and foaming agents than are soy proteins and other plant proteins. Furthermore, average hydrophobicity of most proteins fall within a narrow range, yet they exhibit remarkable differences in their surface activity. It must be concluded, therefore, that differences in surface activity are related primarily to differences in protein conformation. The conformational factors of importance include stability/flexibility of the polypeptide chain, ease of adaptability to changes in the environment, and

distribution pattern of hydrophilic and hydrophobic groups on the protein surface. All these conformational factors are interdependent, and they collectively have a large influence on the surface activity of proteins.

It has been shown that desirable surface-active proteins have three attributes: (1) ability to rapidly adsorb to an interface, (2) ability to rapidly unfold and reorient at an interface, and (3) an ability, once at the interface, to interact with the neighboring molecules and form a strong cohesive, viscoelastic film that can withstand thermal and mechanical motions [57,58].

Formation and stabilization of foams and emulsions require the presence of a surfactant that can effectively reduce the interfacial tension between the air/oil and aqueous phases. This can be achieved using either small molecule surfactants, such as lecithin, monoacylglycerol, and Tween 20, or macromolecules, such as proteins. At equivalent concentration at an interface, proteins are generally less effective than small molecule surfactants in decreasing the interfacial tension. Typically, most proteins decrease the tension at air–water and oil–water interfaces by about 15 mN m^{-1} at saturated monolayer coverage, compared to 30–40 mN m^{-1} for small molecule surfactants. The inability of proteins to greatly reduce the interfacial tension is related to their complex structural properties. Though proteins contain hydrophilic and hydrophobic groups in their primary structure, there are no clearly defined hydrophilic head and hydrophobic tail as found in lecithin or monoacylglycerol. These groups are randomly spread all over the primary structure of proteins, and in the tertiary folded conformation some of the hydrophobic residues exist as segregated patches on the protein surface, while a majority of them are in fact buried in the interior of the protein.

The pattern of distribution of hydrophilic and hydrophobic patches on a protein surface affects the rapidity of its adsorption to the air–water or oil–water interface. If the protein surface is extremely hydrophilic and contains no discernable hydrophobic patches, anchoring of the protein at the interface probably will not take place because the protein surface will have a lower free energy in the aqueous phase than at the interface. If a protein has several hydrophobic patches on its surface, spontaneous adsorption to an interface becomes more probable (Figure 5.25). Single hydrophobic residues randomly distributed on the protein surface do not constitute a hydrophobic patch, nor do they possess sufficient interaction energy to strongly anchor the protein at an interface. Even though more than 40% of a typical globular protein's overall accessible surface is covered with nonpolar residues, they will not enhance protein adsorption unless they exist as segregated regions or patches. In other words, the molecular characteristics of the protein surface have an enormous influence on whether a protein will spontaneously adsorb to an interface and how effective it will be as a stabilizer of dispersions.

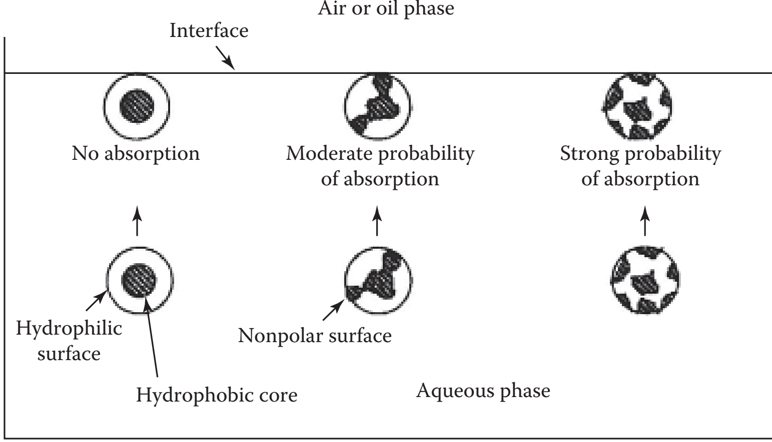


FIGURE 5.25 Schematic representation of the role of surface hydrophobic patches on the probability of adsorption of proteins at the air–water interface. (From Damodaran, S., *J. Food Sci.*, 70, R54, 2005.)

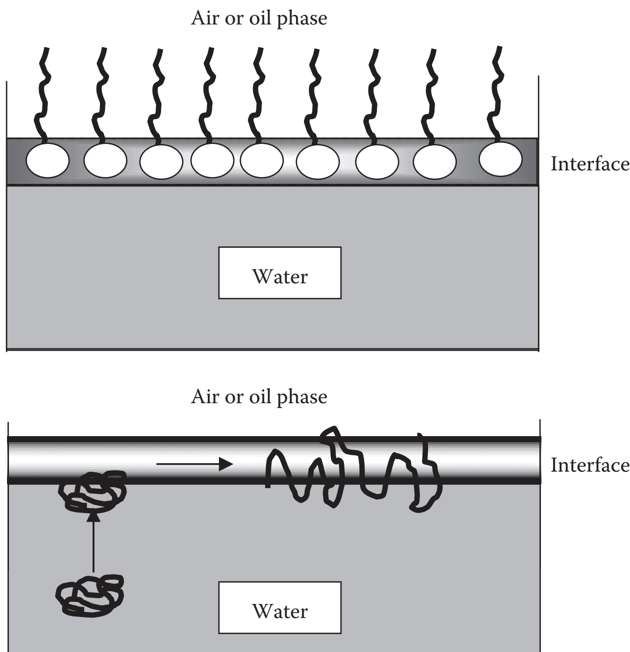


FIGURE 5.26 Difference in the mode of adsorption of a small molecule surfactant and a protein at the air–water or oil–water interface.

The mode of adsorption of proteins at an interface is different from that of small-molecular-weight surfactants. In the case of small molecule surfactants, such as phospholipids and monoacylglycerols, conformational constraints for adsorption and orientation do not exist because hydrophilic and hydrophobic moieties are present at the ends of the molecule. In the case of proteins, however, the distribution pattern of hydrophobic and hydrophilic patches on the surface and the structural rigidity of the molecule impose constraints to adsorption and orientation. Because of the bulky, folded nature of proteins, once adsorbed, a large portion of the molecule remains in the bulk phase and only a small portion is anchored at the interface (Figure 5.26). The tenacity with which this small portion of the protein molecule remains attached at the interface depends on the number of peptide segments anchored to the interface, and the energetics of interaction between these segments and the interface. The protein will be retained at the interface only when the sum of negative free energy changes of segment interactions is much greater than the thermal kinetic energy of the protein molecule. The number of peptide segments anchored at the interface depends, in part, on the conformational flexibility of the molecule. Highly flexible molecules, such as caseins, can undergo rapid conformational changes once they are adsorbed at the interface, enabling additional polypeptide segments to bind to the interface. On the other hand, rigid globular proteins such as lysozyme and soy protein cannot undergo extensive conformational changes at the interface.

At interfaces, polypeptide chains assume three distinct configurations: trains, loops, and tails (Figure 5.27). The trains are segments that are in direct contact with the interface, loops are segments of the polypeptide that are suspended in the aqueous phase, and tails are N- and C-terminal segments of the protein that are usually located in the aqueous phase. The relative distribution of these three configurations depends on the conformational characteristics of the protein. The greater the proportion of polypeptide segments in a train configuration, the stronger the binding and the lower the interfacial tension.

The single most important molecular property that impacts surface activity of proteins is its molecular flexibility. This relates to a protein's innate ability to undergo rapid conformational

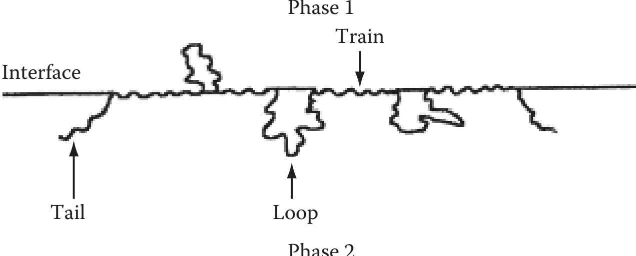


FIGURE 5.27 The various configurations of a flexible polypeptide at an interface. (From Damodaran, S., *J. Food Sci.*, 70, R54, 2005.)

change when it is transferred from one environment to another, for example, from a bulk aqueous phase to an interface. Adiabatic compressibility of proteins is often used as a measure of their molecular flexibility. Investigations on several unrelated proteins have shown that the dynamic surface activity of proteins, that is, the reduction in surface tension caused by 1 mg of protein per cm^2 *during* adsorption from the bulk phase to the air–water interface is positively and linearly correlated to the adiabatic compressibility (viz., flexibility) of proteins (Figure 5.28) [59]. Rapid conformation change at an interface is essential for the protein to reorient its hydrophobic and hydrophilic residues toward the oil and the aqueous phases and also to maximize the exposure and partitioning of these residues toward the two phases. This will ensure a rapid reduction in the interfacial tension, especially during the initial stages of formation of an emulsion.

The mechanical strength of a protein film at an interface depends on cohesive intermolecular interactions. These include attractive electrostatic interactions, hydrogen bonding, and hydrophobic interactions. Interfacial polymerization of adsorbed proteins via disulfide–sulfhydryl interchange reactions also increases their viscoelastic properties. The concentration of protein in the interfacial film is about 20%–25% (w/v), and the protein exists in almost a gel-like state. The balance of various noncovalent interactions is crucial to the stability and viscoelastic properties of this gel-like film. For example, if hydrophobic interactions are too strong, it can lead to interfacial aggregation,

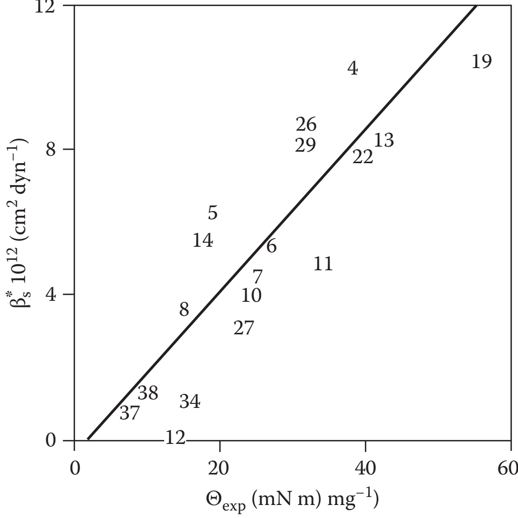


FIGURE 5.28 Relationship between adiabatic compressibility and surface activity of proteins. The numbers in the plot refer to the identities of proteins. (See Reference 59 for further details.)

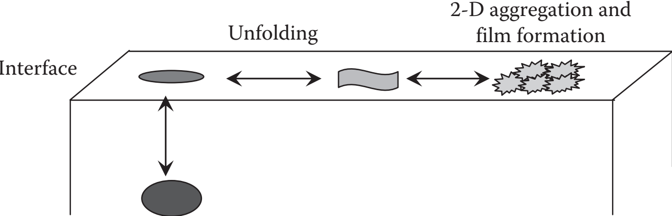


FIGURE 5.29 Schematic illustration of various molecular processes occurring in protein films at interfaces.

coagulation, and eventual precipitation of the protein, which will be detrimental to film integrity. If repulsive electrostatic forces are much stronger than attractive interactions, it may prevent the formation of a condensed, cohesive film. Therefore, to form a stable viscoelastic film, a proper balance of attractive, repulsive, and hydration interactions are required. The various molecular processes that occur during adsorption and formation of protein films at interfaces are summarized in Figure 5.29.

The basic principles involved in the formation and stability of emulsions and foams are very similar. However, since the energetics of these interfaces is different, the molecular requirements for protein functionality at these interfaces are not the same. In other words, a protein that is a good emulsifier may not be a good foaming agent.

It should now be clear that the behavior of proteins at interfaces is very complex and not well understood. Therefore, the following discussion of the emulsifying and foaming properties of food proteins will be largely qualitative in nature.

5.5.3.1 Emulsifying Properties

The physical chemistry of emulsion formation, creaming, flocculation, coalescence, and stability are presented in Chapter 7.

Several natural and processed foods, such as milk, egg yolk, coconut milk, soy milk, butter, margarine, mayonnaise, spreads, salad dressings, frozen desserts, frankfurter, sausage, and cakes, are emulsion-type products where proteins play an important role as an emulsifier. In natural milk, a membrane composed of lipoproteins stabilizes the fat globules. When milk is homogenized, a protein film comprised of casein micelles and whey proteins replaces the lipoprotein membrane. Homogenized milk is more stable against creaming than natural milk because the casein micelle–whey protein film is stronger than the natural lipoprotein membrane.

5.5.3.1.1 Methods for Determining the Emulsifying Properties of Proteins

The emulsifying properties of food proteins are evaluated by several methods such as size distribution of oil droplets formed, emulsifying activity, emulsion capacity (EC), and emulsion stability.

Emulsifying activity index (EAI): The physical and sensory properties of a protein-stabilized emulsion depend on the size of the droplets formed and the total interfacial area created. The average droplet size of emulsions can be determined by several methods, such as use of light microscopy (not very reliable), electron microscopy, and light scattering (photon correlation spectroscopy) or use of a Coulter counter. Knowing mean droplet size, the total interfacial area of an emulsion can be obtained from the relation

$$A = \frac{3\phi}{R} \tag{5.59}$$

where

ϕ is the volume fraction of the dispersed phase (oil)
R is the mean radius of the emulsion particles

If m is the mass of the protein, then the EAI, that is, the interfacial area created per unit mass of protein is

$$\mathrm{EAI} = \frac{3\phi}{\mathrm{Rm}} \tag{5.60}$$

Another simple and more practical method to determine EAI of proteins is the turbidimetric method [60]. The turbidity of an emulsion is given by

$$\mathrm{T} = \frac{2.303\mathrm{A}}{\mathrm{l}} \tag{5.61}$$

where
A is the absorbance
l is the path length

According to the Mie theory of light scattering, the interfacial area of an emulsion is twice its turbidity. If ϕ is the volume fraction of the oil and C is the weight of protein per unit volume of the aqueous phase, then the EAI of the protein is given by

$$\mathrm{EAI} = \frac{2\mathrm{T}}{(1-\phi)\mathrm{C}} \tag{5.62}$$

It should be noted that the term $(1 - \phi)$ in Equation 5.62 refers to the volume of fraction of the aqueous phase in the emulsion and therefore $(1 - \phi)C$ is the total mass of protein in a unit volume of the emulsion.

Although EAI is a simple parameter to evaluate the surface activity of proteins and the turbidimetry method is a simple practical tool to determine EAI, there are two main drawbacks in this concept. First, the method is based on measurement of turbidity at one single wavelength, 500 nm. Since the turbidity of food emulsions is wavelength dependent, the interfacial area obtained from turbidity at 500 nm is not the actual emulsion interfacial area. Therefore, use of Equation 5.62 to estimate mean particle diameter or the number of emulsion particles present in the emulsion gives results that are not very reliable. The second drawback is the way EAI is defined: It is defined as interfacial area created by 1 mg of protein under a set of conditions. It should be noted that, assuming an average molecular weight of 115 for amino acid residues in proteins, while the molar concentration of amino acid residue at a given concentration of various proteins might be the same, the molar concentration of proteins would not be the same since proteins widely differ in their molecular weight. Implicit in the definition of EAI is that all proteins are completely unfolded, and all amino acid residues are completely exposed at the interface. If this is not the case, then EAI would be a function of molar concentration rather than weight concentration of proteins. However, the method can be used for qualitative comparison of emulsifying activities of different proteins, or changes in the emulsifying activity of a protein after various treatments.

Protein load: The amount of protein adsorbed at the oil–water interface of an emulsion has a bearing on its stability. To determine the amount of protein adsorbed, the emulsion is centrifuged, the aqueous phase is separated, and the cream phase is repeatedly washed and centrifuged to remove any loosely adsorbed proteins. The amount of protein adsorbed to the emulsion particles is determined from the difference between the total protein initially present in the emulsion and the amount present in the wash fluid from the

cream phase. Knowing the total interfacial area of the emulsion particles, the amount of protein adsorbed/m^2 of the interfacial area can be calculated. Generally, the protein load is in the range of about 1–3 mg m^{-2} of interfacial area. As the volume fraction of the oil phase is increased, the protein load decreases at constant protein content in the total emulsion. For high-fat emulsions and small-sized droplets, more protein is obviously needed to adequately coat the interfacial area and stabilize the emulsion.

Emulsion capacity: EC is the volume (mL) of oil that can be emulsified per gram of protein before phase inversion (a change from oil-in-water emulsion to water-in-oil) occurs. This method involves addition of oil or melted fat at a constant rate and temperature to an aqueous protein solution that is continuously agitated in a food blender. Phase inversion is detected by an abrupt change in viscosity or color (usually a dye is added to the oil) or by an increase in electrical resistance. For a protein-stabilized emulsion, phase inversion usually occurs when ϕ is about 0.65–0.85. Inversion is not instantaneous, but is preceded by formation of a water-in-oil-in-water double emulsion. Since EC is expressed as volume of oil emulsified per gram protein at phase inversion, it decreases with increasing protein concentration once a point is reached where unadsorbed protein accumulates in the aqueous phase. Therefore, to compare emulsion capacities of different proteins, EC versus protein concentration profiles should be used instead of EC at a specific protein concentration.

Emulsion stability: Protein-stabilized emulsions are often stable for days. Thus, a detectable amount of creaming or phase separation is usually not observed in a reasonable amount of time when samples are stored at atmospheric conditions. Therefore, drastic conditions, such as storage at elevated temperature or separation under centrifugal force is often used to evaluate emulsion stability. If centrifugation is used, stability is then expressed as percent decrease in interfacial area (i.e., turbidity) of the emulsion, or percent volume of cream separated, or as the fat content of the cream layer. More often, however, emulsion stability is expressed as

$$\text{ES} = \frac{\text{Volume of cream layer}}{\text{Total volume of emulsion}} \times 100 \tag{5.63}$$

where the volume of the cream layer is measured after a standardized centrifugation treatment. A common centrifugation technique involves centrifugation of a known volume of emulsion in a graduated centrifuge tube at *1300 g* for 5 min. The volume of the separated cream phase is then measured and expressed as percentage of the total volume. Sometimes, centrifugation at a relatively low gravitational force (*180 g*) for a longer time (15 min) is used to avoid coalescence of droplets.

The turbidimetric method (see earlier) can also be used to evaluate emulsion stability. In this case, stability is expressed as emulsion stability index, which is defined as the time to achieve a turbidity of the emulsion that is one-half of the original value.

The methods used to determine emulsion stability are very empirical. The most fundamental quantity that defines stability is the change in interfacial area with time, but this is difficult to measure directly.

5.5.3.1.2 Factors Influencing Emulsification

The properties of protein-stabilized emulsions are affected by several factors. These include intrinsic factors, such as pH, ionic strength, temperature, the presence of low-molecular-weight (LMW) surfactants, sugars, oil phase volume, type of protein, and the melting point of the oil used, and extrinsic factors such as type of equipment, rate of energy input, and rate of shear. Standardized methods for systematically evaluating the emulsifying properties of proteins have not emerged.

Therefore, results among laboratories cannot be accurately compared, and this has hampered the understanding of the molecular factors that affect the emulsifying properties of proteins.

The general forces involved in the formation and stabilization of emulsion were discussed in Chapter 7. Therefore, only the molecular factors that affect protein-stabilized emulsions need be discussed here.

Solubility plays a role in emulsifying properties, but 100% solubility is not an absolute requirement. While highly insoluble proteins do not perform well as emulsifiers, no reliable relationship exists between solubility and emulsifying properties in the 25%–80% solubility range. However, since the stability of a protein film at the oil–water interface is dependent on favorable interactions with both the oil and aqueous phases, some degree of solubility is likely to be necessary. The minimum solubility requirement for good performance may vary among proteins. In meat emulsions, such as in sausage and frankfurter, solubilization of myofibrillar proteins in 0.5 M NaCl enhances their emulsifying properties. Some commercial soy protein isolates that are isolated by thermal processing have poor emulsifying properties because of their very low solubility.

The formation and stability of protein-stabilized emulsions are affected by pH. Several mechanisms are involved. Generally, proteins that have high solubility at the isoelectric pH (e.g., serum albumin, gelatin, and egg-white proteins) show maximum emulsifying activity and EC at that pH. The lack of net charge and electrostatic repulsive interactions at the isoelectric pH helps maximize protein load at the interface and promotes formation of a highly viscoelastic film, both of which contribute to emulsion stability. However, the lack of electrostatic repulsive interactions among emulsion particles can, in some instances, promote flocculation and coalescence and thus decrease emulsion stability. On the other hand, if the protein is highly hydrated at the isoelectric pH (unusual), then hydration repulsion forces between emulsion particles may prevent flocculation and coalescence and thus stabilize the emulsion. Because most food proteins (caseins, commercial whey proteins, meat proteins, soy proteins) at their isoelectric pH are sparingly soluble and poorly hydrated and lack electrostatic repulsive forces, they are generally poor emulsifiers at this pH. These proteins may, however, be effective emulsifiers when moved away from their isoelectric pH.

The emulsifying properties of proteins show a weak positive correlation with surface hydrophobicity, but not with mean residue hydrophobicity (i.e., kcal mol^{-1} $residue^{-1}$). The ability of various proteins to decrease interfacial tension at the oil–water interface and to increase the EAI is related to their surface hydrophobicity values (Figure 5.30). However, this relationship is by no means perfect. The emulsifying properties of several proteins, such as β-lactoglobulin, α-lactalbumin, and soy proteins, do not show a strong correlation with surface hydrophobicity. Some probable reasons are provided in Section 5.5.3.1.1.

The surface hydrophobicity of proteins is usually determined by measuring the amount of a hydrophobic fluorescent probe, such as *cis*-parinaric acid, which can bind to the protein [61]. Although this method provides some information on the hydrophobicity of the protein surface, it is questionable whether the measured value truly reflects the "hydrophobicity" of the protein surface. The true definition of surface hydrophobicity is that portion of the nonpolar surface of the protein that makes contact with the surrounding bulk water. However, *cis*-parinaric acid is capable of binding only to hydrophobic cavities. These protein cavities are accessible to nonpolar ligands, but they are not accessible to water and may not be accessible to either phase in an oil–water emulsion, unless the protein is able to undergo rapid conformational rearrangement at the interface. The poor correlation of surface hydrophobicity (as measured by *cis*-parinaric acid binding) with the emulsifying properties of some proteins may be related to the fact that *cis*-parinaric acid provides no indication of molecular flexibility. Molecular flexibility at the oil–water interface may be the most important determinant of the emulsifying properties of proteins.

Partial denaturation of proteins prior to emulsification, which does not result in insolubilization, usually improves their emulsifying properties. This is due to increased molecular flexibility and surface hydrophobicity. In the unfolded state, proteins containing free sulfhydryl groups and disulfide bonds undergo slow polymerization via disulfide–sulfhydryl interchange reaction [62]. This leads to formation of a highly viscoelastic film at the oil–water interface. Excessive heat denaturation may impair the emulsifying properties by rendering the protein insoluble.

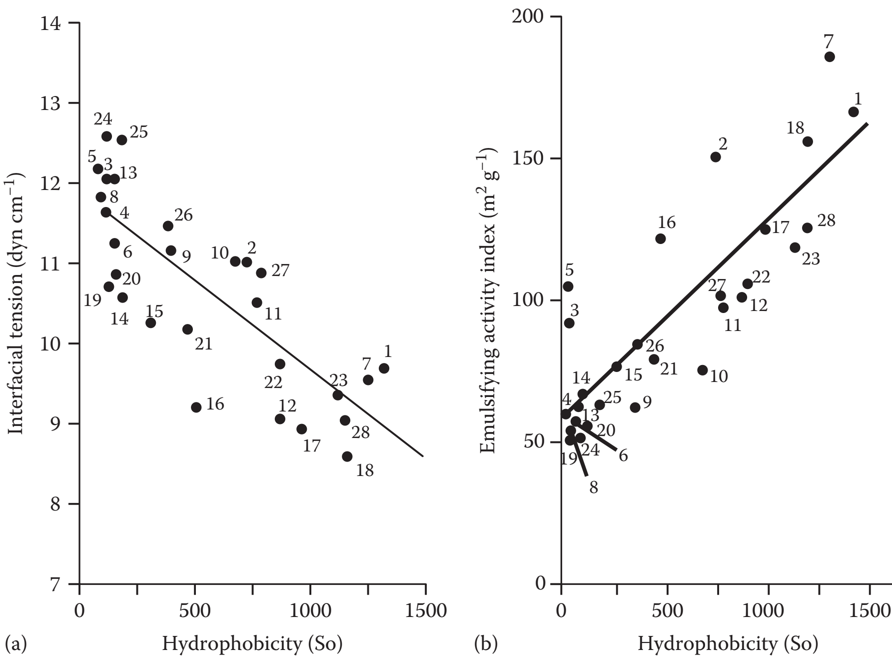


FIGURE 5.30 Correlations of surface hydrophobicity of various proteins with (a) oil–water interfacial tension and (b) emulsifying activity index. Surface hydrophobicity was determined from the amount of hydrophobic fluorescent probe bound per unit weight of protein. The numbers in the plots represent (1) bovine serum albumin, (2) β-lactoglobulin, (3) trypsin, (4) ovalbumin, (5) conalbumin, (6) lysozyme, (7) κ-casein, and (8–12) ovalbumin denatured by heating at 85°C for 1, 2, 3, 4, or 5 min, respectively; (13–18) lysozyme denatured by heating at 85°C for 1, 2, 3, 4, 5, or 6 min, respectively; (19–23) ovalbumin bound to 0.2, 0.3, 1.7, 5.7, or 7.9 mole dodecyl sulfate per mol protein, respectively; (24–28) ovalbumin bound to 0.3, 0.9, 3.1, 4.8, or 8.2 mollinoleate per mole protein, respectively. (From Kato, A. and Nakai, S., *Biochim. Biophys. Acta*, 624, 13, 1980.)

Small molecule emulsifiers, such as phospholipids, which are generally found in foods, compete with proteins for adsorption at the oil–water interface [63]. Since small molecule surfactants can diffuse rapidly to the interface and lack conformational constraints for reorientation at the interface, they can effectively inhibit adsorption of proteins at high concentrations. If small molecule emulsifiers are added to a protein-stabilized emulsion, they can displace the protein from the interface and cause instability in the emulsion.

Another factor that affects protein-stabilized emulsions is the protein composition. Food proteins in general are mixtures of several protein components. For instance, egg protein is a mixture of five major proteins and several minor protein components. Likewise, whey protein is a mixture of α-lactalbumin and β-lactoglobulin and several other minor proteins. Seed storage proteins, such as soy protein isolate, contain at least two major protein fractions, namely, legumins and vicillins. During emulsification, the protein components of the mixture compete with each other for adsorption to the interface. The composition of the protein film formed at the interface is dependent on relative surface activities of various protein components of the mixture. For instance, when a 1:1 mixture of α- and β-caseins are allowed to adsorb to the oil–water interface, the amount of α-casein in the protein film at equilibrium is almost twice that of β-casein [64]. At the air–water interface, however, an exactly opposite behavior is observed [65]. Variations in the protein composition of the bulk phase would affect protein composition of the adsorbed film and possibly the stability of the emulsion.

At high concentration, protein mixtures generally exhibit incompatibility of mixing in solution [66]. In mixed protein films at the oil–water interface, where the local protein concentration is in the range of 15%–30%, it is likely that two-dimensional phase separation of the proteins can occur

with storage time. Evidence for this at the air–water [67,68] and oil–water [64] interfaces has been reported. If distinct phase separation of proteins occurs in mixed protein films around oil droplets, it is conceivable that the interface of such phase-separated regions might act as source of instability in emulsions. However, a direct correlation between thermodynamic incompatibility of mixing of proteins in mixed protein films at the oil–water interface and the kinetic stability of emulsions made of protein mixtures is yet to be determined.

5.5.3.2 Foaming Properties

Foams consist of an aqueous continuous phase and a gaseous (air) dispersed phase. Many processed foods are foam-type products. These include whipped cream, ice cream, cakes, meringue, bread, souffles, mousses, and marshmallow. The unique textural properties and mouthfeel of these products stem from the dispersed tiny air bubbles. In most of these products, proteins are the main surface-active agents that help in the formation and stabilization of the dispersed gas phase.

Generally, bubbling, whipping, or shaking a protein solution creates protein-stabilized foams. The foaming property of a protein refers to its ability to form a thin tenacious film at gas–liquid interfaces so that large quantities of gas bubbles can be incorporated and stabilized. Foaming properties are evaluated by several means. The *foamability* or *foaming capacity* of a protein refers to the amount of interfacial area that can be created by the protein. It can be expressed in several ways, such as *overrun* (or steady state foam volume) or *foaming power* (or foam expansion). Overrun is defined as

$$\text{Overrun} = \frac{\text{Volume of foam}}{\text{Total volume of inital liquid}} \times 100 \qquad (5.64)$$

The foaming power, FP, is expressed as

$$\text{FP} = \frac{\text{Volume of gas incorporated}}{\text{Total volume of liquid}} \times 100 \qquad (5.65)$$

Foaming power generally increases with protein concentration until a maximum value is attained. It is also affected by the method used for foam formation. FP at a given protein concentration is often used as a basis for comparing the foaming properties of various proteins (Table 5.15).

TABLE 5.15
Comparative Foaming Power of Protein Solutions

Protein Type	Foaming Power[a] at 0.5% Protein Conc. (w/v)
Bovine serum albumin	280%
Whey protein isolate	600%
Egg albumen	240%
Ovalbumin	40%
Bovine plasma	260%
β-Lactoglobulin	480%
Fibrinogen	360%
Soy protein (enzyme hydrolyzed)	500%
Gelatin (acid-processed pigskin)	760%

Source: Poole, S. et al., *J. Sci. Food Agric.*, 35, 701, 1984.
[a] Calculated according to Equation 5.60.

"Foam stability" refers to the ability of protein to stabilize foam against gravitational and mechanical stresses. Foam stability is often expressed as the time required for 50% of the liquid to drain from foam or for a 50% reduction in foam volume. These are very empirical methods, and they do not provide fundamental information about the factors that affect foam stability. The most direct measure of foam stability is the reduction in foam interfacial area as a function of time. This can be done as follows. According to the Laplace equation, the internal pressure of a foam bubble is greater than the external (atmospheric) pressure, and under stable conditions the pressure difference, ΔP, is

$$\Delta P = p_i - p_o = \frac{4\gamma}{r} \tag{5.66}$$

where

p_i and p_o are the internal and external pressures, respectively
r is the radius of the foam bubble
γ is the surface tension

According to this equation, the pressure inside a closed vessel containing foam will increase when the foam collapses. The net change in the pressure is given by [69]

$$\Delta P = \frac{-2\gamma\Delta A}{3V} \tag{5.67}$$

where

V is the total volume of the system
ΔP is the pressure change
ΔA is the net change in interfacial area resulting from collapse of the foam

The initial interfacial area of the foam is given by

$$A_o = \frac{3V\Delta P_\infty}{2\gamma} \tag{5.68}$$

where ΔP_∞ is the net pressure change when the entire foam is collapsed. The A_o value is a measure of foamability. Assuming that the collapse of a foam follows the first-order kinetics, the rate of collapse of a foam can be expressed as

$$\frac{A_o - A_t}{A_o} = -kt \tag{5.69}$$

where

A_t is the area of the foam and time t
k is the first-order rate constant

The first-order rate constant can be used to compare the stability of foams created by different proteins. This approach has been used to study the foaming properties of food proteins [70,71].

The *strength* or *stiffness* of the foam refers to the maximum weight a column of foam can withstand before it collapses. Measuring foam viscosity also assesses this property.

5.5.3.2.1 Environmental Factors Influencing Foam Formation and Stability

pH: Several studies have shown that protein-stabilized foams are more stable at the isoelectric pH of the protein than at any other pH, provided there is no insolublization of the protein at pI. At or near the isoelectric pH region, the lack of repulsive interactions promotes favorable protein–protein interactions and formation of a viscous film at the interface. In addition, an increased amount of protein is adsorbed to the interface at the pI because of lack of repulsion between the interface and the adsorbing molecules. These two factors improve both foamability and foam stability. If the protein is sparingly soluble at pI, as most food proteins are, then only the soluble protein fraction will be involved in foam formation. Since the concentration of this soluble fraction is very low, the amount of foam formed will be less, but the stability will be high. Although the insoluble fraction does not contribute to foamability, adsorption of these insoluble protein particles may stabilize the foam, probably by increasing cohesive forces in the protein film. Generally, adsorption of hydrophobic particles increases the stability of foams. At pH other than pI, foamability of proteins is often good, but foam stability is poor. Egg-white proteins exhibit good foaming properties at pH 8–9 and at their isoelectric pH 4–5.

Salts: The effects of salts on the foaming properties of proteins depend on the type of salt and the solubility characteristics of the protein in that salt solution. The foamability and foam stability of most globular proteins increase with increasing concentration of NaCl. This behavior is usually attributed to neutralization of charges by salt ions. However, some proteins, such as whey proteins, exhibit the opposite effect: both foamability and foam stability decrease with increasing concentration of NaCl (Table 5.16) [72]. This is attributed to salting-in of whey proteins, especially β-lactoglobulin. Proteins that are salted-out in a given salt solution generally exhibit improved foaming properties, whereas those that are salted-in generally exhibit poor foaming properties. Divalent cations, such as Ca^{2+} and Mg^{2+}, dramatically improve both foamability and foam stability at 0.02–0.4 M concentration. This is primarily due to cross-linking of protein molecules and creation of films with better viscoelastic properties [73].

Sugars: Addition of sucrose, lactose, and other sugars to protein solutions often impairs foamability, but improves foam stability. The positive effect of sugars on foam stability is due to increased bulk phase viscosity, which reduces the rate of drainage of the lamella fluid. The depression in foam overrun is mainly due to enhanced stability of protein structure in sugar solutions. Because of this, the protein molecule is less able to unfold upon

TABLE 5.16
Effect of NaCl on Foamability and Stability of Whey Protein Isolate

NaCl Concentration (M)	Total Interfacial Area (cm^2 mL^{-1} of Foam)	Time for 50% Collapse of Initial Area(s)
0.00	333	510
0.02	317	324
0.04	308	288
0.06	307	180
0.08	305	165
0.10	287	120
0.15	281	120

Source: Compiled from Zhu, H. and Damodaran, S., *J. Food Sci.*, 59, 554, 1994.

adsorption at the interface. This decreases the ability of the protein to reduce interfacial tension and produce large interfacial areas and large foam volume during whipping. In sugar-containing foam-type dessert products, such as meringues, souffles, and cakes, it is preferable to add sugar after whipping when possible. This will enable the protein to adsorb, unfold, and form a stable film, and then the added sugar will increase foam stability by increasing the viscosity of the lamella fluid.

Lipids: Lipids, especially phospholipids, when present at concentrations greater than 0.5%, markedly impair the foaming properties of proteins. Because lipids are more surface active than proteins, they readily adsorb at the air–water interface and inhibit adsorption of proteins during foam formation. Since lipid films lack the cohesive and viscoelastic properties necessary to withstand the internal pressure of the foam bubbles, the bubbles rapidly expand and then collapse during whipping. Thus, lipid-free whey protein concentrates (WPCs) and isolates, soy proteins, and egg proteins without egg yolk display better foaming properties than do lipid-contaminated preparations.

Protein concentration: Several properties of foams are influenced by protein concentration. The higher the protein concentration, the stiffer is the foam. Foam stiffness results from small bubble size and high viscosity. The stability of the foam is enhanced by greater protein concentrations because this increases viscosity and facilitates formation of a multilayer cohesive protein film at the interface. Foamability generally reaches a maximum value at some point during increase of protein concentration. Some proteins, for example, serum albumin, are able to form relatively stable foams at 1% protein concentration, whereas WPI and soy proteins require a minimum of 2%–5% to form relatively stable foam. Generally, most proteins display maximum foamability at 2%–8% concentration. The interfacial concentration of proteins in foams is about 2–3 mg m^{-2}.

Partial heat denaturation improves the foaming properties of proteins. For instance, heating of WPI at 70°C for 1 min improves, whereas heating at 90°C for 5 min decreases, foaming properties even though the heated proteins remain soluble in both instances [71]. The decrease in foaming properties of WPI heated at 90°C is due to extensive polymerization of the protein via disulfide–sulfhydryl interchange reactions. The highly cross-linked and polymerized protein is unable to adsorb to the air–water interface during foaming.

The method of foam generation influences the foaming properties of proteins. Air introduction by bubbling or sparging usually results in "wet" foam with a relatively large bubble size. Whipping at moderate speed generally results in foam with small-sized bubbles because the shearing action results in partial denaturation of the protein before adsorption occurs. However, whipping at high shear rate or "overbeating" can decrease foaming power because of extensive denaturation, aggregation, and precipitation of proteins.

Some foam-type food products, such as marshmallow, cakes, and bread, are heated after the foam is formed. During heating, expansion of air and decreased viscosity can cause bubble rupture and collapse of the foam. In these instances, the integrity of the foam depends on gelation of the protein film at the interface, so sufficient mechanical strength is developed to stabilize the foam. Gelatin, gluten, and egg white, which display good foaming and gelling properties, are highly suitable for this purpose.

5.5.3.2.2 Molecular Properties Influencing Foam Formation and Stability

For a protein to perform effectively as a foaming agent or an emulsifier, it must meet the following basic requirements: (1) it must be able to rapidly adsorb to the air–water interface, (2) it must readily unfold and rearrange at the interface, and (3) it should be able to form a viscous cohesive film through intermolecular interactions. The molecular properties that affect foaming properties are molecular flexibility, charge density and distribution, and hydrophobicity.

The excess free energy of the air–water interface is significantly greater than that of the oil–water interface. Therefore, to stabilize the air–water interface during foam formation, a protein must have

the ability to rapidly adsorb to the freshly created interface and instantaneously decrease the interfacial tension to a low value. The lowering of interfacial tension is dependent on the ability of the protein to rapidly unfold, rearrange, and expose hydrophobic groups at the interface. Random-coil-type proteins, such as β-casein, perform well in this manner. On the other hand, tightly folded globular proteins, such as lysozyme, adsorb very slowly, only partially unfold, and reduce the surface tension only marginally [74]. Lysozyme is, therefore, a poor foaming agent. Thus, molecular flexibility at the interface is quintessential for good performance as a foaming agent.

Apart from molecular flexibility, hydrophobicity also plays a role in foamability of proteins. The foaming power of proteins is positively correlated with the *mean* hydrophobicity. However, the foaming power of proteins varies curvilinearly with *surface* hydrophobicity, and a significant correlation does not exist between these two properties at hydrophobicity values of greater than 1000 [75]. This indicates that a surface hydrophobicity of at least 1000 is needed for initial adsorption of proteins at the air–water interface, whereas, once adsorbed, the ability of the protein to create more interfacial area during foam formation depends on the mean hydrophobicity of the protein.

A protein that displays good foamability need not be a good foam stabilizer. For example, although β-casein exhibits excellent foamability, the stability of the foam is poor. On the other hand, lysozyme exhibits poor foamability, but its foams are very stable. Generally, proteins that possess good foaming power do not have the ability to stabilize foams, and proteins that produce stable foams often exhibit poor foaming power. It appears that foamability and stability are influenced by two different sets of molecular properties of proteins that are often antagonistic. Whereas foamability is affected by the rate of adsorption, flexibility, and hydrophobicity, stability depends on the rheological properties of the protein film. The rheological properties of films depend on hydration, thickness, protein concentration, and favorable intermolecular interactions. Proteins that only partially unfold and retain some degree of folded structure usually form thicker, denser films and more stable foams (e.g., lysozyme and serum albumin) than do those that completely unfold (e.g., β-casein) at the air–water interface. In the former case, the folded structure extends into the subsurface in the form of loops. Noncovalent interactions, and possibly disulfide cross-links, between these loops promote formation of a gel network, which has excellent viscoelastic and mechanical properties. For a protein to possess good foamability and foam stability, it should have an appropriate balance between flexibility and rigidity, should easily undergo unfolding, and should engage in abundant cohesive interactions at the interface. However, what extent of unfolding is desirable for a given protein is difficult, if not impossible, to predict. In addition to these factors, foam stability usually exhibits an inverse relationship with the charge density of proteins. High charge density apparently interferes with formation of a cohesive film.

Most food proteins are mixtures of various proteins, and therefore their foaming properties are influenced by interaction between the protein components at the interface. The excellent whipping properties of egg white are attributed to interactions between its protein components, such as ovalbumin, conalbumin, and lysozyme. Several studies have indicated that the foaming properties of acidic proteins can be improved by mixing them with basic proteins, such as lysozyme and clupeine [76]. This enhancing effect seems to be related to the formation of an electrostatic complex between the acidic and basic proteins.

Limited enzymatic hydrolysis of proteins generally improves their foaming properties. This is because of increased molecular flexibility and greater exposure of hydrophobic groups. However, extensive hydrolysis impairs foamability because LMW peptides cannot form a cohesive film at the interface.

5.5.4 Flavor Binding

Proteins themselves are odorless. However, they can bind flavor compounds and thus affect the sensory properties of foods. Several proteins, especially oilseed proteins and WPCs, carry undesirable flavors, which limits their usefulness in food applications. These off-flavors are due mainly to

aldehydes, ketones, and alcohols generated by oxidation of unsaturated fatty acids. Upon formation, these carbonyl compounds bind to proteins and impart characteristic off-flavors. For example, the beany and grassy flavor of soy protein preparations is attributed to the presence of hexanal. The binding affinity of some of these carbonyls is so strong that they resist even solvent extraction. A basic understanding of the mechanism of binding of off-flavors to proteins is needed so that appropriate methods can be developed for their removal.

The flavor-binding property of proteins also has desirable aspects, because they can be used as flavor carriers or flavor modifiers in fabricated foods. This is particularly useful in meat analogs containing plant proteins, where successful simulation of a meat-like flavor is essential for consumer acceptance. In order for a protein to function as a good flavor carrier, it should bind flavors tightly, retain them during processing, and release them during mastication of food in the mouth. However, proteins do not bind all flavor compounds with equal affinity. This leads to uneven and disproportionate retention of some flavors and undesirable losses during processing. Because protein-bound flavorants do not contribute to taste and aroma unless they are released readily in the mouth, knowledge of the mechanisms of interaction and binding affinity of various flavorants is essential if effective strategies for producing flavor–protein products or for removing off-flavors from protein isolates are to be devised.

5.5.4.1 Thermodynamics of Protein–Flavor Interactions

In water–flavor model systems, addition of proteins causes a reduction in the headspace concentration of flavor compounds. This is due to binding of flavors to proteins. The mechanism of flavor binding to proteins depends upon the moisture content of the protein sample, but interactions are normally noncovalent. Dry protein powders bind flavors mainly via van der Waals, hydrogen bonding, and electrostatic interactions. Physical entrapment within capillaries and crevices of dry protein powders may also contribute to flavor properties of dry protein powders. In liquid or high-moisture foods, the mechanism of flavor binding by proteins primarily involves interaction of the nonpolar flavor compounds (ligands) with hydrophobic patches/cavities on the protein surface. In addition to hydrophobic interactions, flavor compounds with polar head groups, such as hydroxyl and carboxyl groups, may also interact with proteins via hydrogen bonding and electrostatic interactions. After binding to the surface hydrophobic regions, aldehydes and ketones may be able to diffuse into the hydrophobic interior of the protein molecule.

Flavor–protein interaction is usually completely reversible. However, aldehydes can covalently bind to the amino group of lysine side chains and this interaction is nonreversible. However, only the noncovalently bound fraction can contribute to aroma and taste of the protein product.

The extent of flavor binding by hydrated proteins depends on the number of hydrophobic binding regions available on the protein surface [77]. The binding sites are usually made up of groups of hydrophobic residues segregated in the form of a well-defined cavity. Single nonpolar residues on the protein surface are less likely to act as binding sites. Under equilibrium conditions, the reversible noncovalent binding of a flavor compound with proteins follows the Scatchard equation:

$$\frac{\upsilon}{[L]} = nK - \upsilon K \tag{5.70}$$

where

υ is the mole of ligand bound per mole of protein
n is the total number of binding sites per mole of protein
[L] is the free ligand concentration at equilibrium
K is the equilibrium binding constant (M^{-1})

TABLE 5.17
Thermodynamic Constants for Binding of Carbonyl Compounds to Proteins

Protein	Carbonyl Compound	n (Moles per Mole)	K (M^{-1})	ΔG (kcal mol^{-1})
Serum albumin	2-Nonanone	6	1800	−4.4
	2-Heptanone	6	270	−3.3
β-Lactoglobulin	2-Heptanone	2	150	−3.0
	2-Octanone	2	480	−3.7
	2-Nonanone	2	2440	−4.7
Soy protein				
Native	2-Heptanone	4	110	−2.8
	2-Octanone	4	310	−3.4
	2-Nonanone	4	930	−4.1
	5-Nonanone	4	541	−3.8
	Nonanal	4	1094	−4.2
Partially denatured	2-Nonanone	4	1240	−4.3
Succinylated	2-Nonanone	2	850	−4.0

Sources: Compiled from Damodaran, S. and Kinsella, J.E., *J. Agric. Food Chem.*, 28, 567, 1980; Damodaran, S. and Kinsellam, J.E., *J. Agric. Food Chem.*, 29, 1249, 1981; O'Neill, T.E. and Kinsella, J.E., *J. Agric. Food Chem.*, 35, 770, 1987.

n, number of binding sites in native state; K, equilibrium binding constant.

According to the this equation, a plot of υ/[L] versus υ will be a straight line; the values of K and n can be obtained from the slope and the intercept, respectively. The free energy change for binding of ligand to protein is obtained from the equation $\Delta G = -RT \ln K$, where R is the gas constant and T is the absolute temperature. The thermodynamic constants for the binding of carbonyl compounds to various proteins are presented in Table 5.17. The binding constant increases by about threefold for each methylene group increment in chain length, with a corresponding free energy change of −0.55 kcal mol^{-1} per CH_2 group. This indicates that the binding is hydrophobic in nature.

It is assumed in the Scatchard relationship that all ligand-binding sites in a protein have the same affinity and that no conformational changes occur upon binding of the ligand to these sites. Contrary to the latter assumption, proteins generally do undergo a modest conformational change upon binding of flavor compounds. Diffusion of flavor compounds into the interior of the protein may disrupt hydrophobic interactions between protein segments and thus destabilize the protein structure. Flavor ligands with reactive groups, such as aldehydes, can covalently bind to the ε-amino groups of lysine residues, change the net charge of the protein, and thus cause protein unfolding. Unfolding generally results in exposure of new hydrophobic sites for ligand binding. Because of these structural changes, Scatchard plots for protein are generally curvilinear. In the case of oligomeric proteins, such as soy proteins, conformational changes may involve both dissociation and unfolding of subunits. Denatured proteins generally exhibit a large number of binding sites with weak association constants. Methods for measuring flavor binding can be found in References 77 and 78.

5.5.4.2 Factors Influencing Flavor Binding

Since volatile flavors interact with hydrated proteins mainly via hydrophobic interactions, any factor that affects hydrophobic interactions or surface hydrophobicity of proteins will influence flavor binding. Temperature has very little effect on flavor binding, unless there is significant thermal unfolding of the protein. This is because the association process is primarily entropy driven, not enthalpy driven. Thermally denatured proteins exhibit increased ability to bind flavors; however,

the binding constant is usually low compared to that of native proteins. The effects of salts on flavor binding are related to their salting-in and salting-out properties. Salting-in-type salts, which destabilize hydrophobic interactions, decrease flavor binding, whereas salting-out-type salts increase flavor binding.

The effect of pH on flavor binding is generally related to pH-induced conformational changes in proteins. Flavor binding is usually enhanced more at alkaline pH than at acid pH; this is because proteins tend to denature more extensively at alkaline pH than at acid pH. Breakage of protein disulfide bonds, which occurs at alkaline pH and causes unfolding of proteins, usually increases flavor binding. Proteolysis, which disrupts and decreases the number of hydrophobic regions in proteins, decreases flavor binding. This can be used as a way of removing off-flavors from oilseed proteins.

5.5.5 Viscosity

The consumer acceptability of several liquid and semisolid-type foods (e.g., gravies, soups, beverages) depends on the viscosity or consistency of the product. The viscosity of a solution relates to its resistance to flow under an applied force (or shear stress). For an ideal solution, the shear stress (i.e., force per unit area, F/A) is directly proportional to the shear rate (i.e., the velocity gradient between the layers of the liquid, dv/dr). This is expressed as

$$\frac{F}{A} = \eta \frac{dv}{dr} \tag{5.71}$$

The proportionality constant η is known as the viscosity coefficient. Fluids that obey this expression are called Newtonian fluids.

The flow behavior of solutions is greatly influenced by solute type. Large-molecular-weight soluble polymers greatly increase viscosity even at very low concentrations. This again depends on several molecular properties such as size, shape, flexibility, and hydration. Solutions of randomly coiled macromolecules display greater viscosity than do solutions of compact folded macromolecules of same molecular weight.

Most macromolecular solutions, including protein solutions, do not display Newtonian behavior, especially at high concentrations. For these systems, the viscosity coefficient decreases when the shear rate increases. This behavior is known as "pseudoplastic" or "shear thinning" and follows the relationship

$$\frac{F}{A} = m\left(\frac{dv}{dr}\right)^n \tag{5.72}$$

where

m is the consistency coefficient

n is an exponent known as the "flow behavior index"

The pseudoplastic behavior of protein solutions arises because of the tendency of protein molecules to orient their major axes in the direction of flow. Dissociation of weakly held dimers and oligomers into monomers also contribute to shear thinning. When shearing or flow is stopped, the viscosity may or may not return to the original value depending on the rate of relaxation of the protein molecules to random orientation. Solutions of fibrous proteins, for example, gelatin and actomyosin, usually remain oriented and thus do not quickly regain their original viscosity. On the other hand, solutions of globular proteins, for example, soy proteins and whey proteins, rapidly regain their viscosity when flow is stopped. Such solutions are called "thixotropic."

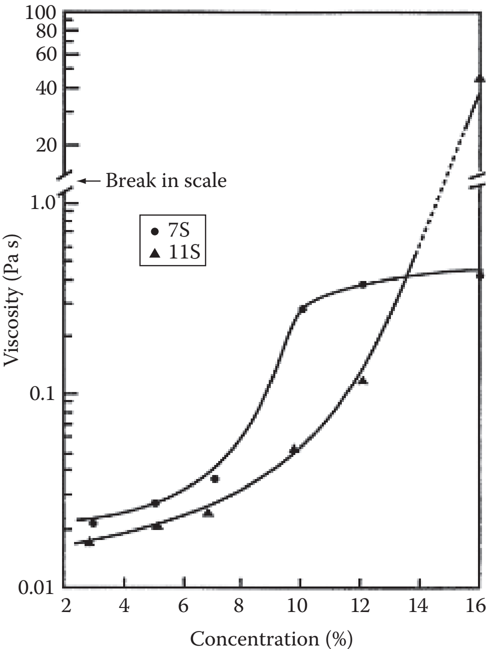


FIGURE 5.31 Effect of concentration on viscosity (or consistency index) of 7S and 11S soy protein solutions at 20°C. (From Rao, M.A. et al., Flow properties of 7S and 11S soy protein fractions, in: *Food Engineering and Process Applications*, Le Maguer, M. and Jelen, P. (eds.), Elsevier Applied Science, New York, 1986, pp. 39–48.)

The viscosity (or consistency) coefficient of most protein solutions follows an exponential relationship with protein concentration because of both protein–protein interactions and interactions between the hydration spheres of protein molecules. An example involving soy protein fractions is shown in Figure 5.31 [79]. At high protein concentrations or in protein gels, where protein–protein interactions are numerous and strong, proteins display plastic viscoelastic behavior. In these cases, a specific amount of force, known as "yield stress," is required to initiate flow.

The viscosity behavior of proteins is a manifestation of complex interactions among several variables, including size, shape, protein–solvent interactions, hydrodynamic volume, and molecular flexibility in the hydrated state. When dissolved in water, proteins absorb water and swell. The volume of the hydrated molecules is much larger than their unhydrated volume. The protein-associated water induces long-range effects on the flow behavior of the solvent. The dependence of viscosity on shape and size of protein molecules follows the relationship

$$\eta_{sp} = \beta C(\upsilon_2 + \delta_1 \upsilon_1) \tag{5.73}$$

where

- η_{sp} is the specific viscosity
- β is the shape factor
- C is the concentration
- υ_2 and υ_1 are specific volumes of unhydrated protein and solvent, respectively
- δ_1 is the gram of water bound per gram of protein

Here, υ_2 is also related to molecular flexibility; the greater the specific volume of the protein, the greater is its flexibility.

The viscosity of dilute protein solutions is expressed in several ways. *Relative viscosity* η_{rel} refers to the ratio of viscosity of the protein solution to that of the solvent. It is measured in an Ostwald–Fenske-type capillary viscometer and is expressed as

$$\eta_{rel} = \frac{\eta}{\eta_0} = \frac{\rho t}{\rho_0 t_0} \tag{5.74}$$

where

ρ and ρ_0 are densities of protein solution and solvent, respectively

t and t_0 are times of flow for a given volume of protein solution and solvent, respectively, through the capillary

Other forms of expressing viscosity can be obtained from the relative viscosity. *Specific viscosity* is defined as

$$\eta_{sp} = \eta_{rel} - 1 \tag{5.75}$$

Reduced viscosity is

$$\eta_{rel} = \frac{\eta_{sp}}{C} \tag{5.76}$$

where C is the protein concentration, and *intrinsic viscosity* is

$$[\eta] = \text{Lim} \frac{\eta_{sp}}{C} \tag{5.77}$$

The intrinsic viscosity, [η], is obtained by extrapolating a plot of reduced viscosity versus protein concentration to zero protein concentration (Lim). Since protein–protein interactions are nonexistent at infinite dilution, intrinsic viscosity accurately depicts the effects of shape and size on the flow behavior of individual protein molecules. Changes in the hydrodynamic shape of proteins that result from heat and pH treatments can be studied by measuring their intrinsic viscosities.

5.5.6 Gelation

A gel is an intermediate phase between a solid and a liquid. Technically, it is defined as "a substantially diluted system that exhibits no steady state flow." It is made up of polymers cross-linked via either covalent or noncovalent bonds to form a network that is capable of entrapping water and other small-molecular-weight substances (see Chapter 7).

Protein gelation refers to transformation of a protein from the "sol" state to a "gel-like" state. Heat, enzymes, or divalent cations under appropriate conditions facilitate this transformation. All these agents induce formation of network structure; however, the types of covalent and noncovalent interactions involved and the mechanism of network formation can differ considerably.

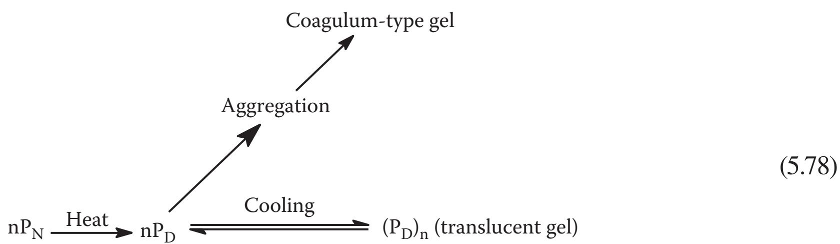
Most food protein gels are prepared by heating a moderately concentrated protein solution. In this mode of gelation, the protein in a "sol" state is first transformed into a "progel" state by denaturation. In the "sol" state the number of noncovalent bonding groups available in proteins for network structure formation is limited. The progel state however is usually a viscous liquid state in which some degree of protein denaturation and polymerization has already occurred. Also, in the progel state, a critical number of functional groups, such as hydrogen bonding and hydrophobic

groups that can form intermolecular noncovalent bonds, become exposed so that the second stage, that is, formation of a protein network, can occur. The conversion of sol to progel is irreversible because many protein–protein interactions occur between the unfolded molecules. When the progel is cooled to ambient or refrigeration temperature, the decrease in the thermal kinetic energy facilitates formation of stable noncovalent bonds among exposed functional groups of the various molecules and this constitutes gelation.

The interactions involved in network formation are primarily hydrogen bonds and hydrophobic and electrostatic interactions. The relative contributions of these forces vary with the type of protein, heating conditions, the extent of denaturation, and environmental conditions. Hydrogen bonding and hydrophobic interactions contribute more than electrostatic interactions to network formation except when multivalent ions are involved in cross-linking. Since proteins generally carry a net charge, electrostatic repulsion occurs among protein molecules, and this is not usually conducive to network formation. However, charged groups are essential for maintaining protein–water interactions and water-holding capacity of gels.

Gel networks that are sustained primarily by noncovalent interactions are thermally reversible; that is, upon reheating they will melt to a progel state, as is commonly observed with gelatin gels. This is especially true when hydrogen bonds are the major contributors to the network. Since hydrophobic interactions are strong at elevated temperatures, gel networks formed primarily by hydrophobic interactions are thermally irreversible, for example, egg-white gels. Proteins that contain both cysteine and cystine groups can undergo polymerization via sulfhydryl–disulfide interchange reactions during heating and form a continuous covalent network upon cooling. Such gels are usually thermally irreversible. Examples of gels of this type are ovalbumin, β-lactoglobulin, and whey protein gels.

Proteins form two types of gels, that is, coagulum (opaque) gels and translucent gels. The type of gel formed by a protein is dictated by its molecular properties and solution conditions. Proteins containing large amounts of nonpolar amino acid residues undergo



(5.78)

P_N, native state; P_D, unfolded state; n, number of protein molecules taking part in cross-linking

hydrophobic aggregation upon denaturation. These insoluble aggregates then randomly associate and set into an irreversible coagulum-type gel. Since the rate of aggregation and network formation is faster than the rate of denaturation, proteins of this type readily set into a gel network even while being heated. The opaqueness of these gels is due to light scattering caused by the unordered (isotropic) network of insoluble protein aggregates. Coagulum-type gels are generally weak and are prone to synersis.

Proteins that contain small amounts of nonpolar amino acid residues form soluble complexes upon denaturation. Since the rate of association of these soluble complexes is slower than the rate of denaturation, and the gel network is predominantly formed by hydrogen bonding interactions, they often do not set into a gel until heating followed by cooling has occurred (typically 8%–12% protein concentration is used). Upon cooling, the slow rate of association of the soluble complexes facilitates formation of an ordered translucent gel network.

At the molecular level, coagulum-type gels tend to form when the sum of Val, Pro, Leu, Ile, Phe, and Trp residues of the protein exceeds 31.5 mol% [80]. Those that contain less than 31.5 mol% of the hydrophobic residues mentioned earlier usually form translucent gels when water is the solvent. However, this dictum is not obeyed when salt solutions are used as solvents. For example, the hydrophobic amino acid content of β-lactoglobulin is 32 mol%, yet it forms a translucent-type gel in water. However, when NaCl is included, it forms a coagulum-type gel even when the salt concentration is as low as 50 mM. This occurs because of charge neutralization by NaCl, which promotes hydrophobic aggregation upon heating. Thus, the balance between attractive hydrophobic interactions and repulsive electrostatic interactions fundamentally controls gelation mechanism and the gel appearance. These two forces in effect control the balance between protein–protein and protein–solvent interactions in a gelling system. If the former is much greater than the latter, a precipitate is likely to form. If protein–solvent interactions predominate, the system may not gel. A coagulum gel or a translucent gel results when the magnitude of hydrophobic and hydrophilic forces are somewhere in between these two extremes.

Protein gels are highly hydrated systems, containing up to 98% water in some cases (e.g., gelatin gels). The water entrapped in these gels has activity close to that in dilute aqueous solutions, but lacks fluidity and cannot be easily pressed out. The mechanism by which liquid water can be held in a semisolid state in gels is not well understood. However, the fact that translucent gels, formed primarily by hydrogen bonding interactions, hold more water than coagulum-type gels and are less prone to syneresis, suggests that much of the water is hydrogen bonded to C=O and N−H groups of the peptide bonds, is associated with charged groups in the form of hydration shells, and/or exists in extensively hydrogen-bonded icelike water–water networks. It is also possible that within the restricted environment of the microstructure of the gel network, water may exist as a hydrogen-bonding cross-linker between C=O and N−H groups of peptide segments. This may restrict the flow ability of water within each cell, the more so as the cell size decreases. It is also likely that some water may be held as capillary water in the pores of the gel structure, especially in coagulum gels.

The stability of a gel network against thermal and mechanical forces is dependent on the number and types of cross-links formed per monomer chain. Thermodynamically, a gel network would be stable only when the sum of the interaction energies of a monomer in the gel network is greater than its thermal kinetic energy. This is dependent on several intrinsic (such as the size, net charge) and extrinsic factors (such as pH, temperature, ionic strength). The square root of the hardness of protein gels exhibits a linear relationship with molecular weight [81]. Globular proteins with molecular weight <23,000 Da cannot form a heat-induced gel at any reasonable protein concentration, unless they contain at least one free sulfhydryl group and a disulfide bond. The sulfhydryl groups and disulfide bonds facilitate polymerization and thus increase the effective molecular weight of polypeptides to >23,000 Da. Gelatin preparations with effective molecular weights of less than 20,000 Da cannot form a gel.

Another critical factor is protein concentration. To form a self-standing gel network, a minimum protein concentration, known as least concentration endpoint (LCE), is required [82]. The LCE is 8% for soy proteins, 3% for egg albumin, and about 0.6% for gelatin. Above this minimum concentration, the relationship between gel strength, G, and protein concentration, C, usually follows a power law:

$$G \alpha \left(C - C_o\right)^n \tag{5.79}$$

where C_o is the LCE. For proteins, the value of n varies from 1 to 2.

Several environmental factors, such as pH, salts, and other additives, also affect gelation of proteins. At or near isoelectric pH, proteins usually form coagulum-type gels. At extremes of pH, weak gels are formed because of strong electrostatic repulsion. The optimum pH for gel formation is about 7–8 for most proteins.

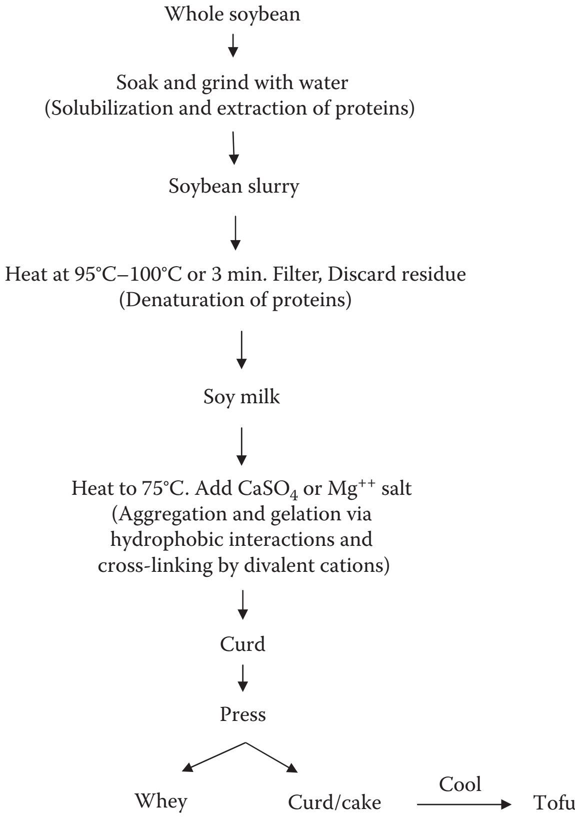


FIGURE 5.32 A typical commercial process for tofu manufacture.

Formation of protein gels can sometimes be facilitated by limited proteolysis. A well-known example is cheese. Addition of chymosin (rennin) to casein micelles in milk results in the formation of a coagulum-type gel. This is achieved by cleavage of κ-casein, a micelle component, causing release of a hydrophilic portion, known as the glycomacropeptide. The remaining so-called para-casein micelles possess a highly hydrophobic surface that facilitates formation of a weak gel network.

Enzymatic cross-linking of proteins at room temperature can also result in the formation of a gel network. Transglutaminase is the enzyme often used to prepare these gels. This enzyme catalyzes formation of ε-(γ-glutamyl)lysyl cross-links between the glutamine and lysyl groups of protein molecules. Using this enzymatic cross-linking method, highly elastic and irreversible gels can be formed even at low protein concentration.

Divalent cations, such as Ca^{2+} and Mg^{2+}, can also be used to form protein gels. These ions form cross-links between negatively charged groups of protein molecules. A good example of this type of gel is tofu from soy proteins. Alginate gels also can be formed by this means. A general method for making tofu is outlined in Figure 5.32.

5.5.7 Texturization

Texturization connotes transformation of a protein from a globular state to a fibrous physical structure that has meat-like mouthfeel characteristics. The various functional properties that texturized protein products are expected to possess include chewiness, elasticity, softness, and juiciness. Vegetable proteins are often the preferred protein source for texturization, primarily because they

lack other desirable functional properties that proteins of animal origin display. Textured vegetable proteins are manufactured using two different processes, namely, *spun-fiber texturization*, and *thermoplastic extrusion*.

5.5.7.1 Spun-Fiber Texturization

In this process, a highly concentrated (~20% w/v) soy protein isolate solution is adjusted to pH 12–13 and aged until the viscosity of the solution increases to 50,000–100,000 centipoise as a result of protein denaturation and certain alkali-induced cross-linking reactions. This highly viscous "dope" is then pumped through a spinneret, a device with a plate containing thousands of micron-size holes. The fibrous extrudate is passed through a bath containing phosphoric acid and salt at pH 2.5. The protein coagulates instantaneously in this bath and becomes a fibrous mass. The fiber is then "towed" through steel rolls where it is compressed and stretched to enhance its strength. The fiber is then passed through a washing bath where excess acidity and salt are removed. The washed fibers are then passed through a series of tanks containing fat, flavors, colors, and binders depending on the final product. The fiber is then heated at 80°C–90°C to induce gelation of the binder protein. Egg white is often used as a binder because of its excellent heat coagulation properties. The final product is dried and sized. A process flow chart for the spun-fiber texturization process is outlined in Figure 5.33.

5.5.7.2 Extrusion Texturization

In this process, defatted soy flour or soy protein concentrate with high protein solubility index is conditioned with steam and the moisture content is adjusted to 20%–25%. This solid mass is then fed to an extruder, which is mainly a rotating screw housed in a tapered cylindrical barrel in which

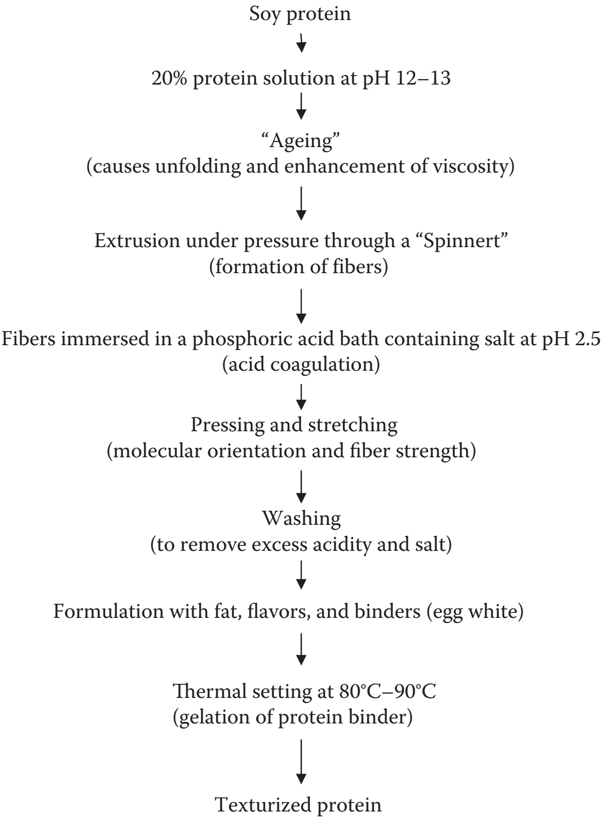


FIGURE 5.33 A typical spun-fiber process for texturization of soy proteins.

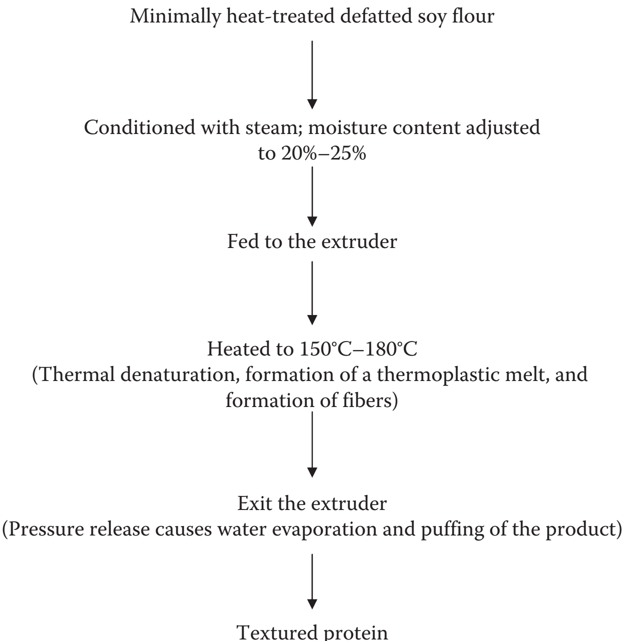


FIGURE 5.34 Extrusion texturization of soy flour.

the space between the screw and the barrel decreases progressively along the screw axis. As the protein mass advances through the screw, it is rapidly heated to 150°C–180°C. This high temperature and the progressive buildup of pressure as the mass moves down the screw results in pressure cooking, and as a result the protein mass melts and the proteins are denatured. In technical terms this is known as thermoplastic melt. The denatured proteins become aligned in fiber form as the mass moves through the screw. When the mass exits the die, sudden release of pressure evaporates water and puffs the product. Adjusting the pressure and temperature can control puffing. If a dense product is desired, the mass is cooled before it exits the die. The extrudate is then cut to pieces and processed further depending on its use. A general process flow chart for extrusion texturization of proteins is shown in Figure 5.34.

The general principles involved in both these methods are thermal or alkaline denaturation of proteins, realignment of the denatured proteins in the form of a fibrous network, binding of the fibers using a protein binder, and flavoring of the final product. Texturized vegetable proteins are increasingly being used as meat extenders in comminuted meat products (meat patties, sauces, burgers, etc.) and as meat analogs or "imitation meat."

5.5.8 Dough Formation

When a mixture of wheat flour and water (about 3:1 ratio) is kneaded, it forms a viscoelastic dough suitable for making bread and other bakery products. These unusual dough characteristics are mainly attributable to the proteins in wheat flour.

Wheat flour contains several soluble and insoluble protein fractions. The soluble proteins, comprising about 20% of the total proteins, are primarily albumin- and globulin-type enzymes and certain minor glycoproteins. These proteins do not contribute to the dough forming properties of wheat flour. The major storage protein of wheat is gluten. Gluten is a heterogeneous mixture of proteins, mainly gliadins and glutenins, with limited solubility in water. When mixed with water, gluten forms viscoelastic dough capable of entrapping gas during fermentation.

TABLE 5.18
Amino Acid Composition of Glutenin and Gliadin

Amino Acid	Glutenin (mol%)	Gliadin (mol%)
Cys	2.6	3.3
Met	1.4	1.2
Asp	3.7	2.8
Thr	3.4	2.4
Ser	6.9	6.1
Glx[a]	28.9	34.6
Pro	11.9	16.2
Gly	7.5	3.1
Ala	4.4	3.3
Val	4.8	4.8
Ile	3.7	4.3
Leu	6.5	6.9
Tyr	2.5	1.8
Phe	3.6	4.3
Lys	2.0	0.6
His	1.9	1.9
Arg	3.0	2.0
Trp	1.3	0.4

Source: MacRitchie, F. and Lafiandra, D., Structure-function relationships of wheat proteins, in: *Food Proteins and Their Applications*, Damodaran, S. and Paraf, A., eds., Marcel Dekker, New York, 1997, pp. 293–324.

[a] Glx corresponds to mixture of Glu and Gln. Most of Glx in wheat protein is in the form of Gln.

Gluten has a unique amino acid composition, with Gln and Pro accounting for more than 40% of the amino acid residues (Table 5.18). The low water solubility of gluten is attributable to its low content of Lys, Arg, Glu, and Asp residues, which together amount to less than 10% of the total amino acid residues. About 30% of gluten's amino acid residues are hydrophobic, and the residues contribute greatly to its ability to form protein aggregates by hydrophobic interactions and to bind lipids and other nonpolar substances. The high glutamine and hydroxyl amino acid (~10%) contents of gluten are responsible for its water binding properties. In addition, hydrogen bonding between glutamine and hydroxyl residues of gluten polypeptides contributes to its cohesion–adhesion properties. Cysteine and cystine residues account for 2–3 mol% of gluten's total amino acid residues. During formation of the dough, these residues undergo sulfhydryl–disulfide interchange reactions, resulting in extensive polymerization of gluten proteins [83].

Several physical and chemical transformations occur when wheat flour or isolated wheat gluten is mixed with water and kneaded to form dough: water binds to various hydrophilic and charged groups in gluten. In the dry state, the major secondary structural element in gluten is β-sheet structure [84]. These β-sheets are not the regular intersheet hydrogen-bonded parallel and antiparallel pleated β-sheets commonly found in globular proteins, as there is no endothermic heat flow in the differential scanning calorimetry profile of gluten [85]. Upon water absorption, gluten undergoes a major structural transformation involving conversion of β-sheet structures into β-turn structure [84,85]. It is known that glutenin polypeptides in gluten contain repeat sequences of PGQGQQ and GYYPTSLQQ [86], and these sequences can readily form consecutive β-turns, which assume

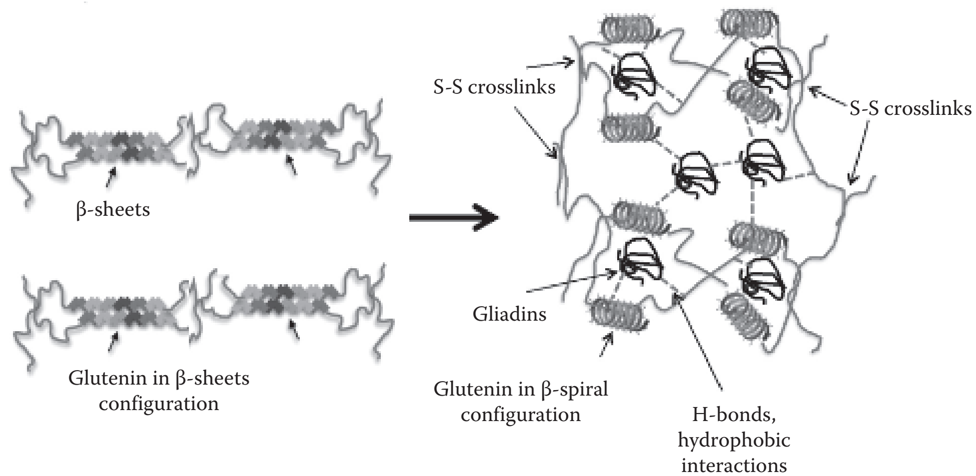


FIGURE 5.35 Proposed mechanism of formation of gluten network structure in wheat dough.

a β-spiral-type structure. The diameter of this β-spiral is 19.5 Å and the pitch is 14.9 Å [87]. The β-spiral structure effectively behaves like a stretchable spring, and it is implicated as one of the structural elements responsible for the viscoelasticity of dough [88]. In addition to this major structural transformation, the glutenin polypeptides undergo sulfhydryl–disulfide interchange reactions during the kneading process, which results in formation of threadlike polymers. These linear polymers in turn interact with each other, presumably via hydrogen bonding, hydrophobic associations, and disulfide cross-linking, to form a network-like film capable of entrapping gas (Figure 5.35). Because of these transformations in gluten, the resistance of the dough increases with time until a maximum is reached, and this is followed by a decrease in resistance indicative of a breakdown in the network structure. The breakdown involves alignment of polymers in the direction of shear and some scission of disulfide cross-links, which reduces the polymer size. The time it takes to reach maximum dough strength (R_{max}) during kneading is used as a measure of wheat quality for bread making—a longer time indicating better quality.

The viscoelasticity of wheat dough is related to the extent of sulfhydryl–disulfide interchange reactions. This view is supported by the fact that when reductants, such as cysteine, or sulfhydryl blocking agents, such as *N*-ethylmaleimide (NEM), are added to dough, viscoelasticity decreases greatly. On the other hand, addition of oxidizing agents, such as iodates and bromates, increase the elasticity of the dough. This implies that wheat gluten rich in SH and S–S groups might possess superior bread making qualities, but this relationship is unreliable. Thus, interactions other than disulfide cross-links, such as hydrogen bonding and hydrophobic interactions, also play a vital role in viscoelasticity of wheat dough.

Differences in bread making qualities of different wheat cultivars may be related to differences in the composition of gluten itself. As mentioned earlier, gluten is made up of gliadins and glutenins. Gliadins are comprised of four groups, namely, α-, β-, γ-, and ω-gliadins. In gluten, these exist as single polypeptides with molecular weights ranging from 30,000 to 80,000 Da. Gliadins contain even number of cysteine residues. They exist as intramolecular disulfide bonds. The disulfide bonds are buried in the interior of the protein so that they do not take part in sulfhydryl–disulfide interchange reactions with other proteins. The disulfide bonds appear to remain as intramolecular disulfides during dough making. Thus, dough made from isolated gliadins and starch is viscous but not viscoelastic.

Glutenins, on the other hand, are heterogeneous polypeptides with molecular weights ranging from 12,000 to 130,000 Da. These are further classified into high-molecular-weight (mw > 90,000, HMW)

and low-molecular-weight (mw < 90,000, LMW) glutenins. In gluten, these glutenin polypeptides are present as polymers joined by disulfide cross-links, with molecular weights ranging into the millions. Because of their ability to polymerize extensively via sulfhydryl–disulfide interchange reactions, glutenins contribute greatly to the elasticity of dough. Some studies have shown a significant positive correlation between HMW glutenin content and bread making quality in some wheat varieties [89]. Available information indicates that a specific pattern of disulfide cross-linked association between LMW and HMW glutenins in gluten structure may be far more important to bread quality than the amount of HMW protein. For example, association/polymerization among LMW glutenins gives rise to a structure similar to that formed by HMW gliadin. This type of structure contributes to viscosity of the dough, but not to elasticity. In contrast, the dough elasticity increases when LMW glutenins cross-link to HMW glutenins via disulfide cross-links (in gluten). It is possible that in good quality wheat varieties, more of the LMW glutenins may polymerize to HMW, whereas in poor quality wheat varieties, most of the LMW glutenins may polymerize among themselves. These differences in associated states of glutenins in gluten of various wheat varieties may be related to differences in their conformational properties, such as surface hydrophobicity, and reactivity of sulfhydryl and disulfide groups.

In summary, hydrogen bonding among amide and hydroxyl groups, hydrophobic interactions, and sulfhydryl–disulfide interchange reactions all contribute to the development of the unique viscoelastic properties of wheat dough. However, culmination of these interactions into good dough making properties may depend on the structural properties of each protein and the proteins with which it associates in the overall gluten structure.

Because polypeptides of gluten, especially the glutenins, are rich in proline, they have very little ordered secondary structure. Whatever ordered structure initially exists in gliadins and glutenins is lost during mixing and kneading. Therefore, no additional unfolding occurs during baking.

Supplementation of wheat flour with albumin- and globulin-type proteins, for example, whey proteins and soy proteins, adversely affects the viscoelastic properties and baking quality of the dough. These proteins decrease bread volume by interfering with formation of the gluten network. Addition of phospholipids or other surfactants to dough counters the adverse effects of foreign proteins on loaf volume. In this case, the surfactant/protein film compensates for the impaired gluten film. Although this approach results in acceptable loaf volume, the textural and sensory qualities of the bread are less desirable than normal.

Isolated gluten is sometimes used as a protein ingredient in nonbakery products. Its cohesion–adhesion properties make it an effective binder in comminuted meat and surimi-type products.

5.6 PROTEIN HYDROLYSATES

Partial hydrolysis of proteins using proteolytic enzymes is one of the strategies for improving the functional properties. Functional properties such as solubility, dispersibility, foaming, and emulsification can be potentially improved by limited proteolysis of proteins. Protein hydrolysates have many uses in speciality foods such as geriatric foods, nonallergenic infant formula, sports drinks, and diet foods. Because protein hydrolysates can be readily digested, they are particularly useful in infant formula and geriatric foods.

Proteolysis denotes enzymatic hydrolysis of peptide bonds in proteins.

$$-NH-\underset{\substack{|\\R_1}}{CH}-CO-NH-\underset{\substack{|\\R_2}}{CH}-CO- + H_2O \xrightarrow{\text{Protease}} -NH-\underset{\substack{|\\R_1}}{CH}-COOH + H_2N-\underset{\substack{|\\R_2}}{CH}-CO- \quad (5.80)$$

In this reaction, for every peptide bond cleaved by the enzyme, one mole each of carboxyl group and amino group is liberated. When the reaction is allowed to completion, the final product is a mixture

TABLE 5.19
Specificity of Various Proteases

Protease	Type	Specificity
Elastase	Endoproteinase	Ala—aa; Gly—aa
Bromelain	Endoproteinase	Ala—aa; Tyr—aa
Trypsin	Endoproteinase	Lys—aa; Arg—aa
Chymotrypsin	Endoproteinase	Phe—aa; Trp—aa; Tyr—aa
Pepsin	Endoproteinase	Leu—aa; Phe—aa
V-8 protease	Endoproteinase	Asp—aa; Glu—aa
Thermolysin	Endoproteinase	aa—Phe; aa—Leu
Alcalase	Endoproteinase	Non specific
Papain	Endoproteinase	Lys—aa; Arg—aa; Phe—aa; Gly—aa
Prolylendopeptidase	Endoproteinase	Pro—aa
Subtilisin A	Endoproteinase	Nonspecific

aa: Refers to any of the 20 amino acid residues.

of all constituent amino acids of the protein. Incomplete proteolysis results in liberation of a mixture of polypeptides from the original protein. The functional properties of the protein hydrolysate are dependent upon the degree of hydrolysis (DH) and the physicochemical properties, that is, size and solubility, of the polypeptides in the hydrolysate.

The DH is defined as the fraction of peptide bonds cleaved and it is often expressed as percentage:

$$\%DH = \frac{n}{n_T} \times 100 \quad (5.81)$$

where

n_T is the total number of moles of peptide bonds present in 1 mole of protein
n is the number of moles of peptide bonds cleaved per of mole of protein

When molar mass of a protein is not known or the protein sample is a mixture of various proteins, n and n_T are expressed as the number of peptide bonds per gram of protein.

The DH is generally monitored using the pH-stat method. The principle behind this method is that when a peptide bond is hydrolyzed, the newly formed carboxyl group completely ionizes at pH > 7, which releases H^+ ion. As a result, the pH of the protein solution progressively decreases with time of hydrolysis. In the pH range 7–8, the number of moles of H^+ ion released is equivalent to the number of moles of peptide bonds hydrolyzed. In the pH-stat method, the pH of the protein solution is maintained at a constant pH by titrating with NaOH. The number of moles of NaOH consumed during proteolysis is equivalent to the number of moles of peptide bonds cleaved.

Several proteases can be potentially used for preparing protein hydrolysates. Some of these proteases are site-specific enzymes (Table 5.19). Because of their specificity, the types of polypeptide fragments released in the hydrolysate differ between proteases. Alcalase from *Bacillus licheniformis* is a major commercial enzyme used in the manufacture of protein hydrolysate. This enzyme belongs to a family of subtilisins, which are serine proteases.

5.6.1 Functional Properties

The functional properties of protein hydrolysates depend on the type of enzymes used in their preparation. This is primarily because of differences in the size and other physicochemical

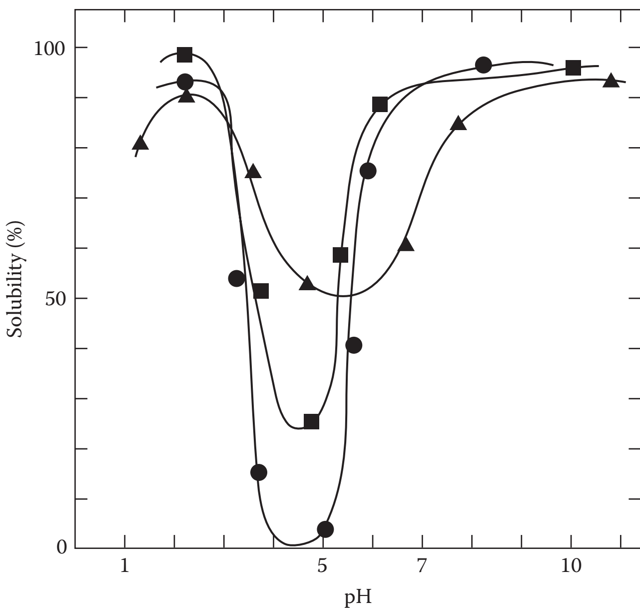


FIGURE 5.36 pH–solubility profiles of native casein and of *Staphylococcus aureus* V-8 protease-modified casein. The solubility was expressed as percent of total protein in solution. ●, native casein; ■, 2% DH; ▲, 6.7% DH. (From Adler-Nissen, J., *J. Agric. Food Chem.*, 27, 1256, 1979.)

properties of the polypeptides released during hydrolysis. Generally, solubility of most proteins improves after hydrolysis regardless of the enzyme used. The greater the DH, the higher is the solubility. However, the net increase in solubility depends on the type of enzyme used. Shown in Figure 5.36 is the pH-solubility profile of casein before and after hydrolysis with V-8 protease. It should be noted that the solubility of casein at its isoelectric pH is significantly increased after partial hydrolysis. This type of behavior is also observed with other proteins. Higher protein solubility is particularly important in acidic protein drinks in which precipitation and sedimentation are undesirable.

Since solubility of a protein is essential for its foaming and emulsifying properties, partially hydrolyzed proteins generally show improved foaming and emulsifying properties. However, this improvement is dependent on the type of enzyme used and the DH. Generally, the foaming and emulsifying capacity improve up to DH < 10% and decrease at DH > 10%. On the other hand, the stabilities of foams and emulsions made with protein hydrolysates are generally lower than that of the intact protein. One of the reasons for this is the inability of the short polypeptides to form a cohesive viscoelastic film at the air–water and oil–water interfaces.

Protein hydrolysates generally do not form heat-induced gels. One exception is gelatin. Gelatin is produced from collagen by acid or alkaline hydrolysis. Gelatin is a heterogeneous mixture of polypeptides. The weight-average-molecular weight of polypeptides in a gelatin sample depends on the DH. This profoundly affects their gel strength. The higher the weight-average-molecular weight, the higher the gel strength is. Gelatin samples with weight-average-molecular weight less than 20,000 Da do not form gels at all gelatin concentration. The gelling properties of commercial gelatin products are expressed in terms of bloom rating measured using a bloom gelometer. The bloom rating is defined as the weight in grams required for driving a plunger of a gelometer 4 cm into a 6.67% (w/v) gelatin gel that has been incubated for 17 h in a water bath at 10°C. Table 5.20 shows the bloom rating requirements for various types of gelatin-based food products.

TABLE 5.20
Bloom Rating Requirements for Some Gelatin-Based Food Products

Product	Bloom Rating (g)	Concentration Used in Food (%)
Jelly beans	220	7–8
Fruit jelly	100–120	10–12
Marshmallow	220	2–3
Lozenges	50–100	1

5.6.2 Allergenicity

Several food proteins, including cow's milk, soy proteins, gluten, egg proteins, and peanut proteins, cause severe allergic reactions in children and adults. However, hydrolysates of these proteins possess lower allergenicity than their native counterparts [90,91]. Allergenicity of intact proteins arises because of the presence of allergenic sites (epitopes) that bind to IgE. In protein hydrolysates, both conformational and sequence-specific (linear) epitopes are destroyed by proteolytic cleavage. For instance, hydrolysis of casein up to 55% DH using pancreatin (mixture of pancreatic enzymes) decreases its allergenicity by about 50% [92]. Similarly, hydrolysis of whey proteins using a combination of pepsin and α-chymotrypsin effectively reduced its allergenicity [93]. Thus, protein hydrolysates are the preferred source of essential amino acids for infants and children who are predisposed or at high risk of developing allergic reaction to food proteins.

The net reduction in allergenicity of protein hydrolysates depends on the type of protease used. Nonspecific proteases or a mixture of proteases are more effective than a site-specific protease in reducing the allergenicity of proteins. The DH also plays a role: the higher the DH, the greater is the reduction of allergenicity. For these reasons, the efficacy of proteases in reducing allergenicity of a protein is often expressed as allergenicity reduction index (ARI). ARI is defined as the ratio of % reduction in allergenicity to %DH.

5.6.3 Bitter Peptides

One of the most undesirable properties of protein hydrolysates is their bitter flavor. The bitterness arises from certain peptides released during hydrolysis. There is ample evidence that bitterness of peptides is related to hydrophobicity. Peptides with a mean residue hydrophobicity of less than 1.3 kcal mol^{-1} are not bitter (see Chapter 11). On the other hand, peptides with a mean residue hydrophobicity of greater than 1.4 kcal mol^{-1} are bitter [94]. The mean residue hydrophobicity of the peptides is calculated using the free energies of transfer of amino acid residues from ethanol to water (see Table 11.1). Formation of bitter peptides in protein hydrolysates depends on the amino acid composition and sequence and the type of enzymes used. Hydrolysates of highly hydrophobic proteins such as casein, soy proteins, and corn protein (zein) are very bitter, whereas hydrolysates of hydrophilic proteins such as gelatin are less bitter. Caseins and soy proteins hydrolyzed with several commercial proteases produce several bitter peptides. The bitterness can be reduced or eliminated by using a mixture of endo- and exo-peptidases, which further break down bitter peptides into fragments that have less than 1.3 kcal mol^{-1} mean residue hydrophobicity.

5.7 NUTRITIONAL PROPERTIES OF PROTEINS

Proteins differ in their nutritive value. Several factors, such as essential amino acids content and digestibility, contribute to these differences. The daily protein requirement therefore depends on the type and composition of proteins in a diet.

5.7.1 Protein Quality

The "quality" of a protein is related mainly to its essential amino acid's content and digestibility. High-quality proteins are those that contain all the essential amino acids at levels greater than the FAO/WHO/UNU [95] reference levels, and a digestibility comparable to or better than those of egg white or milk proteins. Animal proteins are of better "quality" than plant proteins.

Proteins of major cereals and legumes are often deficient in at least one of the essential amino acids. While proteins of cereals, such as rice, wheat, barley, and maize, are very low in lysine and rich in methionine, those of legumes and oilseeds are deficient in methionine and rich or adequate in lysine. Some oilseed proteins, such as peanut protein, are deficient in both methionine and lysine contents. The essential amino acids whose concentrations in a protein are below the levels of a reference protein are termed "limiting amino acids." Adults consuming only cereal proteins or legume proteins have difficulty maintaining their health; children below 12 years of age on diets containing only one of these protein sources cannot maintain a normal rate of growth. The essential amino acid contents of various food proteins are listed in Table 5.21.

Both animal and plant proteins generally contain adequate or more than adequate amounts of His, Ile, Leu, Phe + Tyr, and Val. These amino acids usually are not limiting in staple foods. More often, Lys, Thr, Trp, and the sulfur containing amino acids are the limiting amino acids. The nutritional quality of a protein that is deficient in an essential amino acid can be improved by mixing it with another protein that is rich in that essential amino acid. For example, mixing of cereal proteins with legume proteins provides a complete and balanced level of essential amino acids. Thus, diets containing appropriate amounts of cereals and legumes (pulses) and otherwise nutritionally complete are often adequate to support growth and maintenance. A poor quality protein also can be nutritionally improved by supplementing it with essential free amino acids that are underrepresented. Supplementation of legumes with Met and cereals with Lys usually improves their quality.

The nutritional quality of a protein or protein mixture is ideal when it contains all of the essential amino acids in proportions that produce optimum rates of growth and/or optimum maintenance capability. The ideal essential amino acid patterns for children and adults are given in Table 5.22. However, because actual essential amino acid requirements of individuals in a given population vary depending on their nutritional and physiological status, the essential amino acid requirements of preschool children (age 2–5) are generally recommended as a safe level for all age groups [96].

Overconsumption of any particular amino acid can lead to "amino acid antagonism" or toxicity. Excessive intake of one amino acid often results in an increased requirement for other essential amino acids. This is due to competition among amino acids for absorption sites on the intestinal mucosa. For example, high levels of Leu decrease absorption of Ile, Val, and Tyr even if the dietary levels of these amino acids are adequate. This leads to an increased dietary requirement for the latter three amino acids. Overconsumption of other essential amino acids also can inhibit growth and induce pathological conditions.

5.7.2 Digestibility

Although the content of essential amino acids is the primary indicator of protein quality, true quality also depends on the extent to which these amino acids are utilized in the body. Thus, digestibility (bioavailability) of amino acids can affect the quality of proteins. Digestibilities of various proteins in humans are listed in Table 5.23. Food proteins of animal origin are more completely digested than those of plant origin. Several factors affect digestibility of proteins.

5.7.2.1 Protein Conformation

The structural state of a protein influences its hydrolysis by proteases. Native proteins are generally less completely hydrolyzed than partially denatured ones. For example, treatment of phaseolin (a protein from kidney beans) with a mixture of proteases results only in limited cleavage of the protein resulting in liberation of a 22,000 Da polypeptide as the main product. When heat-denatured phaseolin is

TABLE 5.21
Essential Amino Acid Contents and Nutritional Value of Proteins from Various Sources (mg g^{-1} Protein)

	Protein Source												
Property (mg g^{-1} Protein)	**Egg**	**Cow's Milk**	**Beef**	**Fish**	**Wheat**	**Rice**	**Maize**	**Barley**	**Soybean**	**Field Bean (Boiled)**	**Pea**	**Peanut**	**French Bean**
Amino acid concentration (mg g^{-1} protein)													
His	22	27	34	35	21	21	27	20	30	26	26	27	30
Ile	54	47	48	48	34	40	34	35	51	41	41	40	45
Leu	86	95	81	77	69	77	127	67	82	71	70	74	78
Lys	70	78	89	91	23[a]	34[a]	25[a]	32[a]	68	63	71	39[a]	65
Met + Cys	57	33	40	40	36	49	41	37	33	22[b]	24[b]	32	26
Phe + Tyr	93	102	80	76	77	94	85	79	95	69	76	100	83
Thr	47	44	46	46	28	34	32[b]	29[b]	41	33	36	29[b]	40
Trp	17	14	12	11	10	11	6[b]	11	14	8[a]	9[a]	11	11
Val	66	64	50	61	38	54	45	46	52	46	41	48	52
Total essential amino acids	512	504	480	485	336	414	422	356	466	379	394	400	430
Protein content (%)	12	3.5	18	19	12	7.5	—	—	40	32	28	30	30
Chemical score (%) (based on FAO/WHO, 1985 pattern)	100	100	100	100	40	59	43	55	100	73	82	67	
PER	3.9	3.1	3.0	3.5	1.5	2.0	—	—	2.3	—	2.65	—	—
BV (on rats)	94	84	74	76	65	73	—	—	73	—	—	—	—
NPU	94	82	67	79	40	70	—	—	61	—	—	—	—

Sources: FAO/WHO/UNU, Energy and protein requirements, Report of a joint FAO/WHO/UNU Expert Consultation, World Health Organization Technical Report Series 724, WHO, Geneva, Switzerland, 1985; Eggum, B.O. and Beames, R.M., The nutritive value of seed proteins, in: *Seed Proteins*, Gottschalk, W. and Muller, H.P., eds., Nijhoff/Junk, The Hague, the Netherlands, 1983, pp. 499–531.

PER, protein efficiency ratio; BV, biological value; NPU, net protein utilization.

[a] Primary limiting acid.

[b] Secondary limiting acid.

TABLE 5.22
Recommended Essential Amino Acid Pattern for Food Proteins

	Recommended Pattern (mg g^{-1} Protein)			
Amino Acid	**Infant (2–5 Years)**	**Preschool Child (10–12 Years)**	**Preschool Child**	**Adult**
Histidine	26	19	19	16
Isoleucine	46	28	28	13
Leucine	93	66	44	19
Lysine	66	58	44	16
Met + Cys	42	25	22	17
Phe + Tyr	72	63	22	19
Threonine	43	34	28	9
Tryptophan	17	11	9	5
Valine	55	35	25	13
Total	434	320	222	111

Source: FAO/WHO/UNU, Energy and protein requirements, Report of a joint FAO/WHO/UNU Expert Consultation, World Health Organization Technical Report Series 724, WHO, Geneva, Switzerland, 1985.

TABLE 5.23
Digestibility of Various Food Proteins in Humans

Protein Source	**Digestibility (%)**	**Protein Source**	**Digestibility (%)**
Egg	97	Millet	79
Milk, cheese	95	Peas	88
Meat, fish	94	Peanut	94
Maize	85	Soy flour	86
Rice (polished)	88	Soy protein isolate	95
Wheat, whole	86	Beans	78
Wheat flour, white	96	Corn, cereal	70
Wheat gluten	99	Wheat, cereal	77
Oatmeal	86	Rice cereal	75

Source: FAO/WHO/UNU, Energy and protein requirements, Report of a joint FAO/WHO/UNU Expert Consultation, World Health Organization Technical Report Series 724, WHO, Geneva, Switzerland, 1985.

treated under similar conditions, it is completely hydrolyzed to amino acids and dipeptides. Generally, insoluble fibrous proteins and extensively denatured globular proteins are difficult to hydrolyze.

5.7.2.2 Antinutritional Factors

Most plant protein isolates and concentrates contain trypsin and chymotrypsin inhibitors (Kunitz type and Bowman–Birk type) and lectins. These inhibitors impair complete hydrolysis of legume and oilseed proteins by pancreatic proteases. Lectins, which are glycoproteins, bind to intestinal mucosa cells and interfere with absorption of amino acids. Lectins and Kunitz-type protease inhibitors are thermolabile, whereas the Bowman–Birk-type inhibitor is stable under normal thermal processing conditions. Thus, heat-treated legume and oilseed proteins are generally more digestible than native protein isolates (despite some residual Bowman–Birk-type inhibitor). Plant proteins also

contain other antinutritional factors, such as tannins and phytate. Tannins, which are condensation products of polyphenols, covalently react with ε-amino groups of lysine residues. This inhibits trypsin-catalyzed cleavage of the polypeptides at lysine sites.

5.7.2.3 Processing

Interaction of proteins with polysaccharides and dietary fiber also reduces the rate and completeness of hydrolysis. This is particularly important in extruded food products where high temperature and pressure is often used. Proteins undergo several chemical alterations involving lysine residues when exposed to high temperatures and alkaline pH. Such alterations reduce their digestibility. Reaction of reducing sugars with ε-amino groups also decreases digestibility of lysine.

5.7.3 Evaluation of Protein Nutritive Value

Since the nutritional quality of proteins can vary greatly and is affected by many factors, it is important to have procedures for evaluating quality. Quality estimates are useful for (1) determining the amount required to provide a safe level of essential amino acids for growth and maintenance and (2) monitoring changes in the nutritive value of proteins during food processing, so that processing conditions that minimize quality loss can be devised. The nutritive quality of proteins can be evaluated by several biological, chemical, and enzymatic methods.

5.7.3.1 Biological Methods

Biological methods are based on weight gain or nitrogen retention in test animals when fed with a protein-containing diet. A protein-free diet is used as the control. The protocol recommended by FAO/WHO [96] is generally used for evaluating protein quality. Rats are the usual test animals, although humans are sometimes used. A diet containing about 10% protein on a dry weight basis is used to ensure that the protein intake is below daily requirements. Adequate energy is supplied in the diet. Under these conditions, protein in the diet is utilized to the maximum possible extent for growth. The number of test animals used must be sufficient to assure results that are statistically reliable. A test period of 9 days is common. During each day of the test period, the amount (g) of diet consumed is tabulated for each animal, and the feces and urine are collected for nitrogen analysis.

The data from animal feeding studies are used in several ways to evaluate protein quality. The *protein efficiency ratio* (PER) is the weight (in grams) gained per gram protein consumed. This is a simple and commonly used expression. Another useful expression is *net protein ratio* (NPR). This is calculated as follows:

$$\text{NPR} = \frac{(\text{Weight gain}) - (\text{Weight loss of protein} - \text{Free group})}{\text{Protein ingested}} \tag{5.82}$$

NPR values provide information on the ability of proteins to support both maintenance and growth. Since rats grow much faster than humans, and a larger percentage of protein is used for maintenance in growing children than in rats, it is often questioned whether PER and NPR values derived from rat studies are useful for estimating human needs [97]. Although this argument is a valid one, appropriate correction procedures are available.

Another approach to evaluating protein quality involves measuring nitrogen uptake and nitrogen loss. This allows calculation of two useful protein quality parameters. *Apparent protein digestibility* or *coefficient of protein digestibility* is obtained from the difference between the amount of nitrogen ingested and the amount of nitrogen excreted in the feces. However, since total fecal nitrogen also includes metabolic or endogenous nitrogen, correction should be made to obtain *true protein digestibility*. True digestibility (TD) can be calculated in the following manner:

$$\text{TD} = \frac{I - (F_N - F_{k,N})}{I} \times 100 \tag{5.83}$$

where
I is the nitrogen ingested
F_N is the total fecal nitrogen
$F_{k,N}$ is the endogenous fecal nitrogen

$F_{k,N}$ is obtained by feeding a protein-free diet. TD gives information on the percentage of nitrogen intake absorbed by the body. However, it does not provide information on how much of the absorbed nitrogen is actually retained or utilized by the body.

Biological value, BV, is calculated as follows:

$$BV = \frac{I - (F_N - F_{k,N}) - (U_N - U_{k,N})}{I - (F_N - F_{k,N})} \times 100 \quad (5.84)$$

where U_N and $U_{k,N}$ are the total and endogenous nitrogen losses, respectively, in the urine.

Net protein utilization (NPU), that is, the percentage of nitrogen intake retained as body nitrogen, is obtained from the product of TD and BV. Thus,

$$NPU = TD \times BV = \frac{I - (F_N - F_{k,N}) - (U_N - U_{k,N})}{I} \times 100 \quad (5.85)$$

The PER, BVs, and NPUs of several food proteins are presented in Table 5.21.

Other bioassays that are occasionally used to evaluate protein quality include assays for enzyme activity, changes in the essential amino acid content of plasma, levels of urea in the plasma and urine, and rate of repletion of plasma proteins or gain in body weight of animals previously fed a protein-free diet.

5.7.3.2 Chemical Methods

Biological methods are expensive and time consuming. Determining its content of amino acids and comparing this with the essential amino acid pattern of an ideal reference protein can obtain quick assessment of a protein's nutritive value. The ideal pattern of essential amino acids in proteins (reference protein) for preschool children (2–5 years) is given in Table 5.22 [95], and this pattern is used as the standard for all age groups except infants. Each essential amino acid in a test protein is given a "chemical score," which is defined as

$$\frac{\text{mg amino acid per g test protein}}{\text{mg same amino acid per g reference protein}} \times 100 \quad (5.86)$$

The essential amino acid that shows the lowest score is the most limiting amino acid in the test protein. The chemical score of this limiting amino acid provides the chemical score for the test protein. As mentioned earlier, Lys, Thr, Trp, and sulfur amino acids are often the limiting amino acids in food proteins. Therefore, the chemical scores of these amino acids are often sufficient to evaluate the nutritive value of proteins. The chemical score enables estimation of the amount of a test protein or protein mix needed to meet the daily requirement of the limiting amino acid. This can be calculated as follows:

$$\text{Required intake of protein} = \frac{\text{Recommended intake of egg or milk protein}}{\text{Chemical score of protein}} \times 100 \quad (5.87)$$

One of the advantages of the chemical score method is that it is simple and allows one to determine the complementary effects of proteins in a diet. This also allows one to develop high-quality protein diets by mixing various proteins suitable for various feeding programs. There are, however, several drawbacks to use of the chemical score method. An assumption underlying chemical score is that all test proteins are fully or equally digestible and that all essential amino acids are fully absorbed. Because this assumption is often violated, correlation between results from bioassays and chemical scores is often not good. However, the correlation improves when chemical scores are corrected for protein digestibility.

The apparent digestibility of proteins can be rapidly determined *in vitro* using a combination of three or four enzymes, such as trypsin, chymotrypsin, peptidase, and bacterial protease.

Another shortcoming of the chemical score is that it does not distinguish between D- and L-amino acids. Since only L-amino acids are usable in animals, the chemical score overestimates the nutritive value of a protein, especially in proteins exposed to high pH, which cause racemization. The chemical score method is also incapable of predicting the negative effects of high concentrations of one essential amino acid on the bioavailability of other essential amino acids, and it also does not account for the effect of antinutritional factors, such as protease inhibitors and lectins, which might be present in the diet. Despite these major drawbacks, recent findings indicate that chemical scores when corrected for protein digestibility correlate well with biological assays for those proteins having biological values above 40%; when the BV is below 40%, the correlation is poor [96].

5.7.3.3 Enzymatic and Microbial Methods

In vitro enzymatic methods are sometimes used to measure the digestibility and release of essential amino acids. In one method, test proteins are first digested with pepsin and then with pancreatin (freeze-dried powder of pancreatic extract) [83]. In another method, a combination of enzymes, namely, pepsin and pancreatin (which is a mixture of trypsin, chymotrypsin, and peptidases), was used to digest proteins under standard assay conditions [98]. These methods, in addition to providing information on innate digestibility of proteins, are useful for detecting processing-induced changes in protein quality.

Growth of several microorganisms, such as *Streptococcus zymogenes*, *Streptococcus faecalis*, *Leuconostoc mesenteroides*, *Clostridium perfringens*, and *Tetrahymena pyriformis* (a protozoan), also have been used to determine the nutritional value of proteins [99]. Of these microorganisms, *T. pyriformis* is particularly useful, because its amino acid requirements are similar to those of rats and humans.

5.8 PROCESSING-INDUCED PHYSICAL, CHEMICAL, AND NUTRITIONAL CHANGES IN PROTEINS

Commercial processing of foods can involve heating, cooling, drying, application of chemicals, fermentation, irradiation, or various other treatments. Of these, heating is most common. This is commonly done to inactivate microorganisms, to inactivate endogenous enzymes that cause oxidative and hydrolytic changes in foods during storage, and to transform an unappealing blend of raw food ingredients into a wholesome and organoleptically appealing food. In addition, proteins such as bovine β-lactoglobulin, α-lactalbumin, and soy protein, which sometimes cause allergenic or hypersensitive responses, can sometimes be rendered innocuous by thermal denaturation. Unfortunately, the beneficial effects achieved by heating proteinaceous foods are generally accompanied by changes that can adversely affect the nutritive value and functional properties of proteins. In this section, both desirable and undesirable effects of food processing on proteins will be discussed.

5.8.1 Changes in Nutritional Quality and Formation of Toxic Compounds

5.8.1.1 Effect of Moderate Heat Treatments

Most food proteins are denatured when exposed to moderate heat treatments (60°C–90°C, 1 h or less). Extensive denaturation of proteins often results in insolubilization, which may impair those functional properties that are dependent on solubility. From a nutritional standpoint, partial denaturation of proteins often improves the digestibility and biological availability of essential amino acids. Several purified plant proteins and egg protein preparations, even though free of protease inhibitors, exhibit poor *in vitro* and *in vivo* digestibility. Moderate heating improves their digestibility without developing toxic derivatives.

In addition to improving digestibility, moderate heat treatment also inactivates several enzymes, such as proteases, lipases, lipoxygenases, amylases, polyphenoloxidase, and other oxidative and hydrolytic enzymes. Failure to inactivate these enzymes can result in the development of off-flavors, rancidity, textural changes, and discoloration of foods during storage. For instance, oilseeds and legumes are

rich in lipoxygenase. During crushing or cracking of these beans for extraction of oil or protein isolates, this enzyme, in the presence of molecular oxygen, catalyzes oxidation of polyunsaturated fatty acids to initially yield hydroperoxides. These hydroperoxides subsequently decompose and liberate aldehydes and ketones, which impart off-flavor to soy flour and soy protein isolates and concentrates. To avoid off-flavor formation, it is necessary to thermally inactivate lipoxygenase prior to crushing.

Moderate heat treatment is particularly beneficial for plant proteins, because they usually contain proteinaceous antinutritional factors. Legume and oilseed proteins contain several trypsin and chymotrypsin inhibitors. These inhibitors impair efficient digestion of proteins and thus reduce their biological availability. Furthermore, inactivation and complexation of trypsin and chymotrypsin by these inhibitors induces overproduction and secretion of these enzymes by the pancreas, which can lead to pancreatic hypertrophy (enlargement of the pancreas) and pancreatic adenoma. Legume and oilseed proteins also contain lectins, which are glycoproteins. These are also known as phytohemagglutinins because they cause agglutination of red blood cells. Lectins exhibit a high binding affinity for carbohydrates. When consumed by humans, lectins impair protein digestion [100] and cause intestinal malabsorption of other nutrients. The latter consequence results from binding of lectins to membrane glycoproteins of intestinal mucosa cells, which alters their morphology and transport properties. Both protease inhibitors and lectins found in plant proteins are thermolabile. Toasting of legumes and oilseeds or moist heat treatment of soy flour inactivates both lectins and protease inhibitors, improves the digestibility and PER of these proteins (Figure 5.37), and prevents pancreatic hypertrophy [101]. These antinutritional factors do not pose problems in home-cooked or industrially processed legumes and flour-based products when heating conditions are adequate to inactivate them.

Milk and egg proteins also contain several protease inhibitors. Ovomucoid, which possesses antitryptic activity, constitutes about 11% of egg albumen. Ovoinhibitor, which inhibits trypsin, chymotrypsin, and some fungal proteases, is present at a 0.1% level in egg albumen. Milk contains several protease inhibitors, such as plasminogen activator inhibitor and plasmin inhibitor, derived from blood. All of these inhibitors lose their activity when subjected to moderate heat treatment in the presence of water.

The beneficial effects of heat treatment also include inactivation of protein toxins, such as botulinum toxin from *Clostridium botulinum* (inactivated by heating at 100°C) and enterotoxin from *Staphylococcus aureus*.

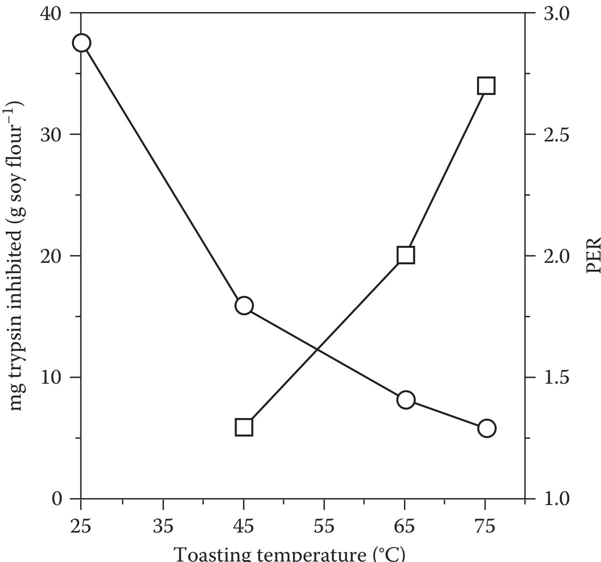


FIGURE 5.37 Effect of toasting on trypsin inhibitory activity and PER of soy flour. Circles represent trypsin inhibition, and squares represent PER. (Adapted from Friedman, M. and Gumbmann, M.R., *Adv. Exp. Med. Biol.*, 199, 357, 1986.)

5.8.1.2 Compositional Changes during Extraction and Fractionation

Preparation of protein isolates from biological sources involves several unit operations, such as extraction, isoelectric precipitation, salt precipitation, thermocoagulation, and ultrafiltration (UF)/diafiltration. It is very likely that some of the proteins in the crude extract might be lost during some of these operations. For example, during isoelectric precipitation, some sulfur-rich albumin-type proteins, which are usually soluble at isoelectric pH, might be lost in the supernatant fluid. Such losses can alter the amino acid composition and nutritional value of protein isolates compared to those of crude extracts. For instance, WPC prepared by UF/diafiltration and ion exchange methods undergo marked changes in their proteose-peptone contents. This markedly affects their foaming properties.

5.8.1.3 Chemical Alteration of Amino Acids

Proteins undergo several chemical changes when processed at high temperatures. These changes include racemization, hydrolysis, desulfuration, and deamidation. Most of these chemical changes are irreversible, and some of these reactions result in formation of modified amino acid types that are potentially toxic.

5.8.1.3.1 Racemization

Thermal processing of proteins at alkaline pH, as is done to prepare texturized foods, invariably leads to partial racemization of L-amino acid residues to D-amino acids [102]. Acid hydrolysis of proteins also causes some racemization of amino acids [103] as does roasting of proteins or protein-containing foods above 200°C [104]. The mechanism at alkaline pH involves initial abstraction of the proton from the α-carbon atom by a hydroxyl ion. The resulting carbanion loses its tetrahedral asymmetry. Subsequent addition of a proton from solution can occur either from the top or bottom of the carbanion. This equal probability results in racemization of the amino acid residue (Equation 5.80) [102]. The rate of racemization of a residue is affected by the electron-withdrawing power of the side chain. Thus, residues such as Asp, Ser, Cys, Glu, Phe, Asn, and Thr are racemized at a faster rate than are other amino acid residues [102]. The rate of racemization is also dependent on hydroxyl ion concentration, but is independent of protein concentration. Interestingly, the rate of racemization is about 10 times faster in proteins than in free amino acids [102], suggesting that intramolecular forces in a protein reduce the activation energy of racemization. In addition to racemization, the carbanion formed under alkaline pH also can undergo β-elimination reaction to yield a reactive intermediate dehydroalanine (DHA).

L-Amino acid residue → (Alkaline pH, toasting at 200°C, ^-OH) → Carbanion → (H^+) D-Amino acid residue; Carbanion → Dehydroalanine (DHA) + X → Several protein cross-linking reactions (5.88)

Racemization of amino acid residues causes a reduction in protein digestibility because the peptide bonds involving D-amino acid residues are less efficiently hydrolyzed by gastric and pancreatic proteases. This leads to loss of essential amino acids that have racemized and impairs the nutritional value of the protein. D-amino acids are also less efficiently absorbed through intestinal mucosa cells, and even if absorbed, they cannot be utilized in *in vivo* protein synthesis. Moreover, some D-amino acids, for example, D-proline, have been found to be neurotoxic in chickens [105].

In addition to racemization and β-elimination reactions, heating of proteins at alkaline pH destroys several amino acid residues, such as Arg, Ser, Thr, and Lys. Arg decomposes to ornithine.

When proteins are heated above 200°C, as is commonly encountered on food surfaces during broiling, baking, and grilling, amino acid residues undergo decomposition and pyrolysis. Several of the pyrolysis products have been isolated and identified from broiled and grilled meat, and they are highly mutagenic as determined by the Ames test. The most carcinogenic/mutagenic products are formed from pyrolysis of Trp and Glu residues [106]. Pyrolysis of Trp residues gives rise to formation of carbolines and their derivatives. Mutagenic compounds are also produced in meats at moderate temperatures (190°C–200°C). These are known as IQ (imidazoquinolines) compounds, which are condensation products of creatine, sugars, and certain amino acids, such as Gly, Thr, Ala, and Lys [107]. The three most potent mutagens formed in broiled fish are as follows:

2-Amino-3-methylimidazo-[4,5-*f*]quinoline (IQ) | 2-Amino-3,4-dimethylimidazo-[4,5-*f*]quinoline (MeIQ) | 2-Amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline (MeIQx) (5.89)

Following heating of foods according to recommended procedures, IQ compounds are generally present only at very low concentrations (μg amounts).

5.8.1.3.2 Protein Cross-Linking

Several food proteins contain both intra- and intermolecular cross-links, such as disulfide bonds in globular proteins, desmosine, and isodesmosine, and di- and tri-tyrosine-type cross-links in fibrous proteins such as keratin, elastin, resilin, and collagen. Collagen also contains ε-*N*-(γ-glutamyl)lysyl and/or ε-*N*-(γ-aspartyl)lysyl cross-links. One of the functions of these cross-links in native proteins is to minimize proteolysis *in vivo*. Processing of food proteins, especially at alkaline pH, also induces cross-link formation. Such unnatural covalent bonds between polypeptide chains reduce digestibility and biological availability of essential amino acids that are involved in, or near, the cross-link.

As discussed in the previous section, heating at alkaline pH or heating above 200°C at neutral pH results in abstraction of the proton from the α-carbon atom resulting in formation of a carbanion, which leads to formation of DHA residue. DHA formation can also occur via a one-step mechanism without the carbanion intermediate. Once formed, the highly reactive DHA residues react with nucleophilic groups, such as the ε-amino group of a lysyl residue, the thiol group of Cys residue, the δ-amino group of ornithine (formed by decomposition of arginine),

or a histidyl residue, resulting in the formation of lysinoalanine (LAL), lanthionine, ornithoalanine, and histidinylalanine cross-links, respectively, in proteins. LAL is the major cross-link commonly found in alkali-treated proteins because of the abundance of readily accessible lysyl residues (Equation 5.82).

Cystine

Cysteine

Dehydroalanine (DHA)

Lysine

Cysteine

Lysinoalanine

Lanthionine

(5.90)

The formation of protein–protein cross-links in alkali-treated proteins decreases their digestibility and biological value. The decrease in digestibility is related to the inability of trypsin to cleave the peptide bond in the LAL cross-link. Moreover, the steric constraints imposed by the cross-links also prevent hydrolysis of other peptide bonds in the neighborhood of the LAL and similar cross-links. Evidence suggests that free LAL is absorbed in the intestine, but the body

does not utilize it and most of it is excreted in the urine. Some LAL is metabolized in the kidney. The inability of the body to cleave the LAL covalent bond reduces the bioavailability of lysine in alkali-treated proteins.

Rats fed 100 ppm of pure LAL or 3000 ppm of protein-bound LAL develop nephrocytomegaly (i.e., kidney disorder). However, such nephrotoxic effects have not been observed in other animal species, such as quails, mice, hamsters, and monkeys. This has been attributed to differences in the types of metabolites formed in rats versus other animals. At levels encountered in foods, protein-bound LAL apparently does not cause nephrotoxicity in humans. Nevertheless, minimization of LAL formation during alkali processing of proteins is a desirable goal.

The LAL contents of several commercial foods are listed in Table 5.24. The extent of formation of LAL is dependent on pH and temperature. The higher the pH, the greater the extent of LAL formation is. High-temperature heat treatment of foods, such as milk, causes a significant amount of LAL to form even at neutral pH. LAL formation in proteins can be minimized or inhibited by adding small-molecular-weight nucleophilic compounds, such as cysteine, ammonia, or sulfites. The effectiveness of cysteine results because the nucleophilic SH group reacts more than 1000 times faster than the ε-amino group of lysine. Sodium sulfite and ammonia exert their inhibitory effect by competing with the ε-amino group of lysine for DHA. Blocking of ε-amino groups of lysine residues by reaction with acid anhydrides prior to alkali-treatment also decreases the formation of LAL. However, this approach results in loss of lysine and may be unsuitable for food applications.

TABLE 5.24
Lysinoalanine Content of Processed Foods

Food	LAL (μg g^{-1} Protein)
Corn chips	390
Pretzels	500
Hominy	560
Tortillas	200
Taco shells	170
Milk, infant formula	150–640
Milk, evaporated	590–860
Milk, condensed	360–540
Milk, UHT	160–370
Milk, HTST	260–1,030
Milk, spray-dried powder	0
Skim milk, evaporated	520
Simulated cheese	1070
Egg-white solids, dried	160–1,820
Calcium caseinate	370–1,000
Sodium caseinate	430–6,900
Acid casein	70–190
Hydrolyzed vegetable protein	40–500
Whipping agent	6,500–50,000
Soy protein isolate	0–370
Yeast extract	120

Source: Swaisgood, H.E. and Catignani, G.L., *Adv. Food Nutr. Res.*, 35, 185, 1991.

Under normal conditions used for processing of several foods, only small amounts of LAL are formed. Thus, toxicity of LAL in alkali-treated foods is not believed to be a major concern. However, reduction in digestibility, loss of bioavailability of lysine, and racemization of amino acids (some of which are toxic) are all undesirable outcomes in alkali-treated foods such as texturized vegetable proteins.

Excessive heating of pure protein solutions or proteinaceous foods low in carbohydrate content also results in formation of ε-*N*-(γ-glutamyl)lysyl and ε-*N*-(γ-aspartyl)lysyl cross-links. These involve a transamidation reaction between Lys and Gln or Asn residues (Equation 5.83). The resulting cross-links are termed isopeptide bonds because they are foreign to native proteins. Isopeptides resist enzymatic hydrolysis in the gut and these cross-linkages therefore impair digestibility of proteins and bioavailability of lysine.

Lysine: $—NH—CH[(CH_2)_4NH_2]—C(=O)—$ + Glutamine: $—NH—CH[CH_2—C(=O)—NH_2]—C(=O)—$ → NH_3 +

$O{=}C—CH(NH)—(CH_2)_4—NH—C(=O)—CH_2—CH(NH)—C{=}O$ (5.91)

ε-*N*-(γ-glutamyl)lysine cross-link

Ionizing radiation of foods results in the formation of hydrogen peroxide through radiolysis of water in the presence of oxygen, and this, in turn, causes oxidative changes in, and polymerization of, proteins. Ionizing radiation also may directly produce free radicals via ionization of water.

$$H_2O \rightarrow H_2O^+ + e^- \quad (5.92)$$

$$H_2O^+ + H_2O \rightarrow H_3O^+ + {}^{X}OH \quad (5.93)$$

The hydroxyl free radical can induce formation of protein free radicals, which in turn may cause polymerization of proteins.

$$P + \mathbf{OH} \rightarrow \mathbf{P}^* + H_2O \quad (5.94)$$

$$P^X + P^* \rightarrow P - P$$
$$P^* + P \rightarrow P - P^* \quad (5.95)$$

Heating of protein solutions at 70°C–90°C and at neutral pH generally leads to sulfhydryl–disulfide interchange reactions (if these groups are present), resulting in polymerization of proteins. However, this type of heat-induced cross-link generally does not have an adverse effect on the digestibility of proteins and bioavailability of essential amino acids because these bonds can be broken *in vivo*.

5.8.1.4 Effects of Oxidizing Agents

Oxidizing agents such as hydrogen peroxide and benzoyl peroxide are used as bactericidal agents in milk; as bleaching agents in cereal flours, protein isolates, and fish protein concentrate; and for detoxification of oilseed meals. Sodium hypochlorite is also commonly used as a bactericidal and detoxifying agent in flours and meals. In addition to oxidizing agents that are sometimes added to foods, several oxidative compounds are endogenously produced in foods during processing. These include free radicals formed during irradiation of foods, during peroxidation of lipids, during photo-oxidation of compounds such as riboflavin and chlorophyll, and during nonenzymatic browning of foods. In addition, polyphenols present in several plant protein isolates can be oxidized by molecular oxygen to quinones at neutral to alkaline pH, and this will lead ultimately to peroxides. These highly reactive oxidizing agents cause oxidation of several amino acid residues and polymerization of proteins. The amino acid residues most susceptible to oxidation are Met, Cys, Trp, and His and to a lesser extent Tyr.

5.8.1.4.1 Oxidation of Methionine

Methionine is easily oxidized to methionine sulfoxide by various peroxides. Incubation of protein-bound methionine or free methionine with hydrogen peroxide (0.1 M) at elevated temperature for 30 min results in complete conversion of methionine to methionine sulfoxide [108]. Under strong oxidizing conditions, methionine sulfoxide is further oxidized to methionine sulfone and in some cases to homocysteic acid.

$$\underset{\text{Methionine}}{\text{—NH—CH(CH}_2\text{—CH}_2\text{—S—CH}_3\text{)—CO—}} \longrightarrow \underset{\text{Methionine sulfoxide}}{\text{—NH—CH(CH}_2\text{—CH}_2\text{—S(}\rightarrow\text{O)—CH}_3\text{)—CO—}} \longrightarrow \underset{\text{Methionine sulfone}}{\text{—NH—CH(CH}_2\text{—CH}_2\text{—S(}\rightarrow\text{O)}_2\text{—CH}_3\text{)—CO—}} \longrightarrow \underset{\text{Homocysteic acid}}{\text{—NH—CH(CH}_2\text{—CH}_2\text{—S(}\rightarrow\text{O)}_2\text{—OH)—CO—}} \quad (5.96)$$

Methionine becomes biologically unavailable once it is oxidized to methionine sulfone or homocysteic acid. Methionine sulfoxide, on the other hand, is reconverted to Met under acidic conditions in the stomach. Further, evidence suggests that any methionine sulfoxide passing through the intestine is absorbed and reduced *in vivo* to methionine. However, *in vivo* reduction of methionine sulfoxide to methionine is slow. The PER or NPU of casein oxidized with 0.1 M hydrogen peroxide (which completely transforms methionine to methionine sulfoxide) is about 10% less than that of control casein.

5.8.1.4.2 Oxidation of Cysteine and Cystine

Under alkaline conditions, cysteine and cystine follow the β-elimination reaction pathway to produce DHA residues. However, at acidic pH, oxidation of cysteine and cystine in simple systems results in formation of several intermediate oxidation products. Some of these derivatives are unstable. Mono- and disulfoxides of L-cystine are biologically available, presumably because they are reduced back to L-cystine in the body. However, mono- and disulfone derivatives of L-cystine are biologically unavailable. Similarly, while cysteine sulfenic acid is biologically available, cysteine sulfinic acid and cysteic acid are not. The rate and extent of formation of these oxidation products in acidic foods are not well documented.

—NH—CH—CO—
| CH_2
| SH

Cysteine

↓ H^+

—NH—CH—CO—
| CH_2
| SOH

Cysteine sulfenic acid

↓ H^+

—NH—CH—CO—
| CH_2
| SO_2H

Cysteine sulfinic acid

↓ H^+

—NH—CH—CO—
| CH_2
| SO_2H

Cysteine sulfonic acid

| CO | HC—CH_2—S—S—CH_2—HC | NH | (CO and NH on each HC)

Cystine

↓ H^+

CO, HC—CH_2—S(→O)—S(→O)—CH_2—HC, NH

Cystine mono-or di-sulfoxide

↓ H^+

CO, HC—CH_2—S(→O)(→O)—S(→O)(→O)—CH_2—HC, NH

Cystine mono- or di-sulfone

(5.97)

5.8.1.4.3 Oxidation of Tryptophan

Among the essential amino acids, Trp is exceptional because of its role in several biological functions. Therefore, its stability in processed foods is of major concern. Under acidic, mild, oxidizing conditions, such as in the presence of performic acid, dimethylsulfoxide, or *N*-bromosuccinimide, Trp is oxidized mainly to β-oxyindolylalanine. Under acidic, severe, oxidizing conditions, such as in the presence of ozone, hydrogen peroxide, or peroxidizing lipids, Trp is oxidized to *N*-formylkynurenine, kynurenine, and other unidentified products.

$$\text{Trp} \xrightarrow{\text{Performic acid, dimethyl sulfoxide or NBS}} \beta\text{-Oxyindoylalanine}$$

$$\text{Trp} \xrightarrow{\text{Ozone or oxygen + light}} N\text{-Formylkynurenine} \longrightarrow \text{Kynurenine} \quad (5.98)$$

Exposure of Trp to light in the presence of oxygen and a photosensitizer, such as

$$\text{Riboflavin} \xrightarrow{\text{Light}} [\text{Riboflavin}]^{*} \longrightarrow \text{Riboflavin radical}; \quad \text{Tryptophan} \rightarrow [\text{Trp radical} \longleftrightarrow \text{Trp radical}] \longrightarrow \text{Photoadduct} \quad (5.99)$$

riboflavin or chlorophyll, leads to formation of *N*-formylkynurenine and kynurenine as major products and several other minor ones. Depending upon the pH of the solution, other derivatives, such as 5-hydroxy-formylkynurenine (pH > 7.0) and a tricyclic hydroperoxide (pH 3.6–7.1), are also formed [109]. In addition to the photooxidative products, Trp forms a photoadduct with riboflavin. Both protein-bound and free tryptophan is capable of forming this adduct. The extent of formation of this photoadduct is dependent on the availability of oxygen, being greater under anaerobic conditions.

The oxidation products of Trp are biologically active. In addition, kynurenines are carcinogenic in animals, and all other Trp photooxidation products as well as the carbolines formed during broiling/grilling of meat products exhibit mutagenic activities and inhibit growth of mammalian cells in tissue cultures. The tryptophan–riboflavin photoadduct shows cytotoxic effects on mammalian cells and exerts hepatic dysfunctions during parenteral nutrition. These undesirable products are normally present in extremely low concentration in foods unless an oxidation environment is purposely created.

Among the amino acid side chains, only those of Cys, His, Met, Trp, and Tyr are susceptible to sensitized photooxidation. In the case of Cys, cysteic acid is the end product. Met is photooxidized first to methionine sulfoxide and finally to methionine sulfone and homocysteic acid. Photooxidation of histidine leads to the formation of aspartate and urea. The photooxidation products of tyrosine are not known. Since foods contain endogenous as well as supplemented riboflavin (vitamin B2) and usually are exposed to light and air, some degree of sensitized photooxidation of the amino acid residues would be expected to occur. In milk, free methionine is converted to methional by light-activated oxidation, which imparts a characteristic flavor to the milk. At equimolar concentrations, the rates of oxidation of the sulfur amino acids and Trp are likely to follow the order Met > Cys > Trp.

5.8.1.4.4 Oxidation of Tyrosine

Exposure of tyrosine solutions to peroxidase and hydrogen peroxide results in oxidation of tyrosine to dityrosine. Occurrence of this type of cross-link has

$$\text{Tyrosine} \xrightarrow{H_2O_2,\ \text{peroxidase}} \text{Dityrosine} \qquad (5.100)$$

been found in natural proteins, such as resilin, elastin, keratin, and collagen, and more recently in doughs.

5.8.1.5 Carbonyl–Amine Reactions

Among the various processing-induced chemical changes in proteins, the Maillard reaction (nonenzymatic browning) has the greatest impact on its sensory and nutritional properties.

The Maillard reaction refers to a complex set of reactions initiated by reaction between amines and carbonyl compounds, which, at elevated temperature, decompose and eventually condense into insoluble brown product known as melanoidins (see Chapter 3). This reaction occurs not only in foods during processing but also in biological systems. In both instances, proteins and amino acids typically provide the amino component, and reducing sugars (aldoses and ketoses), ascorbic acid, and carbonyl compounds generated from lipid oxidation provide the carbonyl component.

Some of the carbonyl derivatives from the nonenzymatic browning sequence react readily with free amino acids. This results in degradation of the amino acids to aldehydes, ammonia, and carbon dioxide and the reaction is known as "Strecker degradation." The aldehydes contribute

$$\underset{\alpha\text{-Dicarbonyl substances}}{R{-}C({=}O){-}C({=}O){-}R} + \underset{\text{Amino acid}}{H_2N{-}CH(R_1){-}COOH} \longrightarrow R{-}C({=}O){-}C(R){=}N{-}CH(R_1){-}COOH + H_2O \tag{5.101}$$

$$\xrightarrow{H_2O} \underset{\text{Aldehyde derivative of the amino acid}}{\mathbf{R_1CHO}} + CO_2 + R{-}CH(OH){-}C({=}O){-}R + NH_3$$

to aroma development during the browning reaction. Strecker degradation of each amino acid produces a specific aldehyde with a distinctive aroma (Table 5.25).

The Maillard reaction impairs protein nutritional value. Some of the products are antioxidants and some may be toxic; but the toxic products probably are not hazardous at concentrations encountered in foods. Because the ε-amino group of lysine is the major source of primary amines in proteins, it is frequently involved in the carbonyl–amine reaction, and it typically suffers a major loss in bioavailability when this reaction occurs. The extent of Lys loss depends on the stage of the browning reaction. Lysine involved in the early stages of browning, including the Schiff's base,

TABLE 5.25
Characteristic Flavor Notes of Aldehydes Produced by Strecker Degradation of Amino Acids

Amino Acid	Typical Flavor
Phe, Gly	Caramel-like
Leu, Arg, His	Bread-like, toasted
Ala	Nutty
Pro	Bakery, cracker
Gln, Lys	Buttery
Met	Broth, beany
Cys, Gly	Smokey, burnt
α-Amino butyric acid	Walnut
Arg	Popcorn-like

is biologically available. These early derivatives are hydrolyzed to lysine and sugar in the acidic conditions of the stomach. However, beyond the stage of ketosamine (Amadori product) or aldosamine (Heyns product), lysine is no longer biologically available. This is primarily because of poor absorption of these derivatives in the intestine. It is important to note that no color has developed at this stage. Although sulfite inhibits formation of brown pigments [110], it cannot prevent loss of lysine availability, because it cannot prevent formation of Amadori or Heyns products.

Biological activity of lysine at various stages of the Maillard reaction can be determined chemically by addition of 1-fluoro-2,4-dinitrobenzene (FDNB), followed by acid hydrolysis of the derivatized protein. FDNB reacts with available ε-amino groups of lysyl residues. The hydrolysate is then extracted with ethyl ether to remove unreacted FDNB, and the concentration of ε-dinitrophenyl-lysine (ε-DNP-lysine) in the aqueous phase is determined by measuring absorbance at 435 nm. Available lysine also can be determined by reacting 2,4,6-trinitrobenzene sulfonic acid with the ε-amino group. In this case, the concentration of ε-trinitrophenyl-lysine (ε-TNP-lysine) derivative is determined from absorbance at 346 nm.

Nonenzymatic browning not only causes major losses of lysine, but reactive unsaturated carbonyls and free radicals formed during the browning reaction cause oxidation of several other essential amino acids, especially Met, Tyr, His, and Trp. Cross-linking of proteins by dicarbonyl compounds produced during browning decreases protein solubility and impairs digestibility of proteins.

Some of Maillard brown products are suspected mutagens. Although mutagenic compounds are not necessarily carcinogenic, all known carcinogens are mutagens. Therefore, the formation of mutagenic Maillard compounds in foods is of concern. Studies with mixtures of glucose and amino acids have shown that the Maillard products of Lys and Cys are mutagenic, whereas those of Trp, Tyr, Asp, Asn, and Glu are not, as determined by the Ames test. It should be pointed out that pyrolysis products of Trp and Glu (in grilled and broiled meat) also are mutagenic (Ames Test). As discussed earlier, heating of sugar and amino acids in the presence of creatine produces the most potent IQ-type mutagens (see Equation 5.81). Although results based on model systems cannot be reliably applied to foods, it is possible that interaction of Maillard products with other small-molecular-weight constituents in foods may produce mutagenic and/or carcinogenic substances.

On a positive note, some Maillard reaction products, especially the reductones, do have antioxidative activity [111,112]. This is due to their reducing power, and their ability to chelate metals, such as Cu and Fe, which are prooxidants. The amino reductones formed from the reaction of triose reductones with amino acids such as Gly, Met, and Val show excellent antioxidative activity.

Besides reducing sugars, other aldehydes and ketones present in foods can also take part in the carbonyl–amine reaction. Notably, gossypol (in cotton seed), glutaraldehyde (added to protein meals to control deamination in the rumen of ruminants), and aldehydes (especially

$$\underset{\text{Protein amino group}}{P{-}NH_2} + \underset{\text{Malondialdehyde}}{OHC{-}CH_2{-}CHO} \longrightarrow \underset{\text{Protein–protein cross-linkage}}{P{-}N{=}CH{-}CH_2{-}CH{=}N{-}P} \tag{5.102}$$

malonaldehyde) generated from the oxidation of lipids may react with amino groups of proteins. Bifunctional aldehydes, such as malonaldehyde, can cross-link and polymerize proteins. This may result in insolubilization, loss of digestibility and bioavailability of lysine, and loss of functional properties of proteins. Formaldehyde also reacts with the ε-amino group of lysine residues; the toughening of cod-type fish muscle during frozen storage is believed to be due to reactions of formaldehyde with fish proteins.

5.8.1.6 Other Reactions of Proteins in Foods

5.8.1.6.1 Reactions with Lipids

Oxidation of unsaturated lipids leads to formation of alkoxy and peroxy free radicals. These free radicals in turn react with proteins, forming lipid–protein free radicals. These lipid–protein conjugated free radicals can undergo polymerization cross-linking of proteins leading to a variety of cross-linked products:

$$LH + O_2 \rightarrow LOO^* \tag{5.103}$$

$$LOO^* + LH \rightarrow LOOH + L^* \tag{5.104}$$

$$LOOH \rightarrow LO^* + HO^* \tag{5.105}$$

$$LO^* + PH \rightarrow LOP \tag{5.106}$$

$$LOP + LO^* \rightarrow {}^*LOP + LOH \tag{5.107}$$

$${}^*LOP + {}^*LOP \rightarrow POLLOP \tag{5.108}$$

or

$$LOO^* + PH \rightarrow LOOP \tag{5.109}$$

$$LOOP + LOO^* \rightarrow {}^*LOOP + LOOH \tag{5.110}$$

$${}^*LOOP + {}^*LOOP \rightarrow POOLLOOP \tag{5.111}$$

$${}^*LOOP + {}^*LOP \rightarrow POLLOOP \tag{5.112}$$

In addition, the lipid free radicals can also induce formation of protein free radicals at cysteine and histidine side chains, which may then undergo cross-linking and polymerization reactions:

$$LOO^* + PH \rightarrow LOOH + P^* \tag{5.113}$$

$$LO^* + PH \rightarrow LOH + P^* \tag{5.114}$$

$$P^* + PH \rightarrow P{-}P^{!} \tag{5.115}$$

$$P{-}P^* + PH \rightarrow P{-}P{-}P^* \tag{5.116}$$

$$P{-}P{-}P^* + P^* \rightarrow P{-}P{-}P{-}P \tag{5.117}$$

Lipid hydroperoxides (LOOH) in foods can decompose, resulting in liberation of aldehydes and ketones, notably malonaldehyde. These carbonyl compounds react with amino groups of proteins via carbonyl–amine reaction and Schiff's base formation. As discussed earlier, reaction of malonaldehyde with lysyl side chains leads to cross-linking and polymerization of proteins. The reaction of peroxidizing lipids with proteins generally has deleterious effects on nutritional value of proteins. Noncovalent binding of carbonyl compounds to proteins also imparts off-flavors.

5.8.1.6.2 Reactions with Polyphenols

Phenolic compounds, such as *p*-hydroxybenzoic acid, catechol, caffeic acid, gossypol, and quercetin, are found in all plant tissues. During maceration of plant tissues, these phenolic compounds can be oxidized by molecular oxygen at alkaline pH to quinones. This can also occur by the action of polyphenoloxidase, which is commonly present in plant tissues. These highly reactive quinones can irreversibly react with the sulfhydryl and amino groups of proteins. Reaction of quinones with SH and α-amino groups (N-terminal) is much faster than it is with ε-amino groups. In addition, quinones can also undergo condensation reactions, resulting in formation of HMW brown color pigments. These brown products remain highly reactive and readily combine with SH and amino groups of proteins. Quinone–amino group reactions decrease the digestibility and bioavailability of protein-bound lysine and cysteine.

5.8.1.6.3 Reactions with Halogenated Solvents

Halogenated organic solvent are often used to extract oil and some antinutritive factors from oilseed products, such as soybean and cottonseed meals. Extraction with trichloroethylene results in formation of a small amount of *S*-dichlorovinyl-L-cysteine, which is toxic. On the other hand, the solvents dichloromethane and tetrachloroethylene do not seem to react with proteins. 1,2-Dichloroethane reacts with Cys, His, and Met residues in proteins. Certain fumigants, such as methyl bromide, can alkylate Lys, His, Cys, and Met residues. All of these reactions decrease the nutritional value of proteins and some are of concern from a safety standpoint.

5.8.1.6.4 Reactions with Nitrites

Reaction of nitrites with secondary amines, and to some extent with primary and tertiary amines, results in formation of *N*-nitrosamine, which is one of the most carcinogenic compounds formed in foods. Nitrites are usually added to meat products to improve color and to prevent bacterial growth. The amino acids (or residues) primarily involved in this reaction are Pro, His, and Trp. Arg, Tyr, and Cys also can react with nitrites. The reaction occurs mainly under acidic conditions and at elevated temperatures.

Trp

Cys

$NaNO_2 \xrightarrow{\text{Acid}}$ **HONO**

Nitrous acid

N-Nitrosotryptophan

Pro

N-Nitrosopyrrolidone

S-Nitrosocysteine

(5.118)

The secondary amines produced during the Maillard reaction, such as Amadori and Heyns products, also can react with nitrites. Formation of *N*-nitrosamines during cooking, grilling, and broiling of meat has been a major concern, but additives, such as ascorbic acid and erythorbate, are effective in curtailing this reaction.

5.8.1.6.5 Reaction with Sulfites

Sulfites reduce disulfide bonds in proteins to yield *S*-sulfonate derivatives. They do not react with cysteine residues.

$$\text{P}-S-S-\text{P} + SO_3^{2-} \longrightarrow \text{P}-S-SO_3^{2-} + \text{P}-S^- \quad (5.119)$$

In the presence of reducing agents, such as cysteine or mercaptoethanol, the *S*-sulfonate derivatives are converted back to cysteine residues. *S*-Sulfonates decompose under acidic (as in stomach) and alkaline pH to disulfides. The *S*-sulfonation does not decrease the bioavailability of cysteine. The increase in electronegativity and the breakage of disulfide bonds in proteins upon *S*-sulfonation causes unfolding of protein molecules, which affects their functional properties.

5.8.2 Changes in the Functional Properties of Proteins

The methods or processes used to isolate proteins can affect their functional properties. Minimum denaturation during various isolation steps is generally desired because this helps to retain acceptable protein solubility, which is often a prerequisite to functionality of these proteins in food products. In some instances, controlled or partial denaturation of proteins can improve certain functional properties.

Proteins are often isolated using isoelectric precipitation. The secondary, tertiary, and quaternary structures of most globular proteins are stable at their isoelectric pH, and the proteins readily become soluble again when dispersed at neutral pH. On the other hand, protein entities such as casein micelles are irreversibly destabilized by isoelectric precipitation. The collapse of micellar structure in isoelectrically precipitated casein is due to several factors, including solubilization of colloidal calcium phosphate and the change in the balance of hydrophobic and electrostatic interactions among the various casein types. The compositions of isoelectrically precipitated proteins are usually altered from those of the raw materials. This is because some minor protein fractions are reasonably soluble at the isoelectric pH of the major component and therefore do not precipitate. This change in composition affects the functional properties of the protein isolate.

UF is widely used to prepare WPCs. Both protein and nonprotein composition of WPC are affected by removal of small solutes during UF. Partial removal of lactose and ash strongly influence the functional properties of WPC. Furthermore, increased protein–protein interactions occur in the UF concentrate during exposure to moderate temperatures (50°C–55°C), and this decreases solubility and stability of the ultrafiltered protein, which in turn changes its water binding capacity and alters its properties with respect to gelation, foaming, and emulsification. Among the ash constituents, variations in calcium and phosphate content significantly affect the gelling properties of WPC. WPIs prepared by ion exchange contain little ash, and because of this they have functional properties that are superior to those of isolates obtained by UF/diafiltration.

Calcium ions often induce aggregation of proteins. This is attributable to formation of ionic bridges involving Ca^{2+} ions and the carboxyl groups. The extent of aggregation depends on calcium ion concentration. Most proteins show maximum aggregation at 40–50 mM Ca^{2+} ion concentration. With some proteins, such as caseins and soy proteins, calcium aggregation leads to precipitation, whereas, in the case of WPI, a stable colloidal aggregate forms (Figure 5.38).

Exposure of proteins to alkaline pH, particularly at elevated temperatures, causes irreversible conformational changes. This is partly because of deamidation of Asn and Gln residues,

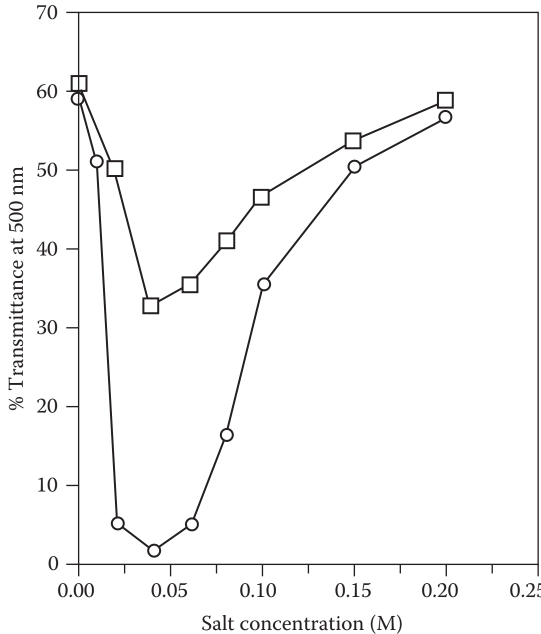


FIGURE 5.38 Salt concentration versus turbidity of whey protein isolate (5%) in $CaCl_2$ (○) and $MgCl_2$ (□) solutions after incubating for 24 h at ambient temperature. (From Zhu, H. and Damodaran, S., *J. Agric. Food Chem.*, 42, 856, 1994.)

and β-elimination of cystine residues. The resulting increase in the electronegativity and breakage of disulfide bonds causes gross structural changes in proteins exposed to alkali. Generally, alkali-treated proteins are more soluble and possess improved emulsification and foaming properties.

Hexane is often used to extract oil from oilseeds, such as soybean and cottonseed. This treatment invariably causes denaturation of proteins in the meal and thus impairs their solubility and other functional properties.

The effects of heat treatments on chemical changes in, and functional properties of, proteins are described in Section 5.6. Scission of peptide bonds involving aspartyl residues during severe heating of protein solutions liberates small-molecular-weight peptides. Severe heating under alkaline and acid pH conditions also causes partial hydrolysis of proteins. The amount of small-molecular-weight peptides in protein isolates can affect their functional properties.

5.9 CHEMICAL AND ENZYMATIC MODIFICATION OF PROTEINS

5.9.1 Chemical Modifications

The primary structure of proteins contains several reactive side chains. The physicochemical properties of proteins can be altered, and their functional properties can be improved by chemically modifying the side chains. However, it should be cautioned that although chemical derivatization of amino acid side chains can improve functional properties of proteins, it can also impair nutritional value, create some amino acid derivatives that are toxic, and pose regulatory problems although similar reactions may occur *in vivo* or *in situ*.

Since proteins contain several reactive side chains, numerous chemical modifications can be achieved. Some of these reactions are listed in Table 5.5. However, only a few of these reactions may be suitable for modification of food proteins. The ε-amino groups of lysyl residues and the SH

group of cyteine are the most reactive nucleophilic groups in proteins. The majority of chemical modification procedures involve these groups.

5.9.1.1 Alkylation

The SH and amino groups can be alkylated by reacting with iodoacetate or iodoacetamide. Reaction with iodoacetate results in elimination of the positive charge of the lysyl residue, and introduction of negative charges at both lysyl and cyteine residues.

$$\text{P}(\text{SH})(\text{NH}_2) + \xrightarrow[\text{pH 8–9}]{\text{Iodoacetate, I—CH}_2\text{—COOH}} \text{P}(\text{S—CH}_2\text{—COOH})(\text{NH—CH—COOH})$$

$$\text{P}(\text{SH})(\text{NH}_2) + \xrightarrow[\text{pH 8–9}]{\text{I—CH}_2\text{—CONH}_2\text{, Iodoacetamide}} \text{P}(\text{S—CH}_2\text{—CONH}_2)(\text{NH—CH}_2\text{—CONH}_2) \quad (5.120)$$

The increase in the electronegativity of the protein may alter the pH-solubility profile of proteins and may also cause unfolding. On the other hand, reaction with iodoacetamide results only in elimination of positive charges. This will also cause a local increase in electronegativity, but the number of negatively charged groups in proteins will remain unchanged. Reaction with iodoacetamide effectively blocks sulfhydryl groups so disulfide-induced protein polymerization cannot occur. Sulfhydryl groups also can be blocked by reaction with NEM.

$$\text{P—SH} + \underset{N\text{-Ethyl}}{\text{maleimide N—C}_2\text{H}_5} \longrightarrow \text{P—S—succinimide N—C}_2\text{H}_5 \quad (5.121)$$

Amino groups can also be reductively alkylated with aldehydes and ketones in the presence of reductants, such as sodium borohydride ($NaBH_4$) or sodium cyanoborohydride ($NaCNBH_3$). In this case, the Schiff base formed by reaction of the carbonyl group with the amino group is subsequently reduced by the reductant. Aliphatic aldehydes and ketones or reducing sugars can be used in this reaction. Reduction of the Schiff base prevents progression of the Maillard reaction, resulting in a glycoprotein as the end product (reductive glycosylation).

$$\text{P—NH}_2 + \underset{\text{Aldehyde}}{\text{R–CHO}} \xrightarrow{\text{Alkaline pH}} \text{P—N=CH—R} \xrightarrow{\text{NaBH}_4} \text{P—NH—CH}_2\text{—R} \quad (5.122)$$

The physicochemical properties of the modified protein will be affected by the reactant used. Hydrophobicity of the protein can be increased if an aliphatic aldehyde or ketone is selected for the reaction, and changing the chain length of the aliphatic group can vary the degree of hydrophobicity. On the other hand, if a reducing sugar is selected as the reactant, then the protein will become more hydrophilic. Since glycoproteins exhibit superior foaming and emulsifying properties (as in the case of ovalbumin), reductive glycosylation of proteins should improve solubility and interfacial properties of proteins.

5.9.1.2 Acylation

The amino groups can be acylated by reacting with several acid anhydrides. The most common acylating agents are acetic anhydride and succinic anhydride. Reaction of protein with acetic anhydride results in elimination of the positive charges of lysyl residues, and a corresponding increase in electronegativity. Acylation with succinic or other dicarboxylic anhydrides results in replacement of positive change with a negative change at lysyl residues. This causes an enormous increase the electronegativity of proteins and causes unfolding of the protein if extensive reaction is allowed to occur.

$$\text{P}{-}\ddot{\text{N}}\text{H}_2 + \underset{\text{Acetic anhydride}}{(\text{CH}_3{-}\text{C}(=\text{O}))_2\text{O}} \xrightarrow{\text{pH} > 7} \text{P}{-}\text{NH}{-}\text{C}(=\text{O}){-}\text{CH}_3 + \text{CH}_3{-}\text{COOH}$$

$$\text{P}{-}\ddot{\text{N}}\text{H}_2 + \underset{\text{Succinic anhydride}}{\text{O}{=}\text{C}{-}\text{CH}_2{-}\text{CH}_2{-}\text{C}{=}\text{O}\ (\text{cyclic, via O})} \xrightarrow{\text{pH} > 7} \text{P}{-}\text{NH}{-}\text{C}(=\text{O}){-}\text{CH}_2{-}\text{CH}_2{-}\text{COOH} \quad (5.123)$$

Acylated proteins are generally more soluble than native proteins. In fact, the solubility of caseins and other less soluble proteins can be increased by acylation with succinic anhydride. However, succinylation, depending on the extent of modification, usually impairs other functional properties. For example, succinylated proteins exhibit poor heat-gelling properties, because of the strong electrostatic repulsive forces. The high affinity of succinylated proteins for water also lessens their adsorptivity at oil–water and air–water interfaces, thus impairing their foaming and emulsifying properties. Also, because several carboxyl groups are introduced, succinylated proteins are more sensitive to calcium-induced precipitation than is the parent protein.

Acetylation and succinylation reactions are irreversible. The succinyl–lysine isopeptide bond is resistant to cleavage catalyzed by pancreatic digestive enzymes. Furthermore, the intestinal mucosa cells poorly absorb succinyl–lysine. Thus, succinylation and acetylation greatly reduce the nutritional value of proteins.

Attaching long chain fatty acids to the ε-amino group of lysyl residues can increase the amphiphilicity of proteins. This can be accomplished by reacting a fatty acylchloride or

$$\text{P}{-}\ddot{\text{N}}\text{H}_2 + \underset{N\text{-hydroxy succinimide ester}}{\text{R}{-}\text{C}(=\text{O}){-}\text{N}(\text{C}(=\text{O}){-}\text{CH}_2{-}\text{CH}_2{-}\text{C}(=\text{O}))} \xrightarrow{\text{pH} > 7} \text{P}{-}\text{NH}{-}\text{C}(=\text{O}){-}\text{R} + \text{HN}(\text{C}(=\text{O}){-}\text{CH}_2{-}\text{CH}_2{-}\text{C}(=\text{O}))$$

$$\text{P}{-}\ddot{\text{N}}\text{H}_2 + \text{Cl}{-}\text{C}(=\text{O}){-}\text{R} \longrightarrow \text{P}{-}\text{NH}{-}\text{C}(=\text{O}){-}\text{R} + \text{HCl} \quad (5.124)$$

N-hydroxy-succinimide ester of a fatty acid with a protein. This type of modification can enhance lipophilicity and fat-binding capacity of proteins and can also facilitate formation of novel micellar structures and other types of protein aggregates.

5.9.1.3 Phosphorylation

Several natural food proteins, such as caseins, are phosphoproteins. Phosphorylated proteins are highly sensitive to calcium-ion-induced coagulation, which may be desirable in simulated cheese-type products. Proteins can be phosphorylated by reacting them with phosphorus oxychloride, $POCl_3$. Phosphorylation occurs mainly at the hydroxyl group of serine and threonine residues and at the amino group of lysyl residues. Phosphorylation greatly increases protein electronegativity. Phosphorylation of amino groups results in addition of two

$$\text{P}(\text{NH}_2)(\text{OH}) + \text{POCl}_3 \longrightarrow \text{P}(\text{NH–PO}_3^{2-})(\text{O–PO}_3^{2-}) \tag{5.125}$$

negative charges for each positive charge eliminated by the modification. Under certain reaction conditions, especially at high protein concentration, phosphorylation with $POCl_3$ can lead to polymerization of proteins as shown in following scheme. Such polymerization reactions tend to minimize the increases in electronegativity and calcium sensitivity of the modified protein. The N−P bond is acid labile. Thus, under the conditions prevailing in the stomach, the *N*-phosphorylated proteins would be expected to undergo dephosphorylation and regeneration of lysyl residues. Thus, the digestibility of lysine is probably not significantly impaired by chemical phosphorylation.

$$\text{P}(\text{NH}_2)(\text{OH}) + \text{POCl}_3 \longrightarrow \text{P}(\text{NH–POCl}_2)(\text{O–POCl}_2) \longrightarrow \text{P}(\text{NH–POCl–O–P–NH}_2)(\text{O–POCl–HN–P–OH}) \longrightarrow \longrightarrow \longrightarrow \text{Polymerization} \tag{5.126}$$

5.9.1.4 Sulfitolysis

Sulfitolysis refers to conversion of disulfide bonds in proteins to *S*-sulfonate derivative using a reduction–oxidation system involving sulfite and copper (Cu^{II}) or other oxidants. The mechanism is shown in the following scheme. Addition of sulfite to protein initially cleaves the

$$\text{P–S–S–P} + \text{SO}_3^{2-} \xrightarrow{\text{Reduction}} \text{P–S–SO}_3^{-} + \text{P–SH} \quad (\text{P–SH} \xrightarrow{\text{Oxidation (Copper)},\ \text{O}_2} \text{P–S–S–P}) \tag{5.127}$$

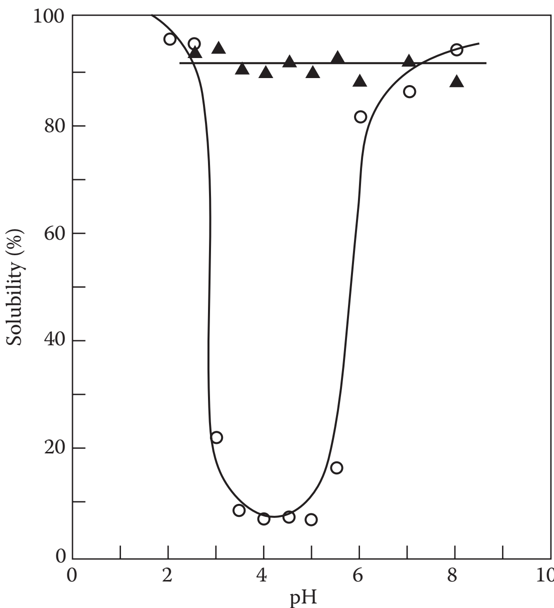


FIGURE 5.39 The pH versus protein solubility profile of (▲) raw sweet whey and (○) sulfonated sweet whey. (From Gonzalez, J.M. and Damodaran, S., *J. Agric. Food Chem.*, 38, 149, 1990.)

disulfide bond, resulting in the formation of one $S{-}SO_3^-$ and one free thiol group. This is a reversible reaction, and the equilibrium constant is small. In the presence of an oxidizing agent, such as copper(II), the newly liberated SH groups are oxidized back to either intra- or intermolecular disulfide bonds, and these, in turn, are again cleaved by sulfite ions present in the reaction mixture. The reduction–oxidation cycle repeats itself until all of the disulfide bonds and sulfhydryl groups are converted to *S*-sulfonate derivative [113].

Cleavage of disulfide bonds and incorporation of SO_3^- groups cause conformational changes in proteins, which affect their functional properties. For example, sulfitolysis of proteins in cheese whey dramatically changes their pH-solubility profiles (Figure 5.39) [114].

5.9.1.5 Esterification

Carboxyl groups of Asp and Glu residues in proteins are not highly reactive. However, under acidic conditions, these residues can be esterified with alcohols. These esters are stable at acid pH, but are readily hydrolyzed at alkaline pH.

5.9.2 Enzymatic Modification

Several enzymatic modifications of proteins/enzymes are known to occur in biological systems. These modifications can be grouped into six general categories, namely, glycosylation, hydroxylation, phosphorylation, methylation, acylation, and cross-linking. Such enzymatic modifications of proteins *in vitro* can be used to improve their functional properties. Although numerous enzymatic modifications of proteins are possible, only a few of them are practical for modifying proteins intended for food use.

5.9.2.1 Enzymatic Hydrolysis

Hydrolysis of food proteins using proteases, such as pepsin, trypsin, chymotrypsin, papain, and thermolysin, alters their functional properties. Extensive hydrolysis by nonspecific proteases,

such as papain, causes solubilization of even poorly soluble proteins. Such hydrolysates usually contain LMW peptides of the order of two to four amino acid residues. Extensive hydrolysis damages several functional properties, such as gelation, foaming, and emulsifying properties. See Section 5.6 for more details.

5.9.2.2 Plastein Reaction

The plastein reaction refers to a set of reactions involving initial proteolysis, followed by resynthesis of peptide bonds by a protease (usually papain or chymotrypsin). The protein substrate, at low concentration, is first partially hydrolyzed by papain. When the hydrolysate containing the enzyme is concentrated (to ~30%–35% solids) and incubated, the enzyme randomly recombines the peptides, generating new peptide bonds [115]. The plastein reaction also can be performed in a one-step process, in which a 30%–35% protein solution (or a paste) is incubated with papain in the presence of L-cysteine. In both cases, however, the molecular weight of the polypeptides formed is typically smaller than the original protein. Thus, the enzyme, especially papain and chymotrypsin, acts both as a protease and an esterase under certain conditions. Since the structure and amino acid sequence of plastein products are different from those of the original protein, they often display altered functional properties. When L-methionine is included in the reaction mixture, it is covalently incorporated into the newly formed polypeptides. Thus, the plastein reaction can be exploited to improve the nutritional quality of methionine or lysine deficient food proteins.

5.9.2.3 Protein Cross-Linking

Transglutaminase catalyses an acyl-transfer reaction that involves reaction between the ε-amino group of lysyl residues (acyl acceptor) and the amide group of glutamine residues (acyl donor), resulting in the formation of an isopeptide cross-link. This

$$\underset{\text{Glutaminyl residue}}{\text{(P)}-(CH_2)_2-\overset{\overset{\displaystyle O}{\|}}{C}-NH_2} + \underset{\text{Lysyl residue}}{H_2N-(CH_2)_4-\text{(P)}} \longrightarrow \text{(P)}-(CH_2)_2-\overset{\overset{\displaystyle O}{\|}}{C}-NH-(CH_2)_4-\text{(P)} + NH_3 \quad (5.128)$$

reaction can be used to cross-link different proteins and to produce new forms of food proteins that might have improved functional properties. At high protein concentration, transglutaminase-catalyzed cross-linking leads to formation of protein gels and protein films at room temperature [116]. This reaction also can be used to improve the nutritional quality of proteins by cross-linking lysine and/or methionine at the glutamine residues.

REFERENCES

1. Böck, A., K. Forchhammer, J. Heider, and C. Baron. 1991. Selenoprotein synthesis: An expansion of the genetic code. *Trends Biochem. Sci.* 16:463–467.
2. Baranov, P. V., R. F. Gesteland, and J. F. Atkins. 2002. Recoding: Translational bifurcations in gene expression. *Gene* 286:187–201.
3. Kryukov, G. V., S. Castellano, S. V. Novoselov, A. V. Lobanov, O. Zehtab, R. Guigó, and V. N. Gladyshev. 2003. Characterization of mammalian selenoproteomes. *Science* 300:1439–1443.
4. Pace, C. N., H. Fu, K. L. Fryar, J. Landua, S. R. Trevino, B. A. Sirley, M. M. Hendricks et al. 2011. Contribution of hydrophobic interactions to protein stability. *J. Mol. Biol.* 408:514–528.
5. Kamtekar, S., J. Schiffer, H. Xiong, J. M. Babik, and M. H. Hecht. 1993. Protein design by binary patterning of polar and nonpolar amino acids. *Science* 262:1680–1685.
6. Mak, A., L. B. Smillie, and G. Stewart. 1980. A comparison of the amino acid sequences of rabbit skeletal muscle and tropomyosins. *J. Biol. Chem.* 255:3647–3655.
7. Damodaran, S. 1988. Refolding of thermally unfolded soy proteins during the cooling regime of the gelation process: Effect on gelation. *J. Agric. Food Chem.* 36:262–269.

8. Pan, K.-M., M. Baldwin, J. Nguyen, M. Gasset, A. Serban, D. Groth, I. Mehlhorn et al. 1993. Conversion of α-helices into β-sheets features in the formation of the crappie prion proteins. *Proc. Natl. Acad. Sci. U.S.A.* 90:10962–10966.
9. Pace, C. N., J. M. Scholtz, and G. R. Grimsley. 2014. Forces stabilizing proteins. *FEBS Lett.* 588:2177–2184.
10. Miller, S., J. Janin, A. M. Lesk, and C. Chothia. 1987. Interior and surface of monomeric proteins. *J. Mol. Biol.* 196:641–656.
11. Graziano, G. 2009a. Dimerization thermodynamics of large hydrophobic plates: A scaled particle theory study. *J. Phys. Chem B.* 113:11232–11239.
12. Graziano, G. 2009b. Role of salts on the strength of pairwise hydrophobic interaction. *Chem. Phys. Lett.* 483:67–71.
13. He, X. M. and D. C. Carter. 1992. Atomic structure and chemistry of human serum albumin. *Nature* 358:209–214.
14. Utsumi, S., Y. Matsumura, and T. Mori. 1997. Structure-function relationships of soy proteins. In *Food Proteins and Their Applications*, S. Damodaran and A. Paraf (Eds.), Marcel Dekker, New York, pp. 257–291.
15. Israelachvili, J. and R. Pashley. 1982. The hydrophobic interaction is long range, decaying exponentially with distance. *Nature* 300:341–342.
16. Richards, F. M. 1977. Areas, volumes, packing, and protein structure. *Annu. Rev. Biophys. Bioeng.* 6:151–176.
17. Graziano, G. 2010. On the molecular origin of cold denaturation of globular proteins. *Phys. Chem. Chem. Phys.* 12:14245–14252.
18. Tanford, C. 1957. Theory of protein titration curves. I. General equations for impenetrable spheres. *J. Am. Chem. Soc.* 79:5333–5339.
19. Caldarelli, G. and P. De Los Rios. 2001. Cold and warm denaturation of proteins. *J. Biol. Phys.* 27(2):229–241.
20. Weber, G. 1992. *Protein Interactions*. Chapman & Hall, New York, pp. 235–270.
21. Ponnuswamy, P. K., R. Muthusamy, and P. Manavalan. 1982. Amino acid composition and thermal stability of proteins. *Int. J. Biol. Macromol.* 4:186–190.
22. Taylor, T. J. and I. I. Vaisman. 2010. Discrimination of thermophilic and mesophilic proteins. *BMC Struct. Biol.* 10(Suppl. 1):S5.
23. Russell, R. J., J. M. Ferguson, D. W. Hough, M. J. Danson, and G. L. Taylor. 1997. The crystal structure of citrate synthase from the hyperthermophilic archaeon pyrococcus furiosus at 1.9 Å resolution. *Biochemistry* 36:9983–9994.
24. Watanabe, K., Y. Hata, H. Kizaki, Y. Katsube, and Y. Suzuki. 1997. The refined crystal structure of *Bacillus cereus* oligo-1,6-glucosidase at 2.0 Å resolution: Structural characterization of proline-substitution sites for protein thermostabilization. *J. Mol. Biol.* 269:142–153.
25. Russell, R. J. M., D. W. Hough, M. J. Danson, and G. L. Taylor. 1994. The crystal structure of citrate synthase from the thermophilic archaeon, *Thermoplasma acidophilum*. *Structure* 2:1157–1167.
26. Bogin, O., M. Peretz, Y. Hacham, Y. Korkhin, F. Frolow, A. J. Kalb, and Y. Burstein. 1998. Enhanced thermal stability of *Clostridium beijerinckii* alcohol dehydrogenase after strategic substitution of amino acid residues with prolines from the homologous thermophilic *Thermoanaerobacter brockii* alcohol dehydrogenase. *Protein Sci.* 7(5):1156–1163.
27. Nakamura, S., T. Tanaka, R. Y. Yada, and S. Nakai. 1997. Improving the thermostability of *Bacillus stearothermophilus* neutral protease by introducing proline into the active site helix. *Protein Eng.* 10:1263–1269.
28. Aguilar, C. F., L. Sanderson, M. Moracci, M. Ciaramella, R. Nucci, M. Rossi, and L. Pearl. 1997. Crystal structure of the-glycosidase from the hyperthermophilic archaeon *Sulfolobus solfataricus. J. Mol. Biol.* 271:789–802.
29. Yip, K. S. P., T. J. Stillman, K. Britton, P. J. Artymium, P. J. Baker, S. E. Sedelnikova, P. C. Engel et al. 1995. The structure of *Pyrococcus furiosus* glutamate dehydrogenase reveals a key role for ion-pair networks in maintaining enzyme stability at extreme temperatures. *Structure* 3:1147–1158.
30. Ahren, T. J. and A. M. Klibanov. 1985. The mechanism of irreversible enzyme inactivation at 100°C. *Science* 228:1280–1284.
31. Wang, C.-H. and S. Damodaran. 1990. Thermal destruction of cysteine and cystine residues of soy protein under conditions of gelation. *J. Food Sci.* 55:1077–1080.
32. Tsukada, H., K. Takano, M. Hattori, T. Yoshida, S. Kanuma, and K. Takashashi. 2006. Effect of sorbed water on the thermal stability of soybean protein. *Biosci. Biotechnol. Biochem.* 70:2096–2103.

33. Damodaran, S. 2012. On the molecular mechanism of stabilization of proteins by cosolvents: Role of Lifshitz electrodynamic forces. *Langmuir* 28:9475–9486.
34. Damodaran, S. 1989. Influence of protein conformation on its adaptability under chaotropic conditions. *Int. J. Biol. Macromol.* 11:2–8.
35. Harwalkar, V. R. and C.-Y. Ma. 1989. Effects of medium composition, preheating, and chemical modification upon thermal behavior of oat globulin and β-lactoglobulin. In *Food Proteins*, J. E. Kinsella and W. G. Soucie (Eds.), American Oil Chemists' Society, Champaign, IL, pp. 210–231.
36. Heremans, K. 1982. High pressure effects on proteins and other biomolecules. *Annu. Rev. Biophys. Bioeng.* 11:1–21.
37. Somkuti, J. and L. Smeller. 2013. High pressure effects on allergen food proteins. *Biophys. Chem.* 183:19–29.
38. Gekko, K. and Y. Hasegawa. 1986. Compressibility-structure relationship of globular proteins. *Biochemistry* 25:6563–6571.
39. Kinsho, T., H. Ueno, R. Hayashi, C. Hashizume, and K. Kimura. 2002. Sub-zero temperature inactivation of carboxypeptidase Y under high hydrostatic pressure. *Eur. J. Biochem.* 269(18):4666–4674.
40. Weber, G. and H. G. Drickamer. 1983. The effect of high pressure upon proteins and other biomolecules. *Q. Rev. Biophys.* 16:89–112.
41. Lado, B. H. and A. E. Yousef. 2002. Alternative food-preservation technologies: Efficacy and mechanisms. *Microbes Infect.* 4(4):433–440.
42. Okomoto, M., Y. Kawamura, and R. Hayashi. 1990. Application of high pressure to food processing: Textural comparison of pressure- and heat-induced gels of food proteins. *Agric. Biol. Chem.* 54:183–189.
43. Buckow, R., A. Sikes, and R. Tume. 2013. Effect of high pressure on physicochemical properties of meat. *Crit. Rev. Food Sci. Nutr.* 53:770–776.
44. Griebenow, K. and A. M. Klibanov. 1996. On protein denaturation in aqueous-organic mixtures but not in pure organic solvents. *J. Am. Chem. Soc.* 118(47):11695–11700.
45. Timasheff, S. N. 1993. The control of protein stability and association by weak interactions with water: How do solvents affect these processes? *Annu. Rev. Biophys. Biomol. Struct.* 22:67–97.
46. Canchi, D. R. and A. E. Garcia. 2013. Cosolvent effects on protein stability. *Annu. Rev. Phys. Chem.* 64:273–293.
47. Timasheff, S. N. 2002. Protein-solvent preferential interactions, protein hydration, and the modulation of biochemical reactions by solvent components. *Proc. Natl. Acad. Sci. U.S.A.* 99:9721–9726.
48. Chalikian, T. V. 2014. Effect of cosolvent on protein stability: A theoretical investigation. *J. Chem. Phys.* 141:22D504–22D5604-9.
49. Schellman, J. A. 2003. Protein stability in mixed solvents: A balance of contact interaction and excluded volume. *Biophys. J.* 85:108–125.
50. Lee, J. C. and S. N. Timasheff. 1981. The stabilization of proteins by sucrose. *J. Biol. Chem.* 256:7193–7201.
51. Damodaran, S. 2013. Electrodynamic pressure modulation of protein stability in cosolvents. *Biochemistry* 52:8363–8373.
52. Taylor, L. S., P. York, A. C. Williams, H. G. M. Edwards, V. Mehta, G. S. Jackson, I. G. Badcoe, and A. R. Clarke. 1995. Sucrose reduces the efficiency of protein denaturation by a chaotropic agent. *Biochim. Biophys. Acta* 1253:39–46.
53. Collins, K. D. 2004. Ions from the Hofmeister series and osmolytes: Effects on proteins in solution and in the crystallization process. *Methods* 34:300–311.
54. Zhang, Y. and Cremer, P. S. 2006. Interactions between macromolecules and ions: The Hofmeister series. *Curr. Opin. Chem. Biol.* 10:658–663.
55. Kuntz, I. D. and W. Kauzmann. 1974. Hydration of proteins and polypeptides. *Adv. Protein Chem.* 28:239–345.
56. Bigelow, C. C. 1967. On the average hydrophobicity of proteins and the relation between it and protein structure. *J. Theoret. Biol.* 16:187–211.
57. Dickinson, E. 1998. Proteins at interfaces and in emulsions: Stability, rheology and interactions. *J. Chem. Soc. Faraday Trans.* 88:2973–2985.
58. Damodaran, S. 2005. Protein stabilization of emulsions and foams. *J. Food Sci.* 70:R54–R66.
59. Razumovsky, L. and S. Damodaran. 1999. Surface activity—Compressibility relationship of proteins. *Langmuir* 15:1392–1399.
60. Pearce, K. N. and J. E. Kinsella. 1978. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *J. Agric. Food Chem.* 26:716–722.

61. Kato, A. and S. Nakai. 1980. Hydrophobicity determined by a fluorescent probe method and its correlation with surface properties of proteins. *Biochim. Biophys. Acta* 624:13–20.
62. Dickinson, E. and Y. Matsummura. 1991. Time-dependent polymerization of β-lactoglobulin through disulphide bonds at the oil-water interface in emulsions. *Int. J. Biol. Macromol.* 13:26–30.
63. Fang, Y. and D. G. Dalgleish. 1996. Competitive adsorption between dioleoylphosphatidylcholine and sodium caseinate on oil-water interfaces. *J. Agric. Food Chem.* 44:59–64.
64. Damodaran, S. and T. Sengupta. 2003. Dynamics of competitive adsorption of s-casein and-casein at the oil-water interface: Evidence for incompatibility of mixing at the interface. *J. Agric. Food Chem.* 51:1658–1665.
65. Anand, K. and S. Damodaran. 1996. Dynamics of exchange betweens1-casein and-casein during adsorption at air-water interface. *J. Agric. Food Chem.* 44:1022–1028.
66. Polyakov, V. L., V. Y. Grinberg, and V. B. Tolstoguzov. 1997. Thermodynamic compatibility of proteins. *Food Hydrocoll.* 11:171–180.
67. Razumovsky, L. and S. Damodaran. 2001. Incompatibility of mixing of proteins in adsorbed binary protein films at the air-water interface. *J. Agric. Food Chem.* 49:3080–3086.
68. Sengupta, T. and S. Damodaran. 2001. Lateral phase separation in adsorbed binary protein films at the air-water interface. *J. Agric. Food Chem.* 49:3087–3091.
69. Nishioka, G. M. and S. Ross. 1981. A new method and apparatus for measuring foam stability. *J. Colloid Interface Sci.* 81:1–7.
70. Yu, M.-A. and S. Damodaran. 1991. Kinetics of protein foam destabilization: Evaluation of a method using bovine serum albumin. *J. Agric. Food Chem.* 39:1555–1562.
71. Zhu, H. and S. Damodaran. 1994. Heat-induced conformational changes in whey protein isolate and its relation to foaming properties. *J. Agric. Food Chem.* 42:846–855.
72. Zhu, H. and S. Damodaran. 1994. Proteose peptones and physical factors affect foaming properties of whey protein isolate. *J. Food Sci.* 59:554–560.
73. Zhu, H. and S. Damodaran. 1994. Effects of calcium and magnesium ions on aggregation of whey protein isolate and its effect on foaming properties. *J. Agric. Food Chem.* 42:856–862.
74. Xu, S. and S. Damodaran. 1993. Comparative adsorption of native and denatured egg-white, human and T4 phage lysozymes at the air-water interface. *J. Colloid Interface Sci.* 159:124–133.
75. Kato, S., Y. Osako, N. Matsudomi, and K. Kobayashi. 1983. Changes in emulsifying and foaming properties of proteins during heat denaturation. *Agric. Biol. Chem.* 47:33–38.
76. Poole, S., S. I. West, and C. L. Walters. 1984. Protein-protein interactions: Their importance in the foaming of heterogeneous protein systems. *J. Sci. Food Agric.* 35:701–711.
77. Damodaran, S. and J. E. Kinsella. 1980. Flavor-protein interactions: Binding of carbonyls to bovine serum albumin: Thermodynamic and conformational effects. *J. Agric. Food Chem.* 28:567–571.
78. Damodaran, S. and J. E. Kinsella. 1981. Interaction of carbonyls with soy protein: Thermodynamic effects. *J. Agric. Food Chem.* 29:1249–1253.
79. Rao, M. A., S. Damodaran, J. E. Kinsella, and H. J. Cooley. 1986. Flow properties of 7S and 11S soy protein fractions. In *Food Engineering and Process Application*s, M. Le Maguer and P. Jelen (Eds.), Elsevier Applied Science, New York, pp. 39–48.
80. Shimada, K. and S. Matsushita. 1980. Relationship between thermo-coagulation of proteins and amino acid compositions. *J. Agric. Food Chem.* 28:413–417.
81. Wang, C.-H. and S. Damodaran. 1990. Thermal gelation of globular proteins: Weight average molecular weight dependence of gel strength. *J. Agric. Food Chem.* 38:1154–1164.
82. Gosal, W. S. and S. B. Ross-Murphy. 2000. Globular protein gelation [Review]. *Curr. Opin. Colloid Interface Sci.* 5(3–4):188–194.
83. Shewry, P. R. and A. S. Tatham. 1997. Disulphide bonds in wheat gluten proteins. *J. Cereal Sci.* 25:207–227.
84. Bock, J. E. and S. Damodaran. 2013. Bran-induced changes in water structure and gluten conformation in model gluten dough studied by Fourier transform infrared spectroscopy. *Food Hydrocoll.* 31:146–155.
85. Bock, J. E., R. K. Connelly, and S. Damodaran. 2013. Impact of bran addition on water properties and gluten secondary structure in wheat flour doughs studied by attenuated total reflectance Fourier transform infrared spectroscopy. *Cereal Chem.* 90:377–386.
86. Van Dijk, A. A., E. De Boef, A. Bekkers, L. L. Van Wijk, E. Van Swieten, R. J. Hamer, and G. T. Robillard. 1997. Structure characterization of the central repetitive domain of high molecular weight gluten proteins. II. Characterization ion solution and in the dry state. *Protein Sci.* 6:649–656.
87. Miles, M. J., H. J. Carr, T. C. McMaster, K. J. I'Anson, P. S. Belton, V. J. Morris, M. Field, P. R. Shewry, and A. S. Tatham. 1991. Scanning tunneling microscopy of a wheat seed storage protein reveals details of an unusual super secondary structure. *Proc. Natl. Acad. Sci. U.S.A.* 88:68–71.

88. Belton, P. S. 1999. On the elasticity of wheat gluten. *J. Cereal Sci.* 29:103–107.
89. Barro, F., L. Rooke, F. Bekes, P. Gras, A. S. Tatham, R. Fido, P. A. Lazzeri, P. R. Shewry, and P. Barcelo. 1997. Transformation of wheat with high molecular weight subunit genes results in improved functional properties. *Nat. Biotechnol.* 15:1295–1299.
90. Bertrand-Harb, C., A. Baday, M. Dalgalarrondo, J. M. Chobert, and T. Haertle. 2002. Thermal modifications of structure and codenaturation of α-lactalbumin and β-lactoglobulin induce changes in solubility and susceptibility to proteases. *Nahrung* 46:283–289.
91. Bonomi, F., A. Fiocchi, H. Frokloiaer, A. Gaiaschi, S. Iametti, P. Rasmussen, P. Restani, and P. Rovere. 2003. Reduction of immunoreactivity of bovine β-lactoglobulin upon combined physical and proteolytic treatment. *J. Dairy Res.* 70:51–59.
92. Mahmoud, M. I., W. T. Malone, and C. T. Cordle. 1992. Enzymatic hydrolysis of casein: Effect of degree of hydrolysis on antigenicity and physical properties. *J. Food Sci.* 57:1223–1227.
93. Kilara, A. and D. Panyam. 2003. Peptides from milk proteins and their properties. *Food Sci. Nutr.* 43:607–633.
94. Adler-Nissen, J. 1986. Relationship of structure to taste of peptides and peptide mixtures. In *Protein Tailoring for Food and Medical Uses*, R. E. Feeney and J. R. Whitaker (Eds.), Marcel Dekker, New York, pp. 97–122.
95. FAO/WHO/UNU. 1985. Energy and protein requirements, Report of a joint FAO/WHO/UNU expert consultation. World Health Organization Technical Report Series 724, WHO, Geneva, Switzerland.
96. FAO/WHO. 1991. Protein Quality Evaluation, Report of a Joint FAO/WHO expert consultation. FAO Food and Nutrition Paper 51, FAO, Geneva, Switzerland, pp. 23–24.
97. Friedman, M. 1996. Nutritional value of proteins from different food sources. A review. *J. Agric. Food Chem.* 44:6–29.
98. Calsamiglla, S. and M. D. Stern. 1995. A three-step in vitro procedure for estimating intestinal digestion of protein in ruminants. *J. Animal Sci.* 73:1459–1465.
99. Ford, J. E. 1981. Microbiological methods for protein quality assessment. In *Protein Quality in Humans: Assessment and In Vitro Estimation*, C. E. Bodwell, J. S. Adkins, and D. T. Hopkins (Eds.), AVI Publishing Co., Westport, CT, pp. 278–305.
100. Vasconcelos, I. M. and J. R. A. Oliveira. 2004. Antinutritional properties of plant lectins. *Toxicon* 44:385–403.
101. Reddy, N. R. and M. D. Pierson. 1994. Reduction in antinutritional and toxic components in plant foods by fermentation. *Food Res. Int.* 27:281–290.
102. Liardon, R. and D. Ledermann. 1986. Racemization kinetics of free and protein-bound amino acids under moderate alkaline treatment. *J. Agric. Food Chem.* 34:557–565.
103. Fay, L., U. Richli, and R. Liardon. 1991. Evidence for the absence of amino acid isomerization in microwave-heated milk and infant formulas. *J. Agric. Food Chem.* 39:1857–1859.
104. Hayase, F., H. Kato, and M. Fujimaki. 1973. Racemization of amino acid residues in proteins during roasting. *Agric. Biol. Chem.* 37:191–192.
105. Cherkin, A. D., J. L. Davis, and M. W. Garman. 1978. D-proline: Stereospecific-sodium chloride dependent lethal convulsant activity in the chick. *Pharmacol. Biochem. Behav.* 8:623–625.
106. Chen, C., A. M. Pearson, and J. I. Gray. 1990. Meat mutagens. *Adv. Food Nutr. Res.* 34:387–449.
107. Kizil, M., Oz, F., and Besier, H. T. 2011. A review on the formation of carcinogenic/mutagenic heterocyclic aromatic amines. *J. Food Process Technol.* 2:120.
108. Stadtman, E. R. and R. L. Levine. 2003. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids* 25:207–218.
109. Rosario, M., M. Domingues, P. Domingues, A. Reis, C. Fonseca, F. M. L. Amado, and J. V. Ferrer-Correia. 2003. Identification of oxidation products and free radicals of tryptophan by mass spectrometry. *J. Am. Soc. Mass Spectrom.* 14:406–416.
110. Wedzicha, B. L., I. Bellion, and S. J. Goddard. 1991. Inhibition of browning by sulfites. In *Nutritional and Toxicological Consequences of Food Process*ing, M. Friedman (Ed.), Advances in Experimental Medicine and Biology, Vol. 289, Plenum Press, New York, pp. 217–236.
111. Somoza, V. 2005. Five years of research on health risks and benefits of Maillard reaction products: An update. *Mol. Nutr. Food Res.* 49:663–672.
112. Yilmaz, Y. and R. Toledo. 2005. Antioxidant activity of water-soluble Maillard reaction products. *Food Chem.* 93:273–278.
113. Gonzalez, J. M. and S. Damodaran. 1990. Sulfitolysis of disulfide bonds in proteins using a solid state copper carbonate catalyst. *J. Agric. Food Chem.* 38:149–153.

114. Gonzalez, J. M. and S. Damodaran. 1990. Recovery of proteins from raw sweet whey using a solid state sulfitolysis. *J. Food Sci.* 55:1559–1563.
115. Gong, M., A. Mohan, A. Gibson, and C. C. Udenigwe. 2015. Mechanisms of plastein formation, and prospective food and nutraceutical applications of the peptide aggregates. *Biotechnol. Rep.* 5:63–69.
116. Kuraishi, C., K. Yamazaki, and Y. Susa. 2001. Transglutaminase: Its utilization in the food industry. *Food Rev. Int.* 17:221–246.
117. Fitch, C. A., G. Platzer, M. Okon, B. Garcia-Moreno, and L. P. McIntosh. 2015. Arginine: Its pK_a value revisited. *Protein Sci.* 24:752–761.
118. Lesser, G. J. and G. D. Ross. 1990. Hydrophobicity of amino acid subgroups in proteins. *Proteins: Struct. Funct. Genet.* 8:6–13.
119. Fauchere, J. L. and Pliska, V. 1983. Hydrophobic parameters—pi of amino acid side-chains from the partitioning of N-acetyl-aminoacid amides. *Eur. J. Med. Chem.* 18:369–375.
120. Bull, H. B. and K. Breese. 1973. Thermal stability of proteins. *Arch. Biochem. Biophys.* 158:681–686.
121. Kinsella, J. E., S. Damodaran, and J. B. German. 1985. Physicochemical and functional properties of oilseed proteins with emphasis on soy proteins. In *New Protein Foods: Seed Storage Proteins*, A. M. Altshul and H. L. Wilcke (Eds.), Academic Press, London, U.K., pp. 107–179.
122. Kuntz, I. D. 1971. Hydration of macromolecules. III. Hydration of polypeptides. *J. Am. Chem. Soc.* 93:514–516.
123. O'Neill, T. E. and J. E. Kinsella. 1987. Binding of alkanone flavors to β-lactoglobulin: Effects of conformational and chemical modification. *J. Agric. Food Chem.* 35:770–774.
124. MacRitchie, F. and D. Lafiandra. 1997. Structure-function relationships of wheat proteins. In *Food Proteins and Their Applications*, S. Damodaran and A. Paraf (Eds.), Marcel Dekker, New York, pp. 293–324.
125. Eggum, B. O. and R. M. Beames. 1983. The nutritive value of seed proteins. In *Seed Proteins*, W. Gottschalk and H. P. Muller (Eds.), Nijhoff/Junk, The Hague, the Netherlands, pp. 499–531.
126. Swaisgood, H. E. and G. L. Catignani. 1991. Protein digestibility: In vitro methods of assessment. *Adv. Food Nutr. Res.* 35:185–236.
127. Lawrence, M. C., E. Suzuki, J. N. Varghese, P. C. Davis, A. Van Donkelaar, P. A. Tulloch, and P. M. Colman. 1990. The three-dimensional structure of the seed storage protein phaseolin at 3 Å resolution. *EMBO J.* 9:9–15.
128. Papiz, M. Z., L. Sawyer, E. E. Eliopoulos, A. C. T. North, J. B. C. Findlay, R. Sivaprasadarao, T. A. Jones, M. E. Newcomer, and P. J. Kraulis. 1986. The structure of-lactoglobulin and its similarity to plasma retinol-binding protein. *Nature* 324:383–385.
129. Scheraga, H. A. 1963. Intramolecular bonds in proteins. II. Noncovalent bonds. In *The Proteins*, H. Neurath (Ed.), 2nd edn., Vol. 1, Academic Press, New York, pp. 478–594.
130. Chen, B. and J. A. Schellman. 1989. Low-temperature unfolding of a mutant of phage T4 lysozyme. 1. Equilibrium studies. *Biochemistry* 28:685–691.
131. Lapanje, S. 1978. *Physicochemical Aspects of Protein Denaturation*. Wiley-Interscience, New York.
132. Creighton, T. E. 1993. *Proteins: Structures and Molecular Properties*. W.H. Freeman & Co., New York, pp. 158–159.
133. Rupley, J. A., P.-H. Yang, and G. Tollin. 1980. Thermodynamic and related studies of water interacting with proteins. In *Water in Polymers*, S. P. Rowland (Ed.), ACS Symposium Series 127, American Chemical Society, Washington, DC, pp. 91–139.
134. Adler-Nissen, J. 1979. Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *J. Agric. Food Chem.* 27:1256–1260.
135. Friedman, M. and M. R. Gumbmann. 1986. Nutritional improvement of legume proteins through disulfide interchange. *Adv. Exp. Med. Biol.* 199:357–390.
136. Yon, J. M. 2001. Protein folding: A perspective for biology, medicine and biotechnology. *Braz. J. Med. Biol. Res.* 34:419–435.
137. Sadi-Carnot. 2015. Energy landscape. *Encyclopedia of Human Thermodynamics, Human Chemistry, and Human Physics*. www.eoht.info/page/Energy+landscape.

6 Enzymes

Kirk L. Parkin

CONTENTS

6.1 Introduction 358
6.2 General Nature of Enzymes 359
6.2.1 Enzymes as Biocatalysts 359
6.2.2 Protein and Nonprotein Nature of Enzymes 359
6.2.3 Catalytic Power of Enzymes 360
6.2.3.1 Collision Theory for Reaction Catalysis 362
6.2.3.2 Transition-State Theory of Enzyme Catalysis 362
6.2.4 Mechanisms of Enzyme Catalysis 364
6.2.4.1 General Nature of Enzyme Active Sites 364
6.2.4.2 Specific Catalytic Mechanisms 365
6.2.5 Kinetics of Enzyme Reactions 373
6.2.5.1 Simple Models for Enzyme Reactions 374
6.2.5.2 Rate Expressions for Enzyme Reactions 375
6.2.5.3 Graphical Analysis of Enzyme Reactions 377
6.2.6 Specificity and Selectivity of Enzyme Action 381
6.2.6.1 Specificity Patterns of Selected Food Enzymes 382
6.2.6.2 Nomenclature and Classification of Enzymes 387
6.3 Uses of Exogenous Enzymes in Foods 389
6.3.1 General Considerations 389
6.3.2 Carbohydrate-Transforming Enzymes 389
6.3.2.1 Starch-Transforming Enzymes 391
6.3.2.2 Sugar Transformation and Applications 398
6.3.2.3 Enzymic Pectin Transformation 401
6.3.2.4 Other Glycosidases 405
6.3.3 Enzymes Transforming Proteins 405
6.3.3.1 Serine Proteases 406
6.3.3.2 Aspartic (Acid) Proteases 406
6.3.3.3 Cysteine (Sulfhydryl) Proteases 407
6.3.3.4 Metalloproteases 408
6.3.3.5 Applications of Proteolytic Action 408
6.3.3.6 Transglutaminase 412
6.3.4 Lipid-Transforming Enzymes 413
6.3.4.1 Lipase 413
6.3.4.2 Lipase Applications 414
6.3.4.3 Lipoxygenases 416
6.3.4.4 Phospholipases 417
6.3.5 Miscellaneous Enzyme Applications 417
6.4 Environmental Influence on Enzyme Action 417
6.4.1 Temperature 418
6.4.1.1 General Responses of Enzyme Action to Temperature 418
6.4.1.2 Optimum Temperature for Enzyme Function 419
6.4.1.3 Summary of Temperature Effects 421

6.4.2 pH Effects 421
6.4.2.1 General Considerations 421
6.4.2.2 Enzyme Stability as a Function of pH 421
6.4.2.3 Effects of pH on Enzyme Activity 422
6.4.2.4 Other Types of pH Behavior 427
6.4.3 Water Relations and Enzyme Activity 428
6.4.3.1 Desiccation and Water Activity Effects 428
6.4.3.2 Osmotic Effects of Desiccation 430
6.4.3.3 Desiccation by Freezing 431
6.4.4 Nonthermal Processing Techniques 433
6.5 Enzymes Endogenous to Foods and Their Control 434
6.5.1 Cellular and Tissue Effects 434
6.5.2 Enzyme Activities Related to Color Quality of Foods 436
6.5.2.1 Phenol Oxidases 436
6.5.2.2 Peroxidases 441
6.5.2.3 Other Oxidoreductases 444
6.5.3 Enzymes Related to Flavor Biogenesis 444
6.5.3.1 Lipoxygenase 444
6.5.3.2 Hydroperoxide Lyase and Related Enzyme Transformations 448
6.5.3.3 Biogenesis of Other Lipid-Derived Flavors 450
6.5.3.4 Origin and Control of Pungent Flavors and Other Bioactive Effects 451
6.5.4 Enzymes Affecting Textural Quality in Foods 455
6.5.4.1 Control of Enzymes Modifying Carbohydrate Polymers 456
6.5.4.2 Control of Enzymes Modifying Proteins 456
6.5.4.3 Mitigation of Texture Defects Using Small Molecules to Control Enzymes 457
References 458
Bibliography 464

6.1 INTRODUCTION

During the 1600s–1800s, the actions of enzymes in living or respiring tissues were referred to as "ferments." Examples representing early food enzymology include alcoholic fermentations of yeast, digestive processes in animals, and malting of grains to evoke "diastatic" activity, causing a conversion of starch into sugar. The term "enzyme" was coined by W. Kühne in 1878 from the Greek term *enzyme*, which translates to "in yeast."

Food enzymes can be generally classified into two categories: those that are added to foods (exogenous sources) to cause a desirable change and those that exist in foods (endogenous sources) and that may or may not be responsible for reactions that affect food quality. Exogenous enzymes can be obtained from a variety of sources, and choices among exogenous enzymes are based on cost and functionality. Appropriate functionality relates to catalytic activity, selectivity, and stability under the conditions that prevail during the specific application. Endogenous enzymes pose greater challenges to control, since they are present in the food matrix at a range of levels, and there are constraints as to how the foodstuff can be handled to modulate enzyme action. In some foods, endogenous enzymes may be responsible for reactions that either improve food quality or detract from it. The goal of this chapter is to provide the chemical basis for understanding how enzymes function and how this understanding can be used to control the action of enzymes for the purposes of transforming foods, producing food ingredients, and maintaining, enhancing, and monitoring food quality.

6.2 GENERAL NATURE OF ENZYMES

6.2.1 Enzymes as Biocatalysts

Enzymes possess three important traits: they are proteins and catalysts, they exhibit selectivity toward substrates, and they are subject to regulation. Enzymes are the most common and ubiquitous form of biological catalysts. They are responsible for life processes and mediate synthetic, turnover, cell signaling, and metabolic functions. The only other known naturally occurring biological catalyst is catalytic RNA or "ribozymes," which are involved in RNA modification and linking of amino acids during protein synthesis (translation). Antibodies can be developed as catalysts when raised against a hapten bearing a transition-state analogue of a desired substrate.

6.2.2 Protein and Nonprotein Nature of Enzymes [30,43,94]

All enzymes are proteins that range in molecular mass from ~8 kDa (about 70 amino acids, e.g., some thioredoxins and glutaredoxins) to 4600 kDa (pyruvate decarboxylase complex). The largest enzymes are comprised of multiple polypeptide chains or subunits and possess quaternary structure. These subunits most often associate through common noncovalent forces (see Chapter 5), and these associations may involve identical (homologous) or dissimilar (heterologous) polypeptide chains. Oligomeric enzymes may possess multiple active sites, and some large enzymes can be comprised of several catalytic activities on a single polypeptide chain. In the latter case, such as the fatty acid synthetase complex of higher organisms, different activities are associated with different protein domains that exist on the polypeptide, and these large polypeptides can associate further as dimers or oligomers. Monomeric enzymes with a single active site can also have different domains within the polypeptide chain, each with a different function related to catalysis or other biological properties.

Some enzymes require nonprotein components called "cofactors," "coenzymes," or "prosthetic groups" to carry out their catalytic function [112]. Most common cofactors include metal ions (metalloenzymes), flavins (flavoenzymes), biotin, lipoate, many of the B vitamins, and nicotinamide derivatives (which are really cosubstrates that are bound tightly and undergo reversible redox reactions). Enzymes replete with an essential cofactor are called "holoenzymes," while those void of an essential cofactor are called "apoenzymes" and lack catalytic function. Other nonprotein components of enzymes include bound lipid (lipoprotein), carbohydrate (at ASN,* glycoprotein), or phosphate (at SER, phosphoprotein), and while these constituents typically do not have a role in catalysis, they do impact physicochemical properties and confer cellular recognition sites for the enzyme. Enzymes synthesized as latent precursors are referred to as "zymogens" and require proteolytic processing to potentiate their activity (such as digestive enzymes and calf chymosin).

Enzymes existing as monomeric proteins (single polypeptide chain) commonly have molecular masses in the range of 13–50 kDa. The majority of cellular enzymes range in mass between 30 and 50 kDa, and oligomeric enzymes typically range from 80 to 100 kDa being comprised of subunits of 20–60 kDa; only ~1%–3% cellular protein is >240 kDa [130]. Oligomeric enzymes are often involved in metabolic processes in the host organism, and the presence of subunits allows for multiple dimensions of regulation by cellular metabolites, allosteric behavior (subunit cooperativity), and interaction with other cellular components or structures.

* Identification of amino acid residues in enzymes will be made using the commonly recognized three-letter codes (uppercase). Position in the protein primary sequence, where appropriate, is indicated by subscripts.

Extracellular and secreted enzymes tend to be smaller and monomeric polypeptides, often with hydrolytic activities and generally greater stabilities relative to intracellular enzymes. These hydrolytic extracellular enzymes help with mobilizing or assimilating nutrients and growth factors from the environment where the (micro)organism would otherwise have little control over factors such as temperature, pH, and composition. Many of the exogenous enzymes used in foods are derived from microorganisms where they can be produced quickly on a large scale by isolation from the fermentation broth. However, enzymes can also be extracted from plant or animal sources and such extracts may be favored in some food applications. Microbial sources of enzymes remain an area of great interest because strain selection and molecular techniques can be used to rapidly select for or modify specific enzyme traits required for certain food processes.

An enzyme can exist as multiple forms that differ slightly in the primary sequence but possess nearly identical catalytic function. These slight differences in sequence may manifest as subtle or even profound differences in substrate/product selectivity and characteristic pH and temperature optima. Such entities are referred to as enzyme "isoforms" (less contemporary terms include isozymes and isoenzymes).

Based on the current wealth of knowledge of protein structure and sequence, enzymes are taxonomically grouped as "families," with members sharing common catalytic function and structural features (with structural elements taking on interesting names such as barrels, propellers, Greek keys, and jelly rolls—terms one may be more likely to hear at a fraternity party than in a discussion of proteins and enzymes). This grouping relates to evolutionary origin and fate. Knowledge of peptide sequence is instrumental to relating enzymes on the basis of similarity in primary structure (homology), and the presence of peptide sequences that are "conserved" as "motifs" helps identify or confirm the existence of the putative active site in mechanistically related enzymes. Understanding how protein structure relates to catalytic function provides the foundation of efforts to improve enzyme use in foods.

6.2.3 Catalytic Power of Enzymes [30,43,54,151]

Catalysts are agents that accelerate the rate of reactions without themselves undergoing any net chemical modification. Catalysts function by reducing the energy barrier required for the transformation of a reactant into a product. This is best illustrated with the use of a hypothetical "reaction coordinate," depicting the free energy change associated with a reaction to yield a product (P) (Figure 6.1). In catalyzed reactions, the substrate (S) is elevated to a transition state ($S^{\ddagger}$) at a reduced expense of free energy ($\Delta G^{\ddagger}_{cat}$) relative to the uncatalyzed reaction ($\Delta G^{\ddagger}_{uncat}$). Figure 6.1 is a simplification since there may be multiple intermediate states in a reaction coordinate. However, there is usually a single, critical or rate-limiting step, possessing either the greatest magnitude or degree of change of $+G$, which generally governs the overall rate for any chemical process. Reactions with a net decrease in free energy ($-\Delta G_{net}$) are favorable, but this does not indicate how *fast* the reaction will go. The reaction rate is dictated thermodynamically by $\Delta G^{\ddagger}$. Examples of the catalytic power of selected enzymes are summarized in Table 6.1.

Terminology relating to enzyme catalysis has been standardized for the purpose of avoiding ambiguity and arbitrary descriptors [3]. One international unit (U) of enzyme activity causes the conversion of 1 μmol substrate per minute under standardized (usually optimized) conditions. The SI unit for enzyme activity is the *katal*, defined as the amount of enzyme causing 1 mol substrate conversion per second under defined conditions. Molecular activity of enzymes is defined as a "turnover number" (k_{cat}), or the number of substrate molecules that can be converted by one enzyme molecule (active site) per minute under defined conditions. The upper limit of k_{cat} observed for enzymes is ~10^7.

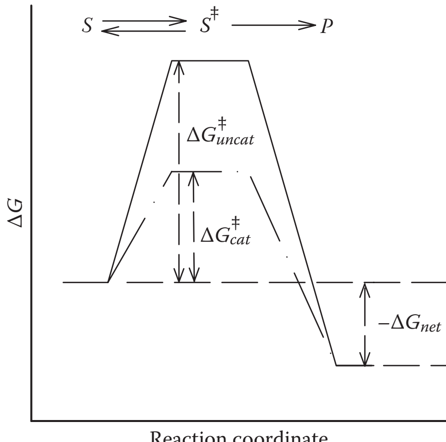


FIGURE 6.1 Comparative reaction coordinates of catalyzed and uncatalyzed reactions.

TABLE 6.1
Examples of Catalytic Power of Enzymes

Reaction	Catalyst	Free Energy of Activation (kcal/mol)	Relative Reaction Rate[a]
$H_2O_2 \rightarrow ½O_2 + H_2O$	None (aqueous)	18.0	1.0
	Iodide	13.5	2.1×10^3
	Platinum	11.7	4.2×10^4
	Catalase (1.11.1.6)	5.5	1.5×10^9
p-Nitrophenyl acetate hydrolysis	None (aqueous)	21.9	1.0
	H^+	18.0	7.2×10^2
	OH^-	16.2	1.5×10^4
	Imidazole	15.9	2.5×10^4
	Serum albumin[b]	15.3	6.9×10^4
	Lipoprotein lipase	11.4	5.0×10^7
Sucrose hydrolysis	H^+	25.6	1.0
	Invertase (3.2.1.26)	11.0	5.1×10^{10}
Urea + $H_2O \rightarrow CO_2 + 2NH_3$	H^+	24.5	1.0
	Urease (3.5.1.5)	8.7	4.2×10^{11}
Casein hydrolysis	H^+	20.6	1.0
	Trypsin (3.4.4.4)	12.0	12.0×10^6
Ethyl butyrate hydrolysis	H^+	13.2	1.0
	Lipase (3.1.1.3)	4.2	4.0×10^6

Sources: O'Connor, C.J. and Longbottom, J.R., *J. Colloid Interface Sci.*, 112, 504, 1986; Sakurai, Y. et al., *Pharm. Res.*, 21, 285, 2004; Whitaker, J.R., Voragen, A.G.J., and D.W.S. Wong (Eds.), *Handbook of Food Enzymology*, Marcel Dekker, New York, 2003.

[a] Relative rates are calculated from $e^{-E_a/RT}$ (Equation 6.1) at 25°C.

[b] Not considered an enzyme reaction.

6.2.3.1 Collision Theory for Reaction Catalysis

There are two approaches to quantitatively account for rates of chemical reactions (kinetics) and catalysis. The simplest one is the *collision theory*, which is expressed as

$$k = PZe^{-E_a/RT} \tag{6.1}$$

where
- k is the reaction rate constant
- P is the probability of reaction (includes molecular orientation as a factor)
- Z is the collision frequency, and the exponential term relates to the proportion of colliding reactants having sufficient energy of activation (E_a) to allow reaction
- R is the gas constant
- T is the temperature

The most important factor dictating reaction rates as a function of temperature in this equation is the exponential term, as a 10°C increase yields only a ~4% increase in "Z," but a 100% increase (doubling) of the $e^{-E_a/RT}$ term if E_a is 12 kcal/mol. E_a of enzyme reactions often ranges 6–15 kcal/mol [122]. The relationship depicted in Equation 6.1 was developed empirically by S. Arrhenius in the late 1800s and finds the greatest utility in integrated form where enzyme response to temperature can be quantitatively assessed (Section 6.4.1).

6.2.3.2 Transition-State Theory of Enzyme Catalysis

Another and mechanistically more meaningful approach to accounting for rates of enzyme reactions is based on the transition-state theory of absolute reaction rates. This theory is largely attributed to H. Eyring (1930s) and is based on the premise that for a reaction of a substrate (S) to product (P) to occur, ground state S must reach an *activated* or transition state ($S^‡$), upon which it becomes *committed* to forming P (Figure 6.1). The distribution of S and $S^‡$ is characterized by a pseudo-equilibrium constant ($K^‡$) as

$$K^‡ = \frac{S^‡}{S} \tag{6.2}$$

and the rate of reaction or decomposition of $S^‡$ to P is characterized as

$$\frac{dP}{dt} = k_d[S^‡] \tag{6.3}$$

where k_d is the first-order rate constant for the decay of $S^‡$ to P. The important thermodynamic parameter is the activation free energy change ($\Delta G^‡$) between S and $S^‡$:

$$\Delta G^‡ = -RT \ln K^‡ \tag{6.4}$$

Combining equivalencies from Equations 6.2 and 6.4 yields

$$[S^‡] = [S]e^{-\Delta G^‡/RT} \tag{6.5}$$

The rate constant k_d (Equation 6.3) is equivalent to the vibrational frequency (v) of the bond undergoing transformation. This is based on the assumption that a molecule in the transition state is so

weakened that decay will occur with the next bond vibration [54]. Decay of $S^‡$ occurs when the bond vibrational energy is equal to the potential energy, and the relationship becomes

$$k_d = \nu = \frac{k_B T}{h} \tag{6.6}$$

where
k_B is the Boltzmann constant
h is Planck's constant

Thus, the theory holds that all transition rates decompose at the same rate and the reaction rate is only influenced by [S], temperature, and the characteristic $\Delta G^‡$ (which defines $K^‡$, Equation 6.4) for an enzyme reaction with a specific S. After substituting for k_d from Equation 6.6 and for $S^‡$ from Equation 6.5, the rate Equation 6.3 now becomes

$$\text{Rate} = \frac{dP}{dt} = k_S[S] = \frac{k_B T}{h} \times [S]^{-\Delta G^‡/RT} \tag{6.7}$$

Thus, over a range of fixed [S], the reaction rate and rate constant $k_S \left(k_S\left[S\right] = k_B T/h \exp^{-\Delta G‡/RT}\right)$ can be experimentally determined, and then $\Delta G^‡$ can be calculated. Once $\Delta G^‡$ is determined, the equation can be rearranged to permit the calculation of the thermodynamic entities, $\Delta H^‡$ and $\Delta S^‡$.

If one knows the reduction in activation free energy that is afforded by a catalyst, one can quantify or predict the extent to which the reaction is accelerated, based on the collision theory (Equation 6.1) or transition-state theory (Equation 6.7) since the result will be the same and is conferred by the exponential free energy term. For example, if an enzyme reduces the energy of activation ($G^‡$ or E_a) of a chemical reaction by 5.4 kcal/mol, which is quite modest, then the relative rate of the enzyme reaction is accelerated by a factor of 250,000 over the noncatalyzed reaction.

The power of the transition-state theory lies in its simplicity in explaining how enzymes function mechanistically, how they evolve to become more efficient catalysts, and how enzymes are distinguished from antibodies (both selectivities recognize ligands). In the context of enzyme catalysis, free substrate (S) must first bind to free enzyme (E) to yield an association complex that is distributed between ground state (ES) and activated state (ES‡). The role of enzyme is to reduce the $\Delta G^‡$, and hence enhance $K^‡$, or the steady-state proportion of S as the activated species $S^‡$, compared to an uncatalyzed reaction. This is indicated for catalysis in general in Figure 6.1, but some key features of enzyme catalysis by transition-state stabilization are better illustrated in a modified reaction coordinate (Figure 6.2a). The association of E and S to form ES has a characteristic free energy of binding (ΔG_S) (often negative for single substrate reactions). Regardless of the magnitude of ΔG_S, this association provides for favorable interactions between E and S, referred to simply as "binding energy," which may be used to facilitate catalysis (Section 6.2.4.2). The next step in catalysis is elevation of S to transition state as ES‡ (all of which forms P and free E). This step is thermodynamically represented as $\Delta G^‡$. The minimum net activation free energy change for reaction to occur (for free $S \rightarrow P$) is ΔG_T. ΔG_T is the sum of the free energies of the individual binding (ΔG_S) and catalytic ($\Delta G^‡$) steps. Using this diagram, it becomes easy to see where the catalytic advantage lies for enzymes as they evolve to recognize substrates. If the enzyme-binding site for substrate evolves *only* in a manner to better recognize (become more complementary to) the ground state of S, affinity between E and S will improve and binding becomes more favorable (more negative ΔG_S; Figure 6.2b). The consequence is no change in ΔG_T, but an increase in $\Delta G^‡$ and a larger energy barrier must be overcome for the step ES → ES‡. Alternatively, if the *only* change in enzyme–substrate recognition is that the binding site becomes more complementary to the structure represented by $S^‡$, then the free energy for both the net reaction (ΔG_T) and the

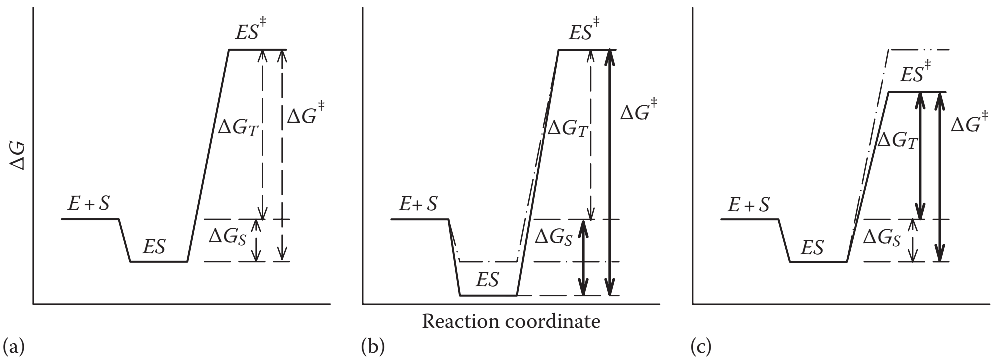


FIGURE 6.2 Reaction coordinate of enzyme reaction and evolutionary advantage. (a) Typical enzyme reaction. (b) Consequence of enzyme evolving to become more complementary to ground state of substrate (*S*). (c) Consequence of enzyme evolving to be complementary to transitions state form of *S*. Bold arrows denote where changes in Δ*G* are evident relative to panel (a). (Adapted from Fersht, A., *Enzyme Structure and Mechanism*, 2nd edn., W.H. Freeman & Company, New York, 1985.)

bond-making/breaking step ($ΔG^‡$) is reduced (Figure 6.2c). It should be clear that the advantage lies in the enzyme recognizing or stabilizing the transition-state form of *S*.*

6.2.4 Mechanisms of Enzyme Catalysis [30,43,151]

On the molecular level, enzymes possess active sites that bind *S* and stabilize $S^‡$. Amino acid residues that form the active site and any required cofactors collectively interact with substrate *via* covalent and/or noncovalent interactions. Enzymes may use a number of mechanisms to catalyze bond-making/breaking and atomic rearrangement processes, and the ability to do this is founded on the specific amino acids and their spatial arrangement within the active site. Aside from the amino acids *essential* for catalysis, other amino acids may *assist* in catalysis through *S* recognition and stabilizing $S^‡$.

6.2.4.1 General Nature of Enzyme Active Sites

Certain amino acids of enzymes are responsible for catalytic activity. Considering the size of proteins, it may seem surprising that only a limited number of amino acids, typically ranging from 3 to 20, are responsible for catalytic function [130], with the number being somewhat proportional to the size of the enzyme. On the other hand, the group of enzymes known as the serine proteases range in size from 185 to 800 amino acid residues, corresponding to 20–90 kDa (most are 25–35 kDa), but contain the same catalytic unit (triad) of HIS-ASP-SER. These comparisons illustrate that enzymes contain many more amino acid residues than are required for catalytic activity, and this prompted the question, "why are enzymes so big?" [130] The catalytic amino acid residues of enzymes are rarely proximal to each other in the primary sequence and are distributed throughout the polypeptide chain. For example, the catalytic triad is HIS_{64}-ASP_{32}-SER_{221} for the *Bacillus subtilis* protease subtilisin and HIS_{257}-ASP_{203}-SER_{144} for the *Rhizomucor miehei* lipase (serine proteases and lipases are mechanistically related) [23,63]. Thus, one function of the noncatalytic portions of the polypeptide chain is to bring the catalytic residues into the same three-dimensional space by virtue of the protein's secondary and tertiary structure. The precise spatial arrangement of the catalytic residues allows them to function as a catalytic unit, and polypeptide folding also brings together other residues contributing binding forces to afford substrate recognition. Thus, polypeptide conformation

* *Note: S* becomes converted to, or stabilized as, $S^‡$ upon binding, through utilization of some of the binding energy and the mechanistic forces involved in enzyme catalysis.

acts as a "scaffold" to correctly position, within a three-dimensional space, the amino acid residues with catalytic and substrate-recognizing functions.

Another role of the polypeptide chain is to provide for close packing of atoms, such that water is largely excluded within the enzyme interior [43]. Limiting water to 25% of the protein volume allows for interior cavities and clefts to form that are relatively nonpolar and devoid of water, and this can enhance dipole forces in facilitating catalysis. Other noncatalytic amino acid residues may participate in overall enzyme functioning by serving as cofactor or affector binding sites and surface recognition sites for interaction with other cellular components or to attract/trap substrate [43,130]. Finally, amino acids not involved in catalysis or substrate recognition may dictate sensitivity of the protein conformation to environmental factors such as pH, ionic strength, and temperature, such that they modulate enzyme activity and confer overall enzyme stability.

6.2.4.2 Specific Catalytic Mechanisms

Mechanisms for how enzymes function as catalysts can be reduced to about four general categories [30,54]. These are approximation, covalent catalysis, general acid–base catalysis, and molecular strain or distortion (Table 6.2). Other forces that contribute to catalysis will be identified where appropriate.

6.2.4.2.1 Role of Binding Energy

Before describing each of the major enzyme mechanisms, it is necessary to expand on the role of binding energy, which was introduced in Section 6.2.3.2, as it contributes to all of the mechanisms described hence. Binding energy is the term used to refer to the favorable interactions derived upon association of substrate and enzyme at the binding site [30,43,151]; binding energy is derived from the complementary features existing between enzyme and substrate. Complementarity (geometric and electronic) may be "preformed" (founded on the old "lock and key" concept of enzyme–substrate recognition advanced by E. Fischer), or be "developed" upon binding, or be a combination of the two. Net binding energy is also defined as the free energy change (often negative) resulting from the desolvation of substrate in exchange for interaction with enzyme. The entropy loss due to enzyme–substrate association is offset by entropy gained by solvent (usually water). Some of this binding energy may be used for productive purposes in catalysis, by being converted to mechanical

TABLE 6.2
Common Mechanisms of Enzyme Catalysis

Mechanism	Forces Involved	Residues and Cofactors Potentially Involved
Approximation	Modeled as intra- vs. intermolecular catalysis	Active site and substrate-recognizing residues
Covalent catalysis	Nucleophilic	SER, THR, TYR, CYS, HIS (base), LYS (base), ASP^-, GLU^-
	Electrophilic	LYS (Schiff base), pyridoxal, thiamine, metals (cations)
General acid–base catalysis	Proton association/dissociation, charge stabilization	HIS, ASP, GLU, CYS, TYR, LYS
Conformational distortion	Induced fit, induced strain, rack mechanism, conformational flexibility	Active site and substrate-recognizing residues

Sources: Copeland, R.A., *Enzymes: A Practical Introduction to Structure, Function, Mechanism, and Data Analysis*, 2nd edn., John Wiley, New York, 2000; Saier, M.H., *Enzyme in Metabolic Pathways: A Comparative Study of Mechanism, Structure, Evolution and Control*, Harper & Row, New York, 1987; Walsh, C., *Enzymatic Reaction Mechanisms*, W.H. Freeman & Company, San Francisco, CA, 1979.

and/or chemical activation energy. It can be used to mobilize S at the active site, or destabilize S, or stabilize $S^{‡}$. The ability of an enzyme to react faster with one substrate over another (defined as "selectivity") may be directly related to how much binding energy can be used to facilitate the catalytic step. Catalytically nonessential amino acid residues at/near the active site often assist catalysis through the use of binding energy.

6.2.4.2.2 Approximation

Approximation is best described as the catalytic units and substrate being proximal to each other in a favorable orientation, facilitating reactivity. Another way to envision the catalytic power of approximation is that since the reactants are localized in the same space within the enzyme active site, their *effective molarity* is greatly enhanced relative to solution concentrations. This mechanism offers the *entropic contribution* to catalysis as it helps overcome the large decrease in entropy otherwise necessary to bring together all participants in a reaction. Thus, the contribution of approximation effects to catalysis is often modeled by effective (enhanced) concentrations in the context of mass action effects on reaction rates.

The lifetime of intermolecular associations between reactants colliding in solution is typically 6 orders of magnitude shorter than that of a complex formed by typical binding of substrate to enzyme [151]. The enzyme-binding pocket affords the "docking" or "anchoring" of substrate at the active site in a water-diminished environment. The longer lifetime of interaction would by itself lead to greater probability of reaching the transition state. Thus, approximation can also be modeled as an *intra*molecular reaction, where all reactants are viewed as existing *within* a single molecule (the enzyme), compared to an *inter*molecular reaction.

The net catalytic effect of approximation is based on rather theoretical calculations but is viewed as yielding up to a 10^4–10^{15} rate enhancement over a chemical reaction involving one to three substrates (greater enhancement for multiple substrate reactions) [151,154]. Approximation is a mechanistic feature that is not conferred by specific amino acids, but rather by the chemical and physical nature of the active site and the constellation of amino acids that comprise it (Table 6.2).

6.2.4.2.3 Covalent Catalysis

Covalent catalysis involves the formation of an enzyme–substrate or cofactor–substrate covalent intermediate, and this mechanism of catalysis is initiated by nucleophilic or electrophilic attack. *(Nucleophilic and electrophilic behavior of enzyme residues/cofactors may also be involved in noncovalent mechanisms.)* Nucleophilic centers are rich in electrons, sometimes negatively charged, and they seek electron-deficient centers (nuclei) with which to react, such as carbonyl carbons, or phosphoryl or glycosyl functional groups. Electrophilic catalysis involves the withdrawal of electrons from reaction centers by electrophiles, also referred to as electron "sinks." While covalent catalysis involves both nucleophilic and electrophilic groups among the reactants, the classification of the reaction is based on which center is initiating the reaction.

Implicit with the formation of a covalent intermediate is the existence of at least two steps along the reaction coordinate, namely, the formation and breakdown of the covalent adduct (Enz-Nu-P_2), each with a characteristic $\Delta G^{‡}$ (Figure 6.3). The multiple stages of catalysis also reflect the presence of multiple enzyme forms, posing a kinetically more complicated reaction coordinate than is depicted in Figure 6.1. Covalent catalysis is common to many classes of enzymes, including the serine and thiol proteases, lipases and carboxylesterases, and many glycosyl hydrolases. The net catalytic effect of covalent catalysis is estimated as yielding up to a 10^2–10^3 rate enhancement over a chemical reaction.

6.2.4.2.3.1 Nucleophilic Catalysis Amino acid residues of enzymes that provide nucleophilic centers are listed in Table 6.2. Generally, nucleophilicity is dependent on basicity of the functional group, which relates to the ability to donate an electron pair to a proton [30,43]. Thus, the nucleophilic rate constant is correlated positively with the pK_a for structurally related compounds

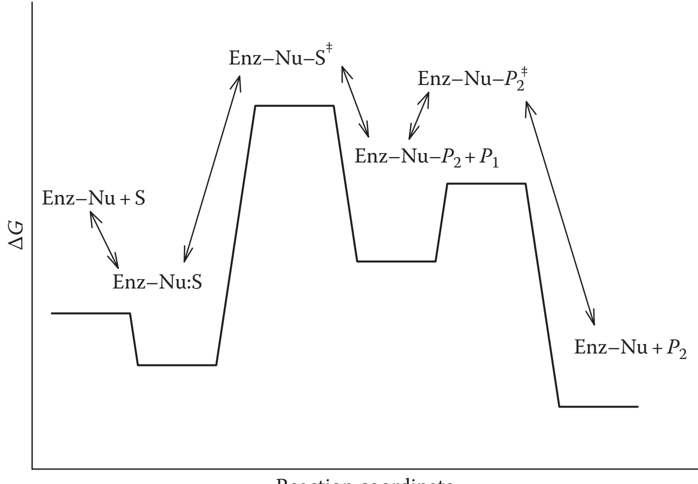


FIGURE 6.3 Reaction coordinate for enzyme reaction by nucleophilic catalysis with covalent intermediate. ENZ-Nu, enzyme with nucleophilic catalytic group; *S*, substrate; P_x, products.

(greater pK_as yield greater reaction rates). However, nucleophilic groups in enzymes typically function over a limited range of pH (often at pH near 7) that is conducive to maintaining enzyme conformational stability. Thus, while ARG can potentially function as a nucleophile, its side chain pK_a value of ~12 dictates it would exist almost exclusively as the conjugate acid form in enzymes under virtually all natural conditions, which explains why it is not listed in Table 6.2. One other factor that impacts the rate of nucleophilic catalysis is the nature of the "leaving group" or the products formed during formation of the covalent intermediate (P_1 in Figure 6.3). The weaker the basicity (lesser pK_a value) of the leaving group, the greater the rate of reaction for a given nucleophile.

The catalytic triad HIS-ASP(GLU)-SER, characteristic of the serine protease and lipase/carboxylesterase families of enzymes, is one of the most studied examples of nucleophilic catalysis. These enzymes catalyze the hydrolysis of amide (peptide) and ester bonds, respectively, *via* a covalent intermediate. The functioning of the HIS-ASP-SER catalytic unit is a classic example of nucleophilic catalysis as a reaction mechanism, but several other mechanistic forces are involved during the course of enzymic catalysis. For the catalytic triad of subtilisin (*B. subtilis* protease, EC 3.4.21.62), SER_{221} acts as a nucleophile, donating electrons to the amide carbon of the peptide bond (Figure 6.4) [23,24]. The nucleophilicity of the SER_{221} oxygen atom is enhanced by HIS_{64} acting as a general base to accept a proton, and the neighboring ASP_{32} residue stabilizes the developing charge on HIS_{64}. This results in the formation of the transient tetrahedral acyl-enzyme intermediate. In the last stage, HIS_{64} acts as a general acid to donate a proton to the N-terminal peptide fragment of the cleaved peptide leaving group, and the covalent acyl-enzyme adduct is formed. Although not shown in this Figure, the completion of the catalytic cycle is achieved when water, acting as a terminal nucleophile, displaces the peptide fragment from SER_{221}, by forming another tetrahedral intermediate using the same catalytic machinery as just described. The ASN_{155} residue is less critical to catalysis but functions to stabilize the developing tetrahedral intermediate (an "oxyanion") within a space in the enzyme referred to as the "oxyanion hole."

The behavior of subtilisin mutants (where specific amino acid residues are replaced by others, using molecular techniques) reveals the importance of the amino acids comprising the triad. The native enzyme has a catalytic efficiency (indexed as k_{cat}/K_M, explained in Section 6.2.5.3) of 1.4×10^5 (Table 6.3). If either of the SER_{221}, HIS_{64}, or ASP_{32} residues is replaced by ALA, catalytic efficiency is reduced by about 10^4–10^6. When any two or all three of these residues are replaced with ALA, little or no further compromise in catalytic efficiency is observed, showing that the three amino

FIGURE 6.4 Reaction mechanism of serine proteases. Substrate peptide backbone in bold. P_1 and P_1' groups denote the side chains of amino acid comprising the respective N- and C-terminal sides of the scissile bond. (Adapted from Carter, P. and Wells, J.A., *Nature*, 332, 564, 1988; Carter, P. and Wells, J.A., *Protein Struct. Funct. Genet.*, 7, 335, 1990.)

TABLE 6.3
Effect of Point Mutations on Catalytic Constants of Subtilisin Protease

Enzyme	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M (s^{-1} M^{-1})
Wild type	6.3×10^1	440	6.3×10^5
$SER_{221} \rightarrow$ ALA	5.4×10^{-5}	650	8.4×10^{-2}
$HIS_{64} \rightarrow$ ALA	1.9×10^{-4}	1300	1.5×10^{-1}
$ASP_{32} \rightarrow$ ALA	1.8×10^{-2}	1400	1.3×10^1
All three mutations	7.8×10^{-3}	730	1.1×10^{-1}

Sources: Carter, P. and Wells, J.A., *Nature*, 332, 564, 1988; Carter, P. and Wells, J.A., *Protein Struct. Funct. Genet.*, 7, 335, 1990.

acid residues act as a *unit*, rather than making accretive contributions to catalytic power. These same amino acid residues make up the catalytic triad of lipases (and most carboxylesterases). For lipases, the same sequence of events as depicted in Figure 6.4 takes place, except that the substrate is an ester (R–CO–OR′), where the acyl group (R–CO–) goes on to form the same acyl-enzyme intermediate, while the liberated alcohol (R′OH) constitutes the leaving group. The catalytic triad of HIS-ASP(GLU)-SER is a highly conserved catalytic unit for lipases and carboxylesterases, whereas proteases may work by any of four distinct catalytic mechanisms (Section 6.3.3). Three carboxylesterases that use alternative catalytic units and mechanisms include secretory phospholipase A_2 (pancreatic, bee and snake venom; HIS/ASP dyad), potato lipid acyl hydrolase (ASP/SER dyad), and pectin methyl esterase (ASP/ASP dyad).

6.2.4.2.3.2 Electrophilic Catalysis Electrophilic catalysis constitutes another type of covalent mechanism, where the characteristic step in the reaction coordinate is electrophilic attack. Amino acid residues in enzymes do not provide adequate electrophilic groups. Instead, electrophiles are drawn from electron-deficient cofactors or a cationic nitrogen derivative formed between substrate and the enzyme catalytic residues to initiate electrophilic catalysis (Table 6.2).

Some of the best characterized enzyme reactions evoking electrophilic catalysis employ pyridoxal phosphate (an essential vitamin nutrient, B_6, Chapter 7) as a cofactor; many such enzymes

FIGURE 6.5 General reaction mechanism of pyridoxal-containing enzymes. (a) Initial steps of transaldimination and removal of α-H atom. (b) Relationship of α-C configuration to types of reactions catalyzed. (Adapted from Fersht, A., *Enzyme Structure and Mechanism*, 2nd edn., W.H. Freeman & Company, New York, 1985; Tyoshimura, T. et al., *Biosci. Biotech. Biochem.*, 60, 181, 1996.)

are involved in amino acid transformation/metabolism [43,140]. A general mechanism of pyridoxal-enzyme reactions involves transfer (transaldimination) of a Schiff base (−C=N−) linked pyridoxal group from an enzyme-LYS residue to a reactive amino acid bound at the enzyme active site (Figure 6.5a). The Schiff base intermediate is stabilized by the pyridine ring, which acts as an electron sink. A residue on the enzyme then acts as a base (B:) to absorb the proton liberated from the substrate as a common first step in the reaction pathway. The substituent group about the chiral center (−R, −H, $-COO^-$) to be cleaved ("lysed") or transferred is conferred by which α-C substituent group is perpendicular to the plane of the pyridinium intermediate, as it has the lowest E_a for transformation/removal (Figure 6.5b).

Some of the active site features shared by many pyridoxal enzymes are illustrated with alliin lyase (EC 4.4.1.4, *S*-alk(en)yl-L-cysteine sulfoxide [ACSO] lyase) action on ACSO (Figure 6.6). This enzyme is commonly referred to as alliinase and is responsible for the generation of pungent flavors of *Allium* vegetables (onion, garlic, leek, chive, etc.) upon initial disruption or cutting of fresh tissues. For the garlic enzyme, LYS_{251} (LYS_{285} in onion; LYS_{280} in chive) coordinates with the pyridoxal cofactor, aided by the "phosphate-binding cup" and additional residues that bind with the pyridinium N and hydroxyl groups [69]. Substrate coordinates with other enzyme residues (ARG_{401}, SER_{63} and GLY_{64} amide, and TYR_{92}) to confer enzyme (stereo) selectivity toward the (+) *S*-alkyl-L-cysteine sulfoxides. Alliinase causes β-cleavage of the substrate, yielding the sulfenic acid (R−S−OH, a good leaving group).

6.2.4.2.4 General Acid–Base Catalysis

Most enzyme reactions involve proton transfer at some point during catalysis and this is often accomplished by amino acid residues that act as general acids to donate a proton and general bases to accept a proton. General acid–base catalysis provides for transfer of protons at the active site as the substrate(s) transitions to products during the catalytic cycle. This can be distinguished from specific acid–base catalysis that requires H^+ or ^-OH derived from the solvent to diffuse to the active site. Amino acid residues that can function as general acids or bases typically have pK_a values in the

FIGURE 6.6 Active site of garlic alliinase. Backbone of *S*-alkyl-L-cysteine sulfoxide substrate in bold. (Adapted from Kuettner, E.B. et al., *J. Biol. Chem.*, 277, 46402, 2002.)

range of the pH optimum for enzyme activity and stability (generally pH 4–10), and such residues appear in Table 6.2. Recall that general acid–base behavior contributes to the nucleophilic mechanism of serine proteases, lipases, and carboxylesterases (Figure 6.4). Indeed, HIS is a residue often involved in general acid–base catalysis, because the pK_a of the imidazole group within proteins is usually in the range of 6–8, making it ideal for functioning as *either an acid or base* under conditions where many enzymes are active.

An example of general acid–base catalysis is found in lysozyme (EC 3.2.1.17, mucopeptide *N*-acetylmuramyl hydrolase, also called muramidase), an enzyme occurring in saliva, tear duct secretion, and hen's egg white. The mechanism evoked by lysozyme applies to glycosyl hydrolases in general (Section 6.3.2), which include the starch, sugar, and pectin hydrolyzing enzymes [126]. Lysozyme may be used as a bacteriocidal agent in foods since it hydrolyzes the peptidoglycan heteropolymers of prokaryotic cell walls (especially of Gram-positive microorganisms, which include many food pathogens). Best illustrated at the near-optimum pH ~5, the mechanism of action relies on the general acid–base nature of active site amino acids GLU_{35} and ASP_{52} [33,126,154].

(6.8)

The proton of GLU_{35} acts as a general acid and donates a proton to the oxygen atom of the scissile glycosidic bond; ASP_{52} carboxylate serves to electrostatically stabilize the developing carboxenium

ion of the substrate by acting as a base.* The incoming water needed to complete hydrolysis (not shown) is partially ionized by the GLU_{35} carboxylate group, to activate the addition of −OH (from water) to C1 of the original glycoside, with H^+ acquired by GLU_{35} to fully cycle the enzyme active site. The exclusion of water and an abundance of hydrophobic residues at the active site cleft of the enzyme create a nonpolar environment proximal to the GLU_{35} residue, rendering it less capable of ionizing and conferring an abnormally high pK_a of 6.1. This allows it to function as a general acid catalyst at pH 5. The relative lack of water to shield charges also allows for fixed dipoles to emerge between the catalytic residues and the developing intermediate. This serves to reduce E_a by ≥9 kcal/mol (corresponding to a rate enhancement of >10^6) relative to the uncatalyzed reaction in water [33].

An example of proton/electron transfer reactions (common in metalloenzymes) in enzymes is found in xylose isomerase (E.C. 5.3.1.5, D-xylose ketol-isomerase), also referred to as glucose isomerase. This enzyme catalyzes an equilibrium reaction between aldose and ketose isomers. Almost all xylose isomerases characterized are homotetramers, yielding two active sites each with a cation cofactor (commonly Mg^{2+}; also Mn^{2+}, Co^{2+}) [154]. A conserved active site sequence (*Streptomyces* spp. enzyme) includes residues binding the cations ($GLU_{180,216}$, $ASP_{244,254,256,286}$, HIS_{219}) and others lining the active site (HIS_{53}, PHE_{93}, TRP_{135}, LYS_{182}, GLU_{185}) [126]. The active site is bifurcated with highly polar and hydrophobic areas (especially TRP_{135}), the latter serving to exclude water. This enzyme has historically been cited as an example of general acid–base catalysis, but a more contemporary view is that it catalyzes a hydride transfer reaction. The specific steps in the reaction sequence include ring opening, rate-limiting hydrogen transfer step, and ring closure [48,49]. Of the two Mg^{2+} ions, Mg_s is structural and coordinates with O2 and O4 of the sugar substrate, and Mg_c is catalytic (Figure 6.7). After ring opening (not shown), $^-$OH is generated from water by ASP_{254} carboxylate acting as a general base to remove an H^+. A proton from O2 is transferred to $^-$OH bound to Mg_c, after which Mg_c is then drawn to the negatively charged O2 (Mg_c actually *moves*) to stabilize the transition state, and this is assisted by H-bonding between LYS_{182} and O1. This movement of Mg_c is synchronous with the transfer of the hydride ($^-$H:) from C2 to C1. This is an equilibrium reaction, and hydride transfer can be reversed by essentially the same steps with the Mg_c: $^-$OH coordinate serving to shuttle H^+ from O1 to O2 alkoxides to facilitate hydride transfer from C1 to C2.

Reaction rate enhancements of 10^2–10^3 are typically contributed by general acid–base catalysis, where the pushing or pulling of electrons is required along the reaction coordinate. In the example of lysozyme, the greater overall rate enhancement is based on other factors (electrostatic stabilization, substrate strain) contributing to catalysis.

FIGURE 6.7 Reaction mechanism of xylose (glucose) isomerase. (Adapted from Garcia-Viloca, M. et al., *J. Am. Chem. Soc.*, 124, 7268, 2002; Garcia-Viloca, M. et al., *Science*, 303, 186, 2004.)

* Many glycosyl hydrolases, including lysozyme, are classified as examples of nucleophilic catalysis because a covalent intermediate is formed [126] although not shown in Equation 6.8. The ASP_{52} carboxylate is a good nucleophile (Table 6.2). Glycosyl hydrolase mechanisms are fully explained in Section 6.3.2.

6.2.4.2.5 Strain and Distortion

This mechanistic explanation is founded on the premise that interacting domains of substrates and enzymes are not as rigid as implied by the *lock and key* conceptualization of enzyme catalysis advanced by E. Fischer in 1894. Distortion or strain as a factor in governing catalysis was offered by J. B. S. Haldane and L. Pauling, as it related to the transition-state theory of enzyme catalysis. Thus, while there is structural and electronic complementarity between enzyme and substrate to provide for attractive forces, this complementarity is not "perfect." If *preformed* complementarity was *perfect*, catalysis would less likely take place because of the large energy barrier required to reach a transition state (recall Figure 6.2b).

Some preformed complementarity between enzyme- and substrate-binding sites provides for substrate recognition, the acquisition of binding energy, and helps orient substrate at the active site. The productive utilization of binding energy arising from enzyme–substrate association may manifest as inducing stress/strain on the enzyme and/or substrate, allowing for complementarity to be further *developed* toward a transition state. The effects on substrate are unlikely to involve bond stretching, twisting, or bending of bond angles because of the large forces estimated to be required for such events [43]. Rather, strain on the substrate more likely occurs as restriction in bond rotational freedom, steric compression, and electrostatic repulsion between enzyme and substrate. Thus, in a true physical sense, substrate may be subjected to *stress* (where distortion does not occur) upon binding to enzyme in a manner that the relief of that stress through utilization of some binding energy helps promote the transition state. An example of this is found in the mechanism of lysozyme, where the transition-state carboxenium ion of the pyranose derivative (Equation 6.8) assumes a half-chair ("sofa") instead of the more stable full-chair conformation.

Enzymes as proteins are considered to possess more flexible structures than small (in)organic substrates. In contrast to preformed complementarity, protein conformational flexibility provides the basis for the "induced fit" hypothesis for enzyme catalysis originally advanced by D. Koshland. Here, conformational perturbations in the enzyme active site upon substrate binding are viewed as facilitating the stabilization of the $ES^{\ddagger}$ complex. In doing so, the conformational modulation in the enzyme active site *upon binding of substrate* may help align reactive groups of both enzyme and substrate to facilitate catalysis.

One example of an induced fit mechanism of catalysis is the surface activation of lipases, where a protein domain constituting a "lid" covering the active site undergoes a conformation shift to allow the fatty acid ester substrate to gain access to the active site and undergo hydrolysis. A more subtle molecular motion in enzymes involves the movement of Mg_c in xylose isomerase just described (Figure 6.7), the estimated acceleration of reaction rate from which is about 10^4 [49]. A third example of induced fit is papain, a sulfhydryl protease, where sterically induced strain upon binding of substrate is relieved upon formation of a tetrahedral intermediate; specificity and mechanism of papain are featured later in Sections 6.2.6 and 6.3.3. It is becoming apparent that many, if not most, enzymes evoke induced fit to some degree during catalytic function. While estimates of the net catalytic effect of strain are difficult to quantify, the extent of rate acceleration ranges 10^2–10^4.

6.2.4.2.6 Other Enzyme Mechanisms

Redox enzymes (oxidoreductases) catalyze electron transfer reactions by cycling between redox states of prosthetic groups. Prosthetic groups can be transition metals (iron or copper) or cofactors such as flavins (nicotinamides, like NAD(P)H, are cosubstrates in redox reactions). Lipoxygenase (linoleate–oxygen oxidoreductase; EC 1.13.11.12) is widely distributed in plants and animals and possesses nonheme iron as the prosthetic group. It is reactive with fatty acids having a 1,4-pentadiene group of polyunsaturated fatty acids (there may be multiple such groups in fatty acids), represented by linoleic acid ($18{:}2_{9c,12c}$). Lipoxygenases initiate oxidative degradation of fatty acids into products, which can collectively impart either undesirable (rancid) or desirable flavors, and they can also bleach pigments through secondary reactions. Lipoxygenase is often isolated from host tissues in the "inactive" $Fe^{(II)}$ state (Figure 6.8). Activation occurs by reaction with a peroxide (there are low

FIGURE 6.8 Reaction mechanism of lipoxygenase. (Adapted from Brash, A.R., *J. Biol. Chem.*, 274, 23679, 1999; Casey, R. and Hughes, R.K., *Food Biotechnol.*, 18, 135, 2004; Sinnott, M. (Ed.), *Comprehensive Biological Catalysis. A Mechanistic Reference*, Vol. III, Academic Press, San Diego, CA, 1998.)

levels of peroxides existing in all biological tissues), yielding the activated HO-$Fe^{(III)}$ complex with the coordinated hydroxyl group serving as the base to abstract an H atom (through a process called "tunneling"*) from the methylenic carbon (these C–H bonds have the lowest bond energy in fatty acids). The free radical adduct is resonance stabilized, and O_2 adds to the alkyl radical at permitted sites at the opposite side of the substrate from Fe (see later discussion on specificity, Section 6.2.6). The resulting peroxyl radical abstracts an H atom from the inactive water–Fe(II) prosthetic group to afford the fatty acid hydroperoxide product (13-*S*-linoleic acid hydroperoxide for the major soybean lipoxygenase) and cycle the enzyme back to an active state.

Metallo- and electrostatic catalysis is often identified as a discrete catalytic mechanism. The author has chosen to illustrate these mechanistic features as a part of the catalytic behavior of other enzymes described in this chapter, including lipoxygenase, xylose isomerase, carboxypeptidase A, and thermolysin.

6.2.4.2.7 Net Effects on Enzyme Catalysis

The net effect of bringing various combinations of mechanisms to bear on enzyme catalysis is estimated to deliver as much as 10^{17}–10^{19} in reaction rate enhancement over uncatalyzed reactions [49,105,154]. Most of this enhancement is by transition-state stabilization (reduction of activation energy) and a small contribution may derive from the process of tunneling, particularly in hydrogen transfer steps.

6.2.5 Kinetics of Enzyme Reactions

The mechanisms of enzyme catalysis just described accounts for the chemistry of substrate transformation, but they do little to characterize the kinetics of enzyme reactions (*how fast they go*).

* Tunneling is a mechanism (modeled as a transmission coefficient) to describe *H* transfer when less energy is required than expected (a shortcut or tunnel is "dug" under the energy barrier). This may involve *H* transfer in two inseparable parts, first the nuclei followed by the electron [49].

Since enzymes are used to hasten reactions in a manner to improve and/or add value to foods, knowledge of how fast enzyme reactions can proceed is a critical factor in deciding if and when an enzyme process should be used. Since enzymes are also selective, knowing *how much more selective* an enzyme is for one substrate over another, or relative to a nonenzymic reaction, may also be a critical factor in governing the choice of using an enzyme process. Rates of any reaction, enzymic or not, depend on intrinsic kinetic factors (related to activation energies; Figures 6.1 and 6.2) and the concentrations of reactants and catalyst (mass action effects). Since concentrations may vary between reaction conditions, it is most valid to compare relative catalytic power on the basis of intrinsic factors such as kinetic constants. If reaction *rate constants* are known for a set of environmental conditions, then *reaction rates can be predicted* for any combination of reactant and catalyst concentrations under those general conditions.

6.2.5.1 Simple Models for Enzyme Reactions [31,122]

Enzymes are fairly unique in the type of kinetics they exhibit. Consider the simplest enzyme reaction, the rapid equilibrium model known as Michaelis–Menten kinetics. Here an enzyme (E) acts upon a single substrate (S) to form a single association complex (ES) (sometimes called the Michaelis complex) that yields a single product (P):

$$E + S \underset{k_1}{\overset{k_{-1}}{\rightleftarrows}} ES \underset{k_{cat}}{\rightarrow} E + P \tag{6.9}$$

The binding of S to E is assumed to represent equilibrium conditions between the association ($E + S \rightarrow ES$) and dissociation steps ($ES \rightarrow E + S$), each with a respective and characteristic second-order (k_1) and first-order (k_{-1}) rate constant. Biochemical convention is to represent binding equilibria as dissociation processes, and thus, the equilibrium condition for the substrate-binding step is expressed as

$$\frac{[E]\times[S]}{[ES]} = \frac{k_{-1}}{k_1} = K_S \quad \text{(the dissociation or affinity constant)} \tag{6.10}$$

Note that a decreasing value of K_S indicates that a greater proportion of enzyme exists in the ES form and a greater binding or affinity exists between E and S. The second stage of the enzyme reaction is the catalytic step of $ES \rightarrow E + P$, characterized by the first-order catalytic rate constant, k_{cat}. Thus, the initial rate or velocity (v) of an enzyme reaction can be represented as

$$v = \frac{dP}{dt} = k_{cat}[ES] \tag{6.11}$$

and the rate of P formation in this model is viewed as not disturbing the binding equilibria between E and S, hence the reference to the rapid equilibrium model for enzyme kinetics.

An alternative kinetic approach assumes that the rate of decomposition of ES to form P can influence the proportion or distribution of enzyme between the free E and ES states. To reconcile this, it can be assumed that over a brief period of time that a reaction is observed, the [ES] does not change or changes negligibly (this is referred to as the steady-state approach, developed by G. Briggs and J. Haldane). Under this scenario:

$$\frac{d[ES]}{dt} \approx 0 \tag{6.12}$$

Thus, the rate of formation of *ES* is equivalent to the rate of disappearance of *ES*. Since *ES* formation comes from binding of *S* with *E* (the k_1 step) and the disappearance of *ES* is accounted for by the sum of the processes of *ES* dissociation (the k_{-1} and k_{cat} steps)

$$k_1\,[E] \times [S] = (k_{-1} + k_{cat})\,[ES] \tag{6.13}$$

This equation can be rearranged as a dissociation process to

$$\frac{[E]\times[S]}{[ES]} = \frac{(k_{-1}+k_{cat})}{k_1} = K_M, \quad \text{the Michaelis constant} \tag{6.14}$$

This equation is similar to Equation 6.10, except that it allows for [*ES*] to be dictated by both the dissociation and catalytic pathways. Also key to the relationship between K_S (Equation 6.10) and K_M (Equation 6.14) is the relative magnitude of k_{-1} and k_{cat}. If k_{cat} is a couple of orders of magnitude or so less than k_{-1}, then k_{cat} can be ignored and the distribution of enzyme between *E* and *ES* is dictated *only* by the binding equilibrium, rendering K_M equivalent to K_S. If on the other hand, k_{cat} is within an order of magnitude or so of k_{-1}, then the predicted binding equilibrium distribution of enzyme between *E* and *ES* will never be reached, because the k_{cat} step is sufficiently fast to deplete *ES* to less than equilibrium levels. Thus, in this case, $K_M \neq K_S$ and K_M does not simply indicate affinity. Enzymes behaving in this manner are considered to conform to steady-state kinetic models. K_M is referred to as a pseudo-dissociation constant for *ES*, and it has the units of molarity (*M*), as does *S* (and K_S). This allows K_M and [*S*] to be directly compared, since they have the same units, and the utility in this relationship will be shown later. In cases when $k_{cat} \gg k_{-1}$, $k_{cat}/K_M = k_1$, which means the reaction is rate limited by the association step. Since association rate constants for enzymes are often ~10^7–10^8 s^{-1} M^{-1}, the existence of steady-state conditions can be diagnosed by the estimated k_{cat}/K_M values being 10^6–10^8 s^{-1} M^{-1} [43,151]. Many oxidation–reduction and isomerizing enzymes exhibit steady-state kinetics, while most (but not all) hydrolytic enzymes do not (thus, for most hydrolytic enzymes $K_M \approx K_s$, and K_M *is* usually a measure of affinity).

6.2.5.2 Rate Expressions for Enzyme Reactions

Enzyme reaction rate expressions can be devised by taking the ratios of two equivalencies: the velocity expression (Equation 6.11) and an expression for the conservation of total enzyme (E_T):

$$\frac{v}{[E_T]} = \frac{k_{cat}\times[ES]}{\big([E]+[ES]\big)} \tag{6.15}$$

The equation is greatly simplified if enzyme species are expressed only in the form [*ES*], which can be done by rearranging Equation 6.14 as $[E] = (K_M \times [ES])/[S]$ and substituting for [*E*] in Equation 6.15. If one considers that the fastest an enzyme reaction can proceed ($V_{\max}$) is when all enzyme is in the *ES* form, then

$$V_{\max} = k_{cat} \times [E_T] \tag{6.16}$$

Equation 6.15 now simplifies to

$$v = \frac{V_{\max}\times[S]}{\big(K_M+[S]\big)} \tag{6.17}$$

This becomes a very powerful relationship in many ways. Since V_{max} and K_M are constants, this equation takes on the form of

$$y = \frac{ax}{(b + x)} \quad (6.18)$$

This equation, where a and b are constants, is defined as a rectangular hyperbola, and simple enzyme kinetics are often referred to as hyperbolic kinetics. Equation 6.17 also helps illustrate how enzyme reaction rates are dependent of substrate, and at low $[S]$, $K_M \gg [S]$, and

$$v = \frac{V_{max} \times [S]}{K_M} \quad (6.19)$$

Thus, when S is at limiting concentrations toward infinite dilution, the rate of the reaction is characterized by the combined constant V_{max}/K_M, the reaction is first order with respect to S, and the enzyme reaction at dilute $[S]$ is depicted as

$$E + S \xrightarrow{V_{max}/K_M} E + P \quad (6.20)$$

This model corresponds to the ability of an enzyme to recognize and then transform a substrate at a dilute state, and this provides for a measure of "catalytic efficiency," which is quantified by the constant V_{max}/K_M (also called the "specificity constant"). Quantitative comparisons of enzyme selectivity toward multiple substrates, based on V_{max}/K_M values, allow inferences to be made as to how the enzyme recognizes substrates (Section 6.2.6). Since V_{max}/K_M are constants, the comparison of selectivity constants is valid at all levels of $[S]$ among competing substrates. At the other extreme, if $[S] \gg K_M$, then Equation 6.17 simplifies to

$$v = V_{max} \quad (6.21)$$

It should be obvious that the reaction rate is zero order with respect to $[S]$, and under this condition all enzyme is *saturated* with substrate, such that the enzyme reaction can be modeled simply as

$$ES \xrightarrow{k_{cat}} E + P \quad (6.22)$$

The importance of this situation is that the reaction rate is dependent only on $[E_T]$ (recall Equation 6.16), and this condition is important to satisfy if one wishes to develop an assay to quantify how much enzyme activity is present, such as the case when enzyme activity is used as indicators of processing efficacy.

There may be cases when enzyme reactions do not conform to conventional Michaelis–Menten, because either the model does not apply or the ability to fit experimental data to the model is obscured by other factors in play (e.g., S inhibition, endogenous inhibitor in S, multiple enzymes causing the same reaction). These and other complexities may be reconciled by more advanced techniques [31,122]. In any case, the use of terms like K_M is reserved only for situations where Michaelis–Menten behavior is validated; otherwise, terms such as $S_{0.5}$ and $K_{0.5}$ are recommended as analogous terms.

Other kinetic models and relationships applied less frequently to enzyme systems in foods will not be discussed in this chapter. However, they are important to identify and include bisubstrate reactions with a compulsory or random order of addition of substrates and/or products, equilibrium reactions, and allosteric enzymes [31,122].

6.2.5.3 Graphical Analysis of Enzyme Reactions

Between the extreme cases of infinite concentration (saturation) and infinite dilution of S, it is easy to predict enzyme reaction rates if one knows the relative values of V_{max}, K_M, and S; the latter two have units of molarity, such that S can be expressed as multiples of K_M (xK_M). And if v is expressed as a proportion of V_{max} (divide both sides of Equation 6.17 by V_{max}), the enzyme reaction rate expression simplifies to

$$\frac{v}{V_{max}} = \frac{xK_M}{\left(K_M + xK_M\right)} \tag{6.23}$$

If one substitutes a series of values (1, 2, 3, etc., and 0.5, 0.33, 0.2, etc.) for "x" in Equation 6.23, one can construct a typical enzyme kinetic relationship as a function of [S] or [S]/K_M, which yields a rectangular hyperbola (Figure 6.9; one asymptote is V_{max}, while the other is at an biologically irrelevant S/K_M value of −1). This figure shows how the reaction is first order with respect to [S] with a slope of the tangent drawn toward infinite dilution of [S] equivalent to V_{max}/K_M as predicted by Equation 6.19. The reaction approaches zero order as [S] increases and enzyme saturation is approached. Furthermore, such a plot can be constructed after V_{max} and K_M are determined for an enzyme reaction, and there should be good fit between observed and predicted behavior. If not, this means the enzyme does not behave strictly according to the Michaelis–Menten model, suggesting greater complexity to the nature of the reaction.*

The determination of V_{max} (proportional to k_{cat}) and K_M is important for any enzyme of interest, because it is these two terms that allow one to predict how fast catalysis will take place over a range

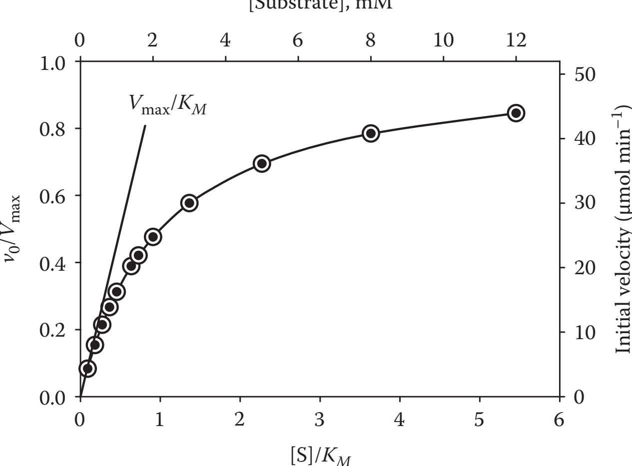


FIGURE 6.9 Michaelis–Menten (hyperbolic) kinetics. Hypothetical enzyme was assumed to have a V_{max} of 52 μmol/min and a K_M of 2.2 mM. Open symbols represent data plotted on the left ordinate/lower axis; closed symbols represent data plotted on the right ordinate and upper axis. Curved line plot represents nonlinear regression fit.

* Many complex enzyme reactions, such as multisubstrate reactions, will exhibit typical hyperbolic kinetics as long as only one substrate is limiting to, or varied for, the reaction, such that it behaves kinetically as a single substrate or "pseudo-first-order" reaction.

of conditions of E and S. A particularly useful application of kinetic parameters in food processing derives from the integrated form of the Michaelis–Menten velocity expression:

$$V_{\max} \times t = K_M \times \ln\left(\frac{S_o}{S}\right) + ([S_o] - [S]) \quad (6.24)$$

where
S_o is the initial substrate concentration
S is the substrate concentration at time t

The time required for a desired fractional conversion (X) of substrate [$X = (S_o - S)/S_o$] is

$$t = SX + K_M \times \frac{\ln[1/(1-X)]}{V_{\max}} \quad (6.25)$$

This relationship can provide a reasonable estimate as to how much enzyme ($V_{\max}$ term) must be added to achieve a specified extent of reaction within a specified time period (such as in a processing situation). This equation can only provide rough estimates as there are many reasons why enzyme activity may depart from the predicted course, and they include depletion of coreactant/substrate, product inhibition, progressive enzyme deactivation, and change in conditions affecting reaction progress, among others.

The rate constants derived from the Michaelis–Menten equation have other meanings. The first-order constant k_{cat} relates only to the behavior of ES and other similar species (other intermediates plus the enzyme-product complex, EP). Recall that this constant is also called the enzyme turnover number. K_M, the *Michaelis constant*, is often referred to as the *apparent dissociation constant*, since this constant may be representative of the behavior of multiple enzyme-bound species (see Figure 6.3 as an example). The "apparent" designation also derives from K_M often being determined by experimental data generating v vs. [S] plots and not by the direct determination of composite rate constants (k_1, k_{-1}, k_{cat}). K_M is the substrate concentration where the enzyme reacts at ½ $V_{\max}$ and where enzyme is half saturated by substrate. K_M is theoretically independent of [E], although anomalous behavior can occur, especially in concentrated and complex enzyme systems. Last, comparing K_M with [S] in a food matrix can be quite revealing. Intermediate metabolites in cellular systems are often present at concentrations in the range of K_M, since this allows for fine reaction control where activity can increase or decrease with a subtle change in [S] [131]. In contrast, if $[S] \gg K_M$ in cellular systems, this implies some barrier to enzyme activity on that substrate must exist (such as physical separation or "compartmentation") for the condition of $[S] \gg K_M$ to persist. While K_M for many enzymes and their substrates is in the range of 10^{-6}–10^{-2} M, some K_M values can be quite high, such as 40 mM for glucose oxidase toward glucose, 250 mM for xylose (glucose) isomerase toward glucose, and 1.1 M for catalase toward H_2O_2 [154]. The apparent second-order rate constant, k_{cat}/K_M (proportional to $V_{\max}/K_M$), relates to properties of the free enzyme (recall Equation 6.20) and is also called the "specificity constant." The magnitude of this constant cannot be greater than any other second-order constant for the enzyme system and, as such, represents a minimum value for the association constant (k_1 step in Equation 6.9) for an enzyme–substrate system.

6.2.5.3.1 *Critical Features of Enzyme Assays*

While understanding that kinetic characterization of enzyme reactions helps guide their use and control in food matrices, it is equally important to understand how to derive such constants with accuracy and confidence. The traditional approach is to collect experimental observations on how reaction velocity (v) varies with [S] (as in Figure 6.9). Reaction progress can be monitored using continuous or discontinuous methods, where P accumulates over time, to yield a collection of reaction

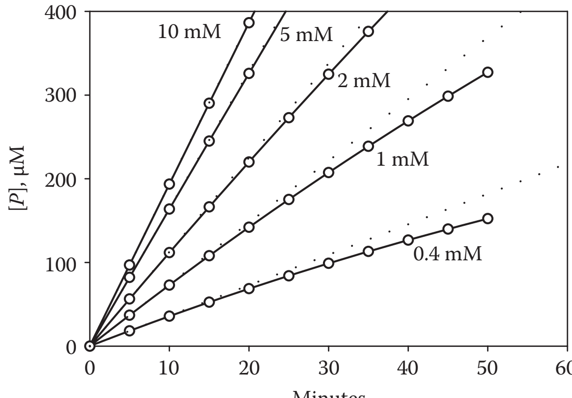


FIGURE 6.10 Progress curves of enzyme reactions as a function of [S]. Reaction progress is based on the hypothetical enzyme parameters in the legend of Figure 6.9 and appears as solid line and symbol plots. Tangents to the initial velocity or "linear" portion of the curves appear as broken-line plots.

rate data (Figure 6.10). One of the most critical issues is to ensure that *linear rates* or *initial velocities* (v_o) are being measured, since the rate expressions developed on the basis of the Michaelis–Menten (and many other kinetic) models are valid only for a specific initial level of substrate [S_o], and not as [S] declines. In practice, this is achieved by allowing for no more than 5%–10% of the original [S] to be consumed during the period of observation [30]. This is especially important at low initial [S] ([S_o] < K_M) where the reaction rate approaches first order with respect to [S]. Even in this case, one can still estimate the linear rate or v_o by drawing a tangent and *linearizing* the initial portion of the reaction progress curve (see Figure 6.10). There is less opportunity at [S_o] ≫ K_M for the reaction to deviate from linearity since the reaction will remain nearly zero order with respect to [S] even after >10% depletion of [S_o]. In addition to the complications of dependence of reaction rates when [S] < K_M, greater *degree of error* is typically encountered when measuring slower reaction rates within a range of [S], based on the limits of sensitivity of the assay (analytical) method.

6.2.5.3.2 Estimation of K_M and V_{max}

A common way to estimate K_M and $V_{\max}$ from experimental rate data is by using any one of three linear transformations of the original Michaelis–Menten rate expression (Equation 6.17, Figure 6.11). Although these transforms take different forms, they are mathematically equivalent and should yield identical results, using accurate data. However, all experimental observations have embedded error, and these errors can differentiate strengths and weaknesses in these alternative linear methods. The most commonly used (and misused) linear transformation is the double reciprocal (Lineweaver–Burk) plot [46,57]. The primary limitation of this plot is that greatest weight is placed on the weakest data points of the set (i.e., the lowest [S] studied are subject to the greatest % error), and the degree of uncertainly (error, along the Y-axis) is further amplified by the reciprocal nature of the coordinates (Figure 6.11b). Thus, even modest error or uncertainty can greatly influence the placement of the regression line. In all fairness, Lineweaver and Burk recognized that appropriate "weighting" of coordinates should be exercised, but this is largely ignored today. The Hanes–Woolf plot is opposite to the double reciprocal plot in that it places greatest emphasis (weight) on data points least encumbered with error (at the highest [S] in the set) (Figure 6.11d). However, this also creates graphical bias within the data set toward the [S] > K_M portion of the curve. Last, the Eadie–Scatchard plot places even weight on each data point of the set but suffers from error (uncertainty) being encountered on both axes, as the dependent variable (v_o) constitutes a factor in each (Figure 6.11c). This linear plot also finds utility in that it allows easier identification of "outlier" data points than the other plots (the point at the lowest v_o stands out).

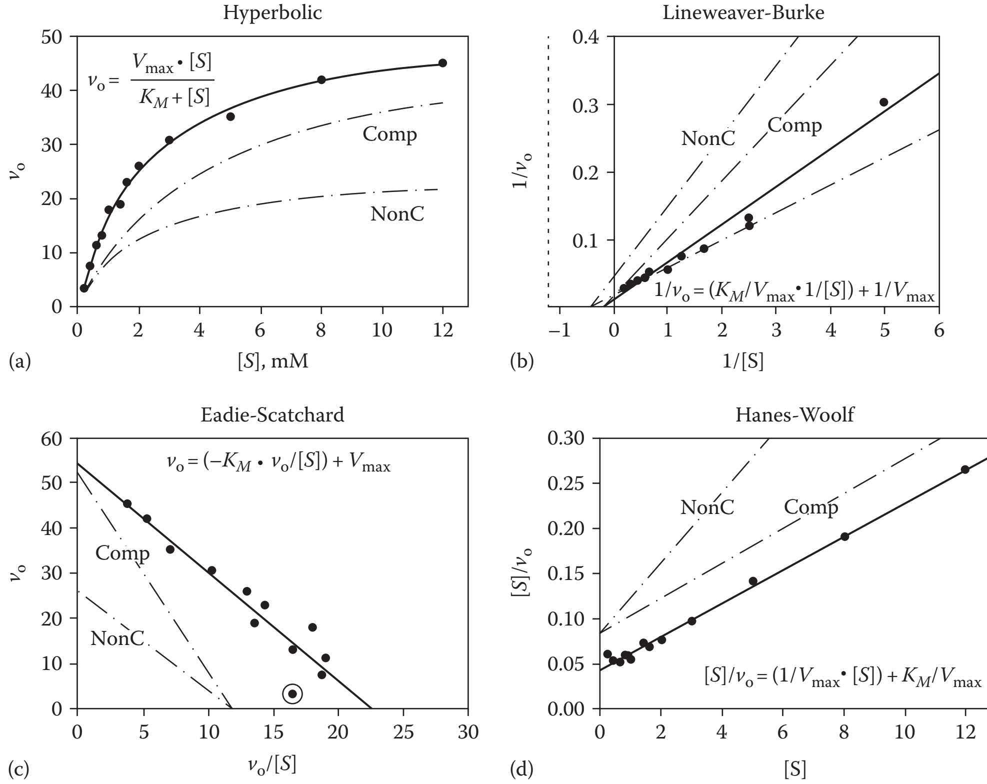


FIGURE 6.11 Hyperbolic and linear transformation plots of enzyme rate data. (a) Hyperbolic, (b) Lineweaver-Burke, (c) Eadie-Scatchard, (d) Hanes-Woolf. Hypothetical experimental observations for an enzyme with kinetic parameters approximating those in the legend of Figure 6.9 appear as best-fit solid line and symbol plots. Equations for all linear plots are expressed in the form of $y = mx + b$. Dot-and-dash broken-line plots are for the types on inhibition modeled in Figure 6.12 and assuming (inhibitor) and K_I values of 0.8 mM and 0.5, respectively, for both competitive (Comp) and noncompetitive (NonC) inhibition. Broken-line (dashed) plot in panel (b) is uninhibited reaction corrected for "outlier" data point observed at the lowest (substrate) evaluated; this outlier point is identified in panel (c).

Regardless of which plot is used, the data set must include observations that comprise a good balance of [S] above, below, and near K_M [31,122]. This prevents the data set from being too biased toward either upper or lower region of the hyperbolic curve (Figure 6.9). More precisely, it is the response of reaction rate to the region where $[S]/K_M$ ranges from 0.2 to 4 that is most important and serves to define the curvature of the plot and how rate depends on [S]. Linear transformations are not the only way to estimate kinetic constants of enzyme reactions. Experimental data can be fitted to a rectangular hyperbola, a specific nonlinear regression fit (Equation 6.17; Figures 6.9 and 6.11a) to obtain estimates of K_M and V_{max} values directly from the original (and nontransformed) data set. This curve also allows for reasonable visual estimates of K_M and V_{max} and how well actual data conform to the fitted curve.

Linear plots also find utility in characterizing action of inhibitors (I) of enzyme reactions (Figure 6.11, broken-line plots). The two common types of inhibition are competitive and noncompetitive (Figure 6.12). Competitive inhibitors have structures that resemble those of substrates and interfere with S binding at the active site, making the enzyme reaction behave as having an elevated K_S or K_M value (without affecting the k_{cat} step or V_{max} value). On the other hand, noncompetitive inhibitors do not interfere with S binding (have no impact of the K_S or K_M value),

$$E + S \underset{}{\overset{K_M}{\rightleftharpoons}} ES \longrightarrow E + P$$

$$I \quad \downarrow\uparrow K_I \qquad EI$$

(a)

$$K_I = \frac{[E] \times [I]}{[EI]}$$

$$E + S \overset{K_M}{\rightleftharpoons} ES \longrightarrow E + P$$

$$I \quad \downarrow\uparrow K_I \qquad I \quad \downarrow\uparrow K_I$$

$$EI + S \overset{K_M}{\rightleftharpoons} SEI$$

(b)

$$K_I = \frac{[E] \times [I]}{[EI]} = \frac{[ES] \times [I]}{[SEI]}$$

FIGURE 6.12 Model for simple (a) competitive and (b) noncompetitive inhibition of enzyme reactions.

but effectively "poison" the enzyme by reducing the V_{max} by a proportion equivalent to the amount of enzyme bound to inhibitor ([EI] + [ESI]) at a given [I] and respective inhibitor dissociation constant (K_I) in the system. The effect of a competitive inhibitor can be ameliorated by adding excess [S] to "out-compete" the inhibitor and pulling the reaction equilibria toward ES and $ES \rightarrow E + P$. In contrast, for the noncompetitive inhibitor, this does not occur because inhibitor can bind to either E or ES, and thus the amount of [$EI + ESI$] is not affected by [S] at a given [I]. Close inspection of the corresponding slopes and intercepts of the lines representing the two types of inhibition in the linear plots (Figure 6.11b through d) reveals that V_{max} remains constant while K_M increases for competitive inhibition and V_{max} decreases and K_M remains constant for noncompetitive inhibition, relative to reactions without inhibitor. Equations for K_I values for these types of inhibition appear on Figure 6.12, and K_M and V_{max} values are modified by the factor of $(1 + [I]/K_I)$ as appropriate [31,122].

Other and lesser common types of inhibition include suicide inhibitors (substrates), which bind to the active site and are transformed by the enzyme to a derivative that reacts with and deactivates the enzyme, and *un*competitive inhibitors, which only bind to the ES species and inhibit enzyme action. Reports of *un*competitive inhibition should be treated with great skepticism, since there are only a few documented cases of this type of behavior [31].

6.2.6 Specificity and Selectivity of Enzyme Action [43]

Although the terms specificity and selectivity are often used interchangeably, these terms relate to the *discriminatory power* of enzyme action. Enzymes can discriminate between competing substrates on the basis of differential binding affinities and facility of catalysis. An enzyme can be *specific* if it reacts only with substrates that have a certain type of chemical bond (e.g., peptide, ester, glycoside) or group (e.g., aldohexose, alcohol, pentadiene), or an enzyme may exhibit (near-) absolute specificity, where a single chemical reaction is catalyzed for a defined substrate(s). In addition, enzymes may also exhibit product specificity and stereochemical specificity. Thus, one can consider *specificity* as denoting the general and/or *exclusive* nature of the type of enzyme reaction catalyzed. The term *selectivity* refers to the relative preference of reactivity of an enzyme toward similar, competing substrates, indexed as V_{max}/K_M (Section 6.2.5). For the casual reader, it is acceptable to use the terms specificity and selectivity interchangeably.

6.2.6.1 Specificity Patterns of Selected Food Enzymes

6.2.6.1.1 Proteolytic Enzymes

Some of the earliest (and considered classic) work on the role of noncatalytic sites of enzymes involved in S recognition involved papain (EC 3.4.22.2), the cysteine protease from papaya latex with commercial application as a meat tenderizing agent. Using a series of synthetic peptide substrates, different sites of the enzyme and substrates were "mapped" [118,119], and the basis of enzyme selectivity was inferred from the relative reactivity of members of this substrate series (Figure 6.13). The formalism developed is now applied to all protease–peptide reactions. The scissile bond of peptide substrates is designated as that linking residues P_1 and P_1', while other substrate amino acid residues are sequentially designated P_2, P_3 … P_i toward the N-terminus and P_2', P_3' … P_i' toward the C-terminus. The corresponding sites of papain that interact with the substrate subsites are designated S and S' with the same numeric codes as the corresponding substrate residues. While the P series of substrate residues corresponds to a single amino acid, one or multiple amino acid residues may share and comprise the same S_x "space" on the enzyme to collectively interact with a corresponding substrate residue. The selectivity data used to "map" the important residues of papain also appear in Figure 6.13.

While papain is considered to have broad selectivity in hydrolyzing peptide bonds, this study showed a clear preference for substrates with PHE (aromatic/nonpolar residue) at the P_2 site of the substrate (other substrates examined are not included in the figure). Consequently, although PHE is not part of the peptide bond hydrolyzed, the enzyme exhibits a preference in recognizing PHE at the S_2 site and this dictates which peptide bond is brought into register as the scissile bond. It is inferred that the S_2 subsite "space" in papain is occupied by similarly hydrophobic residue(s) and that the interaction between the P_2 and S_2 residues makes a major contribution to papain selectivity for peptide bond hydrolysis. The active site of papain is comprised of a deep cleft, with the catalytic residues CYS_{25} and HIS_{159} on opposite sides of the cleft [126] Up to seven nonpolar residues from

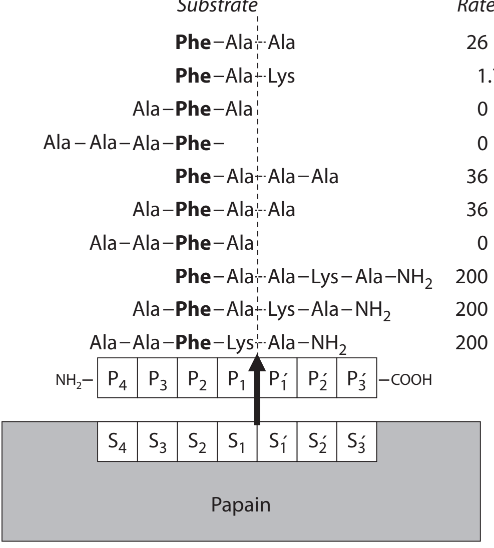


FIGURE 6.13 Substrate *mapping* of the active site of papain by kinetic analysis using peptide substrates. (Data were selected and the figure adapted from Schechter, I. and Berger, A., *Biochem. Biophys. Res. Commun.*, 27, 157, 1967; Schechter, I. and Berger, A., *Biochem. Biophys. Res. Commun.*, 32, 898–912, 1968.) Reaction rates are normalized by the author since reactivity of substrates was determined by end-point analysis after different incubation times (initial velocities were not measured). Arrow and dashed line indicate the register for the scissile peptide bond.

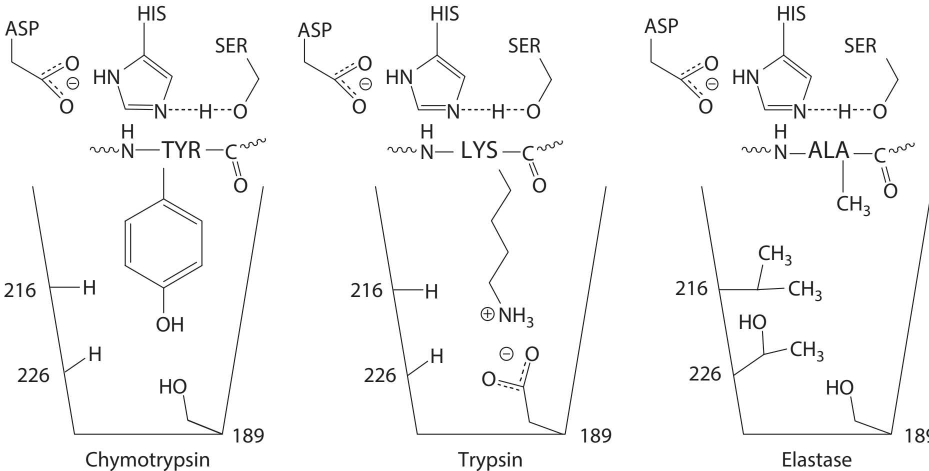


FIGURE 6.14 Substrate-binding pockets for serine proteases. The preferred P_1 amino acid side chain is shown in binding pocket with other amino acid side chains of enzyme at sites 216, 226, and 189. (Adapted from Fersht, A., *Enzyme Structure and Mechanism*, 2nd edn., W.H. Freeman & Company, New York, 1985; Whitaker, J.R., *Principles of Enzymology for the Food Sciences*, 2nd edn., Marcel Dekker, New York, 1994.)

both sides of the cleft are implicated as comprising the S_2 space of papain. By comparison, serine proteases primarily exhibit substrate selectivity through interactions at (sub)sites S_1/P_1, and the critical amino acid residues and the resulting bond selectivity for trypsin, chymotrypsin, and elastase are conferred largely by steric and electrostatic factors as shown in Figure 6.14.

Perhaps no enzyme is better known for its reaction selectivity than is the acid protease chymosin (EC 3.4.23.4, also called rennin), used exclusively for cheese making. The crude enzyme preparation, called "rennet" and obtained from the stomach of young calves, is highly selective for hydrolyzing the PHE_{105}–MET_{106} bond of κ-casein during the initial milk-clotting phase of cheese making. Kinetic studies of chymosin action on synthetic peptides that modeled portions of the κ-casein substrate revealed factors responsible for its selectivity (Table 6.4). First, chymosin was found to be

TABLE 6.4
Enzyme–Substrate Interactions Involved in Chymosin Selectivity for the PHE–MET Bond of κ-Casein

κ-Casein	Peptide: 100 … 105 ↓ 106 … 110	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/K_M (s^{-1} mM^{-1})
Ref	His-Pro-His-Pro-His-Leu-Ser-Phe-Met-Ala-Ile-Pro-Pro-Lys-Lys			
a	Ser-Phe-Met-Ala-Ile-OMe	0.33	8.5	0.04
b	Leu-Ser-Phe-Met-Ala-OMe	0.58	6.9	0.08
c	Leu-Ser-Phe-Met-Ala-Ile-OMe	18.3	0.85	21.6
d	Leu-Ser-Phe-Met-Ala-Ile-Pro-OMe	38.1	0.69	55.2
e	Leu-Ser-Phe-Met-Ala-Ile-Pro-Pro-OMe	43.3	0.41	105
f	Leu-Ser-Phe-Met-Ala-Ile-Pro-Pro-Lys-OH	33.6	0.43	78.3
g	Leu-Ser-Phe-Met-Ala-Ile-Pro-Pro-Lys-Lys-OH	29.0	0.43	66.9
h	His-Pro-His-Pro-His-Leu-Ser-Phe-Met-Ala-Ile-Pro-Pro-Lys-OH	66.2	0.026	2510
i	His-Pro-His-Pro-His-Leu-Ser-Phe-Met-Ala-Ile-Pro-Pro-Lys-Lys-OH	61.9	0.028	2210

Source: Visser, S., *Neth. Milk Dairy J.*, 35, 65, 1981.

a size-selective endopeptidase requiring at least a pentapeptide for activity where neither the PHE or MET could be the terminal residue (data not shown in Table 6.4). Thus, reactivity with peptide fragments (a) and (b) represents a reference or basal level of chymosin activity on the PHE–MET bond in minimally sized peptides. Peptide extension toward the C-terminus of κ-casein (substrates c through g) enhances reaction selectivity (k_{cat}/K_M) toward the PHE–MET bond by 2–3 orders of magnitude over substrate (b), with greater impact on elevating k_{cat} than reducing K_M, although both parameters are affected. This demonstrates the important role that ILE-PRO-PRO$_{108-110}$ residues have on substrate recognition and especially stabilizing the transition state, with the rigidity of the PRO residues playing a pivotal role, perhaps imposing strain/distortion. For the complete κ-casein substrate, PRO may help expose the scissile bond to protease (Chapter 14). Likewise, extending the peptide substrate toward the N-terminus (substrates (*h*) and (*i*)) further increases selectivity by 2 orders of magnitude. This is almost exclusively realized by enhanced affinity (binding) of substrate to the enzyme, as K_M decreases while there is little change in k_{cat}. The positively charged cluster of HIS$_{98,100,102}$ residues at reaction pH helps "freeze" substrate at the active site by coordinating with corresponding electronegative groups on the enzyme at subsites S_8-S_6-S_4, providing for electrostatic attraction. This example demonstrates how substrate structure can enhance reaction selectivity through long-range interactions with enzyme, in this case enhancing selectivity (k_{cat}/K_M) by ~5 orders of magnitude toward the scissile bond. This example also explains why it has been challenging to identify and use "microbial rennets" (chymosin substitutes) for cheese making as these alternative proteases usually have lesser milk clotting–proteolytic activity ratios (0.10–0.52) than chymosin (1.4), and this leads to continued breakdown of the curd (compromising of textural quality) and undesirable bitterness as the cheese ages [73].

6.2.6.1.2 Glycosyl Hydrolases (Glycosidases) [126,154,159]

Glycosyl hydrolases act on glycosidic bonds on di-, oligo-, and polysaccharides. The nature and extent of enzyme–substrate recognition and subsite mapping has been well studied in this group of enzymes. Examples include glucoamylase, an exo-acting hydrolase releasing single glucose units from the nonreducing end of linear α,1→4 linked maltooligosaccharides; lysozyme, an endo-acting hydrolase recognizing a repeating α,1→4-linked heterodimer of [*N*-acetylglucosamine (NAG) → *N*-acetylmuramic acid (NAM)]$_n$; and α-amylase, an endo-acting hydrolase that randomly cleaves linear α,1→4 linked [glucose]$_n$ segments in starch (Figure 6.15). Analogous to active site mapping of proteases, glycosyl hydrolase substrate-binding subsites are mapped as (−*n*…−2, −1, +1, +2 … +*n*) [35]. Hydrolysis occurs at the glycosidic bond of the residue furnishing the carbonyl group at subsite −1 and the alcohol group at subsite +1. Enzyme–substrate interaction at one or both of these subsites (especially −1) may contribute to an unfavorable free energy change of association (+ΔG_S). This should be expected since the substrate bonds to be transformed need to be elevated to a transition state. Rather, interaction at subsites surrounding the transformed residue(s) contributes to the favorable (negative) ΔG_S of binding, and this binding energy may be used to facilitate catalysis. The extent of enzyme–substrate subsite interaction is "mapped" or confined to where further extending the length of the substrate toward +*n* or −*n* subsites has no impact on catalytic parameters. In the specific case of glucoamylase (Figure 6.15a), the +1 to +3 sites particularly enhance both binding and catalysis, whereas other sites serve to enhance binding and have little effect on catalysis.

For lysozyme (Figure 6.15b), interactions with residues at subsites −2 and +1 are similarly pivotal in enhancing reactivity, but even interactions at the more remote subsites of −4 and +2 have considerable effect on catalysis [43,151,159]. H-bonding is a primary factor in enzyme–substrate recognition, especially between substrate residues −4/−3 and ASP$_{101}$. Substrate structure is also important as the bulkier NAM residue is preferred as the −1 subsite; the lactyl moieties of NAM are sterically hindered from occupying enzyme-binding subsites −4, −2, and +1. For α-amylase (Figure 6.15c), the residues immediately adjacent (−2/+2) to the scissile maltose unit (−1/+1) provide for greatest −ΔG_S for binding and acceleration of catalysis. Further degree of polymerization (DP) continues to

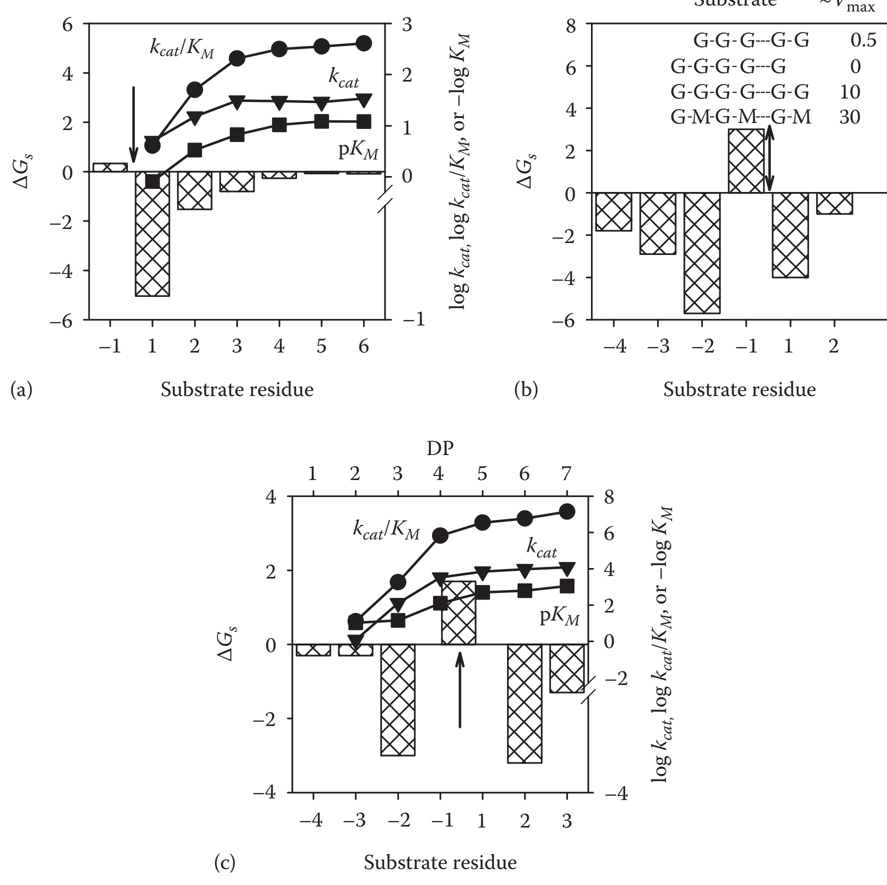


FIGURE 6.15 Substrate subsite mapping of glycosyl hydrolases by kinetic analysis. The activity was analyzed for a series of α-1,4-linked glucose oligomers from 1 to 7 units for (a) glucoamylase and (c) α-amylase. Maltose is the smallest substrate for both enzymes but glucose binding occurs for glucoamylase. For glucoamylase, kinetic constants coincide with substrate length increasing from −1 to n; for α-amylase, kinetic constants coincide with the DP of the oligomers; for (b) lysozyme, kinetic constant is for the model substrates where G = *N*-acetylglucosamine and M = *N*-acetylmuramic acid. Estimates of ΔG_s coincide with each subsite, and arrows indicate scissile bond. (Data obtained from Christophersen, C. et al., *Starch/Stärke*, 50(1, Suppl), 39, 1998; Meagher, M.M. et al., *Biotechnol. Bioeng.*, 34, 681, 1989; Nitta, Y. et al., *J. Biochem.*, 69, 567, 1971.)

enhance binding (K_M) more than catalysis (k_{cat}). In all three examples, remote enzyme–substrate interactions provide the energy required to stabilize the transition state at the active site.

6.2.6.1.3 *Lipid-Transforming Enzymes*

With lipases, binding sites exist for both the acyl and alcohol moieties of the ester to be hydrolyzed, and each site possesses two subsites (Figure 6.16a) [63]. These sites are lined with hydrophobic residues and selectivity is largely conferred by volume of the binding pockets. For example, the sizes of the large (L_A) and medium (M_A) acyl subsites of *Candida rugosa* lipase are compatible with the respective sizes of the C8 and C4 *n*-acyl groups (Figure 6.16b; [96]), giving rise to the marked preference in reactivity for these acyl groups (but not the closely related C6 *n*-acyl group). Many lipases exhibit multiple optima for fatty acyl chain length [2,74,108]. The alcohol group of the ester substrate binds at a site exposed to solvent comprised of subsites to host the large (L_{alc}) and medium (M_{alc}) constitutive groups of the alcohol moiety (and leaving group; Figure 6.16a). At least three amino

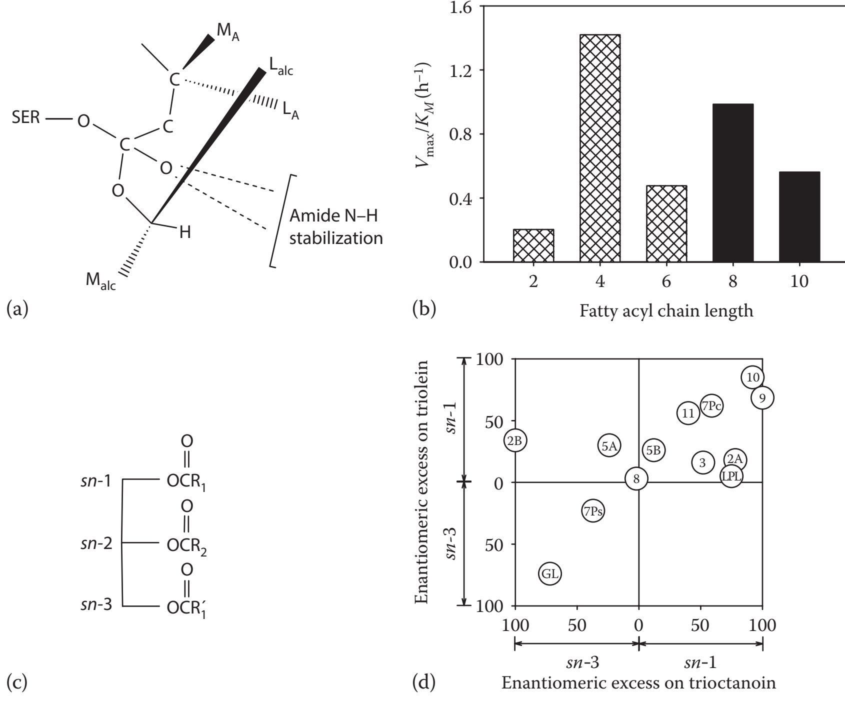


FIGURE 6.16 Features of substrate selectivity of lipases. (Data and figures adapted from Kazlauskas, R.J., *Trends Biotechnol.*, 12, 464, 1994; Parida, S. and Dordick, J.S., *J. Org. Chem.*, 58, 3238, 1993; Rogalska, E. et al., *Chirality*, 5, 24, 1993.) Panels denote: (a) substrate recognition sites, (b) acyl chain length selectivity, (c) stereospecific numbering of glycerolipids, (d) stereoselectivity of some lipases on model substrates. Different shading of bars in panel (b) denotes different stereobias in the reaction among *rac*-α-hydroxylated fatty acid substrates. Numeric coding for lipases in panel (d) appears in Table 6.8, where an accompanying upper case letter refers to an enzyme isoform; LPL, milk lipoprotein lipase; GL, human gastric lipase; 7Ps, *Penicillium simplicissimum* lipase; 7Pc, *Penicillium camemberti* lipase.

acid residues of lipases (adjacent to the catalytic SER/HIS residues and oxyanion-stabilizing amide groups) interact with the M_{alc} group to confer selectivity toward the alcohol group [63]. Other features of substrate-binding sites of lipases, including accessibility, volume, and topography, confer regioselectivity toward ester groups (Figure 6.16c, as *sn*-1,3-regiospecific or nonspecific), as well as fatty acid selectivity (e.g., saturated vs. unsaturated) [2,74,108]. The relative contribution of all these selectivity factors toward acyl and alcohol groups governs stereospecificity (almost all mixed triacylglycerols are chiral), and a survey using two model substrates (triolein and trioctanoin) shows the range of stereoselectivity among lipases and how this can be influenced by substrate structure (Figure 6.16d).

A broad scope of factors confers selectivity of lipoxygenases (LOX), which react exclusively with the 1,4-pentadiene group of polyunsaturated fatty acids, represented by linoleic acid ($18{:}2_{9c,12c}$) [88]. Positional selectivity (regioselectivity) toward oxygenating arachidonic acid ($20{:}4_{5c,8c,11c,14c}$) has emerged as one basis for classifying lipoxygenases (as 5-LOX, 8-LOX, 9-LOX, 11-LOX, 12-LOX, 15-LOX). Lipoxygenases possess two cavities providing access to the active site. One long, funnel-shaped cavity is lined with hydrophobic residues with ILE_{553} and TRP_{500} controlling O_2 access to the active site [88,105]. The other cavity is also lined with neutral and hydrophobic residues and bends to form a "U" or "boot"-shaped pocket near the active center, which hosts the fatty acid substrate (Figure 6.17). Lipoxygenases are selective for oxygenating the carbon of the pentadiene at positions [−2] or [+2] from the methylenic carbon (site of H abstraction), relative to the carboxylic acid terminus. This reflects a basic difference in lipoxygenase *product* specificity in how it "counts carbons"

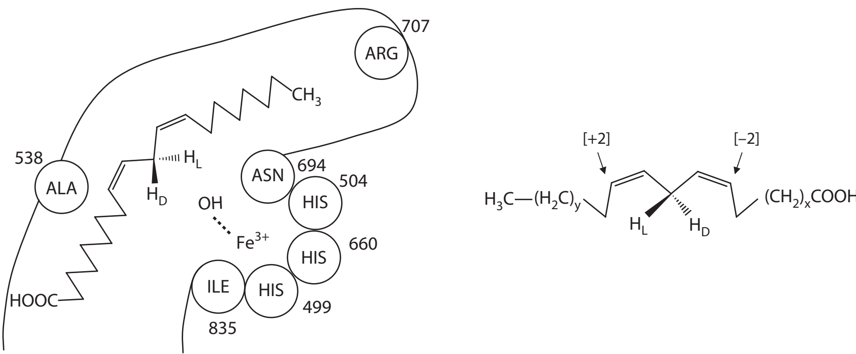


FIGURE 6.17 Active site and positional (stereo)selectivity of lipoxygenase. (Adapted from Boyington, J.C. et al., *Science*, 260, 1482, 1993; Coffa, G. and Brash, A.R., *Proc. Natl. Acad. Sci. U.S.A.*, 101, 15579, 2004; Newcomer, M.E. and Brash, A.R., *Protein Sci.*, 24, 298, 2015; Prigge, S.T. et al., *Biochimie*, 79, 629, 1997.)

based on whether the preferred orientation of substrate binding is carboxylate ([−2] type) or methyl terminus ([+2] type) first entering the binding pocket.

The site of oxygenation also depends on which of possibly multiple 1,4-pentadiene systems ($18{:}3_{9c,12c,15c}$ has two, $20{:}4_{5c,8c,11c,14c}$ has three) is bought in register with the active site iron, and this is partially dependent on the size of the fatty acid–binding pocket. Residues LEU_{546} and ILE_{552} position the methylenic carbon of the pentadiene into register with the catalytic iron. Larger binding pockets accommodate longer portions of the fatty acid substrate and shift positional selectivity toward the carboxyl end (such as 5-LOX) for fatty acids inserting methyl group first. The size of the fatty acid–binding pocket is also controlled by the THR_{709} and SER_{747} residues in the pocket marked by ARG_{707} (Figure 6.17). Finally, the product stereospecificity of lipoxygenases (*S*- or *R*-hydroperoxyfatty acid) is based on a single amino acid residue in the enzyme (residue 542 in soybean LOX-isoform 1) being ALA (R group = CH_3) or GLY (R group = H), respectively [29]. ALA_{542} sterically obstructs O_2 addition to the proximal (pro-*R*, C-9) site and confers 13*S* stereoselectivity, whereas GLY_{542} permits oxygenation at the proximal site, yielding the 9*R* hydroperoxy products (Figure 6.17). This feature applies to all known lipoxygenase structures analyzed to date [88]. LOX reaction selectivity also depends on whether the fatty acid is esterified and in what aggregated form (micelles, detergent complexes or in salt form) and pH (which dictates degree of ionization of the carboxyl group). The pH effect on product selectivity is often explained on the basis of a substrate orientation factor [154]. Soybean LOX-1 exhibits product selectivity at optimum pH ~9 in that the 13-hydroperoxy-octadienoate is preferred over the 9-hydroperoxy-octadienoate by ~10:1, while at pH ~7, the two products are formed in nearly equal proportions. At pH 9, the ionized carboxylate confers positioning of linoleate as shown in Figure 6.17, whereas at pH 7 the protonated linoleic acid may bind in the “inverse” orientation of carboxyl group first, placing the C-9 group in register for the addition of oxygen. This example shows how substrate structure may also influence reaction selectivity.

6.2.6.2 Nomenclature and Classification of Enzymes

Since “trivial” names are often insufficient to represent the precise nature of an enzyme reaction, enzymes are systematically named and catalogued* according to rules of nomenclature as defined by the Enzyme Commission (EC) of the International Union of Biochemists and Molecular Biologists (IUBMB). Although trivial names are still used in referring to enzymes, the assignment of an “EC” number removes ambiguity about the specific reaction being described. The EC number is comprised of 4 integers, each representing some feature of the enzyme reaction (Table 6.5).

* 5684 enzymes were listed as of January 1, 2016: http://www.enzyme-database.org/stats.php.

TABLE 6.5
Systematic Nomenclature Rules and Guidelines for Classification of Enzymes

1st #, Class of Enzyme (Reaction Type)	2nd #, Subclass Substrate, Donor, Bond (Examples)	3rd #, Sub-Subclass Other Distinguishing Group, Substrate, Acceptor, Trait (Examples)	4th #, Bookkeeping Serial Number to Differentiate Enzymes That Share Same First Three # (Examples, Common Names)	Format for Systematic Naming
1. Oxidoreductase (oxidation–reduction)	Group in donor oxidized	Acceptor reduced		Donor–acceptor oxidoreductase
	1. CH–OH group	1. NAD(P)	1.1.1.1 Alcohol dehydrogenase	
	10. Diphenol (or related)	3. O_2	1.10.3.1 Catechol (diphenol) oxidase	
	13. Single donor, O_2	11. 2 atoms O incorporated	1.13.11.12 Lipoxygenase	
	14. Paired donors, O_2	18. 1 atom O incorporated	1.14.18.1 Monophenol monooxygenase	
2. Transferase (group transfer)	Group transferred	Group further delineated		Donor–acceptor grouptransferase
	3. Acyl group	1. Other than amino group	2.3.1.175 Alcohol acyltransferase	
		2. Amino group	2.3.2.13 Transglutaminase	
	4. Glycosyl group	1. Hexosyl group	2.4.1.19 Cyclodextrin glycosyltransferase	
3. Hydrolase (hydrolysis)	Bond hydrolyzed	Substrate class		Hydrolase
	1. Esters	1. Carboxylic ester	3.1.1.3 Lipase	
	2. Glycosidase	1. *O*- or *S*-glycosyl	3.2.1.147 Myrosinase (thioglucosidase)	
	4. Peptide	24. Metallopeptidase	3.4.24.27 Thermolysin	
4. Lyase (elimination)	Bond cleaved	Group eliminated		Substrate group lyase
	1. C–C	2. Aldehyde lyase	4.1.2.32 Trimethylamine-*N*-oxide aldolase	
	2. C–O	2. Act on polysaccharides	4.2.2.10 Pectin lyase	
	4. C–S	1. (None, only 23 enzymes)	4.4.1.4 Alliin lyase	
5. Isomerase (isomerization)	Type of reaction	Substrate, position, chirality		Racemase, epimerase, isomerase, mutase
	2. *cis–trans* isomerase	1. (none, only 10 enzymes)	5.2.1.5 Linoleate isomerase	
	3. Intramolecular redox	1. Aldose–ketose interconvert	5.3.1.5 Xylose isomerase	
6. Ligase (bond formation)	Bond synthesized	Substrate, cosubstrate(s)	6.3.2.3 Glutathione synthetase	X-Y ligase (synthetase)
	4. C–C	2. Acid–amino acid (peptide)		

Source: IUBMB, http://www.chem.qmul.ac.uk/iubmb/.

The first number describes the general class of the reaction. Hydrolases (class 3) are the most important class of enzymes in food, followed by oxidoreductases (class 1). Trivial names for group transferases (class 2) sometimes include the term "synthase," which does not seem very distinct from the term "synthetase," the latter which is reserved for ligases (class 6), the truly synthetic or bond-forming enzymes. Lyases (class 4) are enzymes that break bonds through nonhydrolytic processes, and trivial names for enzymes that cause reverse "lyase" reactions may include "synthase" and "hydratase." Isomerases (class 5) cause intramolecular rearrangement of atoms. The second and third digits go on to further identify the reaction and the substrate(s) and/or bond(s) transformed. Enzyme reactions lacking in sufficient definition have the third digit assigned as "99." The last digit comprises a "bookkeeping" function to differentiate enzymes sharing the same first three digits, while also providing an additional feature of the reaction to distinguish it from all other enzymes known. Several EC numbers have already been identified in earlier portions of this chapter with early or first mention of specific enzymes.

6.3 USES OF EXOGENOUS ENZYMES IN FOODS [3,50,139,155]

6.3.1 General Considerations

The decision of when to employ an enzyme process is based on several considerations [19,98]. Enzymes are favored when (1) mild conditions are permitted to maintain positive attributes of the food, (2) potential by-products of a chemical process are unacceptable, (3) a chemical process is difficult to control, (4) the "natural" designation is to be retained, (5) the food or ingredient is of premium value, (6) a traditional chemical process needs to be replaced or expanded, or (7) reaction specificity is required. Relative cost–benefit ratio is also a critical factor. Some enzymes can be used as "immobilized" preparations, where they remain active while fixed or bound to inert matrices or particles. This allows the enzyme to be packed in a column/bioreactor through which substrate is perfused or recovered after batch reaction with substrate by filtering or settling, such that the enzyme can be used repeatedly until it loses activity beyond an acceptable level. In this manner, enzyme costs are proportionally reduced.

Categorical uses of exogenous enzymes include the production of food ingredients and commodities, such as corn syrups, glucose, high-fructose corn syrup, invert sugar and other sweeteners, protein hydrolysates, and structured lipids; modification of components within a food matrix, such as beer stabilization, milk clotting (cheese making), meat tenderization, citrus juice debittering, and crumb softening; process improvement, such as cheese ripening, juice extraction, juice/wine clarification, fruit and oil seed extraction, beverage (beer/wine) filtration, faster dough mixing, baked product leavening and stabilization; and process control, such as online biosensors and for component analysis. Important uses of exogenous enzymes will be presented on the basis of the nature of the food component undergoing transformation.

6.3.2 Carbohydrate-Transforming Enzymes [126,155,159]

Most enzymes used commercially to act on food carbohydrates are hydrolytic and are collectively referred to as glycosyl hydrolases or glycosidases. Some of these enzymes may catalyze glycosyl group transfers and/or reverse hydrolytic reactions in food processes where substrate levels are often high (30%–40% solids) due to mass action effects. This group of enzymes accounts for about half of enzyme use (cost basis) as processing aids in the food industry, primarily for the production of sweetener and bulking/thickening agents (dextrins) from starch, and for carbohydrate modification in baking applications. Specialty applications for various glycosidases continue to emerge.

Some general properties of this group of enzymes are well established, derived from the structural and sequence analysis of members of over 60 sequence-based families of glycosidases. Glycosyl hydrolases act on glycosidic bonds, and enzymes in this group share many structural

and catalytic properties. Many glycosidases are multidomain proteins, where one portion of the protein functions as the catalytic unit and other domains have alternative functions, one being to bind extended polysaccharide substrates. Glycosidase active sites contain dual carboxyl/carboxylate residues (ASP/GLU) similar to what was shown for the mechanism of lysozyme (Equation 6.8). Mechanistically, this group of enzymes functions by either general acid–base catalysis and/or nucleophilic catalysis (with assistance from electrostatic and strain/distortion effects). In all cases, an acidic residue donates an H^+ to the glycosidic O atom to yield an oxocarbenium ion as the transition state (Figure 6.18). Either the carboxylate residue deprotonates and activates water to yield the nucleophilic −OH to complete the hydrolysis or the carboxylate can act directly as a nucleophile and form a covalent intermediate; in both cases the alcohol residue is released as the leaving group.

Glycosidases can be categorized either as "retaining" or "inverting" types, based on the fate of the anomeric configuration (α or β) of the hydrolyzed glycosidic bond (Figure 6.18). Inverting types have a larger distance between the catalytic acid residues (~9.5Å), allowing the activated water molecule (nucleophile) access to the alternative anomeric site relative to the site of ROH release from the glycosidic bond. Retaining types have shorter spacing between catalytic residues (~5.5Å) such that water enters the active site only *after* the released alcohol group departs the active site (referred to as a double-displacement reaction). In the retaining reaction mechanism, the glycosyl-enzyme covalent intermediate formed with the carboxylate residue serves to direct water (rendered nucleophilic by the general base residue removing H^+) to the same anomeric position that the ROH leaving group formerly occupied, and thus, the anomeric configuration is "retained." Only retaining glycosidases catalyze both hydrolysis and glycosyltransfer reactions, whereas inverting types only

FIGURE 6.18 Mechanistic diversity among glycosyl hydrolases. (Adapted from Sinnott, M. (Ed.), *Comprehensive Biological Catalysis. A Mechanistic Reference*, Vol. I, Academic Press, San Diego, CA, 1998.)

catalyze hydrolysis reactions. Another general distinction among glycosidases is whether they are "endo-" or "exo-"acting. Exo-acting types bind the terminal (mostly, but not always, the nonreducing end) portion of the substrate in register as the scissile bond at the active site, whereas endo-acting types randomly attack interior sites of the substrate. Trivial naming of glycosidases as "α" and "β" (as in amylases and glucosidases) recognizes the anomeric configuration of the liberated reducing group as being axial and equatorial, respectively. A summary of the types and classification of glycosidases of most importance in foods is provided in Table 6.6. Active site/substrate "mapping" was introduced earlier (Figure 6.15), where the scissile glycosidic bond is in register at subsites −1/+1. With few exceptions, one or two hydrophobic residues of the enzyme interact with the C_5-hydroxyl-methylenic group of the −1 substrate residue to provide a transition-state stabilizing "hydrophobic platform" [87].

6.3.2.1 Starch-Transforming Enzymes

Enzymes acting on starch are primarily used for commodity applications, such as the production of corn syrups, dextrins, high-fructose corn syrup, and other sweeteners such as maltose and glucose syrups. Starch transformations are also desirable to a more limited extent in baked goods, and exogenous glycosidases are added for purposes of retarding staling and facilitating yeast leavening.

6.3.2.1.1 α-Amylase [126,143,154,159]

The amylases are used to hydrolyze starch (mostly from corn) into smaller dextrins and thereby "thin" starch suspensions. α-Amylase (EC 3.2.1.1., 1,4-α-D-glucan glucanohydrolase) is an endo-acting, α→α-retaining enzyme principally responsible for rapidly reducing the average molecular weight of starch polymers. It is the representative member of family 13 glycosidases, several of which are used in starch processing. This family is characterized by having at least three separate domains within the protein, one for catalysis, another to serve as a granular starch-binding site, and the third to provide for calcium binding and to link the other two domains. The molecular size of the enzyme from various sources (over 70 sequences have been reported) typically ranges 50–70 kDa (although some can approach 200 kDa). α-Amylases bind Ca^{2+} at multiple sites, the most important being near the active site cleft in a manner that stabilizes secondary and tertiary structure. Ca^{2+} is tightly bound and serves to broaden the pH stability of the enzyme to between pH 6 and pH 10, and the thermal stability of α-amylase is quite dependent on source. The active site is comprised of at least five subsites (positions from −3 to +2, Table 6.6; cf., Figure 6.15c) and requires a substrate of at least 3 glucose units in length. Of three conserved residues at the active site (porcine pancreatic α-amylase), ASP_{197} is the nucleophile that forms the covalent glycosyl-enzyme intermediate, GLU_{233} is situated at the +1 subsite and is the general acid catalyst, and ASP_{300} serves to coordinate with C2-OH and C3-OH of the substrate unit at the −1 subsite to affect substrate strain/stress. Conserved HIS_{299} and HIS_{101} are involved in substrate binding and transition-state stabilization to collectively reduce E_a by 5.5 kcal/mol. HIS_{201} interacts with the catalytic GLU_{233} residue to shift pH optimum from 5.2 to 6.9. Because of the critical contribution of HIS residues to activity and pH–activity profile, HIS was long thought to be involved in the mechanism of α-amylase action. The pH optimum is also dependent on length of the substrate, and maltooligosaccharides that do not fully occupy the five binding subsites react over a narrower optimum pH range. Other conserved nonpolar residues are TRP, TYR, and LEU, which are involved in substrate and starch granule binding through hydrophobic stacking interactions [34,154].

There are several sources of α-amylases, most of which are microbial, although malt (barley or wheat) amylases are available. The typical end products of α-amylase action are branched α-limit dextrins and maltooligosaccharides of 2–12 glucose units, predominantly in the upper end of this range [154,155]. Starch is rapidly reduced in viscosity because of the random nature of hydrolysis, quickly reducing the average molecular mass of amylose/amylopectin chains. Among microbial amylases, optimum parameters are generally found within the ranges of pH 4–7 and 30°C–130°C [95]. Common commercial sources for starch transformation include the α-amylases from *Bacillus*

TABLE 6.6
Catalytic Properties of Glycosyl Hydrolases

Enzyme	Bond Selectivity	Product Selectivity[a]	Catalytic Residues[b]	Substrate Subsite Mapping[c]
α–Amylase	α-1→4 glucose	RET α→α	GLU_{233}, ASP_{300} (acid, nucl/base)	Endo: ☐ ☐ ☐ * −3 −2 −1 +1 +2 * ☐ ☐
β-Amylase	α-1→4 glucose	INV α→β	GLU_{186}, GLU_{380} (acid, nucl/base)	Exo: ☐ ☐ ☐ ☐ ☐ −2 −1 +1 +2 ☐ ☐ ☐
Pullulanase	α-1→6 glucose	Likely RET α→α	GLU_{706}, ASP_{677} (acid, nucl/base)	Endo: ☐ ☐ ☐ −4 −3 −2 −1 +1 +2 ☐ ☐ ☐
Glucoamylase	α-1→4(α-1→6) glucose	INV α→β	GLU_{179}, GLU_{400} (acid, nucl/base)	Exo: ☐ ☐ ☐ ☐ ☐ ☐ −1 +1 +2 +3 +4 +5
Cyclomaltodextrin transferase	α-1→4 glucose	RET α→α	GLU_{257}, ASP_{229} (acid, nucl/base)	Endo: −7 −6 −5 −4 −3 −2 −1 +1 +2 ☐ ☐ ☐
Invertase	β-1→2 fructose	RET β→β	GLU_{204}, ASP_{23} (acid, nucl/base)	β-D-fructofuranosyl = −1; glucose = +1: ☐ ☐ ☐ ☐ ☐ ☐ −1 +1 ☐ ☐ ☐ ☐

(Continued)

TABLE 6.6 (*Continued*)
Catalytic Properties of Glycosyl Hydrolases

Enzyme	Bond Selectivity	Product Selectivity[a]	Catalytic Residues[b]	Substrate Subsite Mapping[c]
β-Galactosidase	β-1→4 galactose	RET β→β	GLU_{461}/Mg^{2+}, GLU_{537} (acid, nucl/base)	β-D-galactopyranosyl = −1; glycone/aglycon = +1 (subsites: −1, +1)
β-Glucosidase	β-1→4, β-1→aglycon glucose	RET β→β	GLU_{170}, GLU_{358} (acid, nucl/base)	Exo (subsites: −1, +1)
Polygalacturonase	α-1→4 galacturonate	INV α→β	ASP_{201} $ASP_{180,202}$ (acid, nucl/base)	Endo (exo-types also exist) (subsites: *, *, −3, −2, −1, +1)
Xylanase	α-1→4 xylose	RET β→β	$GLU_{172,}$ GLU_{78} (acid, nucl/base)	Endo (some exo-types exist, some inverting) (subsites: *, −4, −3, −2, −1, +1, *)
Lysozyme	α-1→4 -NAM-NAG-[d]	RET α→α	GLU_{35}, ASP_{52} (acid, nucl/base)	Endo, NAM-NAG unit binds at −1/+1 (subsites: −4, −3, −2, −1, +1, +2)

[a] RET, retaining; INV, inverting.
[b] Reference enzyme cited in the text; nucl = nucleophile.
[c] *, some enzymes exhibit this subsite.
[d] *N*-acetylmuramate-*N*-acetylglucosamine repeating unit. References cited in the text.

and *Aspergillus* species. The *Bacillus* α-amylases are thermostable and can be used at 80°C–110°C at pH 5–7 and 5–60 ppm Ca^{2+} [155]. Fungal (*Aspergillus*) enzymes function optimally at 50°C–70°C, pH 4–5, and ~50 ppm Ca^{2+} [95,155]. While the fungal α-amylases are also endo-acting, they tend to favor the accumulation of shorter maltooligosaccharides (n = 2–5) as the end products of starch liquefaction [139]. A unique "maltogenic" *Bacillus* α-amylase (EC 3.2.1.133) has also been identified [28], and while maltose production is more commonly associated with the action of β-amylases (see next section), maltogenic α-amylases appear to yield elevated maltose levels either through prolonged (exhaustive) hydrolysis of starch or by multiple ("processive") hydrolytic episodes on a bound amylose chain before it completely dissociates from the active site [34].

Amylases with alkaline pH optima of 9–12 evoke particular interest, potentially as food processing aids (and detergents), and especially how the conserved glycosidase feature of the ASP/GLU active site can function at high pH. Alkaline adaptivity for a *Bacillus* spp. α-amylase was associated with decreased proportion of GLU, ASP, and LYS residues and increases in ARG, HIS, ASN, and GLN. This serves to retain charge balance at alkaline pH and changes active site dynamics that elevate the p*K* of the catalytic ASP/GLU groups [124]. These changes, as well as increased hydrophobic content and compact structure, and reduced water near the active site are observed for many alkaline-adapted glycosyl hydrolases [8].

6.3.2.1.2 β-Amylase [95,126,139,154]

β-Amylase (1,4-α-D-glucan maltohydrolase, EC 3.2.1.2) is an α→β-inverting, exo-acting glycosidase that liberates maltose units from the nonreducing ends of amylose chains and is a member of glycosidase family 14. Extensive action of β-amylase on starch yields a mixture of maltose and β-limit dextrins, the latter of which retain the α-1,6-branch points and remaining linear portions that are inaccessible (by steric constraints) to the enzyme. β-Limit dextrins are of greater average molecular mass than α-limit dextrins because the exo-acting β-amylase cannot act past the α-1,6 branch points, whereas α-amylase can, being an endo-acting enzyme. β-Amylases from soybean, sweet potato, and *Bacillus* spp. are among the best characterized. Plant enzymes are ~56 kDa (sweet potato enzyme is a tetramer), while microbial enzymes range 30–160 kDa. β-Amylase is unique in that it has a single domain structure, instead of the multidomain structure of other amylolytic glycosidases. The catalytic residues (soybean β-amylase) are GLU_{186} (general acid) and GLU_{380} (general base), separated by 10–11 Å within a deep pocket. The binding of substrate causes a lid to close providing for an estimated 22 kcal/mol of favorable binding energy and shielding the active site from solvent. This likely intensifies dipole forces that facilitate catalysis and provides another example of *induced fit* mechanism. There are four substrate-binding subsites with the catalytic GLU residues oriented on opposite faces of subsite −1. HIS_{93} is positioned at subsites −1 and −2 and may confer pH sensitivity on the alkaline side. The equivalent of two maltose units bind at the active site (subsites −2 to +2) and this property may confer how close the enzyme can act toward the branch points in starch. At one time, CYS residues were believed to be involved in catalysis, but point mutations have since revealed them to have little catalytic function, although they may have a role in enzyme conformational stability. While the plant enzymes cannot bind and digest raw starch, some of the microbial enzymes have separate protein domains that confer this ability. β-Amylase is subject to competitive inhibition by α-cyclodextrin, and this appears to be mediated by LEU_{383} forming an inclusion complex and blocking access to the active site. β-Amylases generally have more alkaline pH optima (pH 5.0–7.0) than α-amylases, do not require Ca^{2+}, and exhibit temperature optima in the range of 45°C–70°C, depending on the source (microbial sources being more thermostable).

6.3.2.1.3 Pullulanase [82,143,154,159]

Type I pullulanases (EC 3.2.1.41, pullulan 6-glucanohydrolase) are referred to as "debranching" enzymes or "limit dextrinases," since they hydrolyze dextrins containing the α-1,6 glucosidic bonds constituting the branch points of amylopectin. Pullulanase is present in many bacteria, some yeast, and cereals, and sequence analysis places it in the α-amylase family 13 (α→α-retaining enzymes).

The enzyme is a lipoprotein of 1150 amino acids (MW estimated of 145 kDa) with five domains with five calcium-binding sites. The active site residues (*Klebsiella pneumoniae* enzyme) are GLU_{706} (acid) and ASP_{677} (nucleophile/base) with ASP_{734} assisting (Table 6.6), with substrate subsites of −4 to +2 and features conserved with α-amylase. Pullulanase is characterized (and named trivially) by its ability to act on pullulan, a repeating unit of [α-D-Glc-(1→4)-α-D-Glc-(1→6)-α-D-Glc (1→4)-α-D-Glc]. Pullulanase can act on larger, but not smaller fragments than pullulan, acts slowly on amylopectin, and prefers the limit dextrins that are produced during advanced stages of starch liquefaction and saccharification [159]. Products of pullulanase action are linear glucooligosaccharides as small as maltose. Pullulanases are commonly obtained from *Klebsiella* and *Bacillus* spp. and have masses of ~100 kDa, upper temperature limits of 55°C–65°C, and optimal pH 3.5–6.5 with no known requirement for cofactors (although some are activated by Ca^{2+}). Pullulanases from plant sources are also referred to as limit dextrinases, and germinated or malted grains are the richest sources, especially barley. Type II pullulanases (or amylopullulanases, EC 3.2.1.41 or 3.2.1.1) are principally microbial in origin, have combined α-amylase-pullulanase activity, and can hydrolyze both α-1,4 and α-1,6 linkages in starch. Other related enzymes are neopullulanase (EC 3.2.1.125) and isopullulanase (EC 3.2.1.57), which act on the α-1,4 linkages in pullulan toward the nonreducing and reducing ends of the branch point, respectively, to yield α-1,6-branched trisaccharides panose and isopanose.

6.3.2.1.4 Glucoamylase [95,126,154]

Glucoamylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.3), also known trivially as amyloglucosidase, is an α→β-inverting, exo-acting enzyme solely comprising glycosidase family 15. It hydrolyzes glucose units from nonreducing termini of linear starch fragments. Although glucoamylase is selective for the α-1,4-glucosidic linkage, it can act slowly on the α-1,6 bond characteristic of amylopectin and pullulan. Thus, the exclusive product of exhaustive glucoamylase digestion is glucose. It has structural and mechanistic features similar to α-amylase, including respective acid and base catalytic GLU_{179} and GLU_{400} residues (*Aspergillus* spp. enzyme), a separate starch-binding domain and short linker domain. Some glucoamylases can act on native (raw) granular starch. Two $TRP_{52,120}$ residues assist catalysis by H-bonding to GLU_{179}, enhancing its acidity. The catalytic domain has five subsites other than the scissile glycone residue at −1 (cf., Figure 6.15a), and subsites +1 to +5 all exhibit $-\Delta G$ for binding (favorable), especially at subsite +1. Since the ΔG is accretive for the subsites, the enzyme has greater reaction selectivity for the longer of the C2–C6+ linear glucooligosaccharides. This pattern of selectivity is conducive to obtaining processive and exhaustive hydrolysis of short amylose segments to glucose. The oligomeric substrate must enter a "well" to get access to the active site, and because of these steric constraints, dissociation and rebinding of remaining substrate is the rate-limiting step.

Glucoamylases are sourced primarily from bacteria and fungi [95]. They range in mass from 37 to 112 kDa, can exist as multiple isoforms, have no cofactors, and exhibit optima in the range of pH 3.5–6.0 and 40°C–70°C. The *Aspergillus* glucoamylase is commonly used and it is most active and stable at pH 3.5–4.5, with an optimum temperature range of 55°C–60°C [154]. The *Rhizopus* enzyme is of interest because one isoform can also readily hydrolyze α-1,6-branch points [95]. Glucoamylases are relatively slow acting glycosidases relative to others involved in starch transformation, and processing schedules have evolved to accommodate this feature.

6.3.2.1.5 Cyclomaltodextrin Glucanotransferase [126,154,155]

Cyclomaltodextrin glucanotransferase (CGT, 1,4-α-D-glucan 4-α-D-(1,4-α-D-glucano)-transferase(cyclizing), EC 2.4.1.9) catalyzes hydrolysis as well as intra- and intermolecular transglycosylation reactions. The cyclization reactions yield the hexa-(α), hepta-(β), and octa-(γ) saccharides more commonly known as cyclodextrins. CGT is an α→α-retaining, endo-acting enzyme belonging to family 13 of the glycosidases and has two additional protein domains beyond the three observed for α-amylase, including additional substrate (specifically maltose)-binding sites. The multiple

binding sites allow interaction with raw starch (although CGT is not very active on raw starch) and help guide linear starch fragments into the active site groove. CGTs are from microbial sources and typically monomeric with ~75 kDa mass. The catalytic residues (*Bacillus circulans* enzyme) include ASP_{229} (base/nucleophile) and GLU_{257} (general acid), while ASP_{328} and $HIS_{140,327}$ have roles in substrate binding and transition-state stabilization, ARG_{227} orients the nucleophile, and HIS_{233} coordinates with required Ca^{2+} (as with some α-amylases). There are nine subsites at the active site, −7 to +2, consistent with β-cyclodextrin being the favored product of intramolecular cyclization (Table 6.6).

Although cyclodextrins are the primary commercial products prepared by CGT, CGT is quite promiscuous in its substrate and product selectivity, as it can catalyze a diversity of reactions, including hydrolysis, cyclization, disproportionation, or coupling. For example, it can react with glucose and starch to form maltooligosaccharides of various chain lengths, as well as couple sugars (many monosaccharides are recognized) with alcohol groups such as those of ascorbic acid and flavonoids. These latter processes offer potential for preparing novel compounds of unique functionalities in food systems. CGTs typically exhibit optima at pH 5–6, and temperature optima have been improved from 50°C–60°C to 80°C–90°C in recent years by the introduction of thermostable forms. Different sources of CGT favor different cyclodextrins (hexa-, hepta-, or octaoligomers) as the principal product.

6.3.2.1.6 Applications of Starch Transformation

6.3.2.1.6.1 Starch Hydrolysis Industrial starch transformation begins with a starch slurry substrate of 30%–40% solids at nascent pH of 4.5 (Figure 6.19). "Liquefaction" following pH adjustment

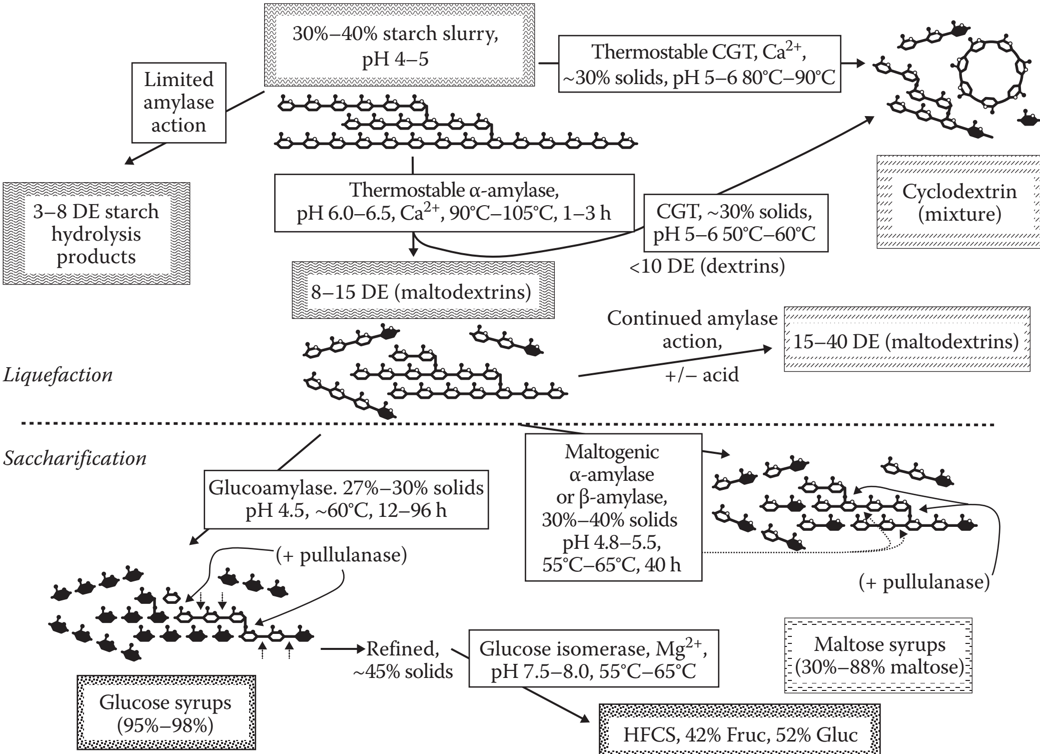


FIGURE 6.19 Commercial starch transformation by enzyme processing. Filled glucose units are reducing ends, and stippled glucose units are nonreducing ends existing in the original starch and evolved during the liquefaction stage. References cited in the text.

to 6.0–6.5 of the starch occurs by briefly heating to 105°C (to gelatinize the starch) and then tempering to 90°C–95°C for 1–3 h in the presence of a thermostable (bacterial) α-amylase and added Ca^{2+}. This yields a mixture of linear and branched dextrins (maltodextrins) with the extent of hydrolysis ranging from 8 to 15 DE (DE = dextrose equivalence), and this is sufficient to prevent gelation of starch upon cooling for subsequent steps (hence the term liquefaction). From this point there are three alternative steps. One is for the production of 15–40 DE maltodextrins (corn syrups, used as thickening, bulking, and viscosity building), which is conducted by further exposure to amylase (in some cases acid [HCl] is used to initially liquefy the gelatinized starch). Two other streams lead to sweetener production, at temperatures of ~60°C and pH 4.5–5.5 to accommodate optimal conditions of the enzymes used. For conversion to a 95%–98% glucose syrup (95 DE), solids are reduced to 27%–30% and treated with glucoamylase (which is often used as an immobilized enzyme column), with or without pullulanase, for 12–96 h. The >95% glucose syrup can then be refined, concentrated to 45% solids, and treated with an immobilized xylose (glucose) isomerase column at pH 7.5–8.0 and 55°C–65°C with added Mg^{2+} to generate a high-fructose corn syrup of 42% fructose (52% glucose), which can be further refined and/or enriched to a 55% fructose syrup. The other sweetener produced from the liquefied starch is facilitated by added fungal (maltogenic) α-amylase or β-amylase, with or without added pullulanase, to yield a range of maltose (30%–88%) syrups for use in confections. Depending on the source of maltogenic amylase selected, the predominant maltooligosaccharides that accumulate in the product mixture range between 2 and 5 glucose units.

Two other types of nonsweetener products prepared from the original starch slurry involve the action of various α-amylases added before the starch is progressively heated to the point of gelatinization. This leads to a controlled (DE 3–8) pattern and degree of hydrolysis that yield large dextrins (generically called starch hydrolysis products) that can form thermoreversible gels and behave as fat mimetics. Much of the details of preparing these products are in the patent literature, but the process generally involves limited amylase action over a range of temperatures [139]. Alternatively, thermostable cyclodextrin glycosyltransferase (CGT) can be added to the native starch slurry after adjusting pH to 5–6 and then incubating at 80°C–90°C. Total yields of cyclodextrin from CGT action on starch are inversely proportional to the concentration of starch and degree of liquefaction [159]. Thus, cyclodextrin production in commercial processes is often conducted at starch levels of ~30% solids (1%–33% have been reported in patent literature [135]) as a compromise between % yield (efficiency) and total yield (production). Thermostable CGT can both hydrolyze native (gelatinized) starch in the presence of added Ca^{2+} and transglycosylate (cyclize) resulting fragments. Nonthermostable CGT also can be used but requires prior and limited digestion of starch to afford liquefaction (to about 10 DE to prevent gelling) after which CGT is added at reduced temperatures (50°C–60°C). Cyclodextrin yields can be enhanced by pre- or cotreatment of starch with a debranching enzyme and by incorporating complexing agents (solvents or detergents) to direct the reaction toward one or more of the cyclodextrin species [135,159].

Going forward, efforts to improve starch processing and transformation will focus on extending pH stability (to pH 4–5) and reducing Ca^{2+} requirement of α-amylase and enhancing the ability to digest raw starch by β-amylases [95,143]. For all enzymes involved, enhancing thermal stability will create further efficiency in processing as well as promote single-step processing. In addition, discovering the determinants of product selectivity of reactions to obtain preferred products or product distributions will remain a priority.

6.3.2.1.6.2 Baking and Baked Goods [28,104,143] Virtually all of the glycosidases discussed earlier have been added for some benefit in baking applications, and the α-amylases have been used the most. Initially amylases were believed to function primarily by mobilizing fermentable carbohydrate for yeast. They are also added to doughs to degrade damaged starch and/or supplement endogenous amylases activities of poor quality (in terms of baking) flours. However, it is now recognized that amylases added directly to the dough will reduce dough viscosity and improve loaf volume, crumb softness (antistaling), and crust color. Most of these effects can be attributed to partial

hydrolysis of starch during baking as the starch gelatinizes. Lowered viscosity (thinning) helps promotes volume and texture by allowing reactions involved in dough conditioning and baking to occur faster (mass transfer effect). The antistaling effect is believed to be conferred by limited hydrolysis of amylose and especially amylopectin chains in a manner that retards the rate at which they can retrograde, and this remains the primary reason why α-amylases are added to baked goods today.* Overdosing of α-amylases leads to gummy or sticky-textured breads, and this is associated with the accumulation of branched maltodextrins of 20–100 DP. Thus, care must be exercised to apportion the right amount of amylase for a specific product, and amylases should not survive the baking process or unwanted residual activity will occur postproduction. This has been done by matching the temperature stability and amount of added amylase to the particular application to control the extent to which the amylase acts and persists during the baking cycle [56]. More recently, the maltogenic types of α-amylases have been recognized as being superior as antistaling agents, since they tend to form shorter maltooligosaccharides (DP 7–9) and large dextrins (which are plasticizers) than those arising from the endo-action by conventional α-amylases. Thus, maltogenic amylases tend to keep the gelatinized starch network in bread intact (soft, but not gummy), and the slight reduction in size of starch chains maintains elasticity of the crumb while being sufficient to retard staling.

6.3.2.1.6.3 Brewery and Fermentations [154,155] Starch hydrolases have long been recognized as essential enzymes in the brewing industry, originating with the 1833 finding of "diastatic" activity in malted (germinated) grains, leading to the commercialization of α- and β-amylases. However, amylases endogenous to malted grain are insufficient to mobilize all of the fermentable carbohydrate because they are of insufficient concentration, they lack thermal stability for the processes involved, and/or there are endogenous inhibitors present in the grains. Thus, α- and β-amylases, glucoamylase and pullulanase, and cell wall–hydrolyzing enzymes are added (almost exclusively from microbial sources) to maximize the availability of fermentable carbohydrate. Glucanases and xylanases (discussed later) are added to hydrolyze glucans (similar to cellulose, but with β-1,3 and β-1,4 linkages) and xylans (predominantly xylose polymers, the major hemicellulose component in cell walls). The added α- and β-amylases are used to complete the degradation of starch to α- and β-limit dextrins that the thermally labile malt amylases cannot achieve. The remaining limit dextrins provide body to the final product. However, limit dextrins can be rendered fermentable by added glucoamylase (and/or pullulase), and beers produced with this enzyme are lower in calorie ("light"). Exogenous enzymes are added during (or right after) the "mashing" step, which is conducted at moderate temperatures (45°C–65°C), and they are destroyed during subsequent the "wort" boiling stage.

6.3.2.2 Sugar Transformation and Applications

6.3.2.2.1 Glucose Isomerization

Xylose (glucose) isomerase (EC 5.3.1.5, D-xylose ketol-isomerase) is one of the most widely recognized enzymes in sweetener production from corn starch, and it has only been found in microorganisms [3,139,154]. Although it is most selective for xylose, it reacts efficiently enough with glucose in an equilibrium isomerization reaction yielding fructose that it has become one of the most important industrial enzymes, used for the production of high-fructose corn syrup (sweetener). The mechanism of this enzyme and important active site residues was discussed in detail in Section 6.2.4.2. The enzyme exists as homotetramers, ranging 170–200 kDa, with two essential metal cofactors per subunit (Mn^{2+}, Mg^{2+}, and Co^{2+} are common). The enzyme is commercially available (principally from *Streptomyces* spp.) as an immobilized form and packed in a column through which glucose syrup is infused. Typical production steps involve ion exchange and charcoal to refine 40%–50% solids of glucose syrup (93% solids as glucose) resulting from starch saccharification (Figure 6.19). The pH is adjusted to ~7.5 (a compromise between maximal stability at pH 5–7

* Estimates of value of disposed baked goods because of staling in the United States in 1990 were about US$1 billion [56].

and maximal activity between 7 and 9), 1.5 mM Mg^{2+} is added, and the syrup is perfused through the reactor for an appropriate residence time to obtain the desired conversion at 55°C–65°C (even though temperature optima are 75°C–85°C). The temperature is a compromise between maximizing enzyme stability (to allow functioning for several weeks to months), reducing viscosity, preventing microbial growth, and limiting Maillard-type reactions (glycation) of enzyme amino side chains, resulting in inactivation. The greatest limitation in the industrial use of glucose isomerase is thermal instability. Depending on process conditions, a glucose syrup (DE ~95) can be converted into a 42%–45% fructose syrup (balance glucose). Operating the enzyme at more elevated temperatures would favor the yield of fructose (based on the temperature dependence of the equilibrium constant), and molecular biology efforts are being used to engineer greater thermal stability.

6.3.2.2.2 Glucose Oxidation [127,154]

Glucose oxidase (EC 1.1.3.4, β-D-glucose–oxygen 1-oxidoreductase) is obtained primarily from *Aspergillus niger*. It is a dimeric glycoprotein of 140–160 kDa, with a deep binding pocket that hosts glucose through 12 H-bonds and multiple hydrophobic interactions, accounting for its sugar specificity. Despite this, the K_M for glucose is rather high at ~40 mM, but this is compensated for by the high turnover/catalytic rate of the reaction. The enzyme is quite stable up to 60°C and over a pH range of 4.5–7.5, allowing a diversity of conditions to employ glucose oxidase as a processing aid. Glucose oxidase is principally used to deplete egg whites of glucose and reduce the potential for Maillard browning upon dehydration and storage. Egg whites must first be adjusted in pH from ~9 to <7 with citric acid before glucose oxidase is added along with H_2O_2 (to serve as a reservoir for O_2 provided by often coexisting catalase activity), at 7°C–10°C for up to 16 h, prior to spray-drying [85]. Other potential uses of using glucose oxidase for removing oxygen in liquids or within packages or generating gluconic acid (an acidic fermentation product and chemical leavening agent) have not been widely adapted. Glucose oxidase can also be used to generate H_2O_2 as an antibacterial agent (in toothpaste and *via* lactoperoxidase action in milk) or as a dough conditioner (strengthener) by providing oxidants where it may serve as a “natural” agent to replace bromates to induce disulfide linkages in gluten [155].

6.3.2.2.3 Sucose Hydrolysis (Inversion) [126,154]

Invertase (EC 3.2.1.26, β-D-fructofuranoside fructohydrolase) has long been the subject of study, and yeast invertase was the enzyme selected by Michaelis–Menten (1913) to generate the data to construct their kinetic model. About 40 invertases have been sequenced, and they exist as isoforms in plant tissues and microorganisms, being monomeric or oligomeric proteins with molecular masses ranging 37–560 kDa. Many are glycoproteins, and plant isoforms are often referred to as acid, or neutral/alkaline type invertases to reflect conditions of optimal activity (pH 4–5 and 7–8, respectively). Invertase is a β→β-retaining glycosyl transferase, and the common name “invertase” reflects the ability of the enzyme to change (“invert”) the optical rotation of a sucrose solution and not the stereochemistry of its action (Table 6.6). The enzyme is unique in that it can withstand, and remain active at, high osmolalities (up to 30 M sucrose). The catalytic residues (yeast enzyme) are GLU_{204} (acid) and ASP_{23} (nucleophile/base). Substrate selectivity is toward β-D-fructofuranosyl glycosides, the most important one being sucrose. Invertase (from yeast) is used as an exogenous enzyme primarily in the production of soft-centered confections and to produce artificial honey from sucrose. For confectionary use, the enzyme can either be injected into coated confections or mixed with the granular sugar mixture (fondant) immediately before being coated. Allowing the confection to stand provides time for invertase to act on sucrose and cause viscous liquefaction of the center.

6.3.2.2.4 Lactose Hydrolysis [62,126,154]

β-D-Galactosidase (EC 3.2.1.23, β-D-galactoside galactohydrolyase, or lactase) is found in mammals (intestinal tract) and microorganisms and belongs to family 2 of the glycosyl hydrolases.

These enzymes typically exist as homotetramers of polypeptide chains ranging in mass from ~90 to 120 kDa, and the enzyme (*lacZ*) from *Escherichia coli* is representative of lactases (monomeric subunit is 1023 amino acids with five structural domains). Each dimeric unit contributes two catalytic units (one from each polypeptide, each of which furnishes a loop to complete the active site of the other). Thus, there are four active sites for each tetramer, and the binding pocket is a deep cleft at the interface of the polypeptide chains. The catalytic dyad involves the GLU_{537} (nucleophile/base, *E. coli*) and GLU_{461} (acid) residues (Table 6.6). Of the several Mg^+ bound by each subunit, two directly relate to activity. The tightly bound active site Mg^+ (or Mn^+) is coordinated with the catalytic GLU_{461} residue, GLU_{416}, HIS_{418}, and three water molecules. The other Mg^+ interacts with GLU_{797} to stabilize the active site loop structure. Both K^+ and Na^+ are bound and confer dimer–dimer stabilization, increase affinity for substrates, and stabilize transition state and covalent intermediate. Many β-D-galactosides are acted upon by lactase, indicating rather strict specificity for the glycone residue (−1 subsite), although a comprehensive analysis of subsite relationships appears to be lacking. H-bonding of enzyme HIS_{540} with C2–OH, C4–OH and C6–OH confers transition-state stabilization and may have a role in glycone specificity. The broad specificity toward the nongalactosyl residue has led to the use of a model, chromogenic substrate, *o*-nitrophenyl β-D-galactoside, for routine and facile assay of the enzyme. Consistent with being a β→β-retaining enzyme, β-D-galactosidase can also catalyze transglycosylation reactions of galactose with other sugars (lactose, galactose, glucose) through β-1,6 linkages, to form unusual oligosaccharides of 2–5 DP.

The enzyme from microbial sources offers a wide range of pH optima (5.5–6.5 for bacteria, 6.2–7.5 for yeasts, and 2.5–5.0 for fungi) for commercial applications. Temperature optima are 35°C–40°C for the bacterial and yeast enzymes and up to 55°C–60°C for the fungal enzymes. The fungal enzyme is the only form not activated by Mg^{2+} or Mn^{2+}. This operational diversity allows for use of microbial β-D-galactosidases in acidic foods (acid whey, fermented dairy foods) as well as in milk and sweet whey. The enzyme is subject to inhibition by product (galactose), for example, Ca^{2+} and Na^+. Lactose hydrolysis can be used to enhance sweetening power, fermentable substrates, and reducing sugars, reduce the incidence of lactose crystallization (e.g., "sandiness" in ice cream), and allow consumption of dairy products by lactose-intolerant individuals (lacking in a gut lactase–phlorizin hydrolase, an enzyme with two active sites and functions). Commercially, lactose-hydrolyzed fluid milk is produced by direct addition (batch processing) of the yeast enzyme, which can achieve ~70% hydrolysis; the enzyme is subsequently destroyed by pasteurization [155]. Whey or whey permeate solids can be processed by immobilized enzyme reactors using the *Aspergillus* β-D-galactosidase, achieving ~90% lactose hydrolysis.

6.3.2.2.5 Other Glycosidases

β-Glucosidases (EC 3.2.1.21; β-D-glucoside glucohydrolase) are a diverse group of enzymes comprising portions of families 1 and 3 of the glycosyl hydrolases. β-Glucosidase is a β→β-retaining enzyme, with the respective acid and nucleophilic residues being GLU_{170} and GLU_{358} (at 5.5 Å spacing, *Alcaligenes faecalis* enzyme) (Table 6.6). β-Glucosidases come from many microbial and plant sources, with the most widely available enzyme being that from almond (also called "emulsin"). β-Glucosidases tend to have broad pH (4–10) stability and are optimally active at pH 5–7, depending on source. The upper practical temperature range is 40°C–50°C, and while the enzyme is sensitive to sulfhydryl reagents (implying a stabilizing role of CYS), its compact structure renders the enzyme quite resistant to proteolytic attack. β-Glucosidases can hydrolyze sugars (such as cellobiase on cellobiose), thioglycosides, and β-D-glucosides of alkyl and aryl groups (which constitute the aglycon portion). The latter types of β-glucosides can generate aromatic compounds in beverages made from fruits (wine and juices) as well as tea [154,156]. Removal of bitterness (naringin) from citrus juices is afforded by β-glucosidases, and such activity may be present in pectinase preparations used in fruit extract/juice preparation. Some endogenous β-glucosidases may be responsible for the emanation of bioactive agents, such as HCN (from cyanogenic glucosides linamarin in cassava and lima beans, dhurrin in sorghum, amygdalin in almonds, peaches, and apricot pits), and the

anticarcinogenic and goiterogenic (and pungent/bitter flavored) isothiocyanates from glucosinolate substrates in Brassicas (by the enzyme myrosinase, discussed later). While some glycosidases in "pectinase" preparations may liberate aromatic flavors from precursors in treated fruit juices, detrimental effects include the loss of anthocyanin-based coloration and the production of adverse ("rotten fruit") flavors from release of ferulic acid [156].

Isomaltulose synthase is an enzyme with both glycosyl hydrolase and transglycosylation activity. The active site residues include ASP$_{241}$ and GLU$_{295}$ (*Klebsiella* spp. LX3 enzyme), as nucleophile/base and acid, respectively [162]. The two-step reaction pathway involves initial hydrolysis of sucrose (α-D-glucosyl-1,2-β-D-fructose), followed by glycosylation of fructose at the C6-OH site to yield isomaltose (α-D-glucosyl-1,6-β-D-fructose). The net effect is an isomerization and both reaction steps occur at a single active site. An industrial process makes use of immobilized bacterial cells [19] to produce isomaltulose (also called isomaltose), a noncariogenic sweetener, potential prebiotic agent, and substrate for hydrogenation to yield the disaccharide sugar alcohol known as Isomalt®.

α-Galactosidase (EC 3.2.1.22) is used to convert raffinose in sugar beet into sucrose to increase process yield by 3% and facilitate sucrose recrystallization. Mycelial pellets of *Mortierella vinacea* are the source of the commercial enzyme [19].

6.3.2.3 Enzymic Pectin Transformation [154]

Pectin-degrading enzymes are categorized into three general types, polygalacturonase, pectate and pectin lyases, and pectin methyl esterase. The specific reactions caused by these three pectinase activities are shown in Figure 6.20. These enzymes are typically found in plants and microorganisms

FIGURE 6.20 Site of action and reaction mechanism of pectin-degrading enzymes. (Adapted from Benen, J.A.E. et al., Structure-function relationships in polygalacturonases: A site-directed mutagenesis approach, in *Recent Advances in Carbohydrate Engineering*, Gilbert, H.J., Davies, G.J., Henrissat, B., and B. Svensson (Eds.), The Royal Society of Chemistry, Cambridge, U.K., pp. 99–106, 1999; Pickersgill, R.W. and Jenkins, J.A., Crystal structure of polygalacturonase and pectin methylesterase, in *Recent Advances in Carbohydrate Engineering*, Gilbert, H.J., Davies, G.J., Henrissat, B., and B. Svensson (Eds.), The Royal Society of Chemistry, Cambridge, U.K., pp. 144–149, 1999; Whitaker, J.R. Voragen, A.G.J., and D.W.S. Wong (Eds.), *Handbook of Food Enzymology*, Marcel Dekker, New York, 2003.)

(especially fungi) and exist as multiple isoforms. Collectively, this group of enzyme activities comprises "pectinase" preparations, often derived from *A. niger*, which are used in most commercial applications for fruit and vegetable tissue processing, juice extraction, and clarification.

6.3.2.3.1 Polygalacturonase [12,101,145,154]

Polygalacturonases (galacturonide 1,4-α-galacturonidase, EC 3.2.1.15 for *endo*-acting form, and EC 3.2.1.67 and 3.2.1.82 for *exo*-acting forms) are α→β-inverting enzymes that belong to family 28 of the glycosyl hydrolases (Table 6.6). The endo-enzyme from *A. niger* has three conserved $ASP_{180,201,202}$ that function as the general acid–base catalytic units, but they appear to be within 4.0–4.5 Å and not at 9.0–9.5 Å apart common to inverting glycosidases. The prevailing view is that the $ASP_{180,201}$ pair activates water as the nucleophile, while ASP_{202} protonates the leaving group and is assisted by HIS_{223} (also conserved), while a conserved TYR_{291} also assists catalysis. From 4 to 6 substrate-binding subsites exist (−5/−3 to +1, depending on isoform) consistent with the endo-acting property, and this feature confers the minimum substrate size. Isoforms with the greatest strength of binding (affinity) at the −5 subsite do not react randomly, but instead react processively by rebinding and repeatedly hydrolyzing a single chain. LYS_{258} is important at the −1 subsite and may confer the requirement for a Gal*p*A substrate residue binding at this site through ionic interaction with the carboxylate group.

Fungal enzymes are most active over a pH range of 3.5–6.0 (as are plant enzymes), 40°C–55°C, and have molecular masses ranging 30–75 kDa. The result of polygalacturonase action is the depolymerization of pectin and the progressive solubilization of polyuronide structures. The practical outcome of such activity is that intercellular barriers (middle lamella) are broken down and viscosity becomes diminished as enzyme action is sustained. While exopolygalacturonases are also of fungal origin and available, they are nonactive when a methylated galacturonic acid residue binds at the +1 subsite, and since they are not efficient at depolymerization and viscosity reduction, they are of limited utility.

6.3.2.3.2 Pectinesterase [101,154]

Pectin methyl esterases (EC 3.1.1.11, pectin pectylhydrolase) have been best characterized from fungi, although they are prevalent also in plant tissues. Collectively, these enzymes exist as multiple isoforms (acidic, neutral, alkaline) from a given source, range 25–54 kDa in mass; can have broad pH stability (within the general range of pH 2–10), and moderate thermal stability (40°C–70°C), depending on source. Fungal enzymes have optima between pH 4 and 6, while plant enzymes have more alkaline optima (pH 6–8) and often require submillimolar levels of Na^+. Being a carboxylesterase, the catalytic units and mechanism are expected to resemble the ASP–HIS–SER triad (as for lipases and serine proteases). However, two $ASP_{178,199}$ and one ARG_{267} residues (*Erwinia chrysanthemi* enzyme) are conserved among pectin methyl esterases. One ASP is unprotonated and serves to activate nucleophilic water to attack the carbonyl carbon, while the other ASP is acidic and protonates an oxygen of the carbonyl (Figure 6.20). A noncovalent tetrahedral intermediate forms and collapses to yield the free acid and methanol. Studies on the *A. niger* enzyme suggest 4–6 glycone-binding sites, and demethylation cannot occur at the nonreducing terminus of a pectin fragment. Substrate/product selectivities vary among pectin methyl esterases (and isoforms) in terms of the preferred degrees of methylation of the pectin substrate, whether continued hydrolysis is favored on a single pectin chain and whether hydrolysis is at random or at closely spaced sites.

6.3.2.3.3 Pectate Lyase [116,154]

Pectate lyase (EC 4.2.2.2, (1→4)-α-D-galacturonan lyase) and pectin lyase (EC 4.2.2.10, (1→4)-6-*O*-methyl-α-D-galacturonan lyase) also depolymerize pectin, with the former recognizing acidic residues adjacent to the scissile bond and the latter recognizing methyl-esterified residues adjacent to the scissile bond (the galacturonate residue to be attacked is positioned at the +1 subsite). Both enzymes occur in multiple isoforms. Pectate lyase has a requirement for as many as four Ca^{2+} to

coordinate the galacturonate group and the multiple and ASP/GLU residues at/proximal to the active site. These enzymes are prevalent in fungi and also found in bacteria (*E. chrysanthemi* enzymes are among the best known), while found to a limited extent in plants. For pectate lyases, pH optima are often in the alkaline range of 8.5–9.5, and low-methoxy pectins are preferred as substrate. Pectin lyases have pH optima ~6, in accord with the preference to act on acidic (protonated) or fully methoxylated forms of pectin, and *Aspergillus* spp. are common sources. Although pectin lyases do not require Ca^{2+}, this cation stimulates activity and shifts pH optimum to a more acidic region. Both lyases are stable to ~50°C, and a conserved ARG_{218} in pectate lyase (*E. chrysanthemi* enzyme Pel1C) acts as the base in the reaction mechanism to abstract the proton from C5 (Figure 6.20). ARG acting as a catalytic base is unusual, but the calculated p*K* of this residue is 9.5 (p*K* depressed by localized Ca^{+}), consistent with the alkaline pH optimum. LYS serves the same purpose as ARG in other pectate lyases. While the specific proton-donor group is unknown, many ASP/GLU residues line the active site and solvent water may also serve this purpose. Exo-acting enzymes have small subsites (limited to −1 or −2 toward the nonreducing terminus) while endo-acting enzymes range in subsite topography from −2/+2 to −7/+3, depending on the isoform. Because of the pH of vegetable and especially fruit tissues that are processed for juice, pectin lyases are generally more applicable as processing aids than are pectate lyases.

6.3.2.3.4 Applications of Pectin-Degrading Enzymes [3,50,85,154,155]

The common uses of pectinase preparations and related enzymes include tissue maceration, tissue liquefaction, enhanced recovery or extraction (juice or oil), clarification, and facilitated peeling (especially citrus fruits). Commercial "pectinase" preparations are often crude mixtures of several types of pectin-, cellulose-, and hemicellulose-degrading enzymes, and the evolution in "pectinase" applications will be toward more specific deployment of enzymes for specific purposes and products. In most cases, enzyme treatments are conducted 20°C–30°C for several hours for mild processes or at 40°C–50°C for 1–2 h for the more rigorous ones (maceration, liquefaction) at the pH of the juice or extract. Tissue processing begins with an initial crushing (milling) or coarse grinding of the tissue (Figure 6.21). The coarse tissue preparation is subjected to enzyme preparations designed to hydrolyze and depolymerize the middle lamella (pectic substances), as this is conducive to liberating individual cells and aggregates with intact cell walls (Figure 6.21a). Thus, endo-polygalacturonase (especially) or endo-pectin lyases are most suitable and the resulting cell suspensions can be used in pulpy juices or nectars, infant foods, or ingredients for other products.

Other processing streams originate with a finer grinding or crushing of tissue (often by a hammer mill), where maximal expulsion of liquid, and sometimes pulpy matter, is the desired outcome. "Extracts" from pome fruits and berries often require addition of enzymes to convert the viscous to semigelled mash of crushed fruit (caused by partial solubilization of pectins and high water-holding capacity of solids) to maximize juice extraction during subsequent pressing (Figure 6.21b). Pectin-degrading enzymes capable of depolymerizing and degrading highly methoxylated pectins are most suitable and include endo-polygalacturonase and pectin methyl esterase in particular, while endo-pectin lyase may also be used. Juices prepared this way may be either clear or cloudy depending on the specific tissue and combination of enzymes used.

Liquefaction of fruit or vegetable material is used to convert the entire mass of tissue to a liquid product, which also limits the need for subsequent filtration or pressing (Figure 6.21c). This is accomplished with a robust combination of pectinases (polygalacturonase, pectin methyl esterase, and pectin lyases), cellulases (both exo- and endo-β-glucanases), and hemicellulases (acting on xylans, mannans, galactans, and arabinans). Once much of the middle lamella and cell wall material is "solubilized" (as much as 80%), the cells are easily burst by osmotic pressure or shear to release liquid contents. Liquefaction is used to convert many pulpy tropical fruits (mango, guava, banana), olives, and stored apples into juices or oleaginous extracts. These juices may be rendered cloudy or clear depending on the tissue and enzymes used.

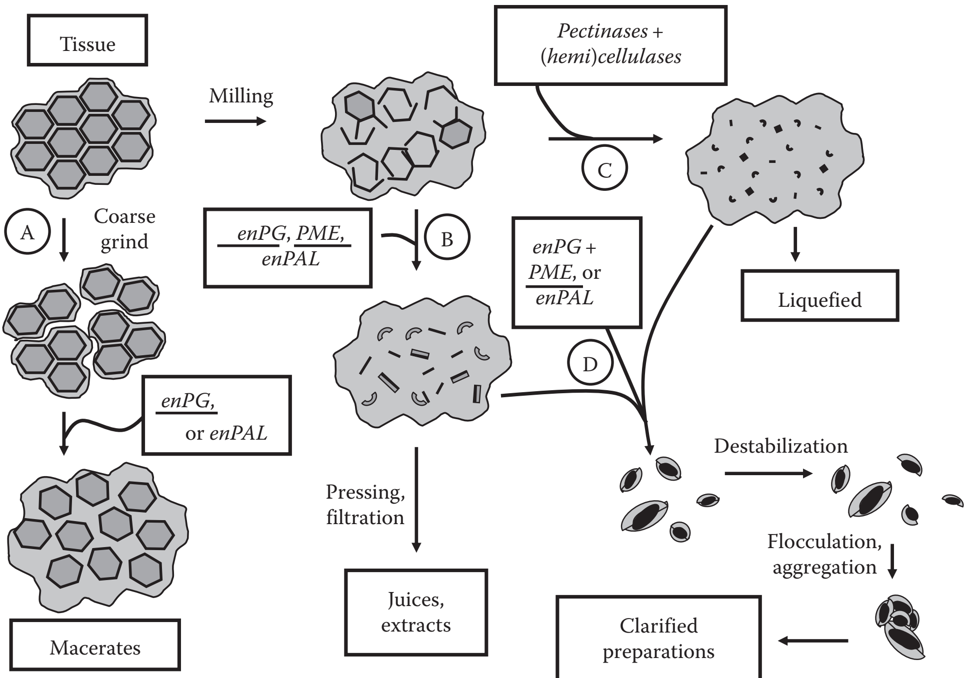


FIGURE 6.21 Commercial processing of fruit and vegetable extracts using pectinases. (Compiled from information contained in Godfrey, T. and West, S. (Eds.), *Industrial Enzymology*, 2nd edn., Stockton Press, New York, 1996; Nagodawithana, T. and Reed, G. (Eds.), *Enzymes in Food Processing*, 3rd edn., Academic Press, New York, 480p, 1993; Whitehurst, R.J. and Law, B.A. (Eds.), *Enzymes in Food Technology*, 2nd edn., CRC Press, Boca Raton, FL, 2002.)

The last major application of exogenous pectin and cell wall–degrading enzymes is for juice or extract clarification (Figure 6.21d). This requires initiating events that destabilize any "cloud" in the juice or extract. Cloud may be desirable in some juices (e.g., orange), but not for those juices such as apple and grape, where transparent juices are preferred. Cloud is conferred by colloidal particles consisting of protein (positively charged at juice pH) coated with pectin (galacturonic acid residues are partially dissociated and negatively charged). Pectin depolymerizing enzymes solubilize and disrupt the pectin layer, allowing protein to electrostatically interact with pectin layers of other particulates, leading to aggregation and flocculation, and providing for easy clarification. The optimum pH for this process has been estimated to be ~3.6, and clarification is most often facilitated by pectin methyl esterase, especially in combination with endo-polygalacturonase, or pectin lyase alone for highly methoxylated pectins (e.g., apples). The role of pectin methyl esterase action is to yield sites for Ca^{2+}-induced cross-linking of particulates, leading to aggregates that easily settle. In some cases, clarified juices may undergo reversion haze. Arabinose comprises ~90% of the polysaccharide material participating in this haze, and haze is minimized if endo-arabinanase is included in the pressing and/or clarification treatment.

In citrus (orange) juices, a process called "pulp wash" calls for pectinases to reduce viscosity of the water extraction of residual pulp before it is added back to the initial juice expressed from the tissue. Citrus can also be enzyme-peeled by scoring the peel and vacuum-infusing pectinase for ~1 h at 20°C–40°C, as this allows the white spongy albedo to be partially digested, leaving the fruit easily peeled and segmented. Specific considerations of which process and enzymes to use for specific fruits and the products to be prepared therefrom exist and are described in detail elsewhere [155].

6.3.2.4 Other Glycosidases

Xylanases (EC 3.2.1.8, β-1,4-D-xylan xylohydrolase) are largely β→β-retaining glycosidases of families 10 and 11 (xylanases in other families also exist), capable of hydrolyzing linear β-1,4-linked polymers of xylose (with various substitutive groups such as arabinose) [126,154] (Table 6.6). Multiple isoforms exist and these enzymes can be endo- or exo-acting (endo-acting are more important in foods). Xylans are a major hemicellulose component and together with cellulose they comprise the bulk of the cell wall material in botanical products. Xylanases are found in plants (especially important in cereals), bacteria, and fungi, and they typically range in molecular mass from 16 to 40 kDa. The *B. circulans* enzyme has catalytic residues of GLU_{78} (nucleophile) and GLU_{172} (general acid/base) with the latter residue cycling between pK_a of 6.7 (free enzyme) and 4.2 (substrate-bound form). The xylanase A from *Pseudomonas fluorescens* has a substrate subsite topography of −4 to +1. In general, subsites range from 4 to 7 residues. Bacterial enzymes are sourced from *Bacillus*, *Erwinia*, and *Streptomyces* spp., while fungal enzymes are sourced from *Aspergillus* and *Trichoderma* spp. Bacterial enzymes have optima of pH 6.0–6.5, while fungal enzymes are most active at pH 3.5–6.0, depending on source, and most xylanases have broad pH stability within the range of pH 3–10. Optimum temperatures for activity range 40°C–60°C.

Xylanase enzymes are beneficial by depolymerizing water-unextractable arabinoxylan into water-soluble pentosans, the latter of which have high water-holding capacity [104]. This increases the viscosity of the dough and leads to increased elasticity, gluten strength, and final loaf volume. Excessive dosing of xylanases or addition of xylanases that preferentially act on water-soluble arabinoxylans either has no effect or result in sticky dough (caused by excessive degradation of water-holding pentosans) with compromised performance. The combination of amylases and xylanases are particularly important in the formulation of frozen doughs [155].

Endo-xylanases are one of the hemicellulases used in fruit and vegetable processing. Xylanases are also used in brewing to reduce viscosity of the wort in brewing, allowing ease in separation/filtration steps, reduced haze formation, and slightly improved process yields. Hemicellulases/xylanases from *Trichoderma* and *Penicillium* spp. have found use in a wet milling process to separate starch from gluten in grains, especially wheat [50].

Other cell wall–degrading enzymes of importance are those that hydrolyze the β-1,4 and −1,3 linkages of glucans, collectively referred to as cellulases and glucanases [154]. These enzymes are added to assist fruit and vegetable tissue liquefaction processes and to brewer's grain to enhance level of fermentable sugars, aid in filtering of spent grains from the wort, and reduce the incidence of "glucan haze" formation [3,155].

Lysozyme has been featured already in terms of mechanism of action (Equation 6.8, Figure 6.15b, and Table 6.6). It has potential for use as an antimicrobial agent, particularly against Gram-positive microorganisms [154]. It is among the smallest enzymes at 14 kDa and the most common source is hen's egg white. It is stable at slightly acidic pH and loses activity in egg white during storage as pH of egg white rises to ~9. It has been used as an antiseptic agent in cheese manufacture [155] and prevents "late blowing" (gas formation) by *Clostridium* spp. in some cheeses [3,50].

6.3.3 Enzymes Transforming Proteins [154]

Proteinases or proteases are used interchangeably to refer to enzymes that hydrolyze proteins. Rules of nomenclature permit the use of these terms, but the preference is to describe such enzymes as exopeptidases or endopeptidases. Proteases are some of the best characterized enzymes in recognition of their vital role in the human digestive system and early commercialization (Christian Hansen marketed a standardized calf rennet for cheese making in 1874). Peptidases that transform food proteins *in situ*, or are added exogenously to cause protein transformation, belong to one of four classes, each of which is described next.

6.3.3.1 Serine Proteases

Among the proteolytic enzymes first studied were the serine proteases secreted by the pancreas, trypsin (EC 3.4.21.4), chymotrypsin (EC 3.4.21.1), and elastase (EC 3.4.21.37); all are involved in human digestion and nutrient assimilation. The serine protease subtilisin (from *B. subtilis*) was featured as an example of nucleophilic mechanism assisted by a charge-relay system in Figure 6.4. Most members of this group have molecular masses of 25–35 kDa, and they are characterized by a surface groove or cleft as the substrate-binding site. Selectivity is conferred by recognizing either the N-terminal (P_1, for the pancreatic enzymes listed earlier) or C-terminal (P_1') residue comprising the scissile peptide bond. Subtilisins from various *Bacillus* spp. are widely used in the preparation of protein hyrolysates, and they tend to exhibit broad selectivity among amino acids comprising the peptide bond (the S_4/P_4 interaction site also confers selectivity). Pancreatic endopeptidases are also used in various applications and their selectivity patterns were illustrated in Figure 6.14.

6.3.3.2 Aspartic (Acid) Proteases

Aspartic proteases are characterized by two highly conserved ASP residues as the catalytic unit, and most are also active under acidic conditions (pH 1–6) with optima near pH 3–4 [154]. Familiar members of this group include the digestive enzyme pepsin, calf chymosin (also called "rennin" or "rennet," used in cheese making), cathepsin (which may be involved in postmortem meat tenderization), and the chymosin-substitute peptidases from *Mucor* spp. These endopeptidases are typically 34–40 kDa in mass and monomeric with two protein domains separated by a deep substrate-binding pocket. Because of their role in nutrient assimilation, pepsins exhibit broad specificity for peptide bonds with a substrate-binding cleft extending from P5 to P3′ [90]. The reaction mechanism for hydrolysis involves a dyad of conserved ASP residues acting as general acid/base ($ASP_{34,216}$ in chymosin) and a noncovalent intermediate. Based on human pepsin, the $ASP_{32,215}$ residues comprise a coplanar platform hosting water in the same plane with ASP_{215} serving as the general base to activate water as nucleophile (Figure 6.22) [39,90]. The ASP_{34} residue enhances electrophilicity of the carbon of the scissile peptide bond by furnishing a low-barrier H-bond to the carbonyl oxygen atom. Attainment of the tetrahedral intermediate (noncovalent with enzyme) is followed by synchronous intermolecular proton transfer to protonate the N atom, leading to cleavage of the peptide bond. Dissociation of the hydrolysis products and H^+ transfer back to the ASP_{32} residue restores the active state pending binding of another water molecule. This molecular architecture and mechanism accounts for the low pH–activity profile of aspartate proteases (pK values of 1.5 and 4.5 for human pepsin) and their ability to cause transpeptidation reactions. The mechanism of transpeptidation remains ambiguous but

FIGURE 6.22 Reaction mechanism of aspartic proteases. (Redrawn from Wlodawer, A. et al., Catalytic pathways of aspartic peptidases, in *Handbook of Proteolytic Enzymes*, 3rd edn., Rawlings, N.D. and G. Salveson (Eds.), Vol. 1, Academic Press, New York, 2013, pp. 19–26.)

must require differential release rates of peptide fragments and binding of another peptide prior to the next water molecule in the catalytic cycle.

Selectivity in protein hydrolysis among aspartic proteases is quite similar in that they recognize nonpolar residues (aromatic, LEU) with broad selectivity (including ASP, GLU) at substrate site P_1. An analysis of the unique features of chymosin specificity toward κ-casein analogues was presented earlier (Table 6.4).

6.3.3.3 Cysteine (Sulfhydryl) Proteases [126,154]

Cysteine proteases are a diverse group of enzymes (over 130 known) present in animals, plants, and microorganisms. Most members of this group belong to the papain family, with other members being chymopapain (EC 3.4.22.6) (multiple isoforms) and caricain (EC 3.4.22.30) from the latex of *Carica papaya*, actinidin (EC 3.4.22.14) from kiwi fruit and gooseberry, ficin (EC 3.4.22.3) from fig (latex), bromelain (EC 4.3.22.4) from pineapple, and lysosomal cathepsins from animal tissues [154]. A unique cysteine protease system in muscle is calpain (multiple isoforms), a two subunit enzyme that is activated by Ca^{2+} and has a role in postmortem tenderization of muscle. Typically, the enzymes of this group are 24–35 kDa in mass, are optimally active at pH 6.0–7.5, and can withstand temperatures up to 60°C–80°C (conferred in part by three disulfide bonds). Conserved residues (papain as reference) include the ion-pair catalytic unit formed by CYS_{25} and HIS_{159}, assisted by ASN_{175}, and GLN_{19} to help stabilize the oxyanion intermediate. Each of the two protein domains contributes one catalytic residue of the ion pair, positioned at a deep cleft between the domains. The mechanism is unique in that nucleophilic and general acid catalysis occur through a thiolate–imidazolium ion pair (Figure 6.23). The thiolate (RS^-) group attacks the electrophilic amide C, yielding an oxyanion covalent intermediate stabilized by the amide NH of CYS_{25} and GLN_{19}. General acid protonation of the leaving amine group by HIS_{159} yields the thioester intermediate,

FIGURE 6.23 Reaction mechanism of cysteine proteases. (Redrawn from Sinnott, M. (Ed.), *Comprehensive Biological Catalysis. A Mechanistic Reference*, Vol. I, Academic Press, San Diego, CA, 1998.)

which is ultimately displaced by HIS_{159}-activated water (through another tetrahedral intermediate). Not shown is the role of ASN_{175}, where the amide oxygen H-bonds with the imidazole $N^{\varepsilon 2}$ atom of HIS_{159}. Cysteine proteases are similar in terms of hydrolytic selectivity. They are considered to have broad selectivity for peptide bonds, with a preference for aromatic and basic amino acids at P_1 and nonpolar substrate residues (especially PHE) at P_2 of the peptide substrate (recall Figure 6.13).

6.3.3.4 Metalloproteases

Metalloproteases constitute the fourth general class of proteolytic enzymes. The most familiar members of this group include exo-acting carboxypeptidase A (peptidyl-L-amino acid hydrolase, EC 3.4.17.1, a digestive enzyme), endo-acting thermolysin (from *Bacillus thermoproteolyticus*, EC 3.4.24.27) originally isolated from a hot spa in Japan, and the neutral endoprotease from *Bacillus amyloliquefaciens* [154]. Most metalloproteases of relevance to quality and processing of food systems are exo-acting and require Zn^{2+} as the metal. They are classified into five families based on the HIS-rich metal-binding motif with a GLU residue, and their primary sequences represent proteins varying in size from 15 to 87 kDa. Both carboxypeptidase A (87 kDa) and thermolysin (35 kDa) have hydrophobic binding pockets that favor nonpolar and aromatic amino acid side chains (especially LEU, PHE) positioned at substrate subsite P_1'. In carboxypeptidase A, a small "hole" is created in part by ARG_{145} and ASN_{144} at enzyme subsite S_1', and this confers the C-terminal, exo-acting nature of this enzyme by coordinating with the P_1'–COO^- group. Thermolysin (an endopeptidase) does not have as constrained a binding pocket as carboxypeptidase and can host a longer segment of the peptide.

A unifying mechanistic model has been proposed for metalloproteases, while leaving room for diversity in catalytic residues [126]. For thermolysin, the Zn^{2+} is coordinated with $^-OH/H_2O$ (coordinate has a pK_a of ~5), which is displaced by binding of substrate (Figure 6.24). HIS_{231} acts as a general base catalyst with a pK_a of ~8 (assisted by ASP_{226}) to activate the nucleophilic water. While GLU_{143} was once thought to be the catalytic base, it is now believed to offer electrostatic stabilization to the δ^+C–O tetrahedral intermediate of the scissile peptide bond; Zn^{2+} also coordinates with the carbonyl δ^-O of the scissile peptide bond. Finally, collapse of the intermediate to yield the product peptides restores the active site. For carboxypeptidase A, the absence of HIS to act as a general base is compensated by the ability of the carboxy terminal residue of the *substrate* to activate nucleophilic water (substrate-assisted catalysis is not rare). Otherwise, the mechanistic features are nearly identical to that for thermolysin. Although the exopeptidase action of carboxypeptidase may be conferred by the small hydrophobic binding pocket, the fact that substrate must provide the general base (carboxylate) function may be equally as important.

6.3.3.5 Applications of Proteolytic Action [76,85,154,155]

Commercial proteases are available at various levels of purity, and some contain multiple proteolytic agents as is typical of *Aspergillus* spp. preparations. Depending on the application, there may be a need for either strict or broad selectivity in protein hydrolysis. Broad selectivity can also be obtained by adding multiple protease preparations. In many cases, proteases secreted by fermentative organisms, whether adventitious or deliberately added as a culture, contribute substantially to proteolysis in food matrices. Some of the important commercial applications of proteolytic enzymes are described in this section.

6.3.3.5.1 Protein Hydrolysates [85,154]

Hydrolysis of proteins by peptidases is done to improve protein/peptide functionality in terms of nutritional, flavor/sensory, textural, and physicochemical (solubility, foaming, emulsifying, gelling) properties, as well as reduced allergenicity (specific examples are cited in Chapter 5). Typically, a protein isolate is treated by a selected endopeptidase as a batch process for a few hours, after which the added enzyme is inactivated by thermal treatment. The major factors that govern the choice of protein as source of the hydrolysate are the value/cost, intrinsic functional properties (which are

FIGURE 6.24 Reaction mechanism of metalloproteases. (Redrawn from Sinnott, M. (Ed.), *Comprehensive Biological Catalysis. A Mechanistic Reference*, Vol. I, Academic Press, San Diego, CA, 1998.)

limited in some fashion to warrant hydrolytic processing), the amino acid composition, and to some extent the primary sequence (if known). These factors are considered in context with the known selectivity of the endopeptidase, especially if there are preferred sites of hydrolysis to obtain the desired functionality. In addition, pH and temperature requirements also impact the suitability of choice among candidate proteins and peptidases to obtain a desired outcome. Endopeptidases are usually employed to attain rapid decreases in average molecular weight of peptides, whereas exopeptidases are used to hydrolyze small oligopeptides to composite amino acids.

Proteins (commonly meat, milk, fish, wheat, vegetable, legume, and yeast sources) may be subjected to a pretreatment that renders them partially denatured as this enhances peptidase access and hydrolytic attack (excessive denaturation may lead to aggregation and hinder hydrolysis). Protein–enzyme levels are poised sufficiently high so the enzyme reacts at nearly V_{max} with limited autodigestion of the enzyme, although product inhibition by accumulating peptides may attenuate reactivity. Protein levels in batch reactions are often 8%–10% provided there are no limitations on solubility, and the amount of enzyme added is generally ~2% on a protein basis, depending on purity. Reaction progress is monitored by one of several means (see Chapter 5), and reaction is quenched when the desired degree of hydrolysis (DH) is achieved. Typically, respective DH values of 3%–6% (average peptide size of 2–5 kDa) is desired for physicofunctionality, DH ~8+% and 1–2 kDa average peptide size for optimal solubility for use in sports and clinical nutrition products, and more exhaustive DH (as high as 50%–70%) to yield small peptides and amino acids of <1 kDa average size for purposes of infant and hypoallergenic foods and savory flavoring ingredient preparations (soups, gravies, sauces). The greater the DH, the greater the opportunity for bitter peptides

(small and hydrophobic) to accumulate, and measures are often required to control this potential flavor defect (discussed later). More recently, there have been reports of preparing bioactive peptides from protein hydrolysates, including Ca^{2+}-binding phosphopeptides from casein for enhanced mineral bioavailability, antioxidant preparations, and peptides that inhibit angiotensin-converting enzyme in human plasma (as a potential intervention to lower blood pressure). Proteases can also be used to isolate residual muscle protein from bones of fish and land animals as protein hydrolysates, and this usually involves incubation at 55°C–65°C for 3–4 h. The final product mixture from enzyme hydrolysis of proteins may require posttreatment refining and/or separation to obtain a product derivative ideally suited for the intended application.

6.3.3.5.2 Milk Clotting [3,154]

Calf chymosin (rennet) and chymosin substitutes are added to milk to cause the initial milk-clotting reaction leading to cheese manufacture. Milk-clotting activity is related to the specific hydrolysis of the PHE_{105}–MET_{106} bond of κ-casein, liberating a glycomacropeptide (the enzymic step) that creates a hydrophobic surface on the micelles prompting them to aggregate (a nonenzymic step). The unique selectivity of chymosin was featured earlier (Table 6.4). Starter cultures are added to milk at 40°C–45°C to cause a pH decline to 5.8–6.5, upon which chymosin is added to initiate clotting. As a result of subsequent steps of cheese manufacture, some enzyme activity remains in the curd and contributes to cheese ripening and flavor development during aging. Proteases in the starter cultures also contribute to sustained proteolysis and flavor development during aging. A recombinant chymosin from *E. coli* K-12 (CHY-MAX®) was the first genetically engineered enzyme to be approved for use in foods and similar commercial preparations are in widespread use. Chymosin substitutes include bovine and porcine pepsins and aspartic endopeptidases from *Rhizomucor* spp. and *Cryphonectria parasitica*, which possess progressively reduced milk clotting–proteolytic activity ratios in the order listed (which leads to reduced process yield and potential bitterness in cheese).

6.3.3.5.3 Meat Tenderization [3,139]

Papain and other sulfhydryl endopeptidases (bromelain and ficin) are applied to muscle or meats that do not become sufficiently tenderized during postmortem aging. These enzymes are effective in this application because they can hydrolyze collagen and elastin, which are connective tissue proteins that contribute to toughness in meat. However, the two drawbacks of tenderization by exogenous endopeptidases are that they can be "overdosed" and the pattern of tenderization is not the same as that which occurs in naturally aged/tenderized meat (proteolysis selectivity patterns are different). Enzyme (usually papain) in a powdered form (using salt or other innocuous material as carrier) can be applied directly to the surface of meats, or the enzyme in dilute saline can be injected or applied as a dip. Antemortem application of enzyme is possible, as a fairly pure solution in saline injected intravenously into animals 2–10 min prior to slaughter, sometimes after stunning; this helps distribute the enzyme throughout the muscle tissues. Injection of inactivated papain (disulfide form) obviates any discomfort among animals, since the enzyme becomes activated by the reducing conditions that soon prevail postmortem. In many cases, owing to the relative thermal stability of these endopeptidases, perhaps as much tenderizing effect occurs during the cooking phase of meat preparation as it does during chilled handling and storage of meat.

6.3.3.5.4 Beverage Processing [85,139]

In beer, a defect referred to as chill haze may be caused by the association (complexing) of tannins and proteins. Papain has long been used (since 1911) to hydrolyze protein and minimize haze formation, although bromelain and ficin, as well as other bacterial and fungal proteases, may now be used for this purpose. The endopeptidase is added postfermentation and prior to final filtering. Papain is ultimately destroyed by typical beer pasteurization, and excessive action of papain may lead to loss in foam stability [155]. Other proteases, particularly the *B. amyloliquefaciens* neutral protease, are added during the mashing step to increase soluble nitrogen from protein to support

subsequent fermentation (and leave less protein to participate in haze formation). Controlled or measured proteolysis in beer is important, since some residual protein is necessary to maintain specific quality attributes.

6.3.3.5.5 Dough Conditioning [3,85,155]

Dough formulations and various types of flour (bread or biscuit quality) confer the strength and rheological properties of the dough and this impacts final product quality. Dough pH is usually ~6.0 but may range widely and approach pH 8.0 in a few cases; available proteases are suitable for the neutral/alkaline pH range with bacterial (*Bacillus* spp.) enzymes and acidic range with fungal (*Aspergillus* spp.) enzymes. Proteases are used to modify and optimize dough strength for a particular product and serve to reduce mixing time to obtain the proper dough viscoelasticity. Proteases can also improve the performance of flours with damaged gluten, which confer less elastic and stiffer doughs. Exogenous proteases are added to affect controlled hydrolysis of the gluten during the dough conditioning stage, although proteases may continue to act during baking until thermally deactivated. Hydrolysis of gluten weakens the gluten network, resulting in enhanced extensibility and viscoelasticity of the developed dough, and these properties are associated with increased bread volume, uniform crumb development, and tenderness of the final product. Controlled hydrolysis is obtained by making use of a protease with moderate peptide bond selectivity (to guard against exhaustive hydrolysis) and dosing at a rate that provides the desired degree of hydrolysis prior to deactivation during the baking cycle. Excessive proteolysis will yield low product volume and textural defects. Use of less specific proteases (or mixtures of proteases) is appropriate when weaker doughs are required for forming into shapes, such as pizza crust, wafers, or biscuits. Choice of protease can be critical to product quality since proteases have different specificities of reaction with the major gluten proteins, gliadin or glutenin (Table 6.7). Such differences can account for different degrees of performance enhancement of doughs by choice of exogenous protease.

6.3.3.5.6 Flavor Modulation (Debittering) [106]

Protein hydrolysates and fermented foods (cheese, cocoa, beer, cured meats, fish sauce, soy) subjected to intermediate degrees of proteolysis by endoproteases may develop bitterness when small hydrophobic peptides accumulate beyond taste thresholds. Exopeptidases are used to “de-bitter” such foods and are available from bacterial, fungal, and plant and animal sources (over 70 are

TABLE 6.7
Hydrolytic Selectivity of Proteases toward Major Gluten Proteins

	Relative Activity Toward		
Protease Preparation	**Glutenin**	**Gliadin**	**Ratio of Activity on Glutenin–Gliadin**
A	1.00	2.17	0.46
B	0.50	0.17	3.0
C	0.69	0.064	11
D	1.30	0.90	1.4
E	0.37	0.19	2.0
F	0.55	0.87	0.63
H	2.07	3.02	0.68
I	2.68	0.38	7.0
G	0.60	0.038	16

Source: Adapted from Tucker, G.A. and Woods, L.F.J. (Eds.), *Enzymes in Food Processing*, 2nd edn., Blackie, New York, 1995.

Note: Individual protease preparations were not specified in the original survey.

catalogued by IUBMB). Exopeptidases may be specific for the C-terminus (carboxypeptidases) or N-terminus (aminopeptidases) and also for liberating a single amino acid, dipeptide, or tripeptide from the substrate. Superimposed on these types of specificity is selectivity for some amino acid residues at the substrate P_1/P_1' or P_2/P_2' site(s), examples being X-PRO-dipeptidyl aminopeptidase and LEU-aminopeptidase. Exopeptidases often require the action of prior and specific endopeptidases to ensure that bitter peptides are efficiently degraded. Exopeptidases from lactic acid bacteria are among the best characterized, and such an understanding allows the strategic use of fermentative or starter cultures or cell-free extracts to control bitterness in fermented or proteolyzed foods. As an example, *Lactobacillus helveticus* CNRZ32 is a commercial strain that reduces bitterness and intensifies flavor development in cheese. It has a complex proteolytic enzyme system that includes endopeptidases with postproline specificity (PRO at S_1) and a general aminopeptidase [17]. Acting in concert, these enzyme activities facilitate degradation of bitter peptides to free amino acids, thereby reducing bitterness.

6.3.3.5.7 Aspartame Synthesis [19,61,154]

Thermolysin, a metalloprotease, is used to synthesize aspartame (L-ASP-L-PHE-OCH_3), a sugar substitute used primarily in low-calorie soft drinks. Thermolysin is a catalyst especially suited for this process: it is stable to 90°C, while being optimally active at 80°C. It is activated >10-fold by high salt levels (1–5 M) allowing it to function well at high osmolarities (high [substrate]), tolerates organic solvents, is selective for preparing the peptide bond at the α-COOH group of ASP (the chemical method can cause reactions at the β-COOH group of ASP, creating a bitter analogue), and does not hydrolyze the methyl ester group of PHE (which is required for sweetening). The synthetic process has evolved to use an immobilized thermolysin in a batch reactor with monophasic ethyl acetate–water as reaction medium, affording yields of >95% at 55°C.

6.3.3.6 Transglutaminase [36,154]

Transglutaminases (EC 2.3.2.12, γ-glutamyl-peptide, amine-γ-glutamyl-transferase) occur in animals, plants, and microorganisms (especially *Streptoverticillium* spp.). In animals they have critical roles in fibrin cross-linking (blood clotting) and keratinization (epidermal tissue development) among other functions; in plants they appear to be involved in cytoskeleton and cell wall formation, while in bacteria they may be involved in coat assembly in sporulating cells. Mammalian transglutaminases (TG) are typically monomeric proteins of 75–90 kDa, while microbial enzymes are about 28–30 kDa. They typically require Ca^{2+} for activity and have neutral to slightly alkaline pH optima. As with endopeptidases, partially denatured or unfolded proteins provide improved access of TG. The types of reactions TG catalyze are (╟ represents the protein backbone) as follows:

$$\text{Cross-linking: } ╟\text{GLN-CO-NH}_2 + \text{NH}_2\text{-LYS}╢ \rightarrow ╟\text{GLN-CO-NH-LYS}╢ + \text{NH}_3 \quad (6.26)$$

$$\text{Acyl transfer: } ╟\text{GLN-CO-NH}_2 + \text{NH}_2\text{-R} \rightarrow ╟\text{GLN-CO-NH-R} + \text{NH}_3 \quad (6.27)$$

$$\text{Deamidation: } ╟\text{GLN-CO-NH}_2 + \text{H}_2\text{O} \rightarrow ╟\text{GLN-COOH} + \text{NH}_3 \quad (6.28)$$

These reactions provide the basis for applications in foods. The most important reaction is cross-linking of proteins by an isopeptide bond (Equation 6.26) that has the capacity to increase the size of the resulting proteins and create a vast network within the food matrix. Examples where this is exploited are the creation of irreversible and temperature-stable gels by cross-linking egg, milk, or soy proteins and gelatin.

Addition of TG during the early stages of yogurt production serves to increase gel strength and reduce syneresis, while in cheese manufacture it may provide greater yield of protein. In baked goods, addition of TG to dough facilitates the formation of a gluten network, enhancing dough stability, gluten strength, and viscoelasticity, leading to improved volume, structure, and crumb of

the final product. For muscle foods, applications of TG revolve around enhancing or controlling the gel strength of surimi products, serving as a binding agent for the creation of formed meat products from low-value small or minced meat fragments, as well as enhancing protein gel strength of ham and sausage products.

6.3.4 Lipid-Transforming Enzymes

6.3.4.1 Lipase

Lipases (EC 3.1.1.3, triacylglycerol acylhydrolase) are distinct from other carboxylesterases in that they act only at the oil–water interface. This requirement is easily seen in the relationship between rate of reaction and increasing levels of substrate (Figure 6.25). While esterases react with soluble substrates by conventional Michaelis–Menten kinetics, lipases do not readily access substrates until they have exceeded their solubility and start to form colloidal aggregates, such as micelles, that pose an interface. Lipases and carboxylesterases almost invariably possess the catalytic triad of GLU(ASP)-SER-HIS as the transforming locus as illustrated for serine proteases (Figure 6.4). Thus, the nucleophilic mechanism involving acyl-enzyme intermediate and two tetrahedral intermediates applies to lipases as well.

While activity of endogenous lipases is often associated with acylglycerol hydrolysis and problems with lipid degradation and/or hydrolytic rancidity (or leading to oxidative rancidity since liberated fatty acids tend to be more prone to oxidation), exogenous lipases are used for beneficial purposes. Currently, commercial uses of lipases involve liberating flavoring (short chain) fatty acids from lipids and rearranging fatty acyl groups along the glycerol backbone to create highly valued and functional triacylglycerols from low-value lipids. Both of these applications are founded on employing lipases with reaction selectivities required to yield the desired products.

Selectivity of lipases was introduced in Figure 6.16 and involves selectivity toward fatty acyl group, ester positional along the *sn*-glycerol backbone, size of the glyceride (mono-, di-, or triacylated), as well as interactions among these factors, which confer characteristic stereoselectivity. The types of selectivity exhibited by many of the commercially relevant or promising lipases of well over 100 characterized sources are shown in Table 6.8. Particularly rare types of selectivity include the preference toward *sn*-2-glycerol sites exhibited by *Candida antarctica* A lipase and a minor

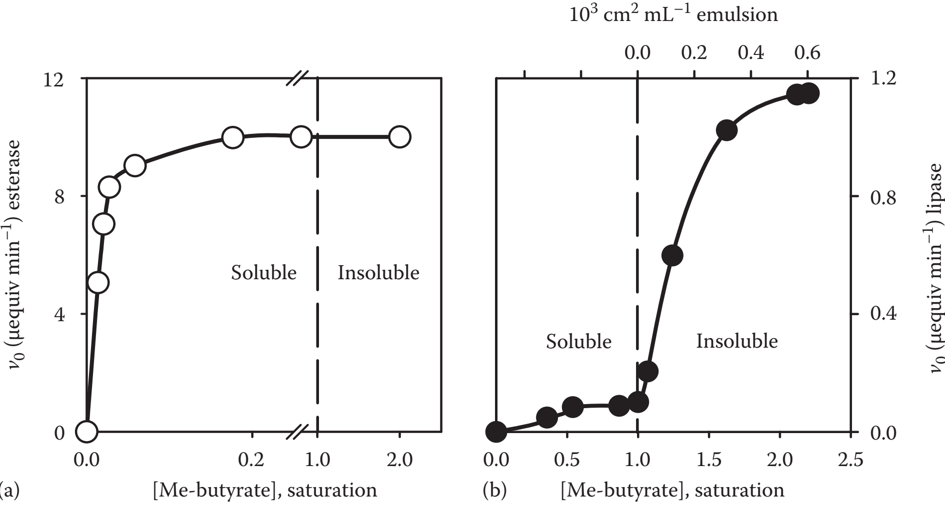


FIGURE 6.25 Differentiation between (a) esterase and (b) lipase on the basis of substrate properties. (Redrawn from Sarda, L. and Desnuelle, P., *Biochim. Biophys. Acta,* 30, 513, 1958.)

TABLE 6.8
Selectivity Patterns of Some Lipases of Commercial Interest or Used in Commercial Applications

Lipase	Preferences Toward			Other Feature or Comment
	sn-Glycerol Sites	Fatty Acid[a]	Glycerolipid[b]	
1. *Aspergillus niger*	*sn*-1,3 ≫ *sn*-2	Short chain, 16	AG	
2. *Candida antarctica* A and B forms	A: *sn*-2 > *sn*-1,3 B: *sn*-1,3 > *sn*-2	Short chain, 18:X 6–10 > broad	AG AG; GL	Fatty acid–binding pocket ~13°C
3. *Candida rugosa*	*sn*-1,3 > *sn*-2; nonspecific	4,8 > broad	AG	Multiple isoforms (formerly *C. cylindracea*) Fatty acid–binding pocket ~17°C
4. *Carica papaya*	*sn*-1,3 > *sn*-2	4, short chain	AG	Latex source containing papain
5. *Geotrichum candidum*	Nonspecific; *sn*-2 > *sn*-1,3	8, long chain, 18:X	AG	Multiple isoforms (minor isoform is *sn*-2 selective)
6. Patatin (potato tuber)	*sn*-1,3 > *sn*-2	8,10	MAG > DAG; GL, PL	General lipid acyl hydrolase
7. *Penicillium* spp.	Nonspecific; *sn*-1,3 > *sn*-2	Long chain	MAG, DAG	Multiple isoforms
8. Pancreatic	*sn*-1,3 (strictly specific)	4 > broad	AG	Fatty acid–binding pocket ~8°C
9. *Pseudomonas* spp.	Nonspecific; *sn*-1,3 > *sn*-2	8,16	AG	*Burkholderia* spp. similar fatty acid–binding pocket ~14°C
10. *Rhizomucor miehei*	*sn*-1,3 ≫ *sn*-2	8–18	AG; PL, GL	Fatty acid–binding pocket ~18°C
11. *Rhizopus arrhizus*	*sn*-1,3 ≫ *sn*-2	8–14	AG; GL, PL	*Rhizopus* spp. lipases almost identical

Sources: Ader, U. et al., Screening techniques for lipase catalyst selection, in *Methods in Enzymology*, Rubin, B. and E.A. Dennis (Eds.), Vol. 286, *Lipases, Part B. Enzyme Characterization and Utilization*, Academic Press, New York, pp. 351–387, 1997; Gunstone, F.D. (Ed.), *Lipid Synthesis and Manufacture*, CRC Press LLC, Boca Raton, FL, 472p., 1999; Lee, C-H. and Parkin, K.L., *Biotechnol. Bioeng.*, 75, 219, 2001; Persson, M. et al., *Chem. Phys. Lipids*, 104, 13, 2000; Pinsirodom, P. and Parkin, K.L. *J. Agric. Food Chem.*, 48, 155, 2000; Pleiss, J. et al., *Chem. Phys. Lipids*, 93, 67, 1998; Rangheard, M-S. et al., *Biochem. Biophys. Acta*, 1004, 20, 1989; Sugihara, A. et al., *Protein Eng.*, 7, 585, 1994; Yamaguchi, S. and Mase, T., *Appl. Microbiol. Biotechnol.*, 34, 720, 1991.

Note: Ambiguities and inconsistencies among compiled observations are common and are founded on the variety of reaction designs in which selectivity patterns are established.

[a] Fatty acids are designated as number of carbons in *n*-acyl chain; 18:X denotes 18C fatty acid with X = 0–3 double bonds.

[b] AG, acylglycerols; GL, glycolipid; PL, phospholipid; MAG, monoacylglycerol; DAG,diacylglycerol.

lipase isoform of *Geotrichum candidum*, although this feature may be linked to the type of substrates (fatty acyl groups) used in studying this trait. Many lipases in Table 6.8 have been analyzed for stereoselectivity (Figure 6.16d). Lipases typically have optimal pH and temperature ranges of 5.0–7.0 and 30°C–60°C, respectively.

6.3.4.2 Lipase Applications

6.3.4.2.1 Flavor Generation

Lipases used to generate age-related "piccante" flavors in cheeses, especially of the Italian and mold-ripened varieties, are selective for hydrolyzing short-chain (C4–C8) fatty acids from triacylglycerols of milkfat and include pregastric lipases from goat, lamb, and calf [3,50,155]. Since these short-chain fatty acids are enriched at the *sn*-3-glycerol position, a lipase that is selective for

this site would also be applicable for this purpose. The lipase in papaya latex is selective for the *sn*-3-glycerol position, but since papaya latex contains papain, it would not be suitable in cheese. Alternatively, some microbial lipases (*C. rugosa*, or *R. miehei* and *A. niger*) are also known to release short-chain and/or *sn*-1,3-linked fatty acids from milkfat (Table 6.8). Most lipases hydrolyze unsaturated fatty acids that may be precursors to oxidative products of ketones and lactones, some resulting from microbial metabolism. Lipases are also used to prepare enzyme-modified cheese for use as processed cheese, spreads, sauces, or flavoring ingredients, and subsequent pasteurization serves to destroy residual enzyme activity. Overdosing of enzyme can lead to soapy or overly pungent flavor.

6.3.4.2.2 Acylglycerol Restructuring

Another major use of lipases is for the strategic rearrangement of fatty acyl groups to yield a predetermined distribution along *sn*-glycerol to create high-value lipids from low-value ones [53]. The intended result is the preparation of "structured lipids." The basic approach to lipase restructuring of lipids is the use of microaqueous (<1% moisture) reaction media comprised primarily of organic solvent or just lipid substrate itself (to serve as "solvent"). Under these conditions, net reactivity of lipids with the lipase is in the direction of ester (re)synthesis and not hydrolysis. The various types of lipase-mediated processes that can be conducted (Figure 6.26) involve reactions of a single triacylglycerol substrate alone (interesterification, path D); between a triacylglycerol substrate and an exogenous source of fatty acid(s) (acidolysis, path A), fatty acyl-ester(s) (transesterification, path B), or alcohol(s) (alcoholysis, path C); or between fatty acid and alcohol cosubstrates (esterification, path E). The most successful applications have strategically exploited the characteristic selectivities of lipases combined with the known distribution of fatty acids within the starting material (natural sources of triacylglycerol), and such applications will be highlighted in the next paragraph.

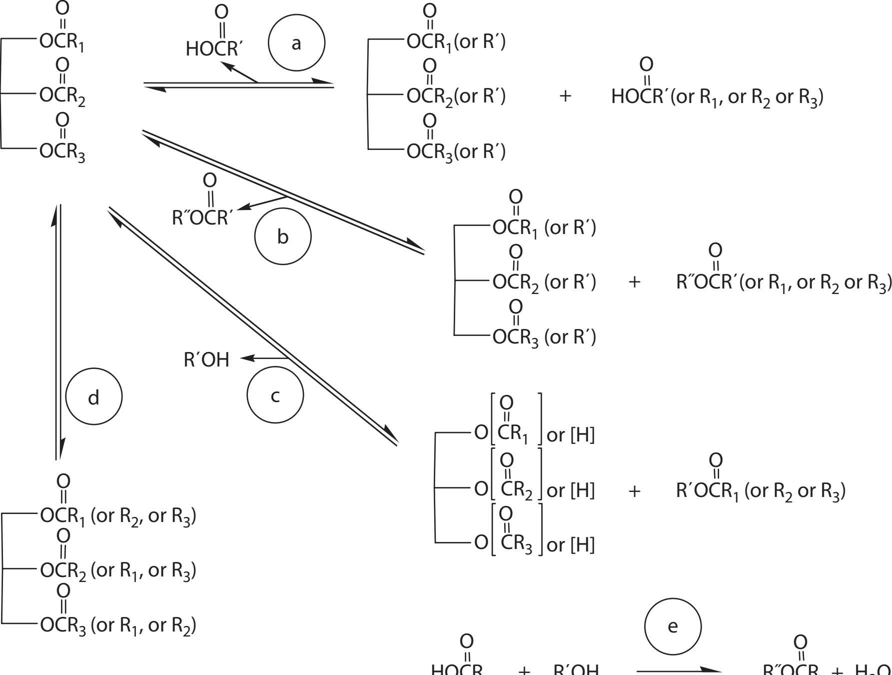


FIGURE 6.26 Types of acyl-restructuring reactions mediated by lipase in microaqueous media: (a) acidolysis, (b) transesterification, (c) alcoholysis, (d) interesterification, and (e) esterification.

Cocoa butter is a premium fat because of its high natural "purity" with >80%–90% of the triacylglycerol molecular species being POSt (38%–44%), StOSt (28%–31%), and POP (15%–18%),* providing a sharp, cooperative melting profile ([53], Chapter 4). Cocoa butter substitutes can be prepared using an *sn*-1,3-regioselective lipase and a palm oil midfraction (58% POSt) combined with exogenous stearic acid using an "acidolysis" approach (Figure 6.26a) in a stirred-tank reactor for 16 h at 40°C. The result is a product that is 32% POSt, 13% StOSt, and 19% POP. The process makes use of *Aspergillus*, *Rhizomucor*, or *Rhizopus* lipases, which can also be immobilized in a packed bed reactor for faster product throughput. The first commercialized enzyme-structured lipid preparation is Betapol®, a fat derivative enriched in OPO, which is the major triacylglycerol in human breast milk [120]. Thus, OPO comprises a nutritional product for use in infant formula. In this application, tripalmitin (PPP; enriched in palm stearin) is a suitable starting material and can be reacted with oleic acid (1:1 w/w) in an acidolysis reaction (Figure 6.26a) with an *sn*-1,3-selective lipase. A two-stage process with an *sn*-1,3-selective lipase involves an initial alcoholysis reaction of PPP with ethanol (Figure 6.26c) to yield an *sn*-2-palmitoylglycerol, followed by an esterification reaction (Figure 6.26e) in the presence of oleic acid. Betapol can also be prepared from native lipid resources of PPP-rich palm oil fraction and high-oleic sunflower or canola oils. Similar approaches can be used to prepare other "structured lipids" with lipases, including medical/dietetic lipids, but the current commercial products are produced by chemical processes.

6.3.4.2.3 Dough Improvement

Lipases are common ingredients in bread doughs [3,139,155]. They supplement endogenous cereal grain lipases and are added as dough improvers, which manifests as increased bread volume, more uniform crumb and air cell size, and lesser tendency to stale, without influencing rheological (mixing) properties of the dough. These improvements are derived from lipase hydrolysis of cereal and/or added lipids, giving rise to emulsifying agents, such as mono- and diacylglycerolipids, which can help incorporate and stabilize small air cells in the dough. Monoacylglycerols can also form inclusion complexes with amylose, and this reduces the tendency for starch to retrograde (stale) after baking. Also the addition of lipases instead of added emulsifiers as ingredients provides a "cleaner" label declaration. Lipases commonly used in baking [50] are sourced from *Rhizomucor* and *Rhizopus* spp., which can hydrolyze glycolipids and phospholipids in addition to acylglycerols (Table 6.8); lyso-phospholipids and lyso-glycolipids are potent surface-active agents. Lipases are also used in noodle formulations as it improves whiteness, an important quality attribute [155]. This effect may result from oxidation of liberated unsaturated fatty acids and bleaching of dough through secondary reactions. Lipase addition also reduces cracking in dried noodles and stickiness upon cooking; this is associated with reduced leakage of starch, perhaps through complexing with fatty acids and lyso-glycerolipids.

6.3.4.3 Lipoxygenases

Lipoxygenase action is generally considered to have detrimental effects on food and lipid quality, and this aspect will be addressed later in this chapter. One beneficial use of lipoxygenase is to provide oxidizing power during dough conditioning [155]. Lipoxygenase oxidizes unsaturated fatty acids (made available by added lipases) generating oxidizing conditions that help strengthen the gluten network by affecting disulfide cross-links within the gluten, enhancing dough viscoelasticity. The addition of soy flour to bread dough is the preferred way of incorporating lipoxygenase and this can lessen or eliminate the need from more conventional oxidizing agents such as bromates. Secondary oxidation reactions can also destroy endogenous carotenoids and affect a bleaching or whitening of the final products, as desired in noodles and some breads.

* Triacyl-*sn*-glycerol species are identified using the shorthand designations of fatty acids (Chapter 4) of St for stearic acid, *P* for palmitic acid, and *O* for oleic acid, listed in order as occurring at the *sn*-1, *sn*-2, *sn*-3 positions.

Phospholipase action Galactolipase action

Phospholipase A_1 Phospholipase A_2 Phospholipase C Phospholipase D

R_2—C(=O)—O— ; —O—C(=O)—R_1 ; —O—P(=O)($O^{\ominus}$)—O—X ; —O— 6-O-βGAL-(1,6-αGAL)$_{0-1}$

FIGURE 6.27 Bond specificity for lipolytic enzymes acting on polar glycerolipids.

6.3.4.4 Phospholipases

Phospholipases are classified as types A_1, A_2, C, and D, each with different and exclusive bond selectivities toward phospholipids (Figure 6.27). One commercial application is the addition of phospholipase A_2 (EC 3.1.1.4) (*Aspergillus* spp. and pancreatic sources are common) to crude oil during the degumming stage to hydrolyze phospholipids at the *sn*-2 site to create the corresponding lyso-phospholipid [50]. This is important for the removal of otherwise nonhydratable phospholipids. Phospholipase A_2 has potential use as an agent to create superior lyso-phospholipid emulsifiers from phospholipid-rich sources, such as egg yolk [3], and this effect may occur *in situ* in bread manufacture by virtue of addition of lipase with phospholipase A_2-like activities (Table 6.8).

6.3.5 Miscellaneous Enzyme Applications

An acid urease (EC 3.5.1.5, urea aminohydrolase) from *Lactobacillus fermentum* is approved for use in wine to prevent accumulation of urea, which can otherwise react with ethanol to form ethylcarbamate, an animal carcinogen. Hexose oxidase (EC 1.1.3.5) has been added to bread dough where multiple hexoses exist and are available as substrate to yield oxidizing equivalents as dough conditioners [3]. Catalase (EC 1.11.1.6, H_2O_2–H_2O_2 oxidoreductase) is specifically added to remove residual H_2O_2 in milk that has been treated with such to reduce microbial loads when refrigeration is not readily accessible [50]. Sulfhydryl oxidase (thiol oxidase, EC 1.8.3.2, thiol–O_2 oxidoreductase) has long been considered as a solution to cooked flavor defect in UHT milk that is caused by thiols formed during processing [154]. Sulfhydryl (thiol) oxidase from *A. niger* has been suggested as possible dough conditioning agent by providing oxidizing power and forming disulfide bonds in gluten [3].

Going forward, as cost of enzyme production is reduced by biotechnological and genetic advances, enhanced competitiveness of enzyme-mediated processes will lead to expanded commercial uses. Space constraints preclude mention and discussion of other enzymes with commercial potential as processing aids. Enhancing range of thermal and pH stabilities will continue as a priority, and recovery of valuables from agricultural waste streams by enzyme processes is likely to attract increasing attention.

6.4 ENVIRONMENTAL INFLUENCE ON ENZYME ACTION

Temperature, pH, and water activity are among the most important environmental factors that influence enzyme activity, and changes in these parameters comprise the principal physical means to control enzyme action in food matrices. This section will examine the basis for how these factors affect enzyme function.

6.4.1 Temperature

6.4.1.1 General Responses of Enzyme Action to Temperature

Temperature has predictable and opposing effects (activation and deactivation) on enzyme activity. Increasing temperature increases free energy in the system; the net result is the lowering of the energy barrier for reactions to occur and they are accelerated. Recall Equation 6.1 (Section 6.2.3.1), and if the Arrhenius frequency factor "A" is substituted for the combined constants "PZ," the log transformation yields

$$\ln k = \ln A - \frac{E_a}{RT} \tag{6.29}$$

Equation 6.29 predicts a linear relationship between ln k and $1/T$ with a slope of $-E_a/R$. Greater E_a values signify greater temperature *dependence* of reactions.

Note that this relationship (Equation 6.29) holds only for examining and predicting rate constants (k_x) or parameters composed of, or directly proportional to, rate constants such as k_{cat}, $V_{\max}$, K_M, and $V_{\max}/K_M$ or K_S, provided that reaction order does not change with temperature. Simply measuring enzyme activity under a specified condition does not satisfy this requirement. "Breaks" or discontinuities in the linear (negatively sloped) portion or nonlinearity of Arrhenius plots have been offered as evidence of major biochemical events, such as lipid phase transitions for membrane enzymes or the presence of multiple enzyme isoforms. It is just as likely that such breaks represent a temperature-dependent shift in the magnitude of a rate constant such as K_M or a change in reaction order, rate-limiting step, or ionization of a critical residue [54,125].

The utility of the Arrhenius plot is that it provides for an estimate of E_a, which is an indicator of catalytic power for an enzyme reaction relative to a corresponding uncatalyzed or chemically catalyzed reaction (cf., Table 6.1). A departure from linearity (but not a "break") on Arrhenius plots for enzyme activity occurs at progressively elevated temperature (at ~0.0030 K^{-1} on the x-axis in Figure 6.28a) because of the second effect of temperature on enzymes which is to cause denaturation. Increases in temperature beyond the maximum or "optimum" for enzyme activity leads to a sharp decline in reaction rate constant, and this positively sloped linear portion of the plot represents an E_a

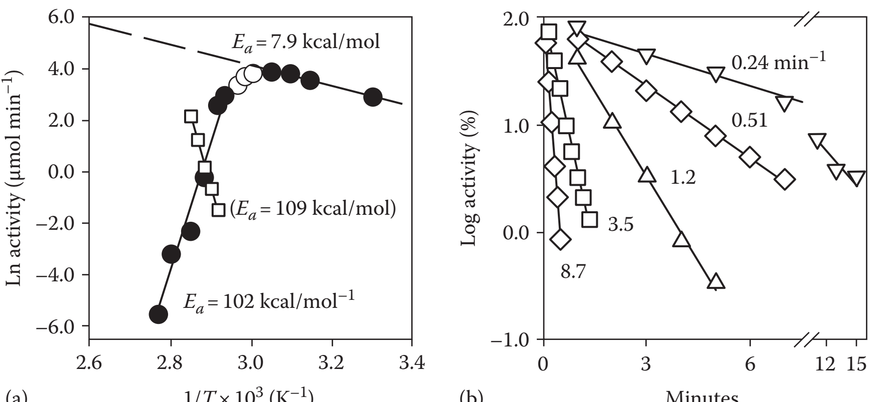


FIGURE 6.28 Thermal sensitivity of tomato fruit pectin methyl esterase. (a) Arrhenius plot (Redrawn from Laratta, B. et al., *Proc. Biochem.*, 30, 251, 1995.), where original data appear as circles and only closed circles are used to construct linear approximations. Open square plots are from data derived from panel (b). (b) First-order deactivation plots (Redrawn from Anthon, G.E. et al., *J. Agric. Food Chem.*, 50, 6153, 2002.), where increasing slopes of plots correspond to incubation temperatures of 69.8°C, 71.8°C, 73.8°C, 75.8°C, and 77.8°C.

for enzyme deactivation (102 kcal/mol in this example). E_a values for enzyme deactivation typically range 40–200 kcal/mol compared to 6–15 kcal/mol for activation. Protein denaturation involves the unfolding of large segments of the polypeptide chain, a global process requiring greater free energy change than that required for stabilization of the transition state at the active site (a localized process).

It can be difficult to accurately determine reaction v_o (i.e., linear rates) at temperatures where the enzyme is initially active but rapidly inactivating, as a means to determine the thermal deactivation of enzyme (as in Figure 6.28a). A more direct way of determining the parameters of thermal inactivation of an enzyme is to incubate the enzyme at various temperatures and test for residual activity remaining under standardized conditions of enzyme assay (usually at optimum pH and a nondeactivating temperature) after various time intervals (Figure 6.28b). The assay for enzyme should use $[S] \gg K_M$,* such that resulting reaction rates are $\sim V_{max}$ ($\propto E_T$) and rate limiting and linear with respect to $[E]$. Since enzyme deactivation is often a first-order process ($[E_o]$ is the initial level)

$$[E] = [E_o]e^{-kt} \quad \text{and} \quad \ln\frac{[E]}{[E_o]} = -k_d t \tag{6.30}$$

Results are interpreted as semilog plots (a factor of 2.303 is used to interconvert log and ln plots), and for each temperature assessed, a corresponding k_d (deactivation rate constant) can be estimated by linear regression (slopes = $-k_d/2.303$) (Figure 6.28b). The collection of k_d values can be transposed to an Arrhenius plot (Figure 6.28a) to estimate E_a for enzyme inactivation, which is E_a of 109 kcal/mol in this example. Thus, good agreement is observed from independent studies using alternative means to determine the thermal sensitivity of pectin methyl esterase of tomato fruit.

6.4.1.2 Optimum Temperature for Enzyme Function

A temperature optimum for enzyme activity results from the net activating and deactivating effects of temperature. While the temperature optimum is where enzyme reaction rate (v_o) is greatest, this condition lasts for a limited duration and over time, progressive denaturation soon dominates, and much of the original activity is lost. An example of typical patterns of thermal behavior of enzymes is provided by pullulanase from *Aerobacter aerogenes* (Figure 6.29a). Note the more

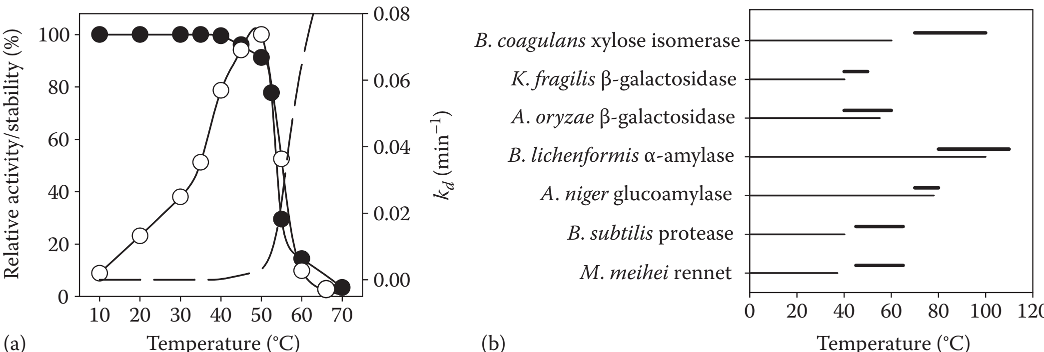


FIGURE 6.29 Thermal sensitivity of (a) pullulanase and (b) various commercial enzymes. (Data selected and figures redrawn from Godfrey, T. and West, S. (Eds.), *Industrial Enzymology*, 2nd edn., Stockton Press, New York, 1996; Ueda, S. and Ohba, R., *Agric. Biol. Chem.*, 36, 2382, 1972.) Closed symbols represent enzyme stability, open symbols represent enzyme activity, and dashed line represents dependence of enzyme deactivation rate constant in panel (a). In panel (b), bold bars represent intrinsic optimum temperature range of the enzyme, and narrow bars indicate process temperatures where these enzymes are typically used.

* Sometimes limits in *S* solubility or other complicating factors render this condition difficult to attain.

gentle progression of upward slope for the activity curve at 10°C–40°C compared to the sharp ascent in deactivation rate constant (k_d) at 50°C–60°C and sharp descent in enzyme activity/stability at 50°C–60°C. These trends of greater thermal dependence (greater E_a values) of enzyme deactivation over temperature activation of reaction can be also be seen from the plots for tomato pectin methyl esterase (Figure 6.28a). Thus, as temperature increases, the acceleration of enzyme deactivation at some point becomes the dominant influence of temperature. The temperature-dependent activity and stability profiles of several food-related enzymes are provided in Figure 6.29b. The practical upper temperature limits of an enzyme reaction in food applications are often 5°C–20°C below the temperature where maximum reaction rate is observed, with the goal to maintain elevated and persistent enzyme activity during the scheduled process.

An analogous plot is reserved for evaluating the temperature influence on equilibrium processes. The plot is similar to Figure 6.28a except that the ordinate is log K and the slope is proportional to ΔH^o, instead of E_a:

$$\frac{d \ln K}{d(1/T)} = \frac{-\Delta H^o}{R} \tag{6.31}$$

An example summarizes the temperature dependence of the equilibrium constant (K_{eq}) for the glucose ⇆ fructose isomerization catalyzed by xylose isomerase (Figure 6.30). This plot finds utility in characterizing temperature dependencies of other equilibria related to optimum enzyme functioning such as $K^‡$ for the transition-state theory, ionization of amino acid side chains (K_a) involved in enzyme activity, or enzyme kinetic functions that represent (pseudo-)equilibria (K_M, K_S).

There are other temperature effects on enzyme activity. Cold deactivation of enzymes may occur for oligomeric enzymes when nonpolar forces are involved in polypeptide association. Low temperature reduces the strength of these interactions (Chapter 5) and may promote dissociation of subunits and compromise activity. Elevated temperature generally reduces aqueous solubility of gases, and reactions that require O_2 may become limiting depending on the K_M for and solubility of dissolved O_2. Some lipid substrates undergo phase transitions over temperature ranges relevant to foods. The presence of solid phase domains, especially in phospholipid bilayers, constitutes a surface defect and creates access for lipolytic enzymes, often leading to enhanced hydrolysis.

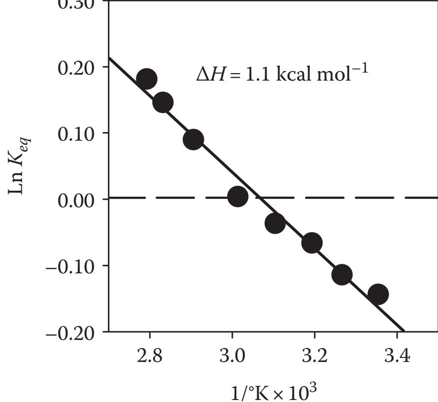


FIGURE 6.30 Thermal sensitivity of reaction equilibrium constant of xylose isomerase. (Figure redrawn from Rangarajan, M. and Hartley, B.S., *Biochem. J.*, 283, 223, 1992.)

6.4.1.3 Summary of Temperature Effects

While each enzyme exhibits unique behavior, some general observations can be made regarding enzyme thermal stability. Ligands (substrates or even inhibitors) improve stability by helping to retain native structure at and around the active site. Other compositional factors in the medium may also enhance or diminish thermal stability. Some general tendencies of enzyme thermal stability are that it is enhanced by the decreasing size of the protein, lesser number of polypeptide chains, increasing number of disulfide linkages and salt bridges, elevated protein levels, and being in a native over *in vitro* environment, for soluble over membranous proteins, and for extracellular over intracellular proteins.

6.4.2 pH Effects

6.4.2.1 General Considerations

All ionizable groups in proteins will undergo pH-dependent transitions based on intrinsic pK_a values of amino acid residues (Table 6.9). Many of these transitions will impact enzyme *stability,* and over a narrow pH range, they may act cooperatively to completely destabilize the enzyme (see Chapter 5). On the other hand, most amino acid side chain ionizations have no or limited impact on enzyme *activity* and they remain "transparent" in the context of enzyme function. Rather, there are a limited number (often 1–5) of amino acid residues for which ionization state confers pH dependence of enzyme activity. Ionization of substrate, product, inhibitor, and cofactors may also have impact on enzyme reactivity, and pH may influence K_{eq} or equilibrium distribution of reactants in an enzyme reaction.

6.4.2.2 Enzyme Stability as a Function of pH

Enzymes have a characteristic dependence of stability on pH; an example is provided by the *A. aerogenes* pullulanase (Figure 6.31a). Two general tendencies are that (1) the pH range of enzyme stability is usually broader than the pH range for enzyme activity and (2) enzyme stability declines rapidly at destabilizing pHs, because pH destabilization is a cooperative process. In contrast, the decline in enzyme activity as a function of pH usually exhibits a more measured transition with features of a titration curve, where 1–3 ionizable groups are the only determinants of activity response to pH where each transition occurs. Stability of enzymes to pH is measured by exposing (preincubating) the enzyme at various pHs and then measuring residual activity at standardized conditions of (near-)optimum pH and a specific, nondenaturing temperature. A plot similar to that used to characterize k_d values for thermal sensitivity of enzymes can be used with pH replacing temperature as

TABLE 6.9
Ionization Properties of Amino Acid Ionizable Groups in Enzymes

Ionizable Group	pK_a (25°C)	ΔH_{ion} (kcal/mol)	Ionizable Group	pK_a (25°C)	ΔH_{ion} (kcal/mol)
Carboxyl			Ammonium		
C-terminal (α)	3.0–3.2	~0 ± 1.5	N-terminal (α)	7.5–8.5	10–13
β/γ- carboxyl (ASP, GLU)	3.0–5.0		ε-amino (LYS)	9.4–10.6	
Imidazolium (HIS)	5.5–7.0	6.9–7.5	Phenolic (TYR)	9.8–10.4	6.0–8.6
Sulfhydryl (CYS)	8.0–8.5	6.5–7.0	Guanidium (ARG)	11.6–12.6	12

Source: Fersht, A., *Enzyme Structure and Mechanism*, 2nd edn., W.H. Freeman & Company, New York, 1985; Segel, I.H., *Enzyme Kinetics. Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*, John Wiley & Sons, Inc., New York, 1975; Whitaker, J.R., *Principles of Enzymology for the Food Sciences*, 2nd edn., Marcel Dekker, New York, 1994.

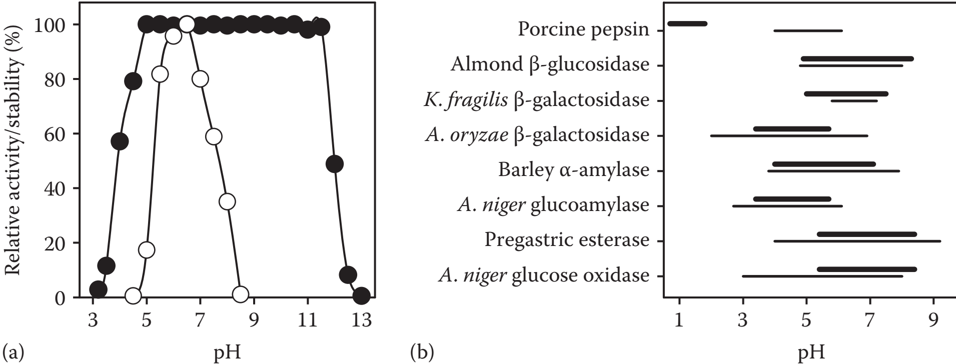


FIGURE 6.31 pH sensitivity of (a) pullulanase and (b) various commercial enzymes. (Data selected and figures redrawn from Godfrey, T. and West, S. (Eds.), *Industrial Enzymology*, 2nd edn., Stockton Press, New York, 1996; Ueda, S. and Ohba, R., *Agric. Biol. Chem.*, 36, 2382, 1972.) Closed symbols represent enzyme stability and open symbols represent enzyme activity in panel (a). In panel (b), bold bars represent where enzyme maintains >80% activity, and narrow bars indicate where enzyme exhibits >80% stability.

the variable of interest (Figure 6.28b). As with temperature sensitivity, enzyme stability to pH may be dependent on medium constituents and conditions; for example, the presence of substrate and other ligands may enhance pH stability, an example being the expansion of the pH stability range where α-amylase is >50% active from pH 4–7 to 4–11 in the presence of Ca^{2+} [153]. In some cases, pH-induced losses in activity may be reversible, but usually within a limited range of destabilizing pH and for a limited duration. Pullulanase is inactive but stable at pH 9–11 for at least 30 min, and within that period of time, activity can be fully recovered by adjustment to pH 6–7 (Figure 6.31a).

Knowing pH stability of enzymes is obviously important for selecting an enzyme compatible with conditions prevailing for a potential application such that the enzyme will persist long enough to fulfill the expected function. It is also important to understand if enzyme destabilization contributes to a decline in activity at a given pH so that an analysis of pH effects on activity can be accurately interpreted (next section). Enzyme pH stability for selected commercial enzymes is shown in Figure 6.31b; pH stability ranges shown here are at temperatures encountered during processing, where stability is more limited than in the pullulanase example (where pH stability was measured at a nondenaturing temperature of 40°C). Likewise, temperature stability becomes reduced at pH ranges away from the optimum for stability of the enzyme. Thus, temperature and pH have coordinative influences on enzyme stability.

6.4.2.3 Effects of pH on Enzyme Activity [43,122,153]

Just like the catalytic locus of an enzyme is comprised of a few critical amino acids, the pH response of enzyme activity is also based on a few ionizable amino acids. The role of these amino acids can be (1) to confer conformational stability at the active site or be involved in (2) substrate binding or (3) substrate transformation, where the ionization state is critical to these roles. The pH range of >80% maximal activity at common processing temperatures for selected food enzymes also appears in Figure 6.31b.

To understand the basis for the effect of pH on enzyme activity, consider a typical "bell-shaped" pH dependence of activity often observed for enzymes (Figure 6.32a). The essential feature of this profile is the presence of separate alkaline-side and acidic-side transitions, referred to as respective H^+-activating and H^+-deactivating steps. Thus, protonation of the alkaline pK_a group allows enzyme to function, and protonation of the acidic pK_a group attenuates enzyme function. Other types of pH behavior shown (Figure 6.32b) include a single pH transition (plot 1), including one with a steeper decline in activity than the other (plot 2), and a case where a pH transition leads to a lesser active (instead of inactive) enzyme state (plot 3).

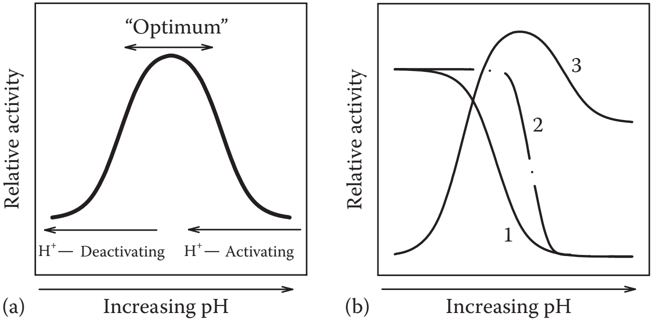


FIGURE 6.32 Typical responses of enzyme activity to pH. See explanations in the text.

The empirical assessment of pH "optimum" of enzyme "activity" under specified conditions of enzyme assay (such as in Figure 6.31a) is rather arbitrary and has limited meaning. It is more informative to ascertain if the pH effect is on conformational stability, substrate binding, or substrate transformation. Thus, analysis of pH dependence of V_{max} and K_M provides insight into how enzyme function responds to pH. The pH behavior of critical enzyme ionizable groups is modeled identically to the ionization status of other weak acids and bases:

$$EH \rightleftarrows E^- + H^+ \quad \text{and} \quad K_a = \frac{[H^+][E^-]}{[EH]} \tag{6.32}$$

Such ionizations for the enzyme exist for both the "free" (E) and "bound" (ES) forms and can be identified for each of the acidic- (K_{a1}) and alkaline-side (K_{a2}) transitions. Such behavior can be represented by three ionization states of the free enzyme:

$$HEH^+ \overset{K_{E1}}{\rightleftarrows} EH + H^+ \overset{K_{E2}}{\rightleftarrows} E^- + H^+ \tag{6.33}$$

$$\text{where } K_{E1} = \frac{[H^+][HE]}{[HEH^+]} \quad \text{and} \quad K_{E2} = \frac{[H^+][E^-]}{[EH]} \tag{6.34}$$

The same pattern of behavior can be envisioned for the ES complex, where

$$HEH^+S \overset{K_{ES1}}{\rightleftarrows} EHS + H^+ \overset{K_{ES2}}{\rightleftarrows} E^-S + H^+ \tag{6.35}$$

$$\text{where } K_{ES1} = \frac{[H^+][EHS]}{[HEH^+S]} \quad \text{and} \quad K_{ES2} = \frac{[H^+][E^-S]}{[EHS]} \tag{6.36}$$

Under this scenario, all ionization and kinetic equilibria can be assembled as just described in context with the catalytic steps of enzyme action (Figure 6.33). In this model, the most active enzyme states are the EH and EHS forms and they are associated with the optimum or "intrinsic" V_{max} and K_M values (consistent with Figure 6.32a). The model can be applied to determine if the decline in "activity" over the acidic or alkaline pH range is caused by certain enzyme forms (HEH^+ and E^-) not binding S or those (HEH^+S and E^-S) incapable of transforming $S \rightarrow P$. The model also accommodates all enzyme species within a specified pH range being partially active (such as plot 3 in Figure 6.32b) with pH-modified kinetic constants ($\alpha/\beta\ K_M^{H^+}$ and $\alpha/\beta\ V_{max}^{H^+}$), with α/β modifiers typically in the range of $1 \rightarrow \infty$ and $1 \rightarrow 0$, respectively, for these kinetic constants. The terms $K_M^{H^+}$ and $V_{max}^{H^+}$ represent the dependence of these kinetic constants on pH relative to intrinsic K_M and V_{max} values at optimum pH.

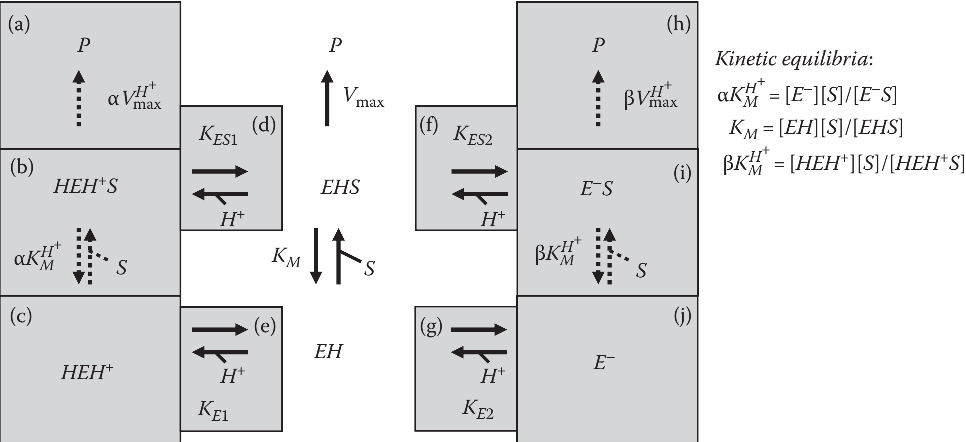


FIGURE 6.33 Kinetic model of enzyme activity response to pH. Panels: (a), (b) and (c) represent catalytic steps in the acid pH range; (h), (i) and (j) represent catalytic steps in the alkaline pH range; (d) and (e) represent ionization equilibria in the acidic pH range; (f) and (g) represent ionization equilibria in the alkaline pH range. (Adapted from Copeland, R.A., *Enzymes: A Practical Introduction to Structure, Function, Mechanism, and data Analysis*, 2nd edn., John Wiley, New York, 2000; Segel, I.H., *Enzyme Kinetics. Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*, John Wiley & Sons, Inc., New York, 1975; Whitaker, J.R., *Principles of Enzymology for the Food Sciences*, 2nd edn., Marcel Dekker, New York, 1994.)

With any enzyme, a reasonable assumption to make (based on the bell-shaped pH–activity curve) is that three ionization states exist, and each has the potential to bind S with only the optimally ionized form capable of transforming $S \rightarrow P$. This assumption would modify the general model (Figure 6.33) by omitting panels "*a*" and "*h*." If combined with the conventional reaction velocity equations applied earlier (Equation 6.15)

$$\frac{v}{E_T} = \frac{k_{cat} \times [EHS]}{[EH]+[HEH^+]+[E^-]+[EHS]+[HEH^+S]+[E^-S]} \tag{6.37}$$

|...... "*E*" species......| |........ "*ES*" species ......|

For the right side of the equation, all enzyme species can be expressed in the form of EHS, using the appropriate ionization (Equations 6.34 and 6.36) and kinetic (Equations in Figure 6.33) equilibria. Since all enzyme species are in equilibrium, any particular enzyme species can be expressed in terms of any other enzyme species. Specifically, the equilibria used to express each enzyme species into the EHS form are as follows:

For EH, K_M; for HEH^+, K_{E1} and then K_M; for E^-, K_{E2} and then K_M.

For HEH^+S, K_{ES1} then K_M; for E^-S, K_{ES2} then K_M.

Next, factoring out EHS, factoring both sides of the equation by E_T (and using Equation 6.16), and then dividing the numerator and denominator of the right-hand side by S/K_M, followed by K_M, yields

$$v = \frac{V_{max} \times [S]}{K_M\left(1+([H^+]/K_{E1})+(K_{E2}/[H^+])\right)+[S]\left(1+([H^+]/K_{ES1})+(K_{ES2}/[H^+])\right)} = f_E f_{ES} \tag{6.38}$$

This equation allows all free "E" species to be expressed collectively as a pH-dependent distribution term (f_E) called a Michaelis pH function, along with an analogous f_{ES} term for all "ES" species.* These functions reflect the quantitative distribution or ratios of the three ionization states of the "E" or "ES" species at any pH as a function of the H^+ and K_a terms (in essence, they yield "titration" curves). In addition, dividing the numerator and denominator of the right side of Equation 6.38 by f_{ES} shows how key kinetic constants are influenced by pH:

$$v = \frac{V_{max}/f_{ES} \times [S]}{K_M(f_E/f_{ES}) + [S]} \tag{6.39}$$

and thus

$$V_{max}^{H^+} = \frac{V_{max}}{\left(1 + ([H^+]/K_{ES1}) + (K_{ES2}/[H^+])\right)} \tag{6.40}$$

and

$$K_M^{H^+} = K_M \times \frac{f_E}{f_{ES}} = K_M \frac{\left(1 + ([H^+]/K_{E1}) + (K_{E2}/[H^+])\right)}{\left(1 + ([H^+]/K_{ES1}) + (K_{ES2}/[H^+])\right)} \tag{6.41}$$

And if the ratio of these modified kinetic constants is taken

$$\frac{V_{max}^{H^+}}{K_M^{H^+}} = \frac{V_{max}}{K_M \times (f_E)} = \frac{V_{max}}{K_M\left(1 + ([H^+]/K_{E1}) + (K_{E2}/[H^+])\right)} \tag{6.42}$$

Thus, the $V_{max}^{H^+}$ term relates *only* to the behavior of all "ES" species (f_{ES}), and the $V_{max}^{H^+}/K_M^{H^+}$ term relates *only* to the behavior of all free "E" species (f_E; also recall Equations 6.19 through 6.22) in how enzymes respond to pH.

Observations obtained for papain can illustrate how pH affects enzyme function (Figure 6.34a through c). A broad pH optimum of 5–7 is observed, and estimates of optimum V_{max} and K_M values allowed the data to be fitted (by the author) to the Equations 6.40 and 6.42 above for $V_{max}^{H^+}$ and $V_{max}^{H^+}/K_M^{H^+}$, yielding pK_a values of 4.0 and 8.2, and 4.2 and 8.2, respectively (Figure 6.34a and b). Values for pK_a can be identified from these plots by dropping perpendiculars from the points on the curves where the ordinate value represents 50% that of the maximum value observed. Since there was little change in K_M† as a function of pH (panel c), the pH-induced ionization of enzyme can be concluded to have negligible effect on substrate binding and can be solely attributed to a pH effect on the catalytic step over the pH region evaluated. To summarize for papain, ionizable group(s) exists for each pH transition, with all ionization states of the free E capable of binding S, but only the EHS form capable of transforming $S \rightarrow P$. Thus, the model assumed leading to Equation 6.37 fits the behavior of papain, and panels *a* and *h* (Figure 6.33) would be omitted from the complete model with $\alpha = \beta = 1$ for $K_M^{H^+}$ to account for papain behavior. The response of papain activity (V_{max}) to pH in Figure 6.34a resembles that in Figure 6.32a.

* Note that these Michaelis pH functions were developed with the EHS species as the reference species; these functions can be developed for any "E" species as reference, and while they will take on different forms, enzyme behavior will be modeled identically for a given set of K_a and $[H^+]$ values.

† Changes in K_M of less than a few multiples are usually considered insignificant and must approach ≥ threefold in magnitude of difference to be practically meaningful in pH response of enzyme action.

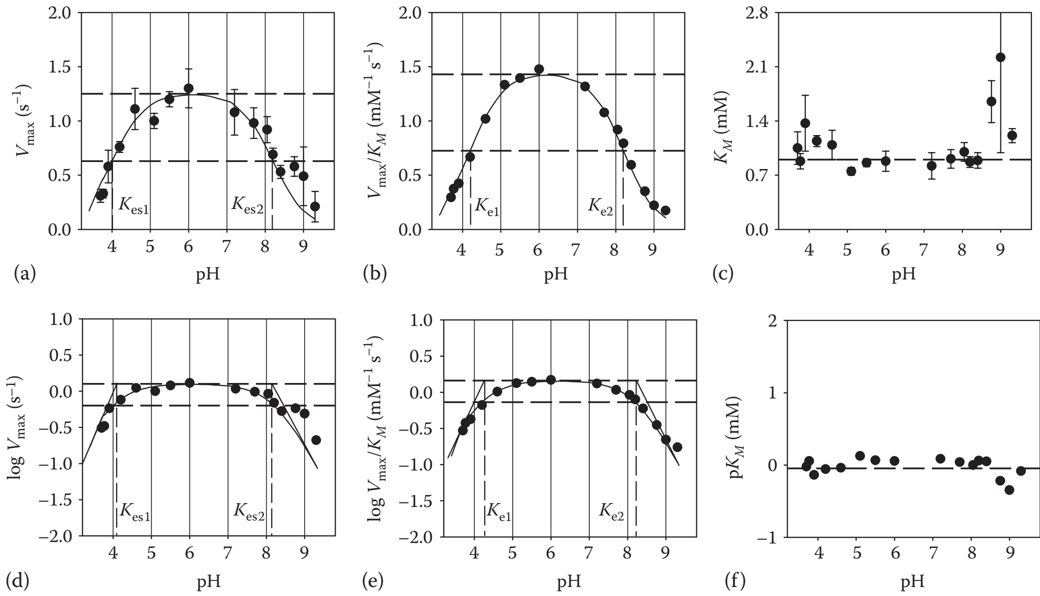


FIGURE 6.34 Analysis of enzyme activity response to pH using papain as an example. Panels: (a) and (d) are responses of V_{max}, (b) and (e) are responses of V_{max}/K_M, and (c) and (f) are responses of K_M. (Data obtained from Lowe, G. and Yuthavong, Y., pH-Dependence and structure-activity relationships in the papain-catalysed hydrolysis of anilides. *Biochem. J.*, 124, 117, 1971.) Line fitting to equations explained in the text.

To allow for more insightful analysis of pH effects [122], the log transforms of Equations 6.40 through 6.42 yield

$$\log V_{max}^{H^+} = \log V_{max} - \log\left[1 + \frac{[H^+]}{K_{ES1}} + \frac{K_{ES2}}{[H^+]}\right] \tag{6.43}$$

$$\log \frac{V_{max}^{H^+}}{K_M^{H^+}} = \log \frac{V_{max}}{K_M} - \log\left[1 + \frac{[H^+]}{K_{E1}} + \frac{K_{E2}}{[H^+]}\right] \quad \text{and} \tag{6.44}$$

$$\log K_M^{H^+} = \log K_M - \log\left[1 + \frac{[H^+]}{K_{ES1}} + \frac{K_{ES2}}{[H^+]}\right] + \log\left[1 + \frac{[H^+]}{K_{E1}} + \frac{K_{E2}}{[H^+]}\right] \tag{6.45}$$

and observations are plotted routinely as "Dixon plots" (for papain behavior in Figure 6.34d through f). Equation 6.45 is not plotted *per se*, but $pK_M^{H^+}$ is instead (p = −log), as this makes any downward deflection of the plot where a pH transition occurs correspond to impaired function, similar to the plots of Equations 6.43 and 6.44. The log forms of the equations make some aspects of enzyme behavior as a function of pH easier to visualize and interpret (Figure 6.34d through f). V_{max} is easily identified by the flat portion (slope ~0) of the plot, and the pH optimum is midpoint between the pK_a values. The slopes for the acidic (+n) and alkaline (−n) transitions at the most steeply ascending and descending portions of the pH response curve represent the number of ionizable amino acid residues involved in each transition. In the case of papain, the Dixon plots yield slopes of +1 and −1, indicating that the ionization state of a single amino acid residue accounts for enzyme response to pH in each transition. Slopes of Dixon plots of enzyme function generally range 1–3, and multiple ionizable groups on the enzyme yield more cooperative transitions (such as plot 2 on Figure 6.32b).

Dixon plots also allow pK_a values to be estimated by two means. Since the point where pH = pK_a represents the condition where the ionizable group(s) is half protonated, this corresponds to where the enzyme activity measured is 50% of the maximum. Thus, on a log scale used in Dixon plots, the pK_a values can be located where the pH response curve intersects a point 0.3 ordinate units below the maximum. Another way to estimate pK_a values is to extend the slopes of the ascending and descending portions to the intersection of the maximum response (a horizontal) and then drop perpendiculars to the axis to identify pK_a. Sometimes the choice of method used is dependent on the nature and extent of the data gathered. For papain (Figure 6.34d through f), estimates by both methods yield close agreement in pK_a values of 4.1 and 8.1, and 4.2 and 8.2, for the respective bound and free enzyme forms. Amino acid residues with ionizable groups that are consistent with these pK_a values are GLU/ASP and CYS (Table 6.9). However, the actual pH behavior of papain is conferred by an imidazole–thiolate (HIS-CYS) ion pair (which acts as a unit, cf., Figure 6.23). The CYS_{25} is active in the dissociated form, while the HIS_{159} residue must be protonated for active site functioning. This behavior provides another example of how ionization properties of amino acid residues in proteins can be widely modulated relative to intrinsic ionization potentials of amino acids in solution (Table 6.9).

With the preceding model, the pH-dependent behavior of enzymes can be quite broadly applied to any enzyme of interest. An analysis of the pH dependence of xylose isomerase indicates that over the pH range of 5–8 (commercial use is at pH 7–8), the ability of the enzyme to transform $S \rightarrow P$ is not affected (log k_{cat} curve is flat, Figure 6.35a). However, the unit slope for the acidic transition indicates that the ionization state of a single ionizable group on the enzyme is responsible for substrate binding (K_M changes, Figure 6.35b), and since k_{cat} does not change, then $\Delta K_M \approx \Delta K_S$ for this analysis. The purpose of identifying pK_a values that represent critical pH-sensitive transitions in enzyme functioning is to insinuate the identity of the amino acid residues involved in that enzyme response. The pK_a value of the ionizable group in xylose isomerase is 5.7–6.1, making it likely to be a HIS residue (Table 6.9). The van't Hoff relationship (Figure 6.30a, Equation 6.31) is often used to further insinuate the participating amino acid residues based on characteristic ΔH_{ion} values. For xylose isomerase, the pK_a of the ionizable group changed as a function of temperature with a ΔH_{ion} value of 5.6 kcal/mol (from slope, Figure 6.35c), also consistent with that observed for imidazole residues (Table 6.9).

6.4.2.4 Other Types of pH Behavior

Other types of pH behavior can affect enzyme reactions. Ionization state of substrate, product, or inhibitor may influence enzyme reactivity depending on the nature of the interactions that allow enzyme to bind and transform these ligands. Likewise, ionization of enzyme amino acid side chains

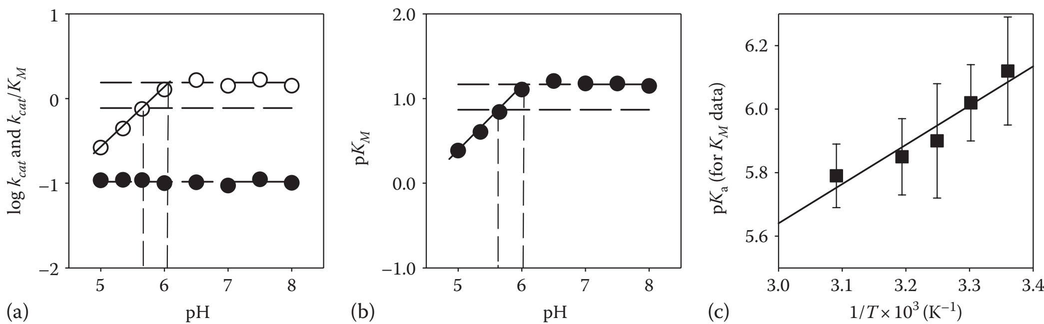


FIGURE 6.35 pH response of xylose isomerase activity. Open circles represent k_{cat}/K_M, and closed circles represent k_{cat} in panel (a). Panels: (a) is response of catalytic steps, (b) is response of K_M, and (c) is temperature response of ionizable group involved in catalysis. (Redrawn from Vangrysperre, W. et al., *Biochem. J.*, 265, 699, 1990.)

may modulate selectivity of reaction among potential substrates. For example, many proteases exhibit different pH optima for hydrolytic activity toward different protein substrates [50].

6.4.3 Water Relations and Enzyme Activity [37,41,121]

Control of the level and disposition of water in foods is a principal form of preservation and can affect enzyme activity and stability. Water impacts rates of reactions by serving as a diffusion medium, controlling dilution or concentrations of solutes, stabilizing and plasticizing proteins, and serving as cosubstrate for hydrolytic reactions. Reducing the amount of bulk or solvent water (by dehydration or freezing) evokes several interrelated compositional and material changes in foods that influence enzyme reactions.

6.4.3.1 Desiccation and Water Activity Effects

The principal effects of reducing bulk or solvent water is to diminish the role of water in acting as a diffusion medium and as cosubstrate. The extent of water reduction is best characterized by the thermodynamic term of water activity (a_w), as it relates to how water behaves with respect to solutes (including enzymes). For lysozyme as an example, at a_w 0–0.1, water is tightly bound (monolayer) to charged and highly polar groups on proteins. At a_w 0.1–0.4, water becomes bound to less polar domains of protein including the peptide backbone. At a_w >0.40 water of condensation contributes to multilayer water and increasingly to the fraction of true bulk or solvent water. The exact a_w values where similar transitions in states of water occur in food matrices are material dependent. The effect of a_w on enzyme reactions was most intensively studied during 1950s–1980s, and a generally applicable example of behavior is illustrated in Figure 6.36a. As a_w is reduced within the range of 0.90–0.35, the progress of hydrolysis reactions is slowed and approaches a near-equilibrium position of more limited extent of hydrolysis. When a_w is then elevated, reaction progress resumes in a manner that is representative to that initially occurring at that a_w. Thus, this effect of water is largely reversible, and in food and biological matrices, such behavior is interpreted as capillary effects that limit the extent of reaction progress at a limiting a_w. Such effects have been shown for lipase, phospholipase, and invertase activity, but they are generally applicable to all enzymes, as polyphenol oxidase activity is reduced by 90%–95% in terms of initial rate and extent of reaction as a_w is reduced from 1.0 to 0.60 [138]. For ester synthesis reactions, lipases from various sources have different and distinct a_w optima (Figure 6.36b).

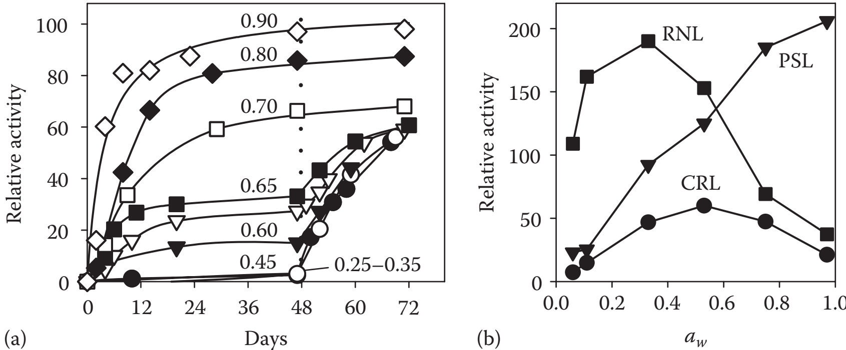


FIGURE 6.36 Response of enzyme activity to a_w. (a) Response of ground barley malt (source of phospholipase) on 2% lecithin at 30°C, with adjustment to a_w of 0.70 after 48 days. (b) Response of ester synthesis activity of various lipases (RNL, *Rhizopus niveus* lipase; PSL, *Pseudomonas* spp. lipase; CRL, *Candida rugosa* lipase). (Redrawn from Acker, L. and Kaiser, H., *Lebensm. Unters. Forsch.*, 110, 349, 1959; Wehtje, E. and Adlercreutz, P., *Biotechnol. Lett.*, 11, 537, 1997.)

TABLE 6.10
A_w Requirements for Activity of Selected Enzymes

Enzyme	Matrix/Substrate	Minimum a_w	Enzyme	Matrix/Substrate	Minimum a_w
Amylases	Rye flour	0.75	Amylases	Starch	0.40–0.76
	Bread	0.36			
Phospholipases	Pasta	0.45	Phospholipases	Lecithin	0.45
Proteases	Wheat flour	0.96	Lipases	Oil, tributyrin	0.025
Phytase	Grains	0.90	Phenol oxidase	Catechol	0.25
Glucose oxidase	Glucose	0.40	Lipoxygenase	Linoleic acid	0.50–0.70

Source: Drapon, R., Modalities of enzyme activities in low moisture media, in *Food Packaging and Preservation. Theory and Practice*, M. Mathlouthi (Ed.), Elsevier Applied Science Publishers, New York, pp. 181–198, 1986.

Enzymes exhibit different minimum a_w for catalytic function. At a_w at or below the monolayer, enzyme plasticity is limited, but some enzymes still exhibit activity. Less than monolayer water may restrict reactivity, but this also enhances thermal stability, since conformational freedom is restricted and there is less tendency for protein unfolding at otherwise denaturing temperatures. The threshold or minimum a_w required for enzyme activity ranges 0.25–0.70 for several oxidoreductases and 0.025–0.96 for several hydrolases, in both food matrices and model systems (Table 6.10). Even low residual enzyme activity may be sufficient to have impact on food quality given the long times that intermediate moisture foods are stored.

Another effect of reducing a_w is to influence equilibria involving water (hydrolysis reactions) through mass action effects. Thus, for $AB + H_2O \leftrightarrows A' + B'$,

$$K_{eq} = \frac{[A'] \times [B']}{[AB] \times [H_2O]} \tag{6.46}$$

As a_w decreases, there is a shift in position of reactants and products toward accumulation of $[AB]$. This principal is exploited commercially by using lipases in microaqueous media (<1% H_2O) to cause various reactions (Figure 6.26) leading to the production of lipids with improved functionality. Similarly, the optimum water content (related to a_w) is ~2%–3% for thermolysin reactions leading to peptide bond formation in the course of aspartame synthesis [86]. Many enzymes exhibit an optimum a_w for activity and it is usually >0.90.

The combined lack of diffusion medium and enzyme plasticity may cause changes in reaction pathways and product distribution [37,121]. For α-amylase action on starch, as a_w is reduced from 0.95 to 0.75, there is a shift in maltooligosaccharide product distribution from a heterogeneous mixture of oligomers of 1–7 glucose units to that favoring products of 1–3 glucose units. This indicates that hydrolysis is less random in nature. Restricted diffusion of enzyme and substrate favors greater processivity in enzyme attack, since the limited mobility of reactants may subject starch segments to multiple hydrolytic actions at proximal sites. Similarly, restricted diffusibility at a_w of 0.65 renders lipoxygenase reaction end products elevated in linoleate condensation products with a corresponding diminution in fatty acid hydroperoxides. The limited ability for diffusion allows the hydroperoxides to achieve elevated local concentrations and participate in bimolecular free radical addition (condensation) reactions.

Reduction in a_w may also change kinetic or equilibrium constants governing enzyme reactivity. For example, the pH optimum of polyphenol oxidase shifts >0.5 pH unit as a_w is decreased from 1.0 to 0.85 [138]. Such a change is consistent with diminished dielectric character of the medium and a corresponding increase in pK_a of important ionizable groups important to enzyme function. Lipase exhibits a minimum K_M at a_w of ~0.4 [37], and this may result from a change in properties of the

enzyme or nature of the substrate interface. Depending on the composition and a_w of some food or model system matrices, glass transitions may occur, where molecular motion is greatly restricted relative to a "rubbery" or more fluid state (Chapter 2). In some cases the glassy state is more stabilizing to enzymes, but enzymes generally exhibit a temperature-dependent sensitivity of stability in low-moisture media, regardless of whether a glassy or rubbery state exists [117]. In terms of enzyme activity, model systems studies have indicated no obvious elevation of enzyme activity as may be expected when a transition from the glassy to rubbery state occurs [27]. Specific compositional factors may modulate enzyme activity and/or stability in low-moisture systems more so than the mere presence of a glassy state.

Finally, as water is removed, there is a corresponding decline in viscosity of the remaining liquid phase, and this may serve to attenuate enzyme reactions by reducing diffusibility of reactants and products. The effect of viscosity has been evaluated in a few cases of enzyme action, using inert "viscogens" (e.g., glycerol, polyols, polymers). Increases in viscosity have been shown to reduce enzyme reactions rates that are diffusion controlled or when increased viscosity causes a change in rate-limiting step, such as to the product dissociation step. Diffusion-controlled ("near-perfect") enzyme reactions are considered those with k_{cat}/K_M values of ~10^8–10^9 M^{-1} s^{-1}, approximating diffusion-limited rates for bimolecular reactions between a large and a small molecule [151]. Early findings of attenuation of invertase reactions at high sucrose were interpreted as an effect of increased viscosity, but it was later shown to be caused largely by substrate inhibition [84]. This example emphasizes the difficulty in trying to isolate the individual effect of an environmental factor as water content is modified, since many other factors are simultaneously modified.

6.4.3.2 Osmotic Effects of Desiccation [41,160]

As water is progressively removed from foods, or as solutes are added to a liquid medium, dissolved solutes become more concentrated in the remaining liquid aqueous phase. Consequently, another outcome of desiccation is increased ionic strength and osmolality. Stability and to a large extent activity of enzymes in hyperosmotic media is influenced by the profile and concentrations of solutes present; specific ionic constituents are generally classified as salting in (destabilizing) or salting out (stabilizing) toward proteins (Chapter 5). Each enzyme exhibits a characteristic response to these solutes and changes in their concentrations as desiccation takes place. Relevant to enzyme behavior in hyperosmotic media are many commercial enzyme processes making use of high levels (10%–40%) of substrate (pectinases, proteases, amylases, and sugar-transforming enzymes). Fortunately, many of these substrates are also protein-stabilizing agents, such as polyols, sugars, and amino acids [160], and high substrate levels help stabilize enzymes to thermal denaturation.

Another consequence of enzyme reactions in high solids media is the favoring of reverse reactions (especially hydrolyses) by mass action effects (recall Equation 6.46). Reverse reactions with lipases provide the means for synthesizing or rearranging esters (Figure 6.26). Plasteins formed by proteases at elevated peptide are mediated by transpeptidation reactions. Such reactions allow the incorporation of nutritionally limiting amino acids. Use of glucoamylase under commercially relevant conditions (Figure 6.19) yields a limited level of undesirable isomaltose (α,1–6 linkage) accumulation through reverse hydrolysis reactions. β-Galactosidase mediates glycosyltransfer reactions at high lactose that yield oligomers of galactose and glucose that have potential use as prebiotics.

Some enzymes are constantly exposed to hyperosmotic stress in nature. Examples of organisms living in hyperosmotic environments include all marine species (saltwater is ~3.5% NaCl), plants and microorganisms inhabiting brackish water, high-salinity soils, mineral springs, and deep-sea vents. Freezing and desiccation also brings about hyperosmotic conditions. Osmoregulatory systems have evolved to mitigate the negative effects of high-osmotic and high-ionic-strength media. Osmoprotectants are compounds such as polyols (glycerol, mannitol, sorbitol), sugars (sucrose, glucose, fructose, trehalose), amino acids (especially GLY, PRO, GLU, ALA, β-ALA), and a series of methylated amines (Figure 6.37). Among these structures, note the frequency of the stabilizing functional groups of −OH (H-bonding capability), NH_4^+, $R_xNH_y^+$, $-CH_2$-COO^-,

Ammonium
Quaternary methyl ammonium
Betaine aldehyde
Sulfate
Dimethylamine
Betaine
Sarcosine
Taurine
Trimethylamine N-oxide
Choline
Proline
Glycerol

FIGURE 6.37 Osmolyte systems.

and SO_3^{2-}, and such groups stabilize proteins by countering or minimizing the effect of destabilizing agents such as Na^+, K^+, Cl^-, urea, and ARG.

The mechanisms by which these osmoprotectants act are believed to include steric repulsion between solute–protein (promoting water structure and protein compactness, promoting the native state) and direct solute–protein interactions (H-bonding). Two examples of osmoprotection deserve special mention, those by methylated amines and trehalose. Tissues of marine organisms can be comprised of up to 100 mM trimethylamine-*N*-oxide (TMAO). This endogenous osmolyte protects tissue enzymes from destabilizing effects (adverse changes in K_M) of salt and even urea (a potent protein denaturant existing in tissues of sharks and rays). A related compound, betaine, relieves inhibitory effects of NaCl on enzymes in saline-stressed plant tissues. Trehalose (glucopyranosyl-α-1,1-glucopyranoside) is among the most effective osmoprotectants known. It appears to H-bond with protein and also promotes structure of water in stabilizing proteins to desiccation and freezing stress [160]. While osmoprotectants preserve enzyme activity in host tissues in water-stressed environments, they can also be added to enzyme preparations to render them more stable. This is done for freezing and freeze-drying of enzyme preparations, many of which are ≤10% active protein with the rest being excipient or carrier material, which may include cryo-/osmoprotectants.

Some enzymes may require ionic constituents to function optimally or they have evolved to function well under conditions of water stress, such as those from halotolerant or halophilic organisms. Some of these enzymes have been identified empirically through the evolution and use of various starter cultures for fermentations where added salt is involved (e.g., cheese, soy sauce). Enzymes of significance to fermentations must be tolerant enough to persist and be sufficiently active to cause the desired change during fermentation. In other cases, salt (osmotic) stimulation of activity has been observed. Thermolysin, used to synthesize the sugar substitute aspartame, is stimulated 12-fold by 4 M NaCl at optimum pH ~7 (Figure 6.38), and stimulation by monovalent cations occurs in descending order: $Na^+ > K^+ > Li^+$ [61]. Stimulation affects only the k_{cat} step and not S binding and shifts the acidic group pK_a from 5.4 to 6.7, while the alkaline group remains at pK_a of ~7.8. The high-salt environment activates the enzyme through electrostatic interactions at the enzyme surface and active site, and this is associated with a conformational change in the protein. This enzyme is ideally suited for peptide synthesis at high cosubstrate levels.

6.4.3.3 Desiccation by Freezing

Freezing is distinct from other desiccation processes on account that bulk water is removed as a solid phase and this is accompanied by lower temperatures (<0°C) than encountered in dried,

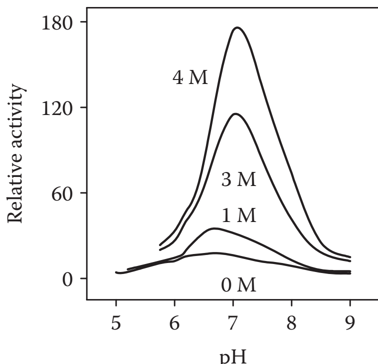


FIGURE 6.38 Salt (NaCl) activation of thermolysin activity. (Redrawn from Inouye, K. et al., *J. Biochem.*, 122, 358, 1997.)

intermediate moisture, or high-osmotic foods. Thus, temperature and increased solute concentration are the principal determinants of enzyme activity in frozen media, with viscosity and diffusion effects embedded in both of these factors. A_w may be a less important factor in frozen compared to desiccated media because it is dictated by the relative vapor pressure of ice and supercooled water at the same temperature; a_w is only suppressed to 0.82 at −20°C (Chapter 2). Freezing effects were examined closely in the 1960s–1980s, although efforts continue because of the sustained interest in cryopreservation of biological systems. Studies have made use of model systems as well as reactions in food matrices.

The combined influence of the two dominant factors accounting for freezing effects on enzyme reactions can be summarized as follows. Reduced temperature will always reduce k_{cat} and predict a declining rate of reaction based on the characteristic E_a for the reaction. Concentration effects are more varied and relate to the initial concentration of agents controlling enzyme activity (substrates, inhibitors, affectors, cofactors, buffering agents) in the unfrozen medium and the collective effects of elevating their concentrations by removal of solvent water as ice. Concentration of solutes may have negative impact or destabilize enzymes through osmotic and/or inhibitor effects, especially if $S > K_M$, and reaction rates will decline as the outcome. The net result would be an overall decrease in reaction rate upon freezing. A limited enhancement of enzyme reactivity can occur, such as through the elevated concentration of *S* or positive affector, but in a manner that may roughly balance the attenuating effect of reduced temperature, resulting in little or no change upon freezing. The third potential outcome is where the substrate-concentration effect substantially enhances reactivity, especially for initially dilute [*S*], such that this effect is dominant over temperature and there is a net increase in reactivity upon freezing.

The physical event of ice crystal formation can have at least three distinct consequences. One is that in cellular systems, ice crystals can disrupt cellular structures and promote mixing of enzyme and solutes that may originate in different cellular compartments. This decompartmentation effect often is responsible for cellular systems exhibiting enhanced reactivity at high, abusive freezing temperatures (−3°C to −12°C), and sometimes as low as −20°C. Ice crystal size, which is primarily a function of how fast freezing occurs (and secondarily through the process of recrystallization), can also have effects on enzyme reactivity in frozen systems. Fast freezing will favor greater homogeneity in ice crystal distribution and smaller, more dispersed "pools" of the remaining reactive, liquid phase. This may retain some segregation between enzyme and reactants, especially if they were originally contained in different cellular compartments, even though the net concentration effect of freezing would be equivalent to slow freezing to the same end-point temperature. The third consequence relates to freezing rate, and while it has long been considered that the faster the freezing in the range of ~1°C–100°C/min, the better enzyme activity/stability is retained, the opposite

seems to be more the general rule [22,132]. Faster freezing creates smaller ice crystals with greater surface area than slow freezing and with less opportunity for pooling of unfrozen liquid media. The small crystals appear to foster surface denaturation of enzymes. Some proteins are not as sensitive as others, and in cellular systems, cellular barriers and compartments may mitigate or exacerbate this phenomenon. In any event, slow to moderate rates of freezing favor stability and retention of enzyme activity during frozen storage. Generally, enzyme activity is lost during sustained frozen storage of aqueous systems, but this occurs to a more limited extent in lyophilized powders, where ice is removed before storage.

Thawing rates have profound influence on retention of enzyme activity in biological media. Progressively slower thawing, from 10°C to 0.1°C/min, leads to increasing losses of several enzymes in model solutions, and the temperature range where most deactivation occurs is from −10°C to thawing [22,44]. Ice recrystallization during thawing may cause additional surface tension and shear to further denature proteins during this process. Slow thawing was also observed to be particularly denaturing to enzymes in food matrices, with onion alliinase serving as an example [149].

Increased viscosity in the liquid phase is another consequence of freezing, with less water available to serve as a diffusion medium. As was observed for reductions in a_w, lower freezing temperatures limit the rate and extent to which reaction can occur (Figure 6.39). More recently, an attempt was made to quantify the effect of viscosity by the study of alkaline phosphatase in frozen sucrose solutions [26]. Alkaline phosphatase is widespread in nature and in milk it is used as a thermal process indicator; it is an efficient enzyme that reacts at a rate (k_{cat}/K_M of 10^6–10^7 $M^{-1}s^{-1}$) near the limit of diffusion. Measurements of catalytic function (k_{cat}/K_M) were in good agreement with the predicted effect of viscosity and could account for behavior in partially frozen solutions. However, other factors may be important for enzymes that react at less than diffusion-controlled rates. One of these other factors not yet discussed includes eutectics, which can cause ionic and compositional (pH) changes that can impact enzyme activity and stability. Also impacting enzyme sensitivity to freezing are enzyme concentration and protein concentration in the medium, with greater concentrations favoring greater degree of retention of active enzyme, likely through stabilizing protein–protein interactions. Last, the presence of cryoprotectant compounds improves enzyme stability, with the same osmoprotectants as discussed earlier being important, particularly trehalose, other polyols, and sugars.

6.4.4 Nonthermal Processing Techniques [137]

The major nonthermal technologies being evaluated for food preservative outcomes include high-hydrostatic-pressure processing (HPP), pulsed electric field, ultrasound, irradiation, ultraviolet

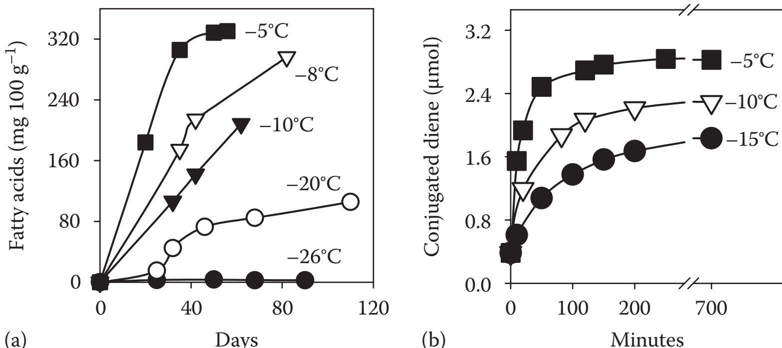


FIGURE 6.39 Effect of freezing on reaction progress of (a) lipase action in unblanched peas and (b) lipoxygenase oxidation of linoleic acid in model reactions. (Redrawn from Bengtsson, B. and Bosund, I., *J. Food Sci.*, 31, 474, 1966; Fennema, O. and Sung, J.C., *Cryobiology*, 17, 500, 1980.)

light, and oxidative processes (ozone, chlorine dioxide). All of these methods target microbial control, but HPP and ultrasound can also deactivate undesirable enzymes in foods, maintaining "freshness" otherwise lost through thermal processing. Sufficiently high pressures will perturb and unfold protein structures and dissociate oligomers, thereby leading to declines in enzyme activity. HPP involves pressures of 100–900 MPa, where activation of enzyme activity can be encountered at pressures up to ~400 MPa, while increased pressures to 900 MPa often cause modest to large-scale inactivation. Enzyme sensitivities to pressure are dependent on the tissue matrix, HPP conditions, and ancillary treatments, such that process development must proceed empirically. Fruit and vegetable products (juice, jams, purees) are most subject to HPP to extend shelf life on the basis of enzyme deactivation. One of the most visually evident commercial successes is the ability to preserve avocado puree for several weeks at refrigerated temperatures because of phenol oxidase inactivation and control.

6.5 ENZYMES ENDOGENOUS TO FOODS AND THEIR CONTROL

The balance of this chapter deals with the characterization and manipulation of enzyme activity endogenous to foods, a continuing challenge to food scientists. The intent here is to provide an understanding of the nature and disposition of enzymes in tissues, the complexity of their behavior and interactions, and how physical and chemical strategies may be employed to attenuate or potentiate enzyme action where necessary or desirable. Complex and interrelated biochemical events such as ripening and postharvest and postslaughter metabolism and genetic manipulation are covered in other chapters.

6.5.1 Cellular and Tissue Effects

Enzymes related to food quality or processing are often studied in purified or partially purified forms to provide an understanding of the intrinsic properties and characteristics of the enzyme. Such *in vitro* studies often make use of enzyme levels of 10^{-7}–10^{-12} M. A quick calculation using a hypothetical food that is 10% protein of 1000 different proteins with an average mass of 100 kDa yields an estimate of the average concentration of any protein species as being 10^{-6} M [122]. Of course some individual proteins are more enriched than others, so the range of concentrations can easily be ±3 orders of magnitude (10^{-3}–10^{-9} M). Thus, on average, the levels of enzymes in foods and biological matrices are several orders of magnitude greater than used in studies to characterize them. Examples of high enzyme levels in foods from various sources are provided in Table 6.11. The levels estimated in this table do not account for any further enrichment conferred by localization (compartmentation) within the cell, which can increase concentrations by another order or magnitude or more. Even nontissue foods like milk and eggs exhibit structural heterogeneity that serves to distribute and concentrate endogenous components among discrete phases.

Compartmentation and *in vivo* concentration of enzymes impact their properties in foods in several ways. Properties of enzymes can be dependent on concentration. This is especially true for oligomeric enzymes where dissociation is favored upon dilution, and thus kinetic character associated with oligomeric enzymes (allosterism) may be diminished. Kinetic relationships between E and S may also change with changes in $[E]$, even though theoretically constants like K_M are independent of $[E]$. A striking example is available for muscle phosphofructokinase (which influences the rate of postmortem glycolysis during the conversion of muscle into meat) (Figure 6.40a). At physiologically relevant levels of enzyme (500 μg/mL, ~10^{-6} M), $S_{0.5}$ is 0.5 mM, whereas at 5 μg/mL (~10^{-8} M), $S_{0.5}$ is ~10-fold greater at 6.4 mM. Furthermore, at the lower level of enzyme, inhibition by ATP (also a cosubstrate) in the presence of an activator, fructose-2,6-biphosphate, was more acute with a K_I of 1.2 mM, compared to a K_I of 10 mM at physiological levels of enzyme. Another dimension of enzyme behavior *in situ* is the fact that other constituents can modulate reactivity. In the presence of fructose bisphosphatase, phosphofructokinase exhibits hyperbolic-type

TABLE 6.11
Examples of High Concentrations of Enzymes in Foods and Tissues

Enzyme	Source	Level Found	Concentration	Comment
Glyceraldehyde -3-phosphate dehydrogenase	Muscle (meat)	>1% weight, wet basis	0.34 mM	Adolase is 0.15 mM; lactate dehydrogenase is 0.11 mM; multienzyme complexes exist.
Peroxidase	Horseradish root	20% of protein	0.2 mM	Isoforms may be cytosolic or plastidic.
Lipid acyl hydrolase	Potato tubers	~30% of protein	0.2 mM	Storage protein, localized at extravacualor membrane, enriched at the bud end of tuber.
Alliinase	Onion bulb Garlic clove	~6% of protein ~10% of protein	0.02 mM 0.2 mM	Cytosolic (onion), or enriched in bundle sheaths (garlic).
Pancreatin (mixture of digestive proteases)	Pancreas	~0.04 g/g dry wt	~1.0 mM total protease	Trypsin, chymotrypsin, and elastase may exist as zymogens and active forms.

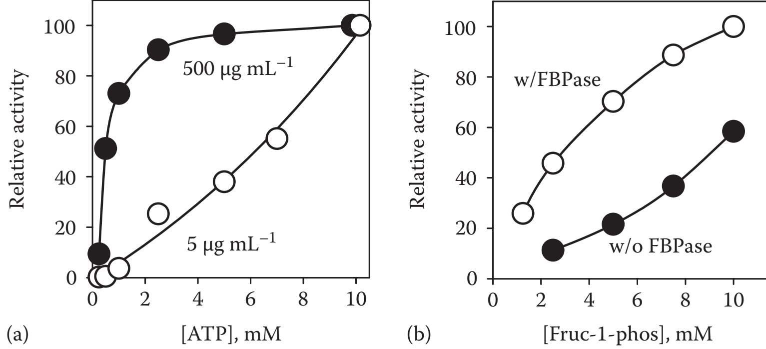


FIGURE 6.40 Effect of simulated *in situ* conditions on functioning of (a) phosphofructokinase and (b) phosphofructokinase in the presence or absence of fructose bisphosphatase (FBPase). (Redrawn from Bär, J. et al., *Biochem. Biophys. Res. Commun.*, 167, 1214, 1990; Ovádi, J. et al., *Biochem. Biophys. Res. Commun.*, 135, 852, 1986.)

kinetics with a $S_{0.5}$ of 2.9 mM, whereas alone, it exhibits allosteric kinetics with an increased $S_{0.5}$ of 9.2 mM (Figure 6.40b). Thus, fructose bisphosphatase may "activate" phosphofructokinase in muscle *in situ* through structural interactions or metabolic effects.

Another factor impacting enzyme reactivity *in situ* is the relative levels of enzymes and substrates and cofactors, the latter two for which multiple enzymes may compete. For example, intermediate metabolites of glycolysis range 20–540 µM, whereas glycolytic enzymes range 32–1400 µM [131]. Thus, substrates may be limiting to reactions for both primary and secondary metabolic pathways. Steady-state levels of NAD^+/NADH are estimated to be ~540/50 µM, and competition and relative K_M values for these cosubstrates among the many oxidoreductases in biological systems often dictate which enzymes are active and which are not (there is virtually no "free" NAD^+/NADH). In contrast, *in vitro* characterization of enzyme activity often makes use of excess (co)substrate(s) and [S] of 10^{-6}–10^{-2} M.

It should be evident by now that compartmentation is a key feature of controlling enzyme action in foods and biological systems. However, compartmentation means more than simply a separation by a membrane structure, within an organelle or some other physical barrier. Enzymes can be

separated from other enzymes or their substrates by being bound to other proteins, membranes, or even polysaccharides. Enzymes can be co-compartmented by interacting and binding to each other and this association allows for metabolic channeling of substrates and intermediates to end products by segregating them from the cytosolic or diffusional metabolic pool in cells. Enzymes may also be functionally compartmented as latent forms by other factors. Examples include localized pH or ionic strength (or gradients), presence of a reversible inhibitor, lack of positive affector or cofactor, or requirement of proteolytic activation of zymogen forms of enzymes.

The disposition of enzymes in foods may be quite easily controlled in some cases. The simple act of disrupting tissue is one means. Whether this improves quality (as in flavor generation) or detracts from it (enzymic browning) depends on the specific food material, its specific quality attributes, and the particular reaction evoked. For example, lipoxygenase action on lipids may yield either rancid or pleasant flavors, and enzymic browning is desirable in tea chemical "fermentation" but not for fresh-cut fruit and vegetables.

6.5.2 Enzyme Activities Related to Color Quality of Foods

6.5.2.1 Phenol Oxidases [129,142,150]

Enzymic browning is caused by enzymes collectively referred to as phenolase, phenoloxidase, polyphenol oxidase, catecholase, cresolase, and tyrosinase. These enzymes are widespread in microorganisms, plants, fungi, and animals, including humans where its action leads to skin pigmentation. These enzymes are related by having the same type 3 (oxidatively coupled) binuclear copper active site architecture and can mediate the latter or both of the following reactions:

$$\text{Monophenol} + O_2 + 2H^+ \rightarrow o\text{-diphenol} + H_2O \quad (6.47)$$

$$o\text{-diphenol} + \tfrac{1}{2}O_2 \rightarrow o\text{-quinone} + H_2O \quad (6.48)$$

The first reaction involves hydroxylation and is classified as monophenol monooxygenase (EC 1.14.18.1) activity, while the second reaction involves oxidation classified as 1,2-benzenediol–oxygen oxidoreductase (EC 1.10.3.1) activity. The former reaction provides the basis for "cresolase activity," since *p*-cresol generally represents monophenols and it is routinely used as a substrate for monophenol hydroxylation (and subsequent oxidation). Catechol is the common name for 1,2-benzenediol (the simplest *o*-diphenol), and thus, cresolase and "catecholase" activities are used to represent the respective hydroxylation and diphenol oxidation steps. Tyrosinase is a term used to generally represent enzymes with both hydroxylation and oxidation reactions, and the name derives from the enzyme abundant in common mushroom (*Agaricus bisporus*), which acts on the endogenous substrate tyrosine. Enzyme action does not form brown pigments directly. Rather, the *o*-quinones resulting from enzyme action undergo chemical condensation reactions (and may involve amines and proteins) to yield diverse, polymeric, and conjugated products called "melanins" that are collectively reddish brown in color.

Each atom of binuclear copper is tightly liganded to three HIS residues (sweet potato catecholase; $HIS_{88,109,118}$ and $HIS_{240,244,274}$), and this feature is the most highly conserved sequence among phenol oxidases and related binuclear copper enzymes [40,129]. Higher plant enzymes tend to be monomeric or homooligomers of 30–45 kDa monomeric mass. Tyrosinases are often glycosylated and exist in multiple isoforms exhibiting different substrate selectivities. The mechanism for tyrosinases involves redox reactions in $2e^-$ steps (Figure 6.41). The state of the enzyme in tissues is typically distributed as ~85% Met (Cu^{II}–Cu^{II}–OH^-) and ~10%–15% Oxy (Cu^{II}–Cu^{II}–O_2^{2-}) forms, and the enzyme is often isolated in the Met form. Oxidation of diphenols is facile with either form, and reactions proceed quickly through the cycle shown on the perimeter. Thus, in one complete cycle, one mole O_2 and $4e^-$ from substrate are used for two moles H_2O produced. In the portion of the

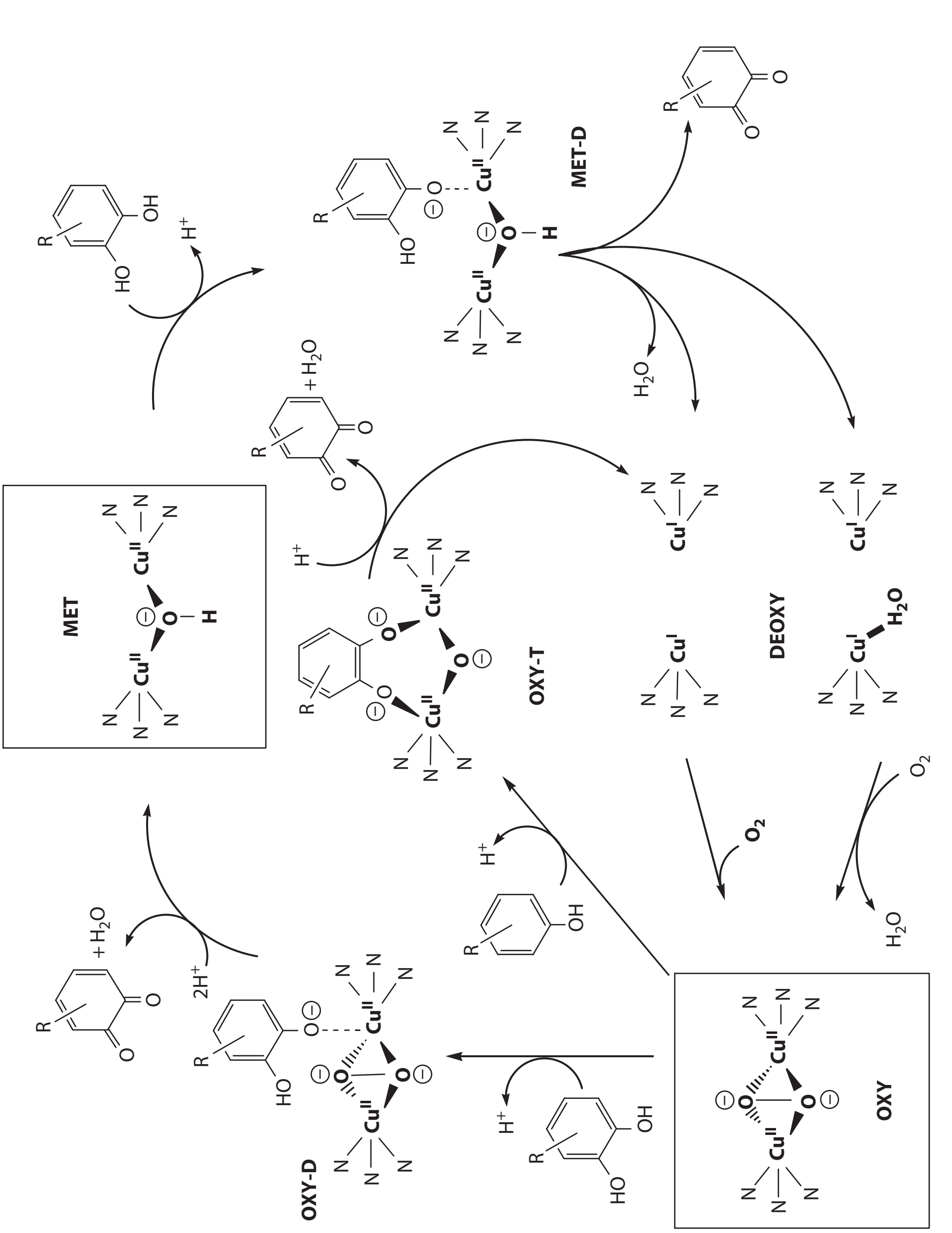


FIGURE 6.41 Reaction mechanism and cycling of polyphenol oxidase. Predominant, naturally occurring enzyme forms appear in boxes. OXY species are coordinated with two mole atoms of O, while MET species are coordinated with ⁻OH. Some species have diphenol (D) or monophenol (T) bound at the active site. (Adapted and redrawn from Eicken, C. et al., *Curr. Opin. Struct. Biol.*, 9, 677, 1999; Solomon, E.I. et al., *Chem. Rev.*, 96, 2563, 1996.)

cycle starting with the deoxy enzyme form, O_2 likely binds before the diphenol and forms a unique peroxide bridge (Oxy form), receiving electrons from Cu^I–Cu^I.

Hydroxylation often exhibits a lag period since it requires the less abundant oxy-enzyme form and substituent groups on the substrate phenol ring may impede reactivity because of steric constraints of *ortho*-hydroxylation [129]. The hydroxylation sequence represents the inner cycle in Figure 6.41 and yields one mole H_2O per mole O_2 consumed. Monophenols appear to undergo both the sequential reactions of hydroxylation and oxidation in a single catalytic episode. Diphenols are activators of enzyme reactivity toward monophenols and reduce the lag period by allowing enzyme to cycle quickly from the Met to Oxy forms (this feature is often expressed in Equation 6.47 as requiring an H-atom donor, BH_2 instead of $2H^+$). The reciprocal competitive inhibition of monophenols on *o*-diphenol oxidation and *o*-diphenols on monophenol *o*-hydroxylation is consistent with shared but partially divergent pathways of enzyme cycling for each activity. Low levels of H_2O_2 can activate tyrosinase by converting the Met form to Oxy form; amounts in excess of this deactivate the enzyme, possibly by a crypto-oxy-radical generated by the binuclear Cu_2-peroxide complex, ultimately destroying the HIS ligands that secure Cu at the active site. Despite earlier reports of enzymes possessing only cresolase activity, it appears that all cresolase-type enzymes have catecholase activity with ratios of activity typically ranging from 1:10 to 1:40 [161]. Most catecholase-type enzymes also have cresolase activity.

Enzymic browning occurs in shrimp and other crustaceans, and the defect is referred to as black spot. Hemocyanin, a copper protein involved in O_2 transport in crustaceans and closely related to tyrosinase, may have some involvement in the development of black spot. Laccases (EC 1.10.3.2) constitute another group of enzymes widespread in plants and fungi that oxidize diphenols but do not exhibit cresolase-type activity. While they may contribute to enzymic browning reactions in foods, their properties are similar enough to *o*-diphenol oxidases (some differences in inhibitor sensitivities exist) that they will not be considered further here.

The role of phenol oxidases in plants is believed to be for defense against pests and pathogens [150]. The action of diphenol oxidases in plant tissue represents a classic decompartmentation mechanism of activation, since the enzyme is largely plastidic (chloroplasts and chromoplasts), can be as much as 95%–99% latent; may be complexed with an inhibitor (e.g., oxalate), and substrates are compartmented elsewhere (vacuoles or in specialized cells) or exist as precursors. The disruption of tissue can activate latent diphenol oxidases by acid and contact with substrates (from vacuoles), by proteolytic processing of zymogen, or by various chemical activators, especially surfactants. The *o*-quinones produced by the enzyme reaction are reactive and can deactivate enzymes secreted by an invading organism, and the polymerization of *o*-quinones (melanosis) may also provide a physical barrier to infestation.

In foods, phenol oxidases are the cause of enzymic browning, which can be desirable in products such as raisins, prunes, cocoa beans, tea, coffee, and apple cider. Phenol oxidases have also been shown to produce dityrosine cross-links and this may be beneficial where protein "texturization" is a desired outcome such as in gel formation and bread dough (gluten) conditioning. *In vivo*, tyrosinase has been implicated as being involved in betalain synthesis. However, in most fruits and vegetables, especially minimally processed products, enzymic browning is associated with color quality loss. The presence of phenol oxidases in grains, such as wheat, is correlated with lack of "whiteness" in noodles, a quality detriment.

Phenol oxidases in fruit and vegetative tissues exhibit optima in the general range of pH 4.0–7.0, and some substrates influence the pH optimum. Effects of pH are mediated by a single ionizable group that affects binding of substrate (K_M step) and not the catalytic (V_{max}) step or overall enzyme conformation. Temperature optima for phenol oxidases are in the range of 30°C–50°C, but temperature stability is comparatively high and characterized by half lives of several minutes in the range of 55°C–80°C depending on source. Thus, during thermal processing, ample opportunity exists for phenol oxidases to become activated, since temperatures ~60°C–65°C evoke cellular leakage (decompartmentation) and mixing of enzyme and substrate at elevated temperature.

FIGURE 6.42 Polyphenol oxidase substrates.

Substrate preferences are dependent on enzyme source and isoform. Among the most common natural or endogenous substrates are caffeoyl-quinic acid, caffeoyl-tartaric and caffeoyl-shikimic acid derivatives, catechin, and others shown in Figure 6.42, where K_M values are in the general range of 0.5–20 mM. Some substrates are inhibitory at sufficiently high levels.

There is much interest in inhibiting enzymic browning and several strategies exist to do so. Dehydration, freezing, and thermal processing are effective as long as the time required to effect the process does not permit intolerable browning and textural changes related to quality retention. Other physical means include packaging in modified atmosphere for minimally processed foods or coating tissue sections with sugar syrups (especially for frozen products) or edible films to limit O_2 cosubstrate availability. This latter approach is made realistic by the K_M for O_2 being ~50 μM, and air-saturated water at 25°C is ~260 μM, providing opportunity for meaningful reduction in dissolved O_2 levels. The limitation for respiring products is that O_2 cannot be depleted to a level that evokes anaerobic metabolism, which often yields off-flavors. While some phenol oxidases undergo reaction inactivation (by reaction with *o*-quinone), the thousands of enzyme turnovers that occur before inactivation happens limit the potential to exploit this feature as a means to control enzymic browning in foods.

Most popular are chemical treatments based on either inhibiting or deactivating enzyme, complexing native substrates, or reducing quinones back to *o*-diphenols and/or conjugating quinones in a manner that prevents melanin formation. For the latter strategy, chemicals that act only as reducing agents will delay browning only to the point where they are depleted and then offer little further protection. Some reducing agents, especially thiols, can chemically conjugate quinones to form nonpolymerizing adducts, but this effect is also of limited duration since the thiol agents are consumed in the process:

(6.49)

Strategies revolving around enzyme inhibition have greater long-term effectiveness and include acidulants, enzyme inhibitors, chelating agents, and enzyme deactivators. Acidulants such as citric, malic, and phosphoric acids exploit the low pH sensitivity of enzyme action provided they are used

Cinnamic acid p-Coumaric acid Ferulic acid Benzoic acid

FIGURE 6.43 Other polyphenol oxidase inhibitors resembling substrates.

at levels without adverse effects. Inhibitors resembling native substrates may competitively occupy the phenolic binding site; such inhibitors appear in Figure 6.43.

Chelators such as EDTA, oxalic, and citric acids (including juices that contain these organic acids, such as lemon and rhubarb) coordinate with copper at the active site, and there is evidence in some cases that a portion of the copper can be removed, although this is not necessarily required for inhibition. HIS binds copper quite tightly (log K_{assoc} of 10–18) and copper-chelating agents (log K_{assoc} of 15–19 for EDTA and 4–9 for oxalate) may not be effective at copper removal from the enzyme active site. Other inhibitors coordinate to the active site copper and competitively inhibit activity; these inhibitors include halide salts, cyanide, CO, and some thiol reagents. Strategies to complex native substrates and limit their availability or access to enzyme reaction have focused on chitosan and cyclodextrin treatments. Prospective use of these agents may be limited to treating fluid products. Polyvinylpyrrolidone (insoluble form) is another phenolic-complexing matrix that is used primarily for research purposes in efforts to isolate phenol oxidases while minimizing the extent of browning occurring during initial extraction from tissue. However, this approach may diminish the nutritional value of juices as the phenols and related compounds are largely viewed as conferring health benefits (Chapter 13).

Reducing agents such as various sulfites, ascorbic acid, and cysteine have multiple effects on inhibiting enzymic browning. They may act by reducing *o*-quinones back to diphenols or chemically conjugating *o*-quinones, thereby delaying melanin formation. This effect would be of limited duration since reducing equivalents would become exhausted during sustained enzyme action. A more important effect of these agents appears to be irreversible, covalent inactivation of phenol oxidases, since enzyme activity is not fully restored by subsequent dialysis after extended preincubation in the absence of substrate [92]. These inhibitors appear to coordinate with active site copper and undergo electron transfer reactions under aerobic conditions to yield “crypto-” oxy-radicals (not easily detected or identified) at the active site. These oxidizing species degrade the active site HIS ligands, inactivating the enzyme and likely releasing copper. The ability of inhibitory agents to function this way in disrupted tissues is based on kinetic factors, that is, how fast and competitively they bind to and inactivate the enzyme relative to how fast enzyme acts on substrates. Sulfites and thiol reagents are of longer-lasting effectiveness as browning inhibitors in disrupted tissues than ascorbic acid, and these distinctions correlate with a faster time frame of enzyme inactivation by the former group [92]. Tropolone and 4-hexylresorcinol are two more recently identified phenol oxidase inhibitors (Figure 6.44).

They both resemble substrate and coordinate tightly with active site copper; these inhibitors are effective in the ~1 μM range. 4-Hexylresorcinol was isolated from an extract of fig used

4-Hexylresorcinol Tropolone Kojic acid

FIGURE 6.44 Other polyphenol oxidase inhibitors resembling substrates.

as a ficin (protease) preparation. It is used primarily to control black spot in crustaceans, as a replacement for sulfites (GRAS exemption by FDA), which are progressively being disallowed because of health-threatening responses of a proportion of humans, particularly asthmatics. Tropolone cannot be added to foods but is useful in discriminating between browning caused by phenol oxidases and peroxidases. Another type of inhibitors of phenol oxidases are peptides in honey and corn seedlings that remain to be identified as well as various small cyclopeptides [150]. Kojic acid was identified from cultures of *Aspergillus* and *Penicillium* spp. as an effective phenol oxidase inhibitor, likely by coordinating to copper at the active site; however, its use may be limited to fermented foods using these organisms since historical data indicates toxicity in animals.

6.5.2.2 Peroxidases [38,142]

Peroxidases are ubiquitous enzymes in plants, animals, and microorganisms and are organized into plant (including microbes) and animal superfamilies. Plant peroxidases are most relevant to food biochemistry, and the various classes (families) of peroxidases include those of prokaryotic origin, secreted fungal peroxidases, and classical plant peroxidases. Plant peroxidases are glycosylated, monomeric, heme (protoporphyrin IX) proteins of 40–45 kDa mass comprised of two like domains, arising from gene duplication. Plant peroxidases are mostly soluble, with others being membrane-associated and covalently bound forms, the latter types being released by cell wall–degrading enzymes. The physiological roles of peroxidases include the formation and degradation of lignin, oxidation of the plant regulator indole acetic acid (involved in ripening and associated catabolic processes), evolution of a defense to pest and pathogens, and removal of cellular H_2O_2. Isoforms are classified as being acidic, neutral, and alkaline based on isoelectric point. The neutral peroxidase C of horseradish root (EC 1.11.1.7, donor–H_2O_2 oxidoreductase) is the most studied member and consequently serves as a model peroxidase; its characteristics are generally applicable to other peroxidases. The general peroxidatic reaction catalyzed is

$$2\text{ AH (electron donor)} + H_2O_2 \rightarrow 2H_2O + 2A^{\bullet} \tag{6.50}$$

The enzyme can exist in five oxidations states, with the resting state being the Fe^{III} form (Figure 6.45). Reaction with H_2O_2 occurs after docking near the heme iron, and HIS_{42} acts as a general base to "pull" an electron to yield the hydroperoxyl anion, a strong nucleophile that coordinates with Fe. The Fe-liganded HIS_{170} residue then acts as a general base to push electrons toward the peroxide and allow heterolytic O-O cleavage to yield H_2O as a leaving group (H^+ coming from HIS_{42} now acting as a general acid), yielding peroxidase compound I ($Fe^V{=}O$). Thus, a net $2e^-$ from heme Fe^{III} is used to reduce H_2O_2 and form H_2O. Two successive $1e^-$ (and H^+) transfer steps from each of two AH donors revert the enzyme back to the resting state (completing the peroxidatic cycle), going through compound II (H^+–$Fe^{IV}{=}O$) and releasing another H_2O as a leaving group. Each of these steps is progressively slower compared to the rate of formation of compound I. Peroxidases are most easily inhibited by chemicals that bind to the heme-prosthetic group, the most common ones being cyanides, NaN_3, and CO, as well as some thiol compounds. However, use of such inhibitors is limited to characterizing peroxidases. Also, the general ambiguity regarding the role of peroxidase in food quality provides little justification for adding specific inhibitors.

Phenols (e.g., *p*-cresol, catechol, caffeic, and coumaric acids; Figures 6.42 and 6.43), ascorbic acid, NADH, and aromatic amines (e.g., *p*-aminobenzoic acid) are common electron donors for the conversion of compound I to compound II and back to ferric peroxidase. The $2A^{\bullet}$ resulting from the peroxidatic cycle can have various fates. If AH is ascorbic acid, then $2A^{\bullet}$ will yield one mole each of ascorbic acid and dehydroascorbate. If AH is guaiacol, then $2A^{\bullet}$ will undergo free radical addition (polymerization) to yield tetramers, and the attendant brown color provides the basis of using guaiacol in the peroxidase assay widely used as a blanching efficacy indicator.

FIGURE 6.45 Reaction mechanism and cycling of peroxidase. P is peroxidatic cycle; C is catalytic cycle; O is oxidatic cycle in bottom scheme. (Redrawn from Dunford, M.B., *Heme Peroxidases*, John Wiley & Sons, New York, 507pp., 1999).

(6.51)

Pyrogallol is another substrate that undergoes free radical homocondensation reactions to yield a purple-colored dimer (purpurogallin). Tocopherol as AH can yield stable free radicals, whereas if tyrosine is used, the free radical adducts may condense to form dimers. Dityrosine cross-links in bread dough (gluten) may promote viscoelasticity and good baking qualities.

In the presence of excess H_2O_2, peroxidase will support a catalytic process (Figure 6.45) by reaction with a second mole of H_2O_2 to H_2O, forming compound III ($H^+–Fe^{II}–O_2$). Peroxidases exhibit maximum activity on AH donors at H_2O_2 levels of 3–10 mM, and these levels of use are important in peroxidase assays serving as blanching indicator tests. Assays using excess H_2O_2 will yield compound III, which is not recycled back to the resting state efficiently, resulting in an underestimation of peroxidase activity.

There are other unique reactions exhibited by peroxidase. One involves NADH, which in the presence of trace H_2O_2 can react in the peroxidatic cycle as AH to yield 2 moles $NAD^•$. $NAD^•$ may have several fates and allow other reactions to occur:

$$NAD^• + O_2 \rightarrow NAD + {}^-O_2^• \quad (6.52)$$

$$^{-}O_2^{\bullet} + 2H^+ \rightarrow H_2O_2 \quad (6.53)$$

$$NAD^{\bullet} + \text{ferric peroxidase} \rightarrow NAD + \text{ferrous peroxidase} \quad (6.54)$$

$$\text{Ferrous peroxidase} + O_2 \rightarrow \text{oxyperoxidase (compound III)} \quad (6.55)$$

$$\text{Oxyperoxidase} \rightarrow \text{ferric peroxidase} + {}^{-}O_2^{\bullet} \text{ (then Equation 6.53 may follow)} \quad (6.56)$$

Thus, using NADH, peroxidase has the ability to generate its own cosubstrate (H_2O_2) when only trace levels exist, making use of both the peroxidatic and oxidatic cycles.

Other types of peroxidase-associated activities, oxidation and hydroxylation, are indirect effects of peroxidase reactivity. The sequence using NADH as AH in the peroxidatic and oxidatic cycles illustrates how peroxidase action can yield reactive oxygen and oxy-radicals. Such oxygen species may cause oxidation reactions. Oxidation reactions can occur if a cosubstrate yields $A^{\bullet}$ species that can abstract H atoms from other components. Such a sequence can initiate other free radical reactions that could possibly lead to polymeric derivatives being formed from phenolic components, reminiscent of phenol oxidase-mediated browning. Thus, reaction of peroxidase with one phenolic substrate may cause indirect (chemical) oxidation of another, potentially obscuring an evaluation of direct peroxidase action on components in a mixed system like foods. Phenolic peroxidase substrates that yield O_2-reactive $A^{\bullet}$ will also form ${}^{-}O_2^{\bullet}$ and H_2O_2, which can further mediate oxidation reactions. Thus, how much a role peroxidases play in browning and other discoloration processes in foods has remained enigmatic. Some of the more recent claims of peroxidase involvement in browning are based on correlative associations of peroxidase activity and levels or incidence of browning; such observations remain short of establishing cause and effect.

Plant peroxidases often exhibit pH optima in the range of pH 4.0–6.0, although the pH range for forming compound I is very broad characterized by terminal pK_a values of ~2.5 and 10.9. The acidic transition is conferred by the HIS_{42} residue, the pK_a of which can vary between 2.5 and 4.1, depending on medium composition. This is an unusually low pK_a for HIS, which must first act as the conjugate base, and it is brought about by multiple H-bonding networks that serve to facilitate H^+-dissociation. The overall pH optima for peroxidase reactions relate to the steps that utilize AH to recycle ferric peroxidase in the peroxidatic cycle. AH species are H-donors (not just e^- donors) and thus must be protonated (if it has a dissociable H^+) to serve as substrate, and pH optima are often substrate dependent.

Peroxidases are among the most ubiquitous and heat-stable enzymes in plant tissues; these characteristics favor their use as blanching indicators. The rationale is that if endogenous peroxidase activity is destroyed, all other quality-deteriorative enzymes must be as well. The limitation of this strategy is that excessive thermal processing is often applied and this may compromise quality in various other ways (e.g., texture, nutrition, component leaching). However, until other specific enzymes are identified as being the most heat stable among those having direct impact on quality of blanched (and the frozen) vegetables and are easy to assay, peroxidase will remain the blanching indicator of choice. Temperature effects on peroxidases vary with the host tissue. Generally, optimal temperature for activity is modest, ranging from 40°C to 55°C. Thermal stability is quite high, and depending on source, complete inactivation may require a several-minute exposure at 80°C–100°C for appropriately sized, intact portions of vegetable tissues. The heme-prosthetic group, glycosylation, four disulfide linkages, and the presence of 2 mol Ca^{2+} with likely participation in salt bridges are factors responsible for peroxidase thermal stability. Thermal stability generally decreases as pH decreases in the range of pH 3–7 and in the presence of increasing ionic strength. Regeneration of peroxidase activity occurs in the range of pH 5.5–8.0, following short durations of thermal processing, such as blanching. Regeneration is believed to involve reconstitution of the heme at the active site that was lost during the initial deactivation. More extensive heating, such as retorting, diminishes the propensity for regeneration of active enzyme because of more extensive conformational

changes and covalent reactions. However, the release of free heme into the medium may provide for catalysis of oxidative reactions and such processes have been implicated as causing off-flavors in canned vegetables. Other reactions catalyzed by peroxidase that impact food quality include the formation of phenoxy radicals that indirectly oxidize lipids and the direct oxidation of capsaicin, the pungent principle of peppers.

While the role of peroxidase in enzymic browning remains open to question, it has been conclusively shown that peroxidase can destroy some pigments, particularly betalains in table beet roots. Peroxidase has also been implicated in the bleaching of chlorophyll under specific conditions.

6.5.2.3 Other Oxidoreductases [38]

Lactoperoxidase is the peroxidase in milk and belongs to the animal superfamily of peroxidases. It is a 78 kDa mass glycoprotein monomer, containing Ca^{2+} and a modified protoporphyrin IX that is covalently bound. Lactoperoxidase has properties similar to horseradish peroxidase C in terms of H_2O_2 reactivity and cycling through peroxidase forms. Lactoperoxidase is particularly distinct from peroxidase C in that it is more reactive with halides (especially I^-) and related species. Of particular interest is the ability to react with thiocyanate (SCN^-), which is normally present in milk, as AH in the peroxidatic cycle where

$$2SCN^- + \text{Enz-}(Fe^{V}{=}O) \rightarrow 2SCN^{\bullet} + \text{Enz-}(Fe^{III}) \rightarrow SCN^- + HOSCN + H^+ \qquad (6.57)$$

The hypothiocyanous acid and conjugate base (pK_a 5.3) hypothiocyanite ($OSCN^-$) are antimicrobial agents. Thus, addition of small amounts of H_2O_2 (and also SCN^-, if not abundant) to milk affords a "cold-pasteurization" process that reduces microbial load in raw milk and this is an important option in (sub)tropical climates where ready access to refrigeration may not be available. The enzyme-generated $OSCN^-$ is more effective than adding exogenous chemical perhaps because lactoperoxidase adsorbs to surfaces and particulates and may afford $OSCN^-$ generation in proximity to microorganisms.

Catalase (EC 1.11.1.6) is a tetrameric heme enzyme that is widespread in nature and is related to peroxidases. Its principal role is to detoxify cells of excess H_2O_2 as the enzyme degrades H_2O_2 to H_2O plus ½O_2. Catalase is rather heat stable and has been considered as a blanching indicator enzyme. It is easy to assay, by taking a small filter paper disk, dipping it into a homogenate of blanched vegetable, and then placing the disk into a test tube of dilute H_2O_2. A positive test for residual catalase is indicated by the disk floating to the surface, buoyed by small, adherent O_2 bubbles formed by any active enzyme absorbed on the disk.

6.5.3 Enzymes Related to Flavor Biogenesis

6.5.3.1 Lipoxygenase [16,25,154]

The role of lipoxygenases in foods and food quality continues to be evaluated, despite these enzymes being characterized over 80 years of prior study. Some of the earliest descriptions referred to "lipoxidase" and "carotene oxidase" activities. Lipoxygenases (and related oxygenases) are widespread and found in plants, animals, and fungi, while once they were believed to exist exclusively in the plant kingdom. Lipoxygenase mechanism and the basis of reaction selectivity were featured earlier in this chapter. This section will focus on the multiplicity of reaction and ancillary pathways of fatty acid transformation and associated roles of lipoxygenase-mediated processes in food quality. Lipoxygenase action may be desirable or undesirable, depending on the specific food material and the context in which it is used, and many examples beyond what appears forth are provided in various reviews [25,154].

Lipoxygenase has long been known to cause quality defects in processed vegetables that have not been sufficiently thermally processed to destroy the enzyme. Legumes (snap beans, soybeans, peas) are particularly susceptible to the development of oxidative rancidity because of high lipoxygenase levels (Table 6.12). The diversity of lipoxygenase-mediated reactions can be accounted for

TABLE 6.12
Properties of Selected Lipoxygenases and Hydroperoxide Lyases

Lipoxygenase Source (Isoform)	Rel. Activity	Opt pH	Lipoxygenase Specificity 9:13, *S/R*	Hydroperoxide Lyase Specificity	Dominant Compounds in Host Tissues
Soybean seed (1)	4200	9.0	4:96 13*S* (pH 9)	*S*-13-LOOH (low levels)	*n*-hexanal, hexenals, off-flavors
			23:77 13*S* (pH 6.6)		
(2)		6.5	50:50 $9R \geq 9S$		
(3)		7.0	65:35 *R*~*S*		
Corn germ	—	6.5	93:7 9*S*	(Trace/low levels)	*n*-hexanal, off-flavors (ketols in corn seed)
Pea seed (3 isoforms)	1800	6.6	67:33 *R*~*S* (pH 6.6)	(Trace/low levels)	Off-flavors
			59:41 13*S*, 9*R* (pH 9)		
Potato tuber	4600	5.5	95:5 9*S*	9/13-LOOH	*trans*-2-cis-6-nonadienal
Tomato fruit (3 isoforms)	360	5.5	96:4 9*S*	13-LOOH (CYP74B)	*trans*-2-hexenal, *cis*-3-hexen-1-ol, *n*-hexanal
Cucumber fruit	30–120	5.5	75:25	9,13-LOOH	*trans*-2-*cis*-6-nonadienal
Green pepper fruit	300	5.5–6.0	Lacking definitive evaluation	13-LOOH (CYP74B)	*cis*-3-hexenal, *trans*-2-hexenal, *n*-hexanal
Pear fruit	Trace	6.0	95:5	9-LOOH	*trans*-2-*cis*-6-nonadienal
Apple fruit	<120	6.0–7.0	15:85	13-LOOH	*n*-hexenal, *trans*-2-hexenal
Mushroom	—	8.0	10:90 13*S*	*See text*	1-octen-3-ol, 1-octen-3-one
Tea leaves	—	6.5	16:84 13*S*	*S*-13-LOOH	*trans*-2-hexenal, *cis*-3-hexenal, *n*-hexanal

Sources: Galliard, T. and Chan, H.W-S. Lipoxygenases, in *The Biochemistry of Plants*: *A Comprehensive Treatise, Volume 4, Lipids: Structure and Function*, P.K. Stumpf (Ed.), Academic Press, New York, pp. 131–161, 1980; Grosch, W., Lipid degradation products and flavour, in *Food Flavours. Part A. Introduction*, Morton, I.D. and A.J. MacLeod (Eds.), Elsevier Scientific Publishing, New York, pp. 325–398, 1982; Kuribayashi, T. et al., *J. Agric. Food Chem.*, 50, 1247, 2002; Matsui, K. et al., *J. Agric. Food Chem.*, 49, 5418, 2001; Vliegenthart, J.F.G. and Veldink, G.A., Lipoxygenases, in *Free Radicals in Biology*, W.A. Pryor (Ed.), Vol. V., Academic Press, New York, pp. 29–64, 1982.

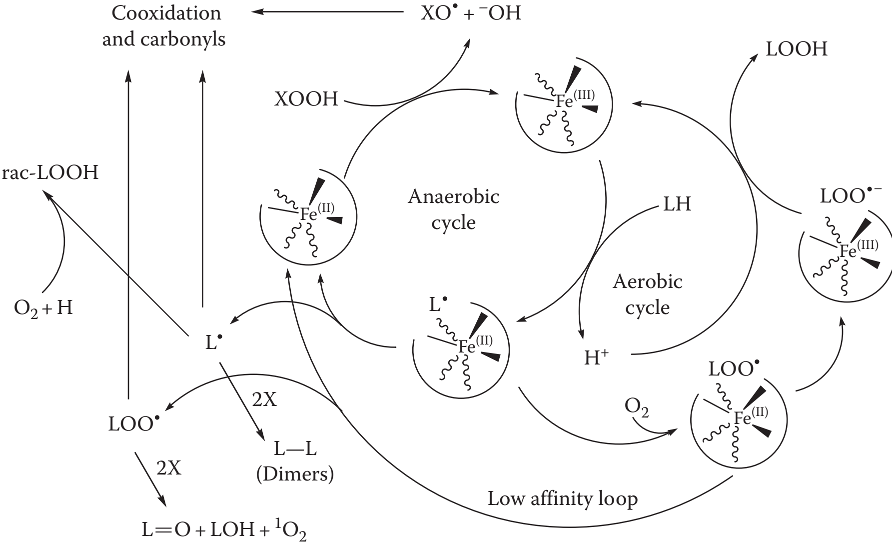


FIGURE 6.46 Reaction pathway and cycling of lipoxygenase. (Adapted and redrawn from Hughes, R.K. et al., *Biochem. J.*, 333, 33, 1998; Whitaker, J.R., Voragen, A.G.J., and D.W.S. Wong (Eds.), *Handbook of Food Enzymology*, Marcel Dekker, New York, 2003; Wu, Z. et al., *J. Agric. Food Chem.*, 47, 4899, 1999.)

by the reaction cycle extending beyond that required to illustrate mechanism (recall Figure 6.8). The anaerobic cycle encompasses reactivity of the enzyme in the absence of O_2 or in O_2-starved media; this portion includes the peroxide activation of enzyme resting state (Fe^{II}) to the active state (Fe^{III}), sometimes referred to as "lipoperoxidase" activity (Figure 6.46). As a result of this activation, an oxy-radical species (XO•) is released, which can propagate free radical reactions; this cycle may continue in the absence of O_2 through which fatty acid radicals (L•) may be formed and released. In cases where XO• is derived from a polyunsaturated fatty acid, it may undergo intramolecular rearrangement and form reactive epoxides. Thus, many lipoxygenases cause secondary, free radical cooxidation reactions when the anaerobic cycle is operative. When O_2 is abundant, the normal reaction mechanism occurs as explained earlier (Figure 6.8). Some lipoxygenases have lesser affinities for fatty acid and reaction intermediates, and the hydroperoxyl radical (LOO•) may dissociate prematurely through the "low-affinity loop" before the normal catalytic cycle is completed (Figure 6.46). This requires that the enzyme become reactivated by peroxide in the anaerobic cycle. The substrate affinity of lipoxygenase isoforms 3 of pea and soybean seeds is 20-fold less than the other respective seed isoforms [7,59]. Thus, pea and soybean isoforms 3 are primarily responsible for yielding LOO• to cause further fatty acid autoxidation and cooxidation reactions through the evolution of reactive oxygen species, including singlet oxygen ($^1O_2$) during the aerobic cycle (most isoforms cause cooxidation only in the anaerobic cycle). The aerobic and anaerobic cycles constitute alternative pathways of enzyme cycling, and both of these pathways may operate simultaneously. The precise O_2 level is not always the sole determinant of the preferred pathway of enzyme cycling. The kinetic characteristics for each step; the relative levels of enzymes, substrates, and intermediates; and the enzyme microenvironment all influence the degree to which each pathway is evoked.

Lipoxygenases from various food sources differ in isoform profiles, optimum pH, and reaction regio- and stereoselectivity (Table 6.12). Lipoxygenases are "soluble" enzymes but different isoforms are found in different cellular compartments, reflecting their unique purpose and role in fatty acid transformation in the host tissue [45]. Soybean seed lipoxygenases are the most studied and historically comprised the basis for classification [7]. Lipoxygenase isoform 1 is the most abundant in soybean seed and is unique in its alkaline pH optimum. This feature and the 13*S*-product

stereoselectivity led it to be classified as a "type I" lipoxygenase. Soybean isoforms 2 and 3 have more neutral pH optima and exhibit less product selectivity; these general features comprise the historical basis for the "type II" classification. It is now clear that most lipoxygenases have near-neutral pH optima and differ widely in degree of product selectivity, rendering the original "type" classification of limited usefulness. Even the classification on the basis of regioselectivity of oxygenation of arachidonic acid (e.g., 5-LOX) described earlier is yielding to a classification on the basis of structural similarities. A selective survey of plant lipoxygenases (Table 6.12) reveals that many are regioselective for oxygenating C9 of linoleic (or linolenic) acid to yield the *S*-configured hydroperoxide (LOOH). Some lipoxygenases (especially soy isoforms 2 and 3 and pea seed isoforms) are lacking in regio- and stereoselectivity. This property is associated with a reduced affinity for fatty acid substrate during the reaction sequence [7,56]. If $L^\bullet$ is released prematurely, chemical combination with molecular O_2 will be random leading to *rac*-LOOH mixtures, whereas oxygenation while substrate is at the enzyme active site will exhibit regio- and stereobias (Figure 6.46).

Lipoxygenase isoforms causing cooxidation reactions yield multiple autoxidation products, including aldehydes and ketones (carbonyls) produced through a free radical mechanism. Cooxidation also bleaches carotenoids, and while this may destroy (pro)nutrients, it is a useful and desirable outcome in whitening of bread dough and related finished baked goods (oxy-radical generation may also improve dough tensile and viscoelastic properties). Both soy and pea seed flours (as well as those from potato and chick pea) may be added to bread dough for their carotene-bleaching and dough-improving effects, since wheat lipoxygenase has low bleaching activity. Lipoxygenases from tomato and green pepper fruit are also capable of cooxidizing carotenoids. Many other plant food sources have multiple lipoxygenase isoforms, including most cereals and grains, as well as snap beans.

The oxidative rancidity defect brought about by lipoxygenase can be attributed to two phenomena (covered in detail in Chapter 4). One is the oxidation of linoleic and linolenic to LOOH, and the ensuing chemical decomposition into various aromatic aldehydes and ketones. The second is the direct enzymic production of fatty acid radicals released to the food matrix that further initiate and propagate cooxidation and free radical autoxidation reactions. *n*-Hexanal confers a beany flavor and is used as a general index of the degree of fatty acid oxidation. Among the sources of lipoxygenase listed (Table 6.12), soybean, corn, and peas are most prone to developing rancidity derived from lipoxygenase action. This requires corn and peas to be blanched to at least a lipoxygenase deactivation end point prior to freezing and storage; in soybeans, lipoxygenase action must be destroyed or attenuated prior to freezing (by blanching), grinding into flour (by drying), or refining into oil and protein isolates.

The rather subtle differences between lipoxygenase isoforms have been exploited in efforts to manage food quality. Isogenic strains of soybean seeds, lacking in certain lipoxygenase isoforms, have been assessed for their propensity to cause rancid flavors in beans, soybean oil, and formulated foods (bread). For homogenized soybean seeds, lipoxygenase 2 appears responsible for producing the greatest levels of *n*-hexanal [58]. The presence of either isoforms 1 or 3, or both, reduced the capacity of isoform 2 to produce *n*-hexanal, suggesting that the fates of fatty acid hydroperoxides are dependent on the isoform that produced it. When flour from soybean strains lacking in specific isoforms was used in bread dough, isoform 1 was associated with greatest increases in bread volume and isoform 2 was associated with greatest increases in dough strength and viscoelasticity [32]. Isoform 2 was also associated with greater levels of objectionable volatiles, and this is why legume flours are added at <1% in bread doughs. These examples show how knowledge of even subtle differences in enzyme action may lead to new strategies to produce foods and manage quality.

Lipoxygenases and related oxygenases (cyclooxygenases) occur in animal tissues, and muscle systems are most relevant to foods [16,25]. Animal lipoxygenases are essentially identical to plant and fungal lipoxygenases in terms of structure and mechanism. One major difference is that arachidonic acid, and longer-chain, higher unsaturates are the natural substrates for animal lipoxygenases, although they are also active on linoleic and linolenic acid. In fresh fish, endogenous lipoxygenase

Nordihydroguaiaretic acid Esculetin Resveratrol

FIGURE 6.47 Lipoxygenase inhibitors.

action is known to form desirable flavors (Chapter 11), but information remains lacking on the relationships between animal lipoxygenases and food quality.

The most effective means to prevent negative consequences of lipoxygenase action is thermal processing to deactivate the enzyme, with pH adjustment being a secondary approach. Many compounds have been identified as inhibitors of the enzyme, and those with typical phenolic-antioxidant properties quench secondary oxidation reactions with little direct effect on the enzyme. Only a few inhibitors have been consistently identified to directly inhibit lipoxygenase (Figure 6.47), and these include nordihydroguaiaretic acid, catechols, and esculetin (all at ~10 μM), which coordinate with active site Fe and/or reduce it to the inactive Fe^{II} state. Also, resveratrol (~10 μM) and $SnCl_2$ (5 mM) are competitive inhibitors.

6.5.3.2 Hydroperoxide Lyase and Related Enzyme Transformations [13,148]

The decomposition of fatty acid LOOH (derived from lipoxygenase action) by *nonspecific*, chemical reactions yield carbonyls that confer rancidity (Chapter 4). In contrast, pleasant aromas arise in the presence of enzymes that *specifically* direct LOOH transformation toward other derivatives. In many fruit and vegetative tissues, this alternative pathway is evoked by hydroperoxide lyases, leading to the accumulation of a limited set of degradation products of 6, 9, and 12 carbons of defined composition (Figure 6.48, Table 6.12). The general sequence of events is the liberation of fatty acid from intact glycerolipid by lipid acyl hydrolase, dioxygenation of fatty acid into 9/13-hydroperoxides by lipoxygenase, cleavage of the 9/13-hydroperoxides by hydroperoxide lyase, and then possible isomerization and final conversion of aldehydes to alcohols by alcohol dehydrogenase activity. The existence of this pathway was first inferred for the origin of banana flavor and the enzymology was definitively established for cucumber and tomato fruit [47,52,148].

The species-specific pathway of transformation of fatty acids into desirable flavors derives from the combined effects and specificity of both lipoxygenase and hydroperoxide lyase and the relative abundance of auxiliary enzymes. For example, for tomato fruit, even though fatty acid 9-LOOH is the dominant product of lipoxygenase action (Table 6.12), the specificity of the hydroperoxide lyase (9:13-LOOH at 1:62 relative rates) dictates that the C6 and C12 fragments are mostly produced in disrupted tissue. In contrast, cucumber fruit hydroperoxide lyase is rather nonspecific (9:13-LOOH at 2:1 relative rates), and the dominance of C9 fragments from directed fatty acid oxidation and fragmentation is dictated largely by lipoxygenase selectivity. Examples listed in Table 6.12 can be grouped as forming principally cucumber-type flavors (nonadienal species), or tea leaf-like, or rich in floral/fruity flavors conferred by hexenal/hexanals/hexenols. The reason why members within a group (C9 or C6 accumulators) do not have the same overall flavor is dependent on several other factors involved in flavor biogenesis. Two factors involve differences in the levels of the composite enzymes in the pathway, as well as ratios of linoleic and linolenic acid substrate liberated by lipid acyl hydrolase action. The pathway (Figure 6.48) is shown for linolenic acid, but the same reactions may occur for linoleic acid to yield analogous products of different flavor character, and differences in enzyme selectivity for these fatty acids will impact final product composition. Differences in tissues among the auxiliary enzymes will also dictate final product composition. While evidence of specific isomerizing enzymes has been obtained for cucumber, flaxseed, wheat germ, barley, and soybean [47,148], these isomerization factors remain ambiguous [5,13]. Hydroperoxide

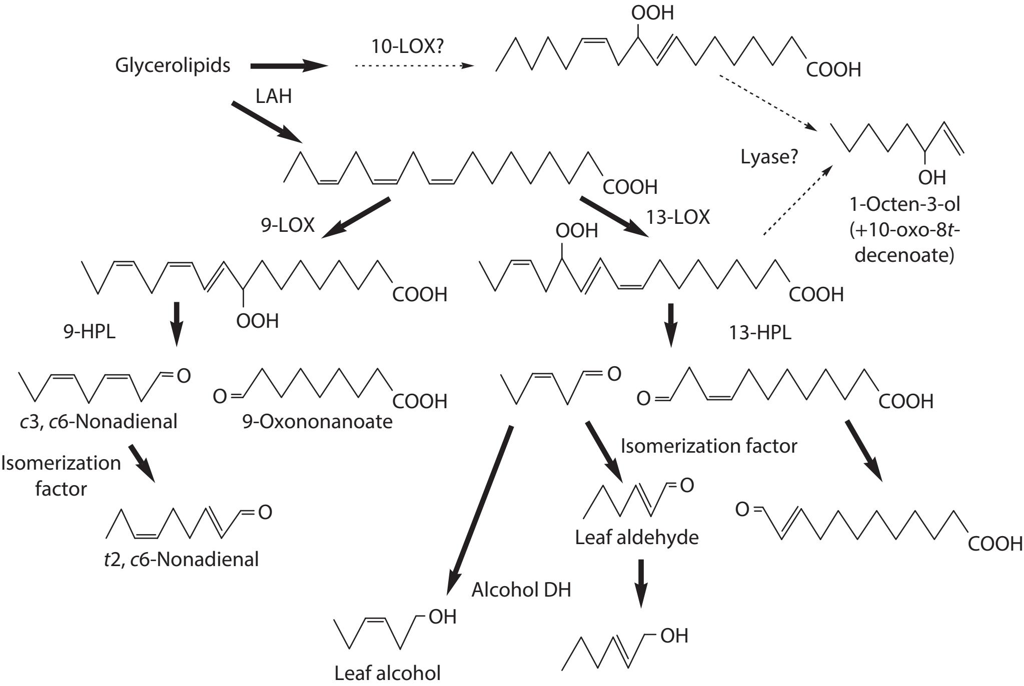


FIGURE 6.48 Coordinated lipoxygenase (LOX), hydroperoxide lyase (HPL), and auxiliary enzyme transformation of fatty acids to yield "green" notes and flavors. (Adapted from Blée, E., *Prog. Lipid Res.*, 37, 33, 1998; Fuessner, I. and Wasternack, C., *Annu. Rev. Plant Biol.*, 53, 275, 2002; Vliegenthart, J.F.G. and Veldink, G.A., Lipoxygenases, in *Free Radicals in Biology*, W.A. Pryor (Ed.), Vol. V, Academic Press, New York, 1982, pp. 29–64.)

isomerase was originally listed as EC 5.3.99.1 (deleted in 1992) but is now accounted for by the action of "hydroperoxide dehydratase," specifically allene-oxide synthase (EC 4.2.1.92) and allene-oxide cyclase (EC 5.3.99.6), although these activities are best known for yielding jasmonates and ketoles [5]. Thus, the nature of an "isomerization factor" remains to be clarified. Little is known of the specific alcohol dehydrogenase(s) involved, although at least three isoforms (*ADH-1, -2, -3* genes) are present in many plant tissues that could contribute this function [13,133]. Each tissue also has other flavoring agents, conferred by other metabolic or enzyme pathways that contribute to or even dominate the overall flavor character of the food sources listed on Table 6.12.

Hydroperoxide lyases (classified as cytochromes, CYP74B and CYP74C) are likely tetramers of 55–60 kDa monomeric units and are membrane bound (plastidic) in tissues [45]. They differ from other cytochromes in that they do not require O_2 and NAD(P)H; instead, they utilize the fatty acid LOOH as both substrate and oxygen donor in forming new C−O bonds. It is not surprising that many lipoxygenase inhibitors (Figure 6.47) also inhibit hydroperoxide lyase since both enzymes bind fatty acid chains. In addition to the four tetrapyrrole ligands, the Fe is coordinated with a CYS [45]. The enzymes from pepper, tomato, and guava fruits are highly 13-LOOH specific and are placed in the CYP74B subfamily, whereas those from cucumber and melon act on both 9/13-LOOH and are placed in the CYP74C subfamily. Other hydroperoxide lyases remain to be fully characterized and classified. A unique set of products is observed for the enzyme system in mushrooms (and other fungi), which forms C10 and C8 fragments from dioxygenated linoleic and linolenic acids (Table 6.12, Figure 6.48). Initially, the presence of a 10-LOX was proposed, but it has been established that mushroom LOX dioxygenates the C-13 site [68]. However, the linoleate 10-LOOH isomer is formed in mushroom, and mushroom protein preparations can cleave the 10-LOOH derivative to

FIGURE 6.49 Reaction mechanism of hydroperoxide lyase. (Adapted and redrawn from Gretchkin, A.N. and Hamberg, M., *Biochim. Biophys. Acta*, 1636, 47, 2004.)

C10 oxo-acid and 1-octen-3-ol. It now appears that psi(growth factors)-producing oxygenases that have a heme-prosthetic group yield 8- and 10-peroxides from linoleic acid [18].

The mechanism of action of hydroperoxide lyase (and the other CYP74 family members) has recently been proposed from studies on the guava fruit enzyme. The homolytic mechanism involves an epoxyallylic radical leading to a hemiacetal, which lyses to yield fragmentation products (Figure 6.49). This mechanism probably applies to all hydroperoxide lyases, and it is consistent with the established mechanism of cytochrome P450s.

Efforts to apply knowledge of the lipoxygenase–hydroperoxide lyase pathway to genetically modified fruits have shown mixed results. Introducing a Δ9 desaturase (to enhance fatty acid substrate) and alcohol dehydrogenase in tomato fruit both enhanced flavor, whereas suppressing lipoxygenase and overexpressing a 9-hydroperoxide lyase had no effect [79]. Commercial opportunities are currently employing highly active 13-specific lipoxygenase and fatty acid 13-LOOH hydroperoxide lyase in combination with an inexpensive source of linolenic acid to produce the "green note" flavors conferred by the 6-C aldehydes and alcohols, a global market estimated at upward of $40 million (US) annually.

6.5.3.3 Biogenesis of Other Lipid-Derived Flavors [114]

Alcohol acyltransferase (EC 2.3.1.84) is responsible for the emanation of aromatic esters in many climacteric fruit, especially during the ripening phase whereupon its tissue levels increase [114]. This enzyme exists in fruits such as apples, strawberries, bananas, melons, and olives, among others, and also in yeast and fungi. The reaction catalyzed is

$$S\text{-acyl-Coenzyme A} + \text{Alcohol} \rightleftarrows \text{Acyl-ester} + \text{Coenzyme A-SH} \qquad (6.58)$$

The typical profile of esters formed in ripening fruit by this enzyme includes acetate and butanoate esters of methyl- and ethyl-branched, phenylethyl, or *n*-alcohol groups, typically of 2–8 carbons.

Other lipid flavors in foods may arise from other pathways. Lipases have been considered as mediators of volatile ester formation, through reverse hydrolysis reactions. There is no evidence of this in fruit tissues, but it occurs to some extent in yeast fermentations, and low levels of fruity flavors may be mediated by fermentative organisms and contribute to overall flavor of aged, fermented foods such as cheese. Terpenoid biosynthesis in plants typically used as herbs and spices occurs through a multistep, complex biosynthetic pathway from isoprene units [114]. A lipoprotein lipase (LPL, stimulated by lipoprotein) occurs in milk at 1–2 mg/L [154] and may give rise to "spontaneous rancidity" if milk is handled poorly prior to pasteurization.

6.5.3.4 Origin and Control of Pungent Flavors and Other Bioactive Effects

6.5.3.4.1 Myrosinase Transformation of Glucosinolates [4,64,83]

Plants of the family Brassicaceae, notably cabbage, broccoli, cauliflower, turnip, kale, Brussels sprouts, radish, mustard, and wasabi, are known for their pungency. Upon tissue disruption, conditions become favorable for the reaction between the enzyme myrosinase (EC 3.2.1.147 [formerly 3.2.3.1], thioglucoside glucohydrolase) and a diverse set of odorless glucosinolate substrates, setting off a "mustard oil bomb." The prevailing view is that myrosinase is localized in vacuoles of specialized cells (idioblasts) called "myrosin cells," and glucosinolates (as high as 100 mM) and ascorbate are contained in vacuoles of other cell types including S-cells (sulfur rich), adjacent to myrosin cells [4,64]. When tissue is disrupted, the ascorbate becomes diluted to the 1–2 mM level that activates the enzyme as it mixes with glucosinolate substrates. Myrosinases are 10%–20% glycosylated and exist as multiple isoforms of 65–70 kDa monomeric mass with structures stabilized by 3 disulfide bridges and by Zn^{2+}; they may exist as homooligomers or in complexes with other proteins. Myrosinases exhibit optimum activity in the ranges of pH 4–8 and 40°C–75°C, depending on source. Proteins in nonmyrosin cells, including epithiospecifier protein (ESP), thiocyanate-forming protein (TFP), and nitrile-specifier protein (NSP), share sequence homology and influence the distribution of products that evolve after initial myrosinase hydrolytic action on glucosinolates [64,67]. The roles of ESP and NSP require Fe^{2+} and their action may be catalytic in nature [15].

Myrosinase is a retaining enzyme of family 1 of the glycosyl hydrolases (Section 6.3.2) and is unique on two accounts. It hydrolyzes β-D-thioglucosides (and not *O*-glycosides), and it has only one of the usual two ASP/GLU catalytic residues [20]. The enzyme has a hydrophobic binding pocket (mustard seed enzyme, $PHE_{331,371,473}$, ILE_{257}, TYR_{330}) to host the R groups of the glucosinolate, which are mostly alk(en)yl chains that may be branched or substituted with S, S=O, keto, or hydroxyl groups [83]. Several residues (GLU_{464}, GLN_{39}, HIS_{141}, ASN_{186}) provide H-bonding to the glucose with TRP_{457} providing the hydrophobic platform for stacking of the pyranose ring [20]. GLN_{409} and $ARG_{194,259}$ coordinate with the sulfate groups of the substrate. GLU_{409} constitutes the enzyme carboxylate nucleophile to displace the *S*-aglycon (a good leaving group) and form an enzyme–glucose covalent intermediate (Figure 6.50). The rate-limiting step is the release of the glucose, and in other glycosyl hydrolases, a second conserved GLU/ASP serves this purpose by activating water as a nucleophile. This function is missing and some assistance to activate water to displace glucose (and retain the β-configuration) may be afforded by H-bonding with GLN_{187}. A long recognized activating effect of ascorbic acid on myrosinase (of few- to several hundred-fold on V_{max}) is now believed to be conferred by ascorbate acting as an "external" cofactor [21]. Ascorbate binds to the enzyme after the aglycon is released (they share the same binding site) and ascorbate seems to function by activating nucleophilic water to displace the glucose. The distance between the GLU_{409} and ascorbate is ~7.0 Å, greater than the 4.5 Å distance of catalytic GLU/ASP of the typical retaining glycosyl hydrolase, but this may be necessary to accommodate the bulkier ascorbate residue. Maximum activation of ascorbate occurs at 0.5–1.5 mM; excess ascorbate competes with glucosinolate binding and impedes enzyme cycling.

The fate of the hydrolyzed glucosinolate also depends on compositional factors and the nature of the glucosinolate structures, some of which are shown in Figure 6.51. Under conditions of pH that typically prevail in vegetative tissues, the isothiocyanate derivatives are formed spontaneously (Figure 6.50). In the presence of Fe^{2+} and an ESP, epithionitriles and nitriles (also if NSP is present) may accumulate at the expense of isothiocyanates. Hydrolysis products of 2-hydroxyalk(en)yl glucosinolates (e.g., progotrin) are unstable as isothiocyanates and undergo rearrangement to oxazolidine-2-thiones. Under acidic conditions in the presence of Fe^{2+} and cysteine, nitriles may accumulate, and under neutral to slightly alkaline conditions, indole (e.g., glucobrassicin) and benzyl glucosinolates decompose to form the corresponding alcohols and cyanate, while allyl (sinigrin) and methylthio (dehydroerucin) derivatives yield the thiocyanates. Thiocyanates can also be formed through the action of TSP [67]. Some of these products have antinutritional effects. Cyanates interfere with

FIGURE 6.50 Reaction mechanism of myrosinase. ESP, epithiospecifier protein; NSP, nitrile specifier protein; TFP, thiocyanate-forming protein. (Adapted from Burmeister, W.P. et al., *Structure*, 5, 663, 1997; Burmeister, W.P. et al., *J. Biol. Chem.*, 275, 39385, 2000; Kissen, R. et al., *Phytochem. Rev.*, 8, 69, 2009; Mithen, R.F. et al., *J. Sci. Food Agric.*, 80, 967, 2000.)

iodine absorption, the 2-hydroxy-3-butenyl-glucosinolates (progoitrin) are associated with hypothyroidism, and nitriles may pose toxicity. Despite these concerns, there is a clear association between consumption of *Brassica* spp. vegetables and reduction in cancer risk, and much of this is attributed to glucosinolates and their transformation products.

Sulforaphane, the isothiocyanate derivative formed from glucoraphanin, is believed to be one of the most potent dietary cancer chemopreventive agents derived from broccoli (Chapter 12). However, the sulforaphane nitrile derivative is formed to a greater extent than sulforaphane in broccoli, and the nitrile form has several orders of magnitude less potency in cancer chemopreventive potential than the isothiocyanate form [80]. Thermal processing was examined as a means to minimize sulforaphane nitrile while maximizing sulforaphane accumulation in broccoli florets (Figure 6.51). A mild thermal treatment of 60°C for 10–20 min retains myrosinase activity and destroys epithiospecifier activity; this preferential deactivation of the latter causes a reversal in ratio of nitrile–isothiocyanate form of sulforaphane from 10:1 to about 1:10. The benefit is to increase the levels of the most potent anticarcinogenic agent in broccoli.

6.5.3.4.2 Alliinases and Related Enzymes [154]

Alliinases (EC 4.4.1.4, alliin alkenyl-sulfenate lyase, or alliin lyase) are the flavor-generating enzymes of members of the genus *Allium*, including onion, garlic, leek, chive, chemotaxonomically related species such as cabbage, and other plants. An alliinase was first reported in shiitake mushroom to be responsible for evolution of the unique flavoring compound lenthionine over four decades ago. However, a recent molecular study indicates the enzyme has little homology with prototypical alliinases and high homology with cysteine desulfurases (EC 2.8.1.7) in fungi; the enzyme is proposed to be a novel desulfurase with broad specificity that includes ACSO substrates [75].

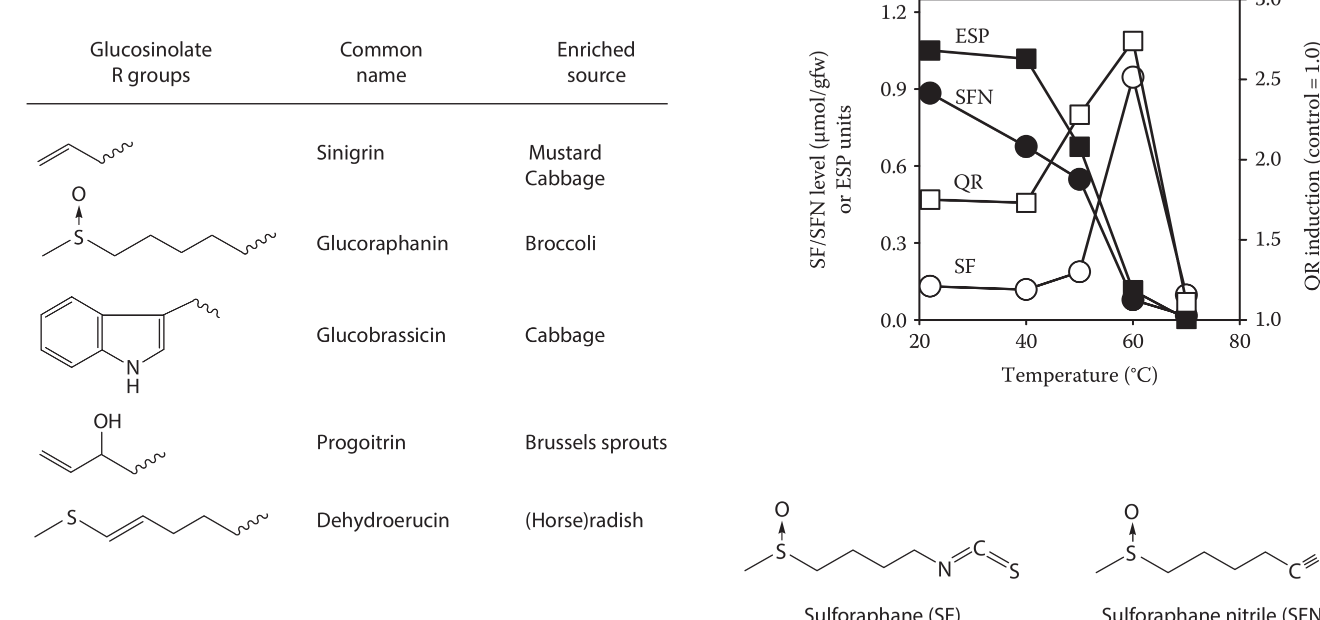
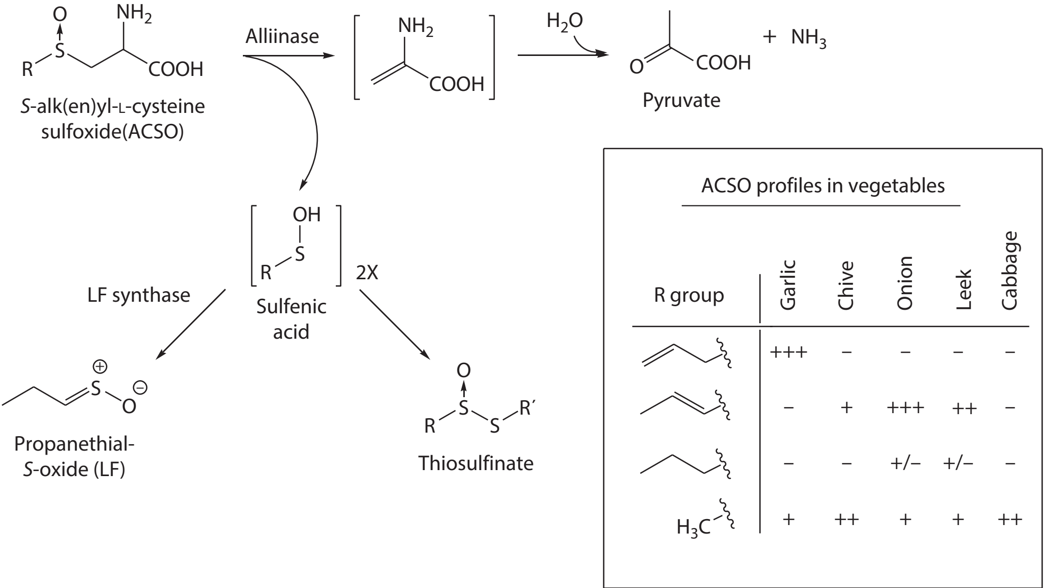


FIGURE 6.51 Representative glucosinolates and control of myrosinase end-product formation. ESP, epithiospecifier protein; QR, quinone reductase. (Redrawn from Matusheski, N.V. et al., *Phytochemistry*, 65, 1273, 2004.)

The active site architecture and mechanism of alliinase was featured earlier as an example of a pyridoxal-phosphate enzyme, and the reaction involves the β-lysis of nonprotein amino acid derivatives, ACSOs (Figure 6.6). The immediate reaction products, the sulfenic acids (R-SOH), condense to form thiosulfinates. In onion and related species (leek, shallot), much of the 1-propenyl sulfenic acid is isomerized to propanethial-*S*-oxide (lachrymatory factor, LF) by LF synthase [78], although for several decades this step was believed to be chemical/spontaneous in nature (Figure 6.52). The mechanism of LF synthase has not been elucidated, but diversity in this enzymic intramolecular rearrangement exists and could involve classic isomerase or dehydrogenase reactions [55]. This reaction is conditional upon rupture of tissues, as the ACSO substrates reside in the cytosol while the enzyme is vacuolar. Most alliinases have similar selectivities with a preference for reaction among ACSO species of descending order: unsaturated (1-propenyl- and 2-propenyl-) > propyl- > methyl-derivatives; reactivity ratios (based on V_{max}/K_M values) of ~10:2:1 [123] represent a middle ground of a wide range of relative selectivity values of alliinases reported in the literature. Consequently, the reaction products and characteristic flavors produced in *Allium* tissues are conferred largely by the relative levels of the various ACSO substrates present (Figure 6.52), rather than by properties of species-specific alliinases.

The enzyme is glycosylated, exists as a limited number of isoforms, and may be oligomeric with monomeric mass typically in the range of 48–54 kDa. One distinction among alliinases is the pH optimum, which is in the range of pH 7–8 for onion, leek, and broccoli enzymes and pH 5.5–6.5 for the garlic and related enzymes. However, this pH optimum difference is of limited practical importance because alliinases are fairly active over the range of pH 4.5–8.5 [65,154]; comprise 6% and 10% of the tissue protein, respectively, in onion and garlic; and there is an abundance of activity in disrupted tissues (where pH ranges 5.2–6.0). From 70% to 90% conversion of ACSO by alliinase to organosulfur products occurs in ruptured cells of onion tissue at room temperature within about 1 min, and nearly ~100% conversion occurs in disrupted cells within 1 h [70,110].



R group	Garlic	Chive	Onion	Leek	Cabbage
(2-propenyl)	+++	–	–	–	–
(1-propenyl)	–	+	+++	++	–
(propyl)	–	–	+/–	+/–	–
H_3C	+	++	+	+	++

FIGURE 6.52 Reaction pathway of alliinase reactions and profile of substrates in various vegetable tissues. LF, lachrymatory factor. (Compiled from Masamura, N. et al., *Biosci. Biotechnol. Biochem.*, 76, 447, 2012; Shen, C. and Parkin, K.L., *J. Agric. Food Chem.*, 48, 6254, 2000; Whitaker, J.R., Voragen, A.G.J., and D.W.S. Wong (Eds.), *Handbook of Food Enzymology*, Marcel Dekker, New York, 2003.)

Aside from the desirable flavors produced upon tissue disruption, there are several features of alliinase reactions that impact the ability to control food quality. Minced and stored or acidified (pickled) *Allium* tissue preparations may discolor and yield pink/red (in onion) and blue-green (in garlic) hues. The 1-propenyl-S(O)S-R thiosulfinates species are implicated as the major cause of such discoloration [66]. Stored (refrigerated) garlic may accumulate low levels of 1-propenyl-ACSO and the allyl-ACSO contributes to discoloration in minced garlic.

Preserving alliinase activity is important for allowing potentiation of the enzyme reaction at a point of choosing for a tissue preparation. As mentioned earlier, freezing preserves alliinase activity provided thawing is fast enough to prevent excessive denaturation [149]. Cryoprotectants such as glycerol and exogenous pyridoxal-phosphate cofactor have been routinely added to alliinase preparations to stabilize enzyme activity. Freeze-drying retains about 75% original activity, whereas low-temperature (55°C) drying retains about 50% original activity [73]. Either of these methods are suitable for preparing *Allium* tissues as dietary supplements where it is desired to have sufficient residual alliinase to generate thiosulfinates *in situ* (in the gut) of humans. This requires the use of enteric-coated capsules or tablets to protect the enzyme from the deactivating effect of gastric acid and enzymes. In contrast, garlic and onion powders prepared for use as spices undergo a more severe thermal treatment and may retain only ~5% residual alliinase activity.

In *Allium* tissues, some ACSO flavor precursors may exist as γ-glutamyl-ACSO peptides, and these peptide-linked ACSO are not recognized as substrates by alliinase. A transpeptidase (EC 2.3.2.2) catalyzes the transfer of the γ-glutamyl-group from ACSO to another amino acid and liberates free ACSO, which can then be acted upon by alliinase and further potentiate flavor. Sprouting *Allium* bulbs and germinating seeds are particularly rich in transpeptidase activity, and use is made of extracts of such tissues to mobilize a secondary pool of flavor precursors in various *Allium* preparations. Such preparations are most useful in dry form such that reconstitution with aqueous milieu elicits enzyme activities and yield enhanced flavor at a time of choosing.

Cystine lyases (EC 4.4.1.8), also known as β-cystathionase, also exist in *Allium*, cruciferous, and leguminous plants, as well as in some bacteria. Cystine lyases are pyridoxal enzymes that catalyze the β-elimination of cystine to yield thiocysteine (Cys-SSH), and this may give rise to sulfurous flavors. In broccoli, multiple isoforms exist, and they are soluble and have optima at pH 8–9. Depending on source, cystine lyases may also react with ACSO, but alliinases do not react with cystine. A similar pyridoxal-enzyme methionine-γ-lyase (EC 4.4.1.11) yields methanethiol (CH_3SH) as a reaction product, and this reaction has been implicated in proper flavor development in some cheeses, likely conferred by starter or adjunct cultures.

6.5.3.4.3 Other Flavor-Related Enzyme Activities

Sweetening through the elevation of maltose in domestically cooked and thermally processed sweet potato products (canned, flakes, puree) is a positive quality trait conferred by endogenous β-amylases [136]. High-maltose sweet potato lines have greater β-amylase activity with adequate thermal stability. During moderate thermal processing (progressively heated at 70°C–90°C over 2 h), a faster and greater degree of starch gelatinization in these same lines allows for sustained β-amylase action on starch, leading to up to fivefold greater maltose levels relative to those observed for the moderate- and low-maltose lines.

6.5.4 Enzymes Affecting Textural Quality in Foods

Textural and rheological changes in foods can be evoked by enzymes that act on high- and low-molecular-weight food components. Examples of some textural and rheological modifications have already been described in the context of using exogenous enzymes to liquefy/thin starch, reduce viscosity and cloud in fruit juices, hydrolyze or induce gelation of proteins, modify bread dough viscoelasticity, etc. This section will focus on controlling endogenous enzymes that can have desirable or undesirable impact on food quality.

6.5.4.1 Control of Enzymes Modifying Carbohydrate Polymers

Perhaps the most long-standing example of controlling endogenous enzyme activity on carbohydrates is the "hot" and "cold" break processes for preparing tomato fruit products. These terms are partly misnomers and a hot break process comprises a rapid heating of tomato tissue to >85°C–90°C with a clear intent to inactivate endogenous polygalacturonase and pectin methyl esterase activities. This preserves pectin levels, promotes viscosity and consistency, and stabilizes juice cloud. In contrast, a *cold* break process makes use of temperatures <70°C where these enzymes are thermally *activated* and will result in pectin depolymerization with a corresponding loss in viscosity, pectin deesterification leading to loss of cloud stability, reduction in consistency, and separation of serum (liquid) from solids. The cold break process may promote greater flavor quality, perhaps by allowing a greater extent of lipoxygenase/hydroperoxide lyase-mediated flavor generation, but this effect has not been consistently observed. Both cold and hot break processes are used for juice and other fruit products, depending on how those products are to be used (either as end products or as ingredients for others). Tomato pastes are best prepared by hot break processes to retain consistency and viscosity. High-temperature processing for pectinase deactivation is also used for other juicing fruits (orange) to maintain cloud stability as a quality attribute.

Another approach to controlling pectinolytic enzymes for texture control is the application of an intermediate and moderate thermal treatment (referred to as low-temperature blanching) to mitigate softening brought about by subsequent thermal processing of intact (or pieces of) fruit and vegetable products. Treatments in the range of 55°C–80°C are intended to stimulate pectin methyl esterase action and "firm" tissue by promoting adhesion among cell wall and middle lamella elements [144]. Hydrolysis of pectin methoxy groups creates carboxylate groups that may form Ca^{2+} bridges between neighboring pectin polymers (see egg-box model, Chapter 3). This can enhance textural firmness and prevent disintegration of tissue pieces ("sloughing") during subsequent thermal processing such as retorting.

One of the earliest successes was registered for potatoes [9], where thermal pretreatments of 30–120 min at 60°C–70°C prior to boiling were effective at preventing excessive softening and almost eliminated sloughing. This effort was initiated to address the needs of preserving high-starch potatoes by canning. A minimum temperature of 55°C–60°C is required to render the tissue "leaky" and permit migration of cations as well as activate pectin methyl esterase, whereas traditional blanching temperatures will deactivate the enzyme before it has sufficient opportunity to act. Thus, potato slices directly boiled (1–2 h to simulate retorting) suffer from 80%–100% disintegration, while those subjected to a 60°C–70°C pretreatment followed by boiling did not slough. The same approach has been shown effective for sweet potatoes, snap beans, cucumbers (for pickle preparation), carrots, and pepper and tomato fruit, and in some cases the firming effect is enhanced by use of Ca^{2+}-containing brines.

6.5.4.2 Control of Enzymes Modifying Proteins

Protein degradation is a major determinant of age tenderization of meat. Cathepsins released from lysosomes and/or calpains are the endogenous muscle proteases that appear to have most impact on tenderization. Other than the temperature and duration of aging and the early postmortem rate of pH decline, there are few means to influence endogenous proteolysis. One process that has received sustained interest is postmortem electrical stimulation of carcasses, which may confer tenderization by reducing cold shortening, disrupting structural elements of muscle, and stimulation of endogenous proteases, in part by Ca^{2+} release into the sarcoplasm [60]. The muscle calpain system, Ca^{2+}-activated CYS-proteases, has been implicated as having a role in early postmortem hydrolysis leading to tenderization. Muscle proteases are just one of several factors that determine tenderness of meats (see Chapter 15).

Endogenous proteases in fish muscle can limit the quality of manufactured gels (surimi products). Proteases in weak gel-forming fish muscle tissue are sensitive to CYS-reactive reagents.

An effective way to manage this problem has proven to be the addition of "cystatin"-like protease inhibitors. Such inhibitors are found in bovine plasma, chicken egg white, and potato and may be added to surimi products to inhibit endogenous protease activity and help maintain gel strength.

Endogenous proteases also occur in milk, the principal one being the plasmin system (derived from the blood). Plasmin (EC 3.4.21.7) is a SER-protease of 81 kDa mass, with reaction optima of pH 7.5–8.0 and 37°C [154]. However, the enzyme is stable over a broad pH range of 4–9 and exhibits 20% maximal activity at 5°C. Plasmin survives pasteurization, owing partly to its multiple disulfide bonds, and it also retains activity after ultrapasteurization. Plasminogen (the zymogen form) is dominant in milk and is transformed by activators (including another SER-protease) to yield the active plasmin. Both plasmin(ogen) and its activators are associated with casein micelles. Inhibitors of plasmin and plasmin activators reside in the serum phase and prevent spontaneous activation and proteolysis in freshly drawn and pasteurized milk. During cheese making, plasmin remains with the casein micelles, and it contributes to proteolysis of particularly α_{s2}- and β-caseins in cheese during aging. In cheese made from ultrafiltered milk, plasmin may be less active because of greater retention of the serum solids (the source of plasmin inhibitors) in the resulting curd. Because of the heat resistance of plasmin, it is a major contributor to proteolysis in cheese subjected to high cook temperatures, which may inactivate plasmin inhibitors. Owing to its heat resistance, plasmin has also been implicated in having a role in gelation of ultrahigh pasteurized milk and creamers.

6.5.4.3 Mitigation of Texture Defects Using Small Molecules to Control Enzymes

In dates, a defect known as "sugar wall" occurs when the sucrose–reducing sugar ratio is sufficiently high (2:1) to cause sucrose crystallization throughout the fruit and yield a sandy, hard texture [128]. Natural and dry dates of premium grade have sugar ratios in the range of 1.1–1.6:1 by comparison, and dates prone to sugar wall tend to have low endogenous invertase levels. To reduce the incidence of this defect, sugar wall dates were subjected to a vacuum-infusion treatment solution of 0.01%–0.10% commercial invertase, with a sample comprising the control treatments sprayed with an equivalent amount of water alone. After the treatment, which resulted in increased moisture contents to 20%–22% (and an increase in a_w), the dates were sealed and stored for 60 days at ~27°C. As expected, the enzyme-infused dates exhibited an "inversion" of 54%–76% of the sucrose, lowering the sucrose–reducing sugar ratio to 0.22–0.44:1. Surprisingly, even in the water-infused dates, sucrose inversion amounted to 53% with a reduction in sucrose–reducing sugar ratio to 0.56:1. The sugar wall defect was not evident after the 60-day treatment period for enzyme- and water-infused samples. This illustrates how facile it can sometimes be to potentiate endogenous activity, in this case, by simple water addition. At the end of the 60-day period, all treated and control dates were dried to the 16%–18% original moisture content and observed for another month. All water-infused samples returned to the sugar wall defect, while the enzyme-infused samples showed 0%–10% incidence of the defect returning, inversely related to the enzyme dose. Thus, permanent elimination of the defect requires treatment with exogenous invertase.

The last example of control of enzyme action in foods deals with TMAO demethylase (EC 4.1.2.32), which causes a reaction in muscle, particularly for fish of the Gadoid (cod) family:

$$(CH_3)_3N \rightarrow O \rightarrow (CH_3)_2NH + HCHO \tag{6.59}$$

The formaldehyde (HCHO) produced causes protein cross-linking, rendering the muscle tissue tough and fibrous when stored frozen as fillets or in blocks/portions. Tissue disruption, by freezing or simple mincing, causes the enzyme reaction to occur by decompartmentation (TMAO can be >100 mM in muscle). TMAO demethylase is not widely distributed but occurs in some bacteria. In fish muscle and organ tissues, it appears to be membrane associated but can be solubilized. Two *cofactor* or cosubstrate systems were shown to mediate reactivity for the isolated membrane

enzyme [97]. One requires NAD(P)H and FMN and functions only anaerobically, while the other involves Fe^{2+}, ascorbate, and/or cysteine and functions independent of oxygen tension but is only 20% as stimulatory as the NAD(P)H/FMN system.

It is commercially important to prevent this reaction in frozen fish blocks (~7 kg of rectangular dimension), which are processed later into fish sticks and portions; "aging" on ice for up to 10 days prior to freezing was evaluated as a practical approach [109]. Rates of HCHO formation ranged 10–25 μmol/100 g day^{-1} blocks prepared from fresh (0 days aged) fish fillets with greatest rates toward the more anaerobic interior, where the NAD(P)H/FMN cofactor systems is most functional However, this depth effect quickly diminished after only 1 day of aging before block preparation and rates ranged 7–12 μmol HCHO formed/100 g day^{-1} (from exterior to interior of the block). After 10 days of aging on ice, rates of HCHO formation ranged 2.1–2.4 μmol/100 g day^{-1} at all locations in the block. An explanation is that the more rate-accelerating anaerobic cofactors (NAD(P)H and FMN) decayed quickly in aging fish muscle and could not be replenished [100]. The HCHO-forming potential remaining after longer aging times was contributed by the lesser reactive cofactor system (iron, ascorbate, cysteine), which also decayed over time leading to an ultimate 80%–90% inhibition of HCHO formation after 10 days of aging. This example illustrates a simple means for controlling enzyme action by strategies that target the disposition of (co-) reactants for enzyme reactions. An alternative approach to managing this specific reaction and associated textural problem was based on a Maine fishermen's suggestion to soak/freeze the fillets in seawater as an intermediate step [71]. This allows for a proportion of the low-molecular-weight constituents, including substrate and cofactors, to be osmotically leached out of the muscle, resulting in ~80% reduction in rate and extent of HCHO formation and less textural deterioration upon subsequent freezing.

REFERENCES

1. Acker, L. and H. Kaiser (1959). Uber den einfluß der feuchtigkeit auf den ablauf enzymatischer reaktionen in wasserarmen lebensmitteln. II. Mitteilung. *Lebensm. Unters. Forsch.* 110:349–356 (in German).
2. Ader, U., Andersch, P., Berger, M., Goergens, U., Haase, B., Hermann, J., Laumen, K. Seemayer, R., Waldinger, C., and M.P. Schneider (1997). Screening techniques for lipase catalyst selection, In *Methods in Enzymology*, Rubin, B. and E.A. Dennis (Eds.), Vol. 286, *Lipases, Part B. Enzyme Characterization and Utilization*, Academic Press, New York, pp. 351–387.
3. Aehle, W. (2004). *Enzymes in Industry. Production and Applications*, 2nd edn., Wiley-VCH, Weinheim, Germany.
4. Andréasson, E. and L.B. Jørgensen (2003). Localization of plant myrosinases and glucosinolates, In *Recent Advances in Phytochemistry—Volume 37. Integrative Phytochemistry: From Ethnobotany to Molecular Ecology*, J.T. Romero (Ed.), Elsevier Scientific Ltd., Oxford, U.K., pp. 79–99.
5. Andreou, A. and I. Feussner (2009). Lipoxygenases—Structure and reaction mechanism. *Phytochemistry* 70:1504–1510.
6. Anthon, G.E., Sekine, Y., Watanabe, N., and D.M. Barrett (2002). Thermal inactivation of pectin methyl esterase, polygalacturonase, and peroxidase in tomato juice. *J. Agric. Food Chem.* 50:6153–6159.
7. Axelrod, B., Cheesebrough, T.M., and S. Laakso (1981). Lipoxygenase from soybeans. In *Methods in Enzymology*, J.M. Lowenstein (Ed.), Vol. 71, *Lipids*, Academic Press, New York, pp. 441–451.
8. Bai, W., Zhou, C., Zhao, Y., Wang, Q., and Y. Ma (2015). Structural insight into and mutational analysis of family 11 xylanases: Implications for mechanisms of higher pH catalytic adaptation. *PLoS One* 10(7):e0132834.
9. Bartolome, L.G. and J.E. Hoff (1972). Firming of potatoes: Biochemical effects of preheating. *J. Agric. Food Chem.* 20:266–270.
10. Bär, J., Martínez-Costa, O.H., and J.J. Aragón (1990). Regulation of phosphofructokinase at physiological concentration of enzyme studied by stopped-flow measurements. *Biochem. Biophys. Res. Commun.* 167:1214–1220.
11. Bengtsson, B. and I. Bosund (1966). Lipid hydrolysis in unblanched frozen peas (*Pisum sativum*). *J. Food Sci.* 31:474–481.

12. Benen, J.A.E., Kester, H.C.M., Armand, S., Sanchez-Torres, P., Parenicova, L., Pages, S., and J. Visser (1999). Structure-function relationships in polygalacturonases: A site-directed mutagenesis approach, In *Recent Advances in Carbohydrate Engineering*, Gilbert, H.J., Davies, G.J., Henrissat, B., and B. Svensson (Eds.), The Royal Society of Chemistry, Cambridge, U.K., pp. 99–106.
13. Blée, E. (1998). Phytooxylipins and plant defense reactions. *Prog. Lipid Res.* 37:33–72.
14. Boyington, J.C., Gaffney, B.J., and L.M. Amzel (1993). The three-dimensional structure of an arachidonic acid 15-lipoxygenase. *Science* 260:1482–1486.
15. Brandt, W., Backenköhler, A., Schulze, E., Plock, A., Herberg, T., Roese, E., and U. Wittstock (2014). Molecular models and mutational analyses of plant specifier proteins suggest active site residues and reaction mechanism. *Plant Mol. Biol.* 8:173–188.
16. Brash, A.R. (1999). Lipoxygenases: Occurrence, functions, catalysis, and acquisition of substrate. *J. Biol. Chem.* 274:23679–23682.
17. Broadbent, J.R. and J.L. Steele (2007). Proteolytic enzymes of lactic acid bacteria and their influence on bitterness in bacterial-ripened cheeses, In *Flavor of Dairy Products*, Caldwaller, K.R., Drake, M.A., and R.J. McGorrin (Eds.), American Chemical Society (Symposium Series), Washington, DC, pp. 193–203.
18. Brodhun, F., Schneider, S., Obel, C.G., Hornung, E., and I. Feussneri (2010). PpoC from *Aspergillus nidulans* is a fusion protein with only one active haem. *Biochem. J.* 425:553–565.
19. Buccholz, K., Kasche, V., and U.T. Bornscheuer (2005). *Biocatalysis and Enzyme Technology*, Wiley-VCH, Weinhein, Germany.
20. Burmeister, W.P., Cottaz, S., Driguez, H., Iori, R., Palmieri, S., and B. Henrissat (1997). The crystal structures of *Sinapis alba* myrosinase and a covalent glycosyl-enzyme intermediate provides insights into the substrate recognition and active-site machinery of an *S*-glucosidase. *Structure* 5:663–675.
21. Burmeister, W.P., Cottaz, S., Rollin, P., Vasella, A., and B. Henrissat (2000). High resolution x-ray crystallography shows that ascorbate is a cofactor for myrosinase and substitutes for the function of the catalytic base. *J. Biol. Chem.* 275:39385–39393.
22. Cao, E., Chen, Y., Cui, Z., and P.R. Foster (2003). Effect of freezing and thawing rates on denaturation of proteins in aqueous solutions. *Biotechnol. Bioeng.* 832:684–690.
23. Carter, P. and J.A. Wells (1988). Dissecting the catalytic triad of a serine protease. *Nature* 332:564–568.
24. Carter, P. and J.A. Wells (1990). Functional interaction among catalytic residues in subtilisin BPN′. *Protein Struct. Funct. Genet.* 7:335–342.
25. Casey, R. and R.K. Hughes (2004). Recombinant lipoxygenase and oxylipin metabolism in relation to food quality. *Food Biotechnol.* 18:135–170.
26. Champion, D., Blond, G., Le Meste, M., and D. Stimatos (2000). Reaction rate modeling in cryoconcentrated solutions: Alkaline phosphatase catalyzed DNPP hydrolysis. *J. Agric. Food Chem.* 48:4942–4947.
27. Chen, Y-H., Aull, J.L., and L.N. Bell (1999). Invertase storage stability and sucrose hydrolysis in solids as affected by water activity and glass transition. *J. Agric. Food Chem.* 47:504–509.
28. Christophersen, C., Otzen, D.E., Norman, B.E., Christensen, S., and T. Schäfer (1998). Enzymatic characterisation of Novamyl®, a thermostable α-amylase. *Starch/Stärke* 50(1, Suppl):39–45.
29. Coffa, G. and A.R. Brash (2004). A single active site residue directs oxygenation stereospecificity in lipoxygenases: Stereocontrol is linked to the position of oxygenation. *Proc. Natl. Acad. Sci. U.S.A.* 101:15579–15584.
30. Copeland, R.A. (2000). *Enzymes. A Practical Introduction to Structure, Function, Mechanism, and Data Analysis*, 2nd edn., John Wiley, New York.
31. Cornish-Bowden, A. (1995). *Fundamentals of Enzyme Kinetics,* Portland Press, Ltd., London, U.K.
32. Cumbee, B., Hildebrand, D.F., and K. Addo (1997). Soybean flour lipoxygenase isozymes effects on wheat flour dough rheological and breadmaking properties. *J. Food Sci.* 62:281–283,294.
33. Dao-Pin, S., Liao, D-I., and S.J. Remington (1989). Electrostatic fields in the active sites of lysozyme. *Proc. Natl. Acad. Sci. U.S.A.* 86:5361–5365.
34. Dauter, Z., Dauter, M., Brzozowski, A.M., Christensen, S., Borchert, T.V., Beier, L., Wilson, K.S., and G.J. Davies (1999). X-ray structure of Novamyl, the five-domain "maltogenic" α-amylase from *Bacillus stearothermophilus*: Maltose and acarbose complexes at 1.7 Å resolution. *Biochemistry* 38:8385–8392.
35. Davies, G.J., Wilson, K.S., and B. Henrissat (1997). Nomenclature for sugar-binding subsites in glycosyl hydrolases. *Biochem. J.* 321:557–559.
36. De Jong, G.A.H. and S.J. Koppelman (2002). Transglutaminase catalyzed reactions: Impact on food applications. *J. Food Sci.* 67:2798–2806.

37. Drapon, R. (1986). Modalities of enzyme activities in low moisture media, In *Food Packaging and Preservation. Theory and Practice*, M. Mathlouthi (Ed.), Elsevier Applied Science Publishers, New York, pp. 181–198.
38. Dunford, M.B. (1999). *Heme Peroxidases*, John Wiley & Sons, New York, 507pp.
39. Dunn, B.M. (2002). Structure and mechanism of the pepsin-like family of aspartic peptidases. *Chem. Rev.* 102:4431–4458.
40. Eicken, C., Krebs, B., and J.C. Sacchettini (1999). Catechol oxidase—Structure and activity. *Curr. Opin. Struct. Biol.* 9:677–683.
41. Fennema, O. (1975). Activity of enzymes in partially frozen aqueous systems, In *Water Relations in Foods*, R. Duckworth (Ed.), Academic Press, New York, pp. 397–413.
42. Fennema, O. and J.C. Sung (1980). Lipoxygenase-catalyzed oxidation of linoleic acid at subfreezing temperature. *Cryobiology* 17:500–507.
43. Fersht, A. (1985). *Enzyme Structure and Mechanism*, 2nd edn., W.H. Freeman & Company, New York.
44. Fishbein, W.N. and J.W. Winkert (1977). Parameters of biological freezing damage in simple solutions: Catalase. I. The characteristic pattern of intracellular freezing damage exhibited in a membraneless system. *Cryobiology* 14:389–398.
45. Fuessner, I. and C. Wasternack (2002). The lipoxygenase pathway. *Annu. Rev. Plant Biol.* 53:275–297.
46. Fukagawa, Y., Sakamoto, M., and T. Ishikura (1981). Micro-computer analysis of enzyme-catalyzed reactions by the Michaelis-Menten equation. *Agric. Biol. Chem.* 49:835–837.
47. Galliard, T. and H.W-S. Chan (1980). Lipoxygenases, In *The Biochemistry of Plants. A Comprehensive Treatise, Volume 4, Lipids: Structure and Function*, P.K. Stumpf (Ed.), Academic Press, New York, pp. 131–161.
48. Garcia-Viloca, M., Alhambra, C., Truhlar, D.C., and J. Gao (2002). Quantum dynamics of hydride transfer by bimetallic electrophilic catalysis: Synchronous motion of Mg^{2+} and H^- in xylose isomerase. *J. Am. Chem. Soc.* 124:7268–7269.
49. Garcia-Viloca, M., Gao, J., Karplus, M., and D.C. Truhlar (2004). How enzymes work: Analysis by modern rate theory and computer simulations. *Science* 303:186–195.
50. Godfrey, T. and S. West (Eds.) (1996). *Industrial Enzymology*, 2nd edn., Stockton Press, New York.
51. Gretchkin, A.N. and M. Hamberg (2004). The "heterolytic lyase" is an isomerase producing a short-lived fatty acid hemiacetal. *Biochim. Biophys. Acta* 1636:47–58.
52. Grosch, W. (1982). Lipid degradation products and flavour, In *Food Flavours: Part A. Introduction*, Morton, I.D. and A.J. MacLeod (Eds.), Elsevier Scientific Publishing, New York, pp. 325–398.
53. Gunstone, F.D. (Ed.) (1999). *Lipid Synthesis and Manufacture*, CRC Press LLC, Boca Raton, FL, 472pp.
54. Gutfreund, H. (1972). *Enzymes: Physical Principles*, John Wiley & Sons, Ltd., London, U.K., 242pp.
55. He. Q., Kubec, R., Jadhav, A.P., and R.A. Musah (2011). First insights into the mode of action of a ''lachrymatory factor synthase''—Implications for the mechanism of lachrymator formation in *Petiveria alliacea*, *Allium cepa* and *Nectaroscordum* species. *Phytochemistry* 72:1939–1946.
56. Hebeda, R.E., Bowles, L.K., and W.M. Teague (1991). Use of intermediate temperature stability enzymes for retarding staling in baked goods. *Cereal Foods World* 36:619–624.
57. Henderson, P.J.F. (1979). Statistical analysis of enzyme kinetic data, in *Techniques in Protein and Enzyme Biochemistry, Part II*, Kornberg, H.L., Metcalfe, J.C., Northcote, D.H., Pogson, C.I., and K.F. Tipton (Eds.), Elsevier/North Holland Biomedical Press, Amsterdam, the Netherlands, pp. B113/1– B113/43.
58. Hildebrand, D.F., Hamilton-Kemp, T.R., Loughrin, J.H., Ali, K., and R.A. Andersen (1990). Lipoxygenase 3 reduces hexanal production from soybean seed homogenates. *J. Agric. Food Chem.* 38:1934–1936.
59. Hughes, R.K., Wu, Z., Robinson, D.S., Hardy, D., West, S.I., Fairhurst, S.A., and R. Casey (1998). Characterization of authentic recombinant pea-seed lipoxygenases with distinct properties and reaction mechanisms. *Biochem. J.* 333:33–43.
60. Hwang, I.H., Devine, C.E., and D.L. Hopkins (2003). The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. *Meat Sci.* 65:677–691.
61. Inouye, K., Lee, S-B., Nambu, K., and B. Tonomura (1997). Effect of pH, temperature, and alcohols on the remarkable activation of thermolysin by salts. *J. Biochem.* 122:358–364.
62. Juers, D.H., Matthews, B.W., and R.E. Huber (2012). LacZ *b*-galactosidase: Structure and function of an enzyme of historical and molecular biological importance. *Protein Sci.* 21:1792–1807.
63. Kazlauskas, R.J. (1994). Elucidating structure-mechanism relationships in lipases: Prospects for predicting and engineering catalytic properties. *Trends Biotechnol.* 12:464–472.
64. Kissen, R., Rossiter, J.T., and A.M. Bones (2009). The 'mustard oil bomb': Not so easy to assemble?! Localization, expression and distribution of the components of the myrosinase enzyme system. *Phytochem. Rev.* 8:69–86.

65. Krest, I., Glodek, J., and M. Keusgen (2000). Cysteine sulfoxides and alliinase activity of some *Allium* species. *J. Agric. Food Chem.* 48:3753–3760.
66. Kubec, R., Hrbáčová, M., Musah, R.A., and J. Velíšek (2004). *Allium* discoloration: Precursors involved in onion pinking and garlic greening. *J. Agric. Food Chem.* 52:5089–5094.
67. Kuchering, J.C., Burow, M., and U. Wittstock (2012). Evolution of specifier proteins in glucosinolate-containing plants. *BMC Evol. Biol.* 12:127 (14pp).
68. Kuribayashi, T., Kaise, H., Uno, C., Hara, T., Hayakawa, T., and T. Joh (2002). Purification and characterization of lipoxygenase from *Pleurotus ostreatus*. *J. Agric. Food Chem.* 50:1247–1253.
69. Kuettner, E.B., Hilgenfeld, R., and M.S. Weiss (2002). The active principle of garlic at atomic resolution. *J. Biol. Chem.* 277:46402–46407.
70. Lancaster, J.E., Shaw, M.L., and W.M. Randle (1998). Differential hydrolysis of alk(en)yl cysteine sulphoxides by alliinase in onion macerates: Flavour implications. *J. Sci. Food Agric.* 78:367–372.
71. Landolt, L.A. and H.O. Hultin (1982). Inhibition of dimethylamine formation in frozen red hake muscle after removal of trimethylamine oxide and soluble proteins. *J. Food Biochem.* 6:111–125.
72. Laratta, B., Fasanaro, G., De Sio, F., Castaldo, D., Palmieri, A., Giovane, A., and L. Servillo (1995). Thermal inactivation of pectin methyl esterase in tomato puree: Implications on cloud stability. *Proc. Biochem.* 30:251–259.
73. Lawson, L.D. and Z.J. Wang (2001). Low allicin release from garlic supplements: A major problem due to the sensitivities of alliinase activity. *J. Agric. Food Chem.* 49:2592–2599.
74. Lee, C-H. and K.L. Parkin (2001). Effect of water activity and immobilization on fatty acid selectivity for esterification reactions mediated by lipases. *Biotechnol. Bioeng.* 75:219–227.
75. Liu, L.Y., Lei, X-Y., Chen, L-F., Bian, Y-B., Yang, H., Ibrahim, S.A., and W. Huang (2015). A novel cysteine desulfurase influencing organosulfur compounds in *Lentinula edodes*. *Sci. Rep.* 5:10047; doi: 10.1038/srep10047.
76. Löffler, A. (1986). Proteolytic enzymes: Sources and applications. *Food Technol.* 40(1):63–70.
77. Lowe, G. and Y. Yuthavong (1971). pH-Dependence and structure-activity relationships in the papain-catalysed hydrolysis of anilides. *Biochem. J.* 124:117–122.
78. Masamura, N., Ohashi, W., Tsuge, N., Imai, S., Ishii-Nakamura, A., Hirota, H., Nagata, T., and H. Kumagai (2012). Identification of amino acid residues essential for onion lachrymatory factor synthase activity. *Biosci. Biotechnol. Biochem.* 76:447–453.
79. Matsui, K., Fukutomi, S., Wilkinson, J., Hiatt, B., Knauf, V., and T. Kajawara (2001). Effect of overexpression of fatty acid 9-hydroperoxide lyase in tomatoes (*Lycopersicon esculentum* Mill.). *J. Agric. Food Chem.* 49:5418–5424.
80. Matusheski, N.V., Juvik, J.A., and E.H. Jeffery (2004). Heating decreases epithiospecifier protein activity and increases sulforaphane formation in broccoli. *Phytochemistry* 65:1273–1281.
81. Meagher, M.M., Nikolov, Z.L., and P.J. Reilly (1989). Subsite mapping of *Aspergillus niger* glucoamylases I and II with malto- and isomaltooligosaccharides. *Biotechnol. Bioeng.* 34:681–688.
82. Mikami, B., Iwamoto, H., malle, D., Yoon, H-J., Demirkan-Sarikaya, E., Mezaki, Y., and Y. Katsuya (2006). Crystal sructure of pullulanase: Evidence for parallel binding of oligosaccharides in the active site. *J. Mol. Biol.* 359:690–707.
83. Mithen, R.F., Dekker, M., Ververk, R., Rabot, S., and I.T. Johnson (2000). The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. *J. Sci. Food Agric.* 80:967–984.
84. Monsan, P. and D. Combes (1984). Effect of water activity on enzyme action and stability. *Ann. NY Acad. Sci.* 434:48–60.
85. Nagodawithana, T. and G. Reed (Eds.) (1993). *Enzymes in Food Processing*, 3rd edn., Academic Press, New York, 480pp.
86. Nakanishi, K., Takeuchi, A., and R. Matsuno (1990). Long-term continuous synthesis of aspartame precursor in a column reactor with an immobilized thermolysin. *Appl. Microbiol. Biotechnol.* 32:633–636.
87. Nerinckx, W., Desmet, T., and M. Claeyssens (2003). A hydrophobic platform as a mechanistically relevant transition state stabilizing factor appears to be present in the active center of *all* glycoside hydrolases. *FEBS Lett.* 538:1–7.
88. Newcomer, M.E. and A.R. Brash (2015). The structural basis for specificity in lipoxygenase catalysis. *Protein Sci.* 24:298–309.
89. Nitta, Y., Mizushima, M., Hiromi, K., and S. Ono (1971). Influence of molecular structures of substrates and analogues on Taka-amylase A catalyzed hydrolyses. *J. Biochem.* 69:567–576.
90. Wlodawer, A., Gustchina, A., and M.N.G. James (2013). Catalytic pathways of aspartic peptidases, In *Handbook of Proteolytic Enzymes*, 3rd edn., Rawlings, N.D. and G. Salveson (Eds.), Vol. 1, Academic Press, New York, pp. 19–26.

91. O'Connor, C.J. and J.R. Longbottom (1986). Studies in bile salt solutions. XIX. Determination of Arrhenius and transition-state parameters for the esterase activity of bile-salt-stimulated human milk lipase: Hydrolysis of 4-nitrophenyl alkanoates. *J. Colloid Interface Sci.* 112:504–512.
92. Osuga, D.T. and J.R. Whitaker (1995). Mechanisms of some reducing compounds that inactivate polyphenol oxidases, in *Enzymatic Browning and Its Prevention*, Lee, C.Y. and J.R. Whitaker (Eds.), symposium series 600, American Chemical Society, Washington, DC, pp. 210–222.
93. Ovádi, J., Aragón, J.J., and A. Sols (1986). Phosphofructokinase and fructose bisphosphatase from muscle can interact at physiological concentration with mutual effects on their kinetic behavior. *Biochem. Biophys. Res. Commun.* 135:852–856.
94. Palmer, T. (1995). *Understanding Enzymes*, 4th edn., Prentice Hall/Ellis Horwood, New York.
95. Pandey, A., Nigam, P., Soccol, C.R., Soccol, V.T., Singh, D., and R. Mohan (2000). Advances in microbial amylases. *Biotechnol. Appl. Biochem.* 31:135–152.
96. Parida, S. and J.S. Dordick (1993). Tailoring lipase specificity by solvent and substrate chemistries. *J. Org. Chem.* 58:3238–3244.
97. Parkin, K.L. and H.O. Hultin (1986). Characterization of trimethylamine-N-oxide (TMAO) demethylase activity from fish muscle microsomes. *J. Biochem.* 100:77–86.
98. Penet, C.S. (1991). New applications of industrial food enzymology: Economics and processes. *Food Technol.* 45(1):98–100.
99. Persson, M., Svensson, I., and P. Adlercreutz (2000). Enzymatic fatty acid exchange in digalactosyldiacylglycerol. *Chem. Phys. Lipids* 104:13–21.
100. Phillippy, B.Q. and H.O. Hultin (1993). Some factors involved in trimethylamine N-oxide (TMAO) demethylation in post mortem red hake muscle. *J. Food Biochem.* 17:251–266.
101. Pickersgill, R.W. and J.A. Jenkins (1999). Crystal structure of polygalacturonase and pectin methylesterase, in *Recent Advances in Carbohydrate Engineering*, Gilbert, H.J., Davies, G.J., Henrissat, B., and B. Svensson (Eds.), The Royal Society of Chemistry, Cambridge, U.K., pp. 144–149.
102. Pinsirodom, P. and K.L. Parkin (2000). Selectivity of Celite-immobilized patatin (lipid acyl hydrolase) from potato (*Solanum tuberosum* L.) tubers in esterification reactions as influenced by water activity and glycerol analogues as alcohol acceptors. *J. Agric. Food Chem.* 48:155–160.
103. Pleiss, J., Fischer, M., and R.D. Schmid (1998). Anatomy of lipase binding sites: The scissile fatty acid binding site. *Chem. Phys. Lipids* 93:67–80.
104. Poutanen, K. (1997). Enzymes: An important tool in the improvement of the quality of cereal foods. *Trends Food Sci. Technol.* 8:300–306.
105. Prigge, S.T., Boyington, J.C., Faig, M., Doctor, K.S., Gaffney, B.J., and L.M. Anzel (1997). Structure and mechanism of lipoxygenases. *Biochimie* 79:629–636.
106. Raksalkulthai, R. and N.F. Haard (2003). Exopeptidases and their application to reduce bitterness in food: A review. *Crit. Rev. Food Sci. Nutr.* 43:401–445.
107. Rangarajan, M. and B.S. Hartley (1992). Mechanism of D-fructose isomerization by *Arthrobacter* D-xylose isomerase. *Biochem. J.* 283:223–233.
108. Rangheard, M-S., Langrand, G., Triantaphylides, C., and J. Baratti (1989). Multi-competitive enzymatic reactions in organic media: A simple test for the determination of lipase fatty acids specificity. *Biochem. Biophys. Acta* 1004:20–28.
109. Reece, P. (1983). The role of oxygen in the production of formaldehyde in frozen minced cod muscle. *J. Sci. Food Agric.* 34:1108–1112.
110. Resemann, J., Maier, B., and R. Carle (2004). Investigations on the conversion of onion aroma precursors *S*-alk(en)yl-L-cysteine sulphoxides in onion juice production. *J. Sci. Food Agric.* 84:1945–1950.
111. Rogalska, E., Cudrey, C., Ferrato, F., and R. Verger (1993). Stereoselective hydrolysis of triglycerides by animal and microbial lipases. *Chirality* 5:24–30.
112. Saier, M.H. (1987). *Enzyme in Metabolic Pathways. A Comparative Study of Mechanism, Structure, Evolution and Control*, Harper & Row, New York.
113. Sakurai, Y., Ma, S-F., Watanabe, H., Yamaotsu, N., Hirono, S., Kurono, Y., Kragh-Hansen, U., and M. Otagiri (2004). Esterase-like activity of serum albumin: Characterization of its structural chemistry using p-nitrophenyl esters as substrates. *Pharm. Res.* 21:285–292.
114. Sanz, C., Olias, J.M., and A.G. Perez (1997). Aroma biochemistry of fruits and vegetables, In *Phytochemistry of Fruit and Vegetables*, Tomás-Barberán, F.A and R.J. Robins (Eds.), Oxford University Press, Oxford, U.K., pp. 125–155.
115. Sarda, L. and P. Desnuelle (1958). Action de la lipase pancréatique sur les esters en émulsion. *Biochim. Biophys. Acta* 30:513–520.

116. Scavetta, R.D., Herron, S.R., Hotchkiss, A.T., Kita, N., Keen, N.T., Benen, J.A., Kester, H.C., Visser, J., and F. Jurnak (1999). Structure of a plant cell wall fragment complexed to pectate lyase C. *Plant Cell* 11:1081–1092.
117. Schebor, C., Burin, L., Burea, M.P., Aguilera, J.M., and J. Chirife (1997). Glassy state and thermal inactivation of invertase and lactase in dried amorphous matrices. *Biotechnol. Prog.* 13:857–863.
118. Schechter, I. and A. Berger (1967). On the size of the active site of proteases. I. Papain. *Biochem. Biophys. Res. Commun.* 27:157–162.
119. Schechter, I. and A. Berger (1968). On the active site of proteases. III. Mapping the active site of papain; specific peptide inhibitors of papain. *Biochem. Biophys. Res. Commun.* 32:898–912.
120. Schmid, U., Bornscheuer, U.T., Soumanou, M.M., McNeill, G.P., and R.D. Schmid (1999). Highly selective synthesis of 1,3-oleoyl,2-palmitoylglycerol by lipase catalysis. *Biotechnol. Bioeng.* 64:678–684.
121. Schwimmer, S. (1980). Influence of water activity on enzyme reactivity and stability. *Food Technol.* 34(5):64–83.
122. Segel, I.H. (1975). *Enzyme Kinetics. Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*, John Wiley & Sons, Inc., New York.
123. Shen, C. and K.L. Parkin (2000). In vitro biogeneration of pure thiosulfinates and propanethial-*S*-oxide. *J. Agric. Food Chem.* 48:6254–6260.
124. Shirai, T., Igarashi, K., Ozawa, T., Hagihara, H., Kobayashi, T., Ozaki, K., and S. Ito (2007). Ancestral sequence evolutionary trace and crystal structure analyses of alkaline α-amylase from *Bacillus* sp. KSM-1378 to clarify the alkaline adaptation process of Proteins. *Proteins: Struct. Funct. Bioinf.* 66:600–610.
125. Silvius, J.R., Read, B.D., and R.N. McElhaney (1978). Membrane enzymes: Artifacts in Arrhenius plots due to temperature dependence of substrate-binding affinity. *Science* 199:902–904.
126. Sinnott, M. (Ed.) (1998). *Comprehensive Biological Catalysis: A Mechanistic Reference*, Vol. I, Academic Press, San Diego, CA.
127. Sinnott, M. (Ed.) (1998). *Comprehensive Biological Catalysis: A Mechanistic Reference*, Vol. III, Academic Press, San Diego, CA.
128. Smolensky, D.C., Raymond, W.R., Hasegawa, S., and V.P. Maier (1975). Enzymatic improvement of date quality. Use of invertase to improve texture and appearance of "sugar wall" dates. *J. Sci. Food Agric.* 26:1523–1528.
129. Solomon, E.I., Sundaram, U.M., and T.E. Machonkin (1996). Multicopper oxidases and oxygenases. *Chem. Rev.* 96:2563–2605.
130. Srere, P.A. (1984). Why are enzymes so big? *Trends Biochem. Sci.* 9:387–390.
131. Srivastava, D.K. and S.A. Bernhard (1986). Metabolite transfer via enzyme-enzyme complexes. *Science* 234:1081–1086.
132. Strambini, G.B. and E. Gabellieri (1996). Protein in frozen solutions: Evidence of ice-induced partial unfolding. *Biophys. J.* 70:971–976.
133. Strommer, J. (2011). The plant ADH gene family. *Plant J.* 66:128–142.
134. Sugihara, A., Shimada, Y., Nakamura, M., Nagao, T., and Y. Tominaga (1994). Positional and fatty acids specificities of *Geotrichum candidum*. *Protein Eng.* 7:585–588.
135. Szejtli, J. (1988). *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht, the Netherlands, 450pp.
136. Takahata, Y., Noda, T., and T. Nagata (1994). Effect of β-amylase stability and starch gelatinization during heating on varietal differences in maltose content in sweet potatoes. *J. Agric. Food Chem.* 42:2564–2569.
137. Terefe, N.S., Buckow, R., and C. Versteeg (2014). Quality-related enzymes in fruit and vegetable products: Effects of novel food processing technologies, part 1: High-pressure processing. *Crit. Rev. Food Sci. Nutr.* 54:24–63.
138. Tome, D., Nicolas, J., and R. Drapon (1978). Influence of water activity on the reaction catalyzed by polyphenoloxidase (E.C.1.14.18.1) from mushrooms in organic liquid media. *Lebensm-Wiss. u.-Technol.* 11:38–41.
139. Tucker, G.A. and L.F.J. Woods (Eds.) (1995). *Enzymes in Food Processing*, 2nd edn., Blackie, New York.
140. Tyoshimura, T., Jhee, K-H., and K. Soda (1996). Stereospecificity for the hydrogen transfer and molecular evolution of pyridoxal enzymes. *Biosci. Biotech. Biochem.* 60:181–187.
141. Ueda, S. and R. Ohba (1972). Purification, crystallization and some properties of extracelullar pullulanase from *Aerobacter aerogenes*. *Agric. Biol. Chem.* 36:2382–2392.
142. Vámos-Vigyázó, L. (1981). Polyphenol oxidase and peroxidase in fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* 21:49–127.

143. Van der Maarel, M.J.E.C., ven der Veen, B., Uitdehaag, J.C.M., Leemhius, H., and L. Dijkhuizem (2002). Properties and applications of starch-converting enzymes of the α-amylase family. *J. Biotechnol.* 94:137–165.
144. Van Dijk, C., Fischer, M., Beekhuizen, J-G., Boeriu, C., and T. Stolle-Smits (2002). Texture of cooked potatoes (*Solanum tuberosum*) 3. Preheating and the consequences for the texture and cell wall chemistry. *J. Agric. Food Chem.* 50:5098–5106.
145. van Santen, Y., Benen, J.A.E., Schröter, K-H., Kalk, K.H., Armand, S., Visser, J., and B.W. Dijkstra (1999). 1.68-Å crystal structure of endopolygalacturonase II from *Aspergillus niger* and identification of active site residues by site-directed mutagenesis. *J. Biol. Chem.* 274:30474–30480.
146. Vangrysperre, W., Van Damme, J., Vandekerckhove, J., De Bruyne, C.K., Cornelis, R., and H. Kersters-Hilderson (1990). Localization of the essential histidine and carboxylate group in D-xylose isomerases. *Biochem. J.* 265:699–705.
147. Visser, S. (1981). Proteolytic enzymes and their action on milk proteins. A review. *Neth. Milk Dairy J.* 35:65–88.
148. Vliegenthart, J.F.G. and G.A. Veldink (1982). Lipoxygenases, In *Free Radicals in Biology*, W.A. Pryor (Ed.), Vol. V., Academic Press, New York, pp. 29–64.
149. Wäfler, U., Shaw, M.L., and J.E. Lancaster (1994). Effect of freezing upon alliinase activity in onion extracts and pure enzyme preparations. *J. Sci. Food Agric.* 64:315–318.
150. Walker, J.R.L. and P.H. Ferrar (1998). Diphenol oxidases, enzyme-catalysed browning and plant disease resistance. *Biotechnol. Genet. Eng. Rev.* 15:457–497.
151. Walsh, C. (1979). *Enzymatic Reaction Mechanisms*, W.H. Freeman & Company, San Francisco, CA.
152. Wehtje, E. and P. Adlercreutz (1997). Lipases have similar water activity profiles in different reactions. *Biotechnol. Lett.* 11:537–540.
153. Whitaker, J.R. (1994). *Principles of Enzymology for the Food Sciences*, 2nd edn., Marcel Dekker, New York.
154. Whitaker, J.R., Voragen, A.G.J., and D.W.S. Wong (Eds.) (2003). *Handbook of Food Enzymology*, Marcel Dekker, New York.
155. Whitehurst, R.J. and B.A. Law (Eds.) (2002). *Enzymes in Food Technology*, 2nd edn., CRC Press, Boca Raton, FL.
156. Wrolstad, R.E., Wightman, J.D., and R.W. Durst (1994). Glycosidase activity of enzyme preparations used in fruit juice processing. *Food Technol.* 48(11):90–98.
157. Wu, Z., Robinson, D.S., Hughes, R.K., Casey, R., Hardy, D., and S.I. West (1999). Co-oxidation of β-carotene catalyzed by soybean and recombinant pea lipoxygenases. *J. Agric. Food Chem.* 47:4899–4906.
158. Yamaguchi, S. and T. Mase (1991). Purification and characterization of mono- and diacylglycerol lipase isolated from *Penicillium camemberti*. *Appl. Microbiol. Biotechnol.* 34:720–725.
159. Yamamoto T. (Ed.) (1995). *Enzyme Chemistry and Molecular Biology of Amylases and Related Enymes*, The Amylase Research Society of Japan, CRC Press, Boca Raton, FL.
160. Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D., and G.N. Somero (1982). Living with water stress: Evolution of osmolyte systems. *Science* 217:1214–1222.
161. Yoruk, R. and M.R. Marshall (2003). Physicochemical properties and function of plant polyphenol oxidase: A review. *J. Food Biochem.* 27:361–422.
162. Zhang, D., Li, N., Lok, S-M., Zhang, L-H., and K. Swaminathan (2003). Isomaltulose synthase (*Pal*I) of *Klebsiella* sp. LX3. *J. Biol. Chem.* 278:35428–35434.

BIBLIOGRAPHY

Aehle, W. (2004). *Enzymes in Industry. Production and Applications*, 2nd edn., Wiley-VCH, Weinheim, Germany, 484pp.

Copeland, R.A. (2000). *Enzymes: A Practical Introduction to Structure, Function, Mechanism, and Data Analysis*, 2nd edn., John Wiley, New York, 397pp.

Fersht, A. (1985). *Enzyme Structure and Mechanism*, 2nd edn., W.H. Freeman & Company, New York, 475pp.

Godfrey, T. and S. West (Eds.) (1996). *Industrial Enzymology*, 2nd edn., Stockton Press, New York, 609pp.

Palmer, T. (1995). *Understanding Enzymes*, 4th edn., Prentice Hall/Ellis Horwood, New York, 398pp.

Segel, I.H. (1975). *Enzyme Kinetics. Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*, John Wiley & Sons, Inc., New York, 957pp.

Sinnott, M. (Ed.) (1998). *Comprehensive Biological Catalysis. A Mechanistic Reference*, Vols. I–IV, Academic Press, San Diego, CA.

Stauffer, C.E. (1989). *Enzyme Assays for Food Scientists*, Van Norstrand Reinhold, New York, 317pp.

Tucker, G.A. and L.F.J. Woods (Eds.) (1995). *Enzymes in Food Processing*, 2nd edn., Blackie, New York, 319pp.

Whitehurst, R.J. and B.A. Law (Eds.) (2002). *Enzymes in Food Technology*, 2nd edn., CRC Press, Boca Raton, FL, 255pp.

Whitaker, J.R. (1994). *Principles of Enzymology for the Food Sciences*, 2nd edn., Marcel Dekker, New York, 625pp.

Whitaker, J.R., A.G.J. Voragen, and D.W.S. Wong (Eds.) (2003). *Handbook of Food Enzymology*, Marcel Dekker, New York, 1108pp.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

7 Dispersed Systems
Basic Considerations

*Ton van Vliet and Pieter Walstra**

CONTENTS

7.1 Introduction 468
 7.1.1 Foods as Dispersed Systems 468
 7.1.2 Characterization of Dispersions 469
 7.1.3 Effects on Reaction Rates 472
 7.1.4 Summary 472
7.2 Surface Phenomena 473
 7.2.1 Interfacial Tension and Adsorption 473
 7.2.2 Surfactants 475
  7.2.2.1 Amphiphiles 475
  7.2.2.2 Polymers 477
 7.2.3 Contact Angles 478
 7.2.4 Curved Interfaces 479
 7.2.5 Interfacial Rheology 481
 7.2.6 Surface Tension Gradients 482
 7.2.7 Functions of Surfactants 483
 7.2.8 Summary 484
7.3 Colloidal Interactions 484
 7.3.1 van der Waals Attraction 485
 7.3.2 Electric Double Layers 485
 7.3.3 Deryagin–Landau, Verwey–Overbeek Theory 487
 7.3.4 Steric Repulsion 488
 7.3.5 Depletion Interaction 489
 7.3.6 Other Aspects 490
 7.3.7 Summary 490
7.4 Liquid Dispersions 491
 7.4.1 Description 491
 7.4.2 Sedimentation 491
 7.4.3 Aggregation 493
 7.4.4 Summary 494
7.5 Soft Solids 495
 7.5.1 Phase Separation of Mixtures of Biopolymers 495
  7.5.1.1 Thermodynamic Incompatibility 496
  7.5.1.2 Complex Coacervation 497
 7.5.2 Gels: Characterization 497
  7.5.2.1 Structure 497
  7.5.2.2 Rheological and Fracture Parameters 499
  7.5.2.3 Modulus 500
  7.5.2.4 Polymer Gels 501
  7.5.2.5 Particle Gels 502

* Deceased on May 29, 2012.

7.5.3 Functional Properties 503
7.5.4 Some Food Gels 505
7.5.4.1 Polysaccharides 506
7.5.4.2 Gelatin 508
7.5.4.3 Caseinate Gels 508
7.5.4.4 Globular Proteins Gels 509
7.5.4.5 Mixed Gels 510
7.5.5 Mouthfeel of Foods 510
7.5.6 Summary 512
7.6 Emulsions 514
7.6.1 Description 514
7.6.2 Emulsion Formation 515
7.6.2.1 Droplet Breakup 515
7.6.2.2 Recoalescence 516
7.6.2.3 Choice of Emulsifier 517
7.6.3 Types of Instability 519
7.6.4 Coalescence 520
7.6.4.1 Film Rupture 520
7.6.4.2 Factors Affecting Coalescence 521
7.6.5 Partial Coalescence 523
7.6.5.1 Ice Cream 526
7.6.6 Summary 526
7.7 Foams 527
7.7.1 Formation and Description 527
7.7.1.1 Via Supersaturation 527
7.7.1.2 By Mechanical Forces 528
7.7.1.3 Foam Structure Evolution 529
7.7.2 Stability 530
7.7.2.1 Ostwald Ripening 530
7.7.2.2 Drainage 531
7.7.2.3 Coalescence 532
7.7.3 Summary 533
Frequently Used Symbols 533
References 535
Further Reading 539

7.1 INTRODUCTION

The subjects discussed in this chapter are rather different from most of the material in this book, in the sense that true chemistry, which concerns reactions involving electron transfer, is hardly involved. Nevertheless, many aspects of dispersed systems are important to an understanding of the properties of most foods and the manufacture of "fabricated foods."

Although the treatment involves some basic theory, we have tried to keep this at a minimum. Most topics treated in this chapter are discussed in more detail in the textbook on *Physical Chemistry of Foods* by P. Walstra (see the "Further Reading" section).

7.1.1 Foods as Dispersed Systems

Most foods are dispersed systems. A few are homogeneous solutions, such as cooking oil and some drinks, but even beer—as consumed—has a foam layer. The properties of a dispersed system cannot be fully derived from its chemical composition, since they also depend on physical structure.

The structure can be very intricate as is the case with foods derived from animal or vegetable tissues; these are discussed in Chapters 15 and 16. Manufactured foods, as well as some natural foods, may have a somewhat simpler structure: beer foam is a solution containing gas bubbles, milk is a solution containing fat droplets and protein aggregates (casein micelles), plastic fats consist of oil-containing aggregated triacylglycerol crystals, a salad dressing may be just an emulsion, and several gels consist of a network of polysaccharide molecules that immobilize a solution. But other manufactured foods are structurally complicated in that they contain several different structural elements of widely varying size and state of aggregation: filled gels, gelled foams, materials obtained by extrusion or spinning, powders, margarine, dough, bread, and so forth.

The existence of a dispersed state has some important consequences:

1. Since different components are in different compartments, there is no thermodynamic equilibrium. To be sure, even a homogeneous food may not be in equilibrium, but for dispersed systems, this is a much more important aspect. It may have significant consequences for chemical reactions, as is briefly discussed in Section 7.1.3.
2. Flavor components may be in separate compartments, which will slow down their release during eating. Moreover, compartmentalization of flavor components may lead to fluctuations in flavor release during eating, thereby enhancing flavor, because it offsets to some extent adaptation of the senses to flavor components. Most *compartmentalized* foods taste quite different from the same food that has been homogenized before eating.
3. If, as is often the case, attractive forces act between structural elements, the system has a certain consistency, which is defined as its resistance against permanent deformation. This may be an important functional property as it is related to attributes such as stand-up, spreadability, or ease of cutting. Moreover, consistency affects mouthfeel, as does any physical inhomogeneity of the food; food scientists often lump these properties under the word texture.
4. If the product has a significant consistency, any solvent present—in most foods, water—will be immobilized against bulk flow. Transport of mass (and generally of heat also) then has to occur by diffusion rather than convection. This may have a considerable effect on reaction rates.
5. The visual appearance of the system may be greatly affected. This is due to the scattering of light by structural elements, provided they are larger than about 50 nm. Large inhomogeneities are visible as such and give rise to what is the dictionary meaning of texture.
6. Since the system is physically inhomogeneous at a microscopic scale, it may be physically unstable. Several kinds of changes can occur during storage, which may be perceived as the development of macroscopic inhomogeneity, such as separation into layers. Moreover, during processing or usage, changes in the dispersed state may occur, which may be desirable, as in the whipping of cream, or undesirable, as in overwhipping of cream, where butter granules are formed.

Some of these aspects will be discussed in this chapter. Large-scale mechanical properties will be largely left out and so will aspects of hydrodynamics and process engineering. Of course, most foods show highly specific behavior, but treating them all would take much space and provide little understanding. Therefore, some general aspects of fairly simple model dispersions will be emphasized.

7.1.2 Characterization of Dispersions

A *dispersion* is a system of discrete particles in a continuous liquid. When the particles are gaseous, we speak of a foam; with liquid particles, we have an emulsion; and with solid particles, we have a suspension (e.g., orange juice containing cell fragments). Emulsions can be of two types: oil in water (o/w) and water in oil (w/o). Most food emulsions are of the o/w type (milk, salad dressings, most soups); they can be diluted with water. Dispersions can contain a number of different particles: milk

also contains small protein aggregates, and soups tend to contain pieces of vegetable tissue. Butter and margarine contain aqueous droplets, but they are not true w/o emulsions, as the oil contains fat crystals that have formed a space-filling network.

The latter is one example of a solid dispersion, that is, a system in which the continuous mass has been given solid-like properties after the dispersion has been made. In a foam omelet, the continuous protein solution has gelled. Liquid chocolate is a dispersion of solid particles (sugar crystals, cacao bean fragments) in oil, and upon cooling, the oil turns into a largely crystalline fat matrix.

If a binary system is solid-like, it can in principle have two continuous "phases." The prime example is a wet sponge, where matrix and water both are continuous. Several foods are bicontinuous systems; for instance, in bread, both the gas and the solid matrix are continuous. If not, the bread would lose most of its volume after baking: the hot gas cells would shrink considerably upon cooling, since they largely consist of water vapor.

A *colloidal system*, often abbreviated as a *colloid*, is usually defined as a dispersion containing particles that are clearly larger than small molecules (say, solvent molecules), yet too small to be visible. This would imply a size range of about 10 nm to 0.1 mm. Two types of colloidal systems are usually distinguished: lyophilic ("solvent loving") and lyophobic ("solvent hating"). The latter type consists of two (or more) phases, such as air, oil, water, or various crystalline materials. Lyophobic colloidal systems do not form spontaneously: it costs energy to disperse the one phase into the (continuous) other phase, and the system formed is not in equilibrium, and hence physically unstable.

A lyophilic colloidal system forms by "dissolving" a material in a suitable solvent, and the system then is in equilibrium. The main examples are macromolecules (polysaccharides, proteins, etc.) and association colloids. The latter are formed from amphiphilic molecules, such as soaps. These have a fairly long hydrophobic "tail" and a smaller polar (i.e., hydrophilic) "head." In an aqueous environment, the molecules tend to associate in such a way that the tails are close to each other and the heads are in contact with water. In this way, micelles or liquid crystalline structures are formed. Micelles will be briefly discussed in Section 7.2.2; liquid crystalline phases [39] are not very prominent in foods.

It may further be noted that an unstable system may appear to be stable (i.e., does not show a significant change in properties during the observation time). This means that the rate of change is very small, which is often due to (1) a high-activation (free) energy for a chemical reaction or a physical change to occur or (2) a very slow motion of molecules or particles due to extremely high viscosity of the system (as in dried foods).

The *size scale* of structural elements in foods can vary widely, spanning a range of six orders of magnitude (Figure 7.1). A water molecule has a diameter of about 0.3 nm, whereas a typical cell in plant or animal tissues will be about 0.3 mm. The shape of the particles is also important, as is their volume fraction ϕ (i.e., the proportion of the volume of the system that is taken up by the particles). All these variables affect product properties. Some effects of size or scale are as follows:

1. *Visual appearance*: An o/w emulsion, for example, will be almost transparent if the droplets have a diameter of 0.03 μm; bluish white if 0.3 μm; white if 3 μm; and the color of the oil (usually yellow) will be discernable for 30 μm droplets.
2. *Surface area*: For a collection of spheres each with a diameter d (in m), the specific surface area is given by

$$A = 6\frac{\phi}{d} \tag{7.1}$$

in $m^2\ m^{-3}$, where ϕ is the volume fraction dispersed particles. The area can thus be large. For an emulsion of $\phi = 0.1$ and $d = 0.3$ μm, $A = 2\ m^2$ mL of emulsion; if 5 mg of protein is adsorbed per m^2 of oil su*r*face, the quantity of adsorbed protein would amount to 1% of the emulsion.

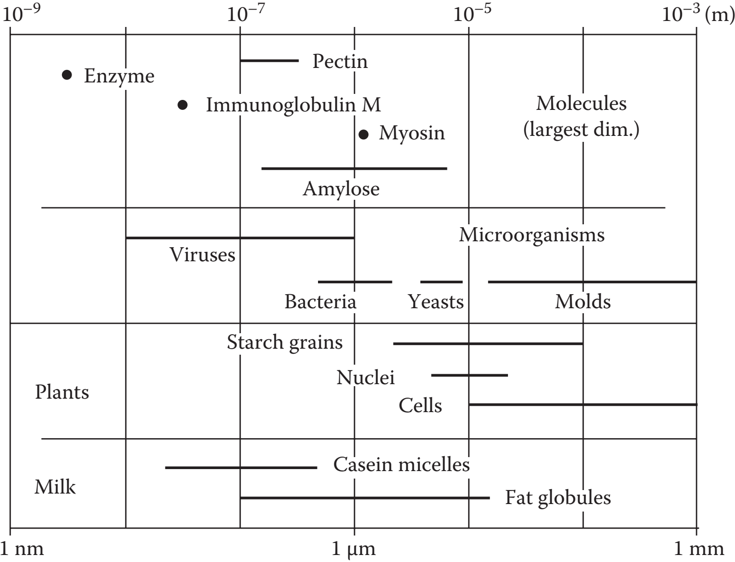


FIGURE 7.1 Approximate size of some structural elements in foods.

3. *Pore size*: Between particles, regions of continuous phase exist, and their size is proportional to particle size and smaller for a larger ϕ. If the dispersed phase forms a space-filling network, pores in this network follow the same rules. The permeability, that is, the ease with which solvent can flow through the pores, is proportional to pore size squared. This is why a polymer gel is far less permeable than a gel made up of fairly large particles (Section 7.5.2).
4. *Time scales involved*: (*Note*: Time scale is defined as the characteristic time needed for an event to occur, for instance, for two molecules to react, for a particle to rotate, and for a bread to be baked.) The larger the particles, the longer are the time scales involved. For example, the root-mean-square value of the diffusion distance (z) of a particle of diameter d, as a function of time t is

$$\langle z^2 \rangle^{0.5} \propto \left(\frac{t}{d}\right)^{0.5} \tag{7.2}$$

In water, a particle of 10 nm diameter will diffuse over a distance equal to its diameter in about 1 μs, a particle of 1 μm in 1 s, and one of 0.1 mm in 12 days. Considering diffusion of a material into a structural element, the relation between diffusion coefficient D, distance l, and time $t_{0.5}$ needed to halve a difference in concentration is

$$l^2 \approx D t_{0.5} \tag{7.3}$$

D of small molecules in water ≈10^{-9} m^2 s^{-1} and in most cases (larger molecules, more viscous solution) it is smaller.
5. *Effect of external forces*: Most external forces acting on particles are proportional to diameter squared, whereas most attractive colloidal forces between particles are proportional to diameter. This implies that small particles are virtually impervious to external influences, like shearing forces or gravity. Large particles often can be deformed or even be disrupted by external forces and also sediment much faster.
6. *Ease of separation*: Some of the points raised earlier imply that it is much more difficult to separate small particles from a liquid than large ones.

Particles rarely are all of the same size. The subject of *size distributions* is a complicated one [2,70], and it will not be discussed here. Suffice it to say that a size range may generally be used to characterize the size distribution and that the volume/surface average diameter d_{vs} or d_{32} can often be seen as typical for the distribution. However, different properties may need different types of averages. The wider the size distribution—width being defined as standard deviation divided by average—the greater the differences between average types (an order of magnitude is not exceptional). It is often very difficult to accurately determine a size distribution [2]. Difficulties in determination and interpretation increase with particles that are more anisometric or otherwise different in properties.

7.1.3 Effects on Reaction Rates

As mentioned earlier, components in a dispersed food may be compartmentalized, and this can greatly affect reaction rates. In a system containing an aqueous (α) and an oil phase (β), a component often is soluble in both. Nernst's distribution or partitioning law then states that the ratio of concentrations (c) in both phases is constant:

$$\frac{c_\alpha}{c_\beta} = \text{Constant} \tag{7.4}$$

The constant will depend on temperature and possibly other conditions. For instance, pH has a strong effect on the partitioning of carboxylic acids, since these acids are oil soluble only when they are in a neutralized state. At high pH, where the acids are fully ionized, almost all acids will be in the aqueous phase, whereas at low pH, the concentration in the oil phase may be considerable. Note that the quantity of a reactant in a phase also depends on the phase volume fraction.

When a reaction occurs in one of the phases present, the reaction rate does not depend on the overall concentration of a reactant but on its concentration in the phase mentioned [102]. This concentration may be equal to or lower than the overall concentration, depending on the magnitude of the partitioning constant (Equation 7.4). Since many reactions in foods actually are cascades of several different reactions, the overall reaction pattern, and thereby the mixture of components formed, may also depend on partitioning. Chemical reactions will often involve transport between compartments and will then depend on distances and molecular mobility. Applying Equation 7.3, it follows that diffusion times for transport into or out of fairly small structural elements, say, emulsion droplets, would mostly be very short. However, if the solvent is immobilized in a network of structural elements, this may greatly slow down reactions, especially if reactants, say, O_2, have to diffuse in from outside. Moreover, some reactions especially occur at the boundary between phases. An example is lipid autoxidation, where the oxidizable material (unsaturated oil) is in oil droplets, and a catalyst, say, Cu ions, is in the aqueous phase. Another example is that of an enzyme present in one structural element and the component on which it acts in another one. In such cases, the specific surface area may be rate determinant. Adsorption of reactive substances onto interfaces between structural elements may diminish their effective concentration and thereby reactivity. Thus, rates of chemical reactions and the mixture of reaction products may be quite different in a dispersed system than in a homogeneous one. Examples in vegetable and animal tissues are well known, but other cases have not been studied in great detail, except for the activity of some additives [102] and, of course, enzymatic lipolysis of the oil in emulsion droplets.

7.1.4 Summary

- Most foods are dispersed systems, which affects properties as speed of chemical changes, flavor, visual appearance, consistency, and physical stability.
- Dispersed systems are characterized by composition, type, and size of inhomogeneities.
- Compartmentalization greatly affects rate of chemical reactions.

7.2 SURFACE PHENOMENA

As mentioned earlier, most foods have a large phase boundary or interfacial area. Often, substances adsorb onto interfaces, and this has a considerable effect on static and dynamic properties of the system. In this section, basic aspects are discussed; applications are discussed later (see [1,3] for general literature).

Various types of interfaces can exist between two phases, the main ones being gas–solid, gas–liquid, liquid–solid, and liquid–liquid. If one of the phases is a gas (mostly air), one usually speaks of a surface, in the other cases of an interface, but these words are often considered to be interchangeable. More important is the distinction between a solid interface, where one of the phases is a solid, and a fluid interface between two fluids (gas–liquid or liquid–liquid). A solid interface is rigid; a fluid interface can be deformed.

7.2.1 Interfacial Tension and Adsorption

An interface between two phases contains an excess of free energy, which is proportional to the interfacial area. Consequently, the interface will try to become as small as possible, to minimize the interfacial free energy. This then means that one has to apply an external force to enlarge the interfacial area. The reaction force in the interface is attractive and acts in the plane of the interface. If the interface is fluid, the force can be measured (see Figure 7.2a) and the force per unit length is called the surface or *interfacial tension*: symbol γ, units N m^{-1}. (γ_{OW} means the tension between oil and water, γ_{AS} between air and a solid, etc.) Also, a solid has a surface tension, but it cannot be measured.

The magnitude of γ depends on the composition of the two phases. Some examples are given in Table 7.1. The interfacial tension also depends on temperature, and it nearly always decreases with increasing temperature.

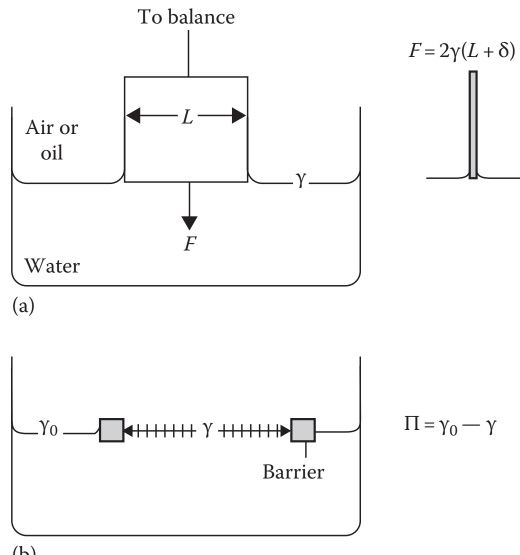


FIGURE 7.2 (a) Measurement of surface or interfacial tension by means of a Wilhelmy plate (width L, thickness δ). The plate is attached to a sensitive balance. F, net force. (b) Illustration of the surface pressure (Π) caused by adsorbed surfactant molecules (depicted by vertical dashes). Between the barriers the surface tension is lowered, and a net two-dimensional pressure of magnitude Π acts on the barriers.

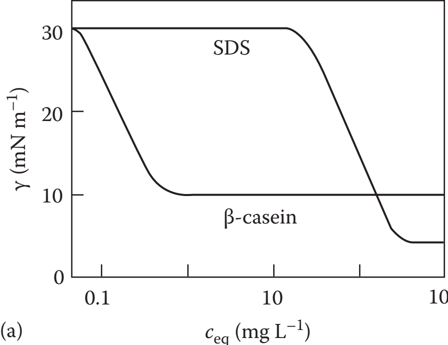
TABLE 7.1
Some Interfacial Tensions

Material	Against Air	Against Water
Water	72	0
Saturated NaCl solution	82	0
0.02 M SDS in water	41	0
0.1 g L^{-1} β-casein[a]	44	0
Ethanol	22	0
Paraffin oil	30	50[b]
Triacylglycerol oil	35	30

Note: Approximate values (mN m^{-1}) at room temperature.
[a] Aging time 1 day [49].
[b] Some buffers give a lower interfacial tension than water.

Some molecules in a solution that is in contact with a phase surface can accumulate at this surface, forming a monolayer. This is called "adsorption." (*Note*: A*d*sorption is to be distinguished from a*b*sorption, where a substance is taken up *in* a material.) A substance that does adsorb is called a "surfactant." It adsorbs because its free energy is lower at the surface than in the bulk phase. When it adsorbs, it also lowers the surface free energy of the solution, and with that it lowers the surface tension. Examples are in Figure 7.3a. It is seen that the decrease in γ depends on the surfactant concentration left in solution after equilibrium has been reached. The lower the value of c_{eq} at which a given decrease in γ is obtained, the higher the *surface activity* of the surfactant.

An important variable is the *surface load*, Γ, that is, the amount (in moles or in mass units) of adsorbed material per unit surface area. For Γ = 0, $\gamma = \gamma_0$, the value for a clean interface. At a relatively high surfactant concentration (c_{eq}), the value of Γ reaches a plateau, where the surfactant has made a packed monolayer. The plateau of Γ corresponds to the surfactant concentration at which γ reaches a plateau value. The magnitude of $\Gamma_{plateau}$ varies among surfactants, for the most part between 1 and 4 mg m^{-2}. The relation between Γ and the equilibrium surfactant concentration is called an "adsorption isotherm." Substances in a gas phase, such as water in air, can also adsorb onto a (solid) surface, and the same relations apply.



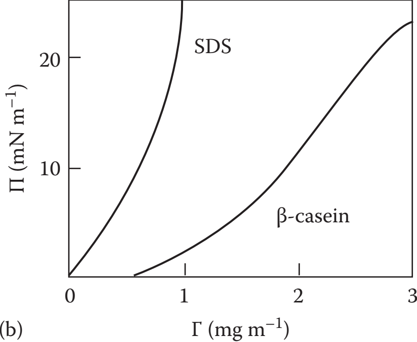


FIGURE 7.3 Absorption of β-casein and SDS at an oil–water interface. (a) Interfacial tension (γ) as a function of equilibrium surfactant concentration (c_{eq}). (b) Relation between surface pressure (Π) and surface load (Γ) (approximate results). (From Walstra, P. et al., *Dairy Science and Technology*, CRC/Taylor & Francis, Boca Raton, FL, 2006.)

Each surfactant has at equilibrium (and at a given temperature) a fixed relation between the magnitude of Γ and the decrease of γ. The latter is called the "surface pressure" $\Pi = \gamma_0 - \gamma$ (cf. Figure 7.2b). The maximum value of Π varies among surfactants; for many surfactants (though not for all) the value is roughly the same for air–water and oil–water interfaces. The relation between Π and Γ is called "surface equation of state." Examples are given in Figure 7.3b.

The *rate of adsorption* of a surfactant depends primarily on its concentration. The surfactant will often be transported to a surface by diffusion. If its concentration is c and the surface load to be obtained Γ, a layer adjacent to the surface of thickness Γ/c will suffice to provide the surfactant. Application of Equation 7.3 and putting $l = \Gamma/c$ leads to

$$t_{0.5} = \frac{\Gamma^2}{Dc^2} \tag{7.5}$$

In aqueous solutions D is generally on the order of 10^{-10} m^2 s^{-1}. Assuming a surfactant concentration of 3 kg m^{-3}, and Γ = 3 mg m^{-2}, then results in $t_{0.5} \approx 10$ ms. Adsorption will be complete in about 10 times $t_{0.5}$, that is, well within a second. If the surfactant concentration is lower, adsorption will take a (much) longer time, but then stirring will markedly enhance adsorption rate. In other words, adsorption will nearly always be fast in practice.

7.2.2 Surfactants

Surfactants come in two main types, polymers and small amphiphilic molecules. (*Note on terminology*: Some workers use the word surfactant for small-molecule amphiphiles only. Also, surfactants are often called emulsifiers.)

7.2.2.1 Amphiphiles

The hydrophobic (lipophilic) part of a small-molecule amphiphile typically is an aliphatic chain. There is a wide diversity of hydrophilic parts. In the classical surfactant, common soap, it is an ionized carboxyl group. Most amphiphilic substances are not highly soluble either in water or oil, and they suffer the smallest repulsive interaction from these solvents when they are partly in a hydrophilic environment (water) and partly in a hydrophobic one (oil), that is the case at an o/w interface (see Figure 7.4) [101]. They also adsorb onto air–water and some solid–water interfaces. In solution, they tend to associate and form micelles (i.e., roughly spherical aggregates in which the hydrophobic tails are in the middle and the hydrophilic heads to the outside) to lessen repulsive interaction with solvent.

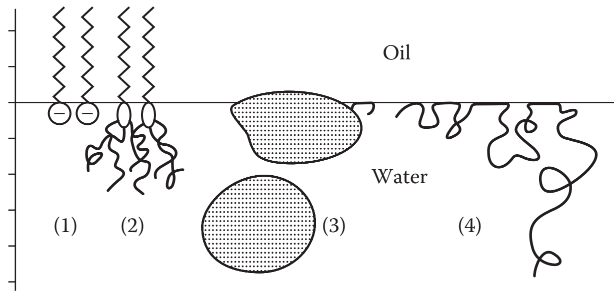


FIGURE 7.4 Mode of absorption of some surfactants at an oil–water interface; at left is a scale of nanometers. (1) A soap, (2) a Tween, (3) a small globular protein (for comparison a molecule in solution is shown), and (4) β-casein. Highly schematic. (From Walstra, P. et al., *Dairy Science and Technology*, CRC/Taylor & Francis, Boca Raton, FL, 2006.)

TABLE 7.2
Some Small-Molecule Surfactants and Their Hydrophilic–Lipophilic Balance Values

Type	Example of Surfactant	HLB Value
Nonionics		
Aliphatic alcohol	Hexadecanol	1
Monoacylglycerol	Glycerol monostearate	3.8
Esters of monoacylglycerols	Lactoyl monopalmitate	8
Spans	Sorbitan monostearate	4.7
	Sorbitan monooleate	7
	Sorbitan monolaurate	8.6
Tween 80	Poly(oxyethylene) sorbitan monooleate	16
Anionics		
Soap	Na oleate	18
Lactic acid esters	Na stearoyl-2-lactoyl lactate	21
Phospholipids	Lecithin (zwitterionic)	Fairly large
Teepol[a]	SDS	40
Cationics[a]		Large

[a] Not used in foods but as detergents.

Some small-molecule surfactants of importance to the food scientist are listed in Table 7.2 [38,64]. They are categorized as nonionic, anionic, and cationic, according to the nature of the hydrophilic part. Also, distinction is made between natural surfactants (e.g., soaps, monoacylglycerols, phospholipids) and synthetic ones. The Tweens are somewhat different from other ones in that the hydrophilic part contains three or four poly(oxyethylene) chains of about five monomers in length. Phospholipids come in a wide range of composition and properties; several are zwitterionic.

An important characteristic of a small-molecule surfactant is its *HLB value*, where HLB stands for hydrophilic–lipophilic balance. It is defined so that a value of 7 means that the substance has about equal solubility in water and oil. Smaller values imply greater solubility in oil, and so forth. Surfactants range in HLB value from about 1 to 40. The relation between HLB value and solubility is in itself useful, but it also relates to the suitability of the surfactant as an emulsifier: surfactants with HLB >7 are generally suitable for making foams and o/w emulsions and those with HLB <7 for w/o emulsions (see also Section 7.6.2 about Bancroft's rule). Surfactants suitable as cleaning agents (detergents) in an aqueous solution have a large HLB number. Several other relations with HLB values have been claimed, but most of these are questionable.

Originally, the HLB value of a surfactant was determined from its solubility in water divided by that in oil. Currently, HLB numbers have been derived for a range of chemical groups. Several authors have given tabulated values (e.g., [28]). The polar group(s) of a surfactant have a positive value and the hydrophobic groups have a negative value. The sum of these values plus 7 gives the HLB number. In general, a longer aliphatic chain yields a lower HLB, and a more polar or a larger polar group a higher HLB. Actually, the HLB number of a surfactant will depend on temperature and on oil type.

As mentioned earlier, many small-molecule amphiphiles tend to form micelles, which occurs above a *critical micellization concentration* (CMC). Beyond that concentration additional surfactant molecules will go into micelles and their thermodynamic activity (or effective concentration, roughly speaking) barely increases. Consequently, the surface load Γ does not further increase and γ does not further decrease. In Figure 7.3a, the CMC for sodium dodecyl sulfate (SDS) is thus reached at a total concentration of about 300 mg L^{-1}. In a homologous series of surfactants, a longer chain

length results in a smaller value of the CMC. For ionic surfactants, the CMC markedly decreases with increasing ionic strength. The CMC can also depend on pH.

At the air–water interface, much of the same pattern is observed, but since γ_0 is higher and Π is roughly the same, γ is much higher. The smallest value of γ obtained at an air–water interface is about 35 mN m^{-1}, whereas at a triacylglycerol oil–water interface it varies from <1 to about 5 mN m^{-1} for most small-molecule surfactants.

It should be realized that commercially available surfactants generally are *mixtures* of several components, varying in chain length and possibly in other properties. These components may differ, for example, in the plateau value of γ. Especially, some trace components may be present that give a lower γ than the main components, and at equilibrium the surfactants yielding the smallest γ will dominate in the interface. Because of their smaller concentration, however, their diffusion to the interface will be slow (see Equation 7.5). This implies that it will take a long time before an equilibrium composition, and thus a steady γ-value, is reached. Another complication is that in actual dispersions the surface-to-volume ratio is very large, whereas this ratio is quite small in situations where γ is commonly measured (i.e., at a macroscopic interface between the phases). This means that the result of such measurements of γ may not be representative for the actual values in a foam or emulsion.

7.2.2.2 Polymers

Several synthetic polymers can be used as surfactants, and a mass of experimental evidence as well as theory is available [27]. Copolymers, where part of the segments are fairly hydrophobic and others hydrophilic, are suitable. They tend to adsorb with "trains," "loops," and "tails" (cf. Figure 7.4, curve 4). There are few natural polymers that adsorb in this way. Surface activity of polysaccharides still is controversial. Most surface active polysaccharides contain a protein moiety that is responsible for this attribute [21]. On the other hand, chemical modification can provide polysaccharides with hydrophobic groups; a well-known example is some cellulose ethers, which can be used as emulsifiers [13].

Proteins often are the surfactants of choice in food technology, especially for foams and o/w emulsions [19,57,93]. (Because of their insolubility in oils, they are not suitable for w/o emulsions.) The mode of adsorption of proteins varies (cf. Figure 7.4). There always is a change in conformation, often considerably so. For instance, most enzymes (with the exclusion of true lipases) completely lose their activity after adsorption at an oil–water interface due to conformational change; some enzymes retain part of their activity after adsorption at an air–water interface [17]. Most globular proteins appear to retain an approximately globular conformation at interfaces, though not the native one. Proteins with little secondary structure, such as gelatin and caseins, tend to adsorb more like a linear polymer. This implies that they protrude much farther into the aqueous phase than most globular proteins. The latter can be denatured prior to absorption (e.g., by heat treatment), which alters their conformation after adsorption; generally, Γ and the protrusion distance are increased. At high bulk protein concentration, multilayer adsorption may occur, but the second and more remote layers are only weakly adsorbed.

In Figure 7.3, adsorption of a protein and an anionic surfactant are compared, and there are three main differences that generally hold for proteins and synthetic high polymers, as compared to small-molecule amphiphiles.

1. The protein is clearly more surface active than the anionic surfactant. Consequently, desorption of adsorbed proteins cannot or can barely be achieved by dilution or "washing." The difficulty of desorption may be enhanced by cross-linking reactions between adsorbed protein molecules; this has especially been shown for proteins containing a free thiol group, where cysteine–cystine interchange can occur in the interface [25].
2. As shown in Figure 7.3b, the surface equation of state differs greatly between the protein and the SDS. For a protein, the value of Γ at which a significant surface pressure can be

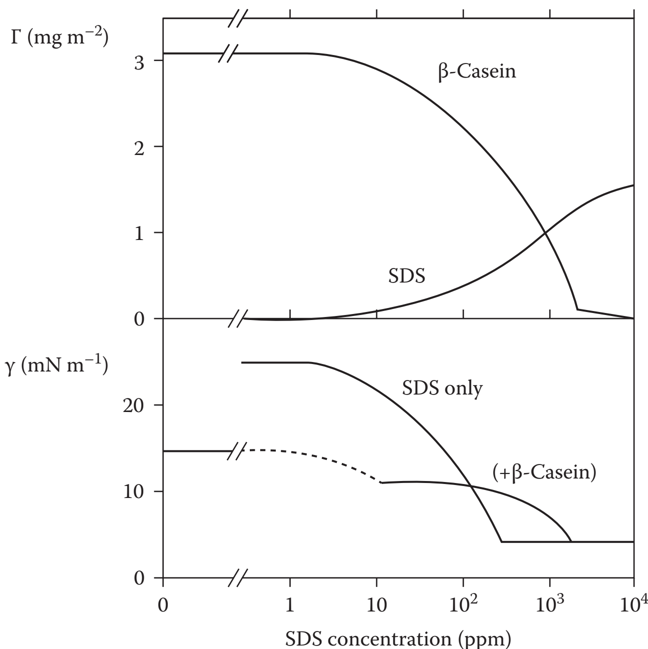


FIGURE 7.5 Surface load (Γ) in an o/w emulsion and interfacial tension (γ) at the o/w interface, for β-casein in the presence of increasing concentration of SDS; γ is also given for SDS only. (From results in Walstra, P. and de Roos, A.L., *Food Rev. Int*., 9, 503, 1993.)

observed is much higher than for SDS. This is because at low values of Γ, the magnitude of Π is proportional to the surface load expressed in moles of surfactant per unit of interfacial area, taking into account that the molar mass of a typical protein is about 100 times that of a typical amphiphile. This has some important consequences for emulsion and foam making (Sections 7.6.2 and 7.7.1).

3. The anionic surfactant yields a lower interfacial tension than the protein at the plateau adsorption. The magnitude of the interfacial tension affects several phenomena, as discussed later on. Here, we will mention one aspect, that is, the displacement of a protein from the interface by an amphiphile that is present in a high concentration [15]. It is illustrated in Figure 7.5. Many foods naturally contain some surfactants (fatty acids, monoacylglycerols, and phospholipids), and these can modify the properties of adsorption layers.

To some extent, proteins can also displace each other in a surface layer, depending on concentration, surface activity, molar mass, molecular flexibility, and so forth. Although protein adsorption appears irreversible in the sense that it is mostly not possible to substantially lower Γ by diluting the system, the occurrence of mutual displacement nevertheless implies that individual protein molecules in the interfacial layer may interchange with those in solution, albeit slowly.

7.2.3 Contact Angles

When two fluids are in contact with a solid and with each other, there is a contact line between the three phases [1]. An example is given in Figure 7.6a for the system air–water–solid. There must be a balance between the surface forces acting in the plane of the solid surface, and this leads to the Young equation:

$$\gamma_{AS} = \gamma_{WS} + \gamma_{AW} \cos\theta \tag{7.6}$$

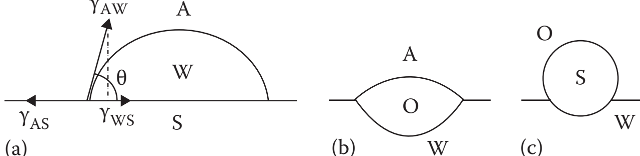


FIGURE 7.6 Contact angles (θ): examples of three-phase systems. A, air; O, oil; S, solid; and W, water. The arrows in (a) indicate the values of the three interfacial tensions. See the text for further explanation. (From Walstra, P. et al., *Dairy Science and Technology*, CRC/Taylor & Francis, Boca Raton, FL, 2006.)

The contact angle θ is conventionally taken in the densest fluid phase. Its value depends on three interfacial tensions. γ_{AS} and γ_{WS} cannot be measured, but their difference can be derived from the contact angle. If $(\gamma_{AS} - \gamma_{WS})/\gamma_{AW} > 1$, Equation 7.6 has no solution, θ = 0, and the solid will be completely wetted by the liquid; an example is water on clean glass. If the quotient mentioned is <–1, there is no wetting at all; an example is water on Teflon or other strongly hydrophobic materials.

In Figure 7.6b the more complicated situation of contact between three fluids is shown. Now there has to be a balance of surface forces in the horizontal as well as in the vertical plane, giving two contact angles. A spreading pressure can be defined as

$$\Pi_S = \gamma_{AW} - (\gamma_{AO} + \gamma_{OW}) \tag{7.7}$$

In Figure 7.6b, $\Pi_S < 0$. If it is >0, the sum of the surface free energies of the A/O and the O/W interfaces is smaller than that of the A/W interface alone, and the oil will spread over the water surface. Use of the values in Table 7.1 leads to the conclusion that for paraffin oil $\Pi_S = -8$ mN m^{-1}, implying that the droplet will not spread (but it does adhere to the a/w interface). For triacylglycerol oil, it follows that $\Pi_S = 7$ mN m^{-1}, and spreading will occur. These aspects are of importance for the interactions between emulsion droplets and foam bubbles. The spreading pressures can, of course, be altered by surfactants. However, most proteins lower γ_{AW} and γ_{OW} by roughly the same amount; therefore, the spreading pressure is not greatly altered.

Figure 7.6c depicts a small solid particle located in an oil–water interface. The Young equation also applies in this case. The contact angle (about 140° in the water phase) would be fairly typical for a triacylglycerol crystal in a triacylglycerol oil–water interface. The contact angle can in such a case be lowered by adding a suitable surfactant (e.g., SDS) to the water phase. Addition of a large quantity of surfactant can even lead to θ = 0 and thus to complete wetting of the crystal by the aqueous phase. This is accomplished in some processes to separate fat crystals from oil. Adherence of crystals to the o/w interface and the associated contact angle may be of importance for emulsion stability (e.g., Section 7.6.5).

It should be noted that the action of gravity can alter the shape of the fluid interfaces depicted in Figure 7.6, but the contact angles remain the same. If the droplets are smaller than about 1 mm, the effect of gravity tends to be quite small.

7.2.4 Curved Interfaces [1]

The pressure at the concave side of a curved phase boundary (interface) is always greater than that at the convex side. The difference is called the "Laplace pressure p_L," given by

$$p_L = \frac{2\gamma}{R} \tag{7.8}$$

where R is the radius of curvature; for a spherical particle, R equals the particle radius r.

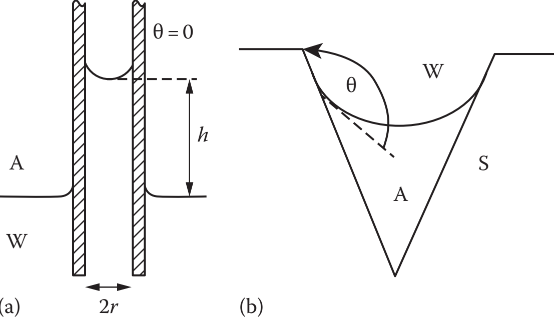


FIGURE 7.7 Some capillary phenomena. (a) Rise of a liquid in a capillary if the contact angle θ = 0. (b) Air pocket in a crevice in a submerged solid in water. See text.

An important consequence is that drops and bubbles tend to be spherical and that it is difficult to deform them, the more so when they are smaller. If a drop is not spherical, the radius of curvature differs with location, which implies a pressure difference within the drop. This causes material in the drop to move from regions with a high pressure to those with a lower one, until a spherical shape is obtained. Only if an external stress is applied can the drop (or bubble) be deformed from the spherical shape. Some examples may be enlightening. For an emulsion droplet of radius 0.5 μm and interfacial tension 0.01 N m^{-1}, the Laplace pressure would be 4×10^4 Pa (0.4 bar) and a considerable external pressure would be needed to cause substantial deformation. For an air bubble of 1 mm radius and $\gamma = 0.05$ N m^{-1}, p_L would be 100 Pa, allowing deformation to occur more easily. These aspects will be discussed further in Sections 7.6.2, 7.6.4, and 7.7.1.

Another consequence of the Laplace pressure is *capillary rise*, illustrated in Figure 7.7a. In a vertical capillary containing a liquid that gives zero contact angle (e.g., water in a glass tube), a concave meniscus is formed. For a capillary of radius r, this implies a pressure difference of magnitude $2\gamma/r$ between the water just below the meniscus and that outside the tube at the same height. The liquid in the tube will then rise, until the pressure due to gravity ($g\rho h$) balances the capillary pressure. For example, pure water in a cylindrical capillary of 0.1 mm internal radius would rise 15 cm. If the contact angle is larger, the rise will be less; if it is >90°, capillary depression occurs.

These aspects are relevant to the dispersion of powders in water. If a heap of powder is placed on water, capillary rise of the water through the pores (voids) between the powder particles must occur for wetting of the particles to occur, and this is a prerequisite for dispersion, hence dissolution, of the powder. It requires a contact angle (between powder material, water, and air) <90°. The effective contact angle in a powder is substantially larger than that at a smooth surface of the powder material, so the latter angle must be distinctly smaller than 90° for wetting of the powder to occur (see [78]).

A third consequence of Laplace pressure is the *enhanced solubility* of the gas in a bubble in the liquid around it. According to Laplace (Equation 7.8), the pressure of a gas in a (small) bubble is enhanced and, according to Henry's law, the solubility of a gas is proportional to its pressure. The effect of curvature of a particle on the solubility of the material in the particle is not restricted to gas bubbles and is in general given by the *Kelvin equation*

$$RT \ln \frac{s(r)}{s_\infty} = \frac{2\gamma M}{\rho r} \tag{7.9}$$

for a spherical particle of radius r; s is solubility, s_∞ solubility at a plane interface (i.e., "normal" solubility), and M and ρ are molar mass and mass density, respectively, of the material in the particle. R is the universal gas constant (J mol^{-1} K^{-1}), and T the absolute temperature (K). Examples of calculations according to Equation 7.9 are in Table 7.3. It is seen that for most systems, particle

TABLE 7.3
Examples of the Increase in Solubility of the Material in a Particle due to Curvature[a]

Variable	Water in Oil	Air in Water	Fat Crystal in Oil	Sucrose Crystal in Saturated Solution
r (m)	10^{-6}	10^{-4}	10^{-8}	10^{-8}
γ (N m^{-1})	0.005	0.05	0.005	0.005
ρ (kg m^{-3})	990	1.2	1075	1580
M (kg mol^{-1})	0.018	0.029	0.70	0.342
s_R/s_∞	1.000073	1.010	1.30	1.091

[a] Calculated according to Equation 7.9 for some arbitrary radii of curvature and some reasonable values of the interfacial tension (temperature 300 K).

radius has to be very small (e.g., <0.1 μm) for a significant effect. However, gas in bubbles of 1 mm has a perceptibly enhanced solubility.

The increased solubility gives rise to *Ostwald ripening*, that is, the growth of large particles in a dispersion at the expense of small ones and thus the eventual disappearance of the smallest particles. However, this only occurs if the material of the particles is at least somewhat soluble in the continuous phase. It may thus occur in foams and in w/o emulsions, but not in triacylglycerol o/w emulsions. The rate of Ostwald ripening is governed by several factors (e.g., see Section 7.7.2).

Ostwald ripening will always occur with crystals in a saturated solution, albeit slowly if the crystals are large. Another effect is that it causes "rounding" of small crystals. At the edge of a crystal, the radius of curvature may be very small, say, some nanometers, and this will lead to a greatly enhanced solubility (Table 7.3, fat crystal in oil). The material near the edge will thus dissolve and be deposited somewhere else. Small ice crystals (say, 20 μm) in partly frozen foods are generally of a fairly isometric shape.

If the surface of a particle is (partly) concave rather than convex, as shown in Figure 7.7b, the solubility is, of course, decreased. If the situation depicted represents a local equilibrium, the gas concentration in the liquid is below saturation. If the gas concentration would be higher, the gas pocket depicted will grow.

7.2.5 Interfacial Rheology [4,5,43,94]

If an interface contains surfactant, it has rheological properties. Two kinds of surface rheology can be distinguished, in shear and in dilation (Figure 7.8). When the interface is sheared (leaving both area and amount of surfactant in the interface constant), one can measure the force in the plane of the interface needed to do this. Often, this is done as a function of the shear rate, and a *surface-shear viscosity* η_{ss} (units N s m^{-1}) is obtained. For most surfactants η_{ss} is negligibly small, but not for several polymeric surfactants. For example, for a Na-caseinate monolayer, a viscosity of 0.002 N s m^{-1} has been observed, for layers of globular proteins values between 0.01 and 1 N s m^{-1}. For most systems, shear rate thinning occurs and the observed viscosity is an apparent viscosity, that is, its value depends on the shear rate. Values reported for globular proteins greatly vary, partly because of experimental uncertainty: the monolayer can yield or rupture, and the measured "viscosity" then will greatly depend on the rupture pattern [48]. For some proteins, the viscosity strongly increases with the age of the monolayer, due to the formation of intermolecular bonds [17].

If the interfacial area is enlarged, leaving its shape unaltered, an increase in interfacial tension will occur, because Γ is decreased. This is usually expressed in the *surface-dilational modulus*, defined as

$$E_{SD} \equiv \frac{d\gamma}{d\ln A} \tag{7.10}$$

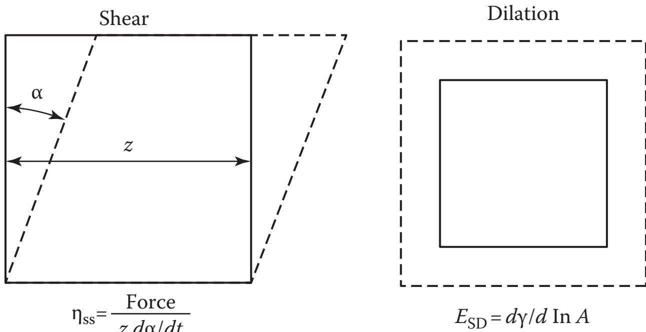


FIGURE 7.8 Illustration of the geometrical changes applied in a surface element when performing surface rheology in simple shear and in dilation.

where A is the interfacial area. E_{SD} is finite for all surfactants, although it will be very small if surfactant activity is high and the rate of surface enlargement is small. In such a case, surfactant from the bulk rapidly diffuses to the enlarged surface, thereby increasing Γ and lowering γ. In other words, the equilibrium between bulk (c_{eq}) and interfacial concentration (Γ) will be rapidly restored. E_{SD}, therefore, strongly decreases with decreasing rate of deformation. For proteins E_{SD} may be large and less dependent on time scale, because proteins adsorb more or less irreversibly. However, the interfacial concentration of protein has a large effect: Figure 7.3b shows that for a protein the value of Γ has to be high before a significant value of Π, hence of E_{SD}, is attained. Changes in protein conformation upon adsorption and dilation can also affect the modulus.

E_{SD} is a property that appears in several equations relating to interfacial phenomena. A problem is, however, that the measurement of E_{SD} is difficult or even impossible, except at relatively long time scales and/or small deformations. By and large, for globular proteins at the a/w interface, values of about 30–100 mN m^{-1} have been observed and for β-casein about 10–20 mN m^{-1} [4,30,56,74]. Values at the o/w interface may be significantly different from those at the a/w interface.

Surface rheological parameters of protein layers naturally depend on pH, ionic strength, solvent quality, temperature, and so forth. Often, moduli and viscosities are at maximum near the isoelectric pH. It may further be noted that one can also measure a surface-dilational viscosity and a surface-shear modulus.

7.2.6 Surface Tension Gradients

If a fluid interface contains a surfactant, surface tension gradients can be created. This is illustrated for the case of an a/w interface in Figure 7.9. In Figure 7.9a, a velocity gradient ($\nabla v = dv_x/dy$) in the water sweeps surfactant molecules downstream, thereby producing a surface tension gradient: γ will now be smaller downstream. This implies that the surface exerts a *tangential stress* $\Delta\gamma/\Delta x$ onto the liquid. When the gradient is large enough, the stress can be equal and opposite to the shear stress $\eta \cdot \nabla v$ (η, viscosity of the liquid), which means that the surface will not move. If there is no surfactant, the surface would move with the flowing liquid; in the case of an o/w interface, the flow velocity would be continuous across the interface.

This has important consequences, especially for foams, as is seen by comparing Frames (c) and (d). In the absence of a surfactant, the liquid between two foam bubbles rapidly streams downward, like a falling drop. In the presence of surfactant, flow is very much slower as the "walls" of the film can now withstand the stress caused by the down flowing liquid. In other words, development of surface tension gradients is essential for the formation of a foam. It also

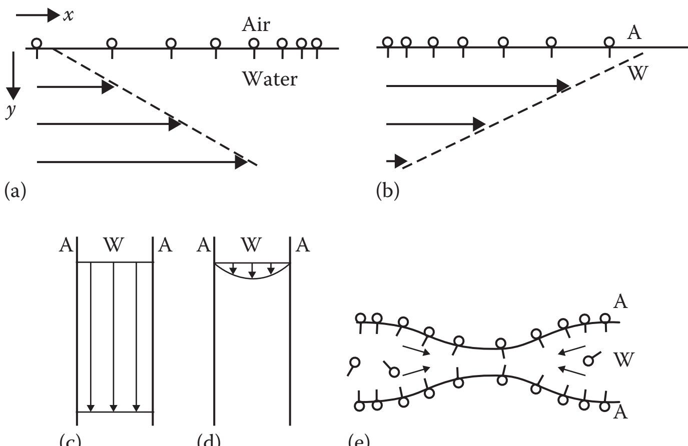


FIGURE 7.9 Surface tension gradients at the a/w interface. (a) Streaming of liquid along a surface causes a surface tension gradient. (b) Marangoni effect: a surface tension gradient cause streaming of the adjacent liquid. (c) Drainage of liquid from a vertical film in the absence or (d) presence of surfactant. (e) Gibbs mechanism of film stability. (After Walstra, P., Principles of foam formation and stability, in: *Foams: Physics, Chemistry and Structure*, Wilson, A.J., ed., Springer, London, U.K., 1989, pp. 1–15.)

means that a small air bubble or an emulsion droplet moving through the surrounding liquid in nearly all cases has an immobile surface, that is, it behaves like a rigid particle. In these cases, the γ-gradient can be quite large because Δx is small.

Figure 7.9b illustrates that liquid adjacent to an interface will move with the interface when the latter exhibits (for some reason, say, local adsorption of surfactant) an interfacial tension gradient. This is called the “Marangoni effect.” It is seen in a glass of wine, where wine drops above the liquid level tend to move upward; here, evaporation of ethanol causes a local increase in γ, thereby producing a γ-gradient.

An important consequence of the Marangoni effect is that it provides stability to a thin film, as illustrated in Figure 7.9e. If the film acquires somehow a thin spot, the surface area of the film is locally increased, hence Γ is lowered, γ is increased, and a γ-gradient is established. This causes adjacent liquid to flow to the thin spot, thereby restoring film thickness. This “Gibbs mechanism” explains the stability of thin liquid films, as in a foam.

Interfacial tension gradients are also paramount in preventing coalescence of newly formed drops during emulsification, as discussed in Section 7.6.2. In all these situations, the effects depend on film or Gibbs elasticity, which is defined as twice the surface-dilational modulus (twice because a film has two surfaces). Thin films typically have a large elasticity, because of the scarcity of dissolved surfactant. In a thick film containing a fairly high concentration of surfactant, surfactant molecules can rapidly diffuse toward a spot with a low surface load and restore the original surface tension. This cannot, or only very slowly, occur in a thin film, implying a large elasticity, except at quite long time scales.

7.2.7 Functions of Surfactants

Surfactants in a food, whether small-molecule amphiphiles or proteins, can produce several effects and these are briefly summarized here:

1. Due to the lowering of γ, the Laplace pressure is lowered and the interface can be more easily deformed. This is important for emulsion and foam formation (Section 7.6.2) and for the occurrence of coalescence (Section 7.6.4).

2. Contact angles are affected, which is important for wetting and dispersion events. The contact angle determines whether a solid particle can adsorb on a fluid interface and to what extent it then sticks out in either fluid phase. These aspects have an important bearing on stability of some emulsions (Section 7.6.5) and foams (Section 7.7.2).
3. A decrease in interfacial free energy will proportionally slow Ostwald ripening. The rate of Ostwald ripening may also be affected by the surface-dilational modulus (Section 7.7.2).
4. The presence of surfactants allows the creation of surface tension gradients and this may be their most important function. It is essential for formation and stability of emulsions and foams (Sections 7.6.2, 7.6.4, 7.7.1, and 7.7.2).
5. Adsorption of surfactants onto particles may greatly modify (colloidal) interparticle forces, mostly enhancing repulsion and thereby stability. This is discussed in Section 7.3.
6. Small-molecule amphiphiles can form micelles, which can harbor some hydrophobic molecules, say, oil molecules, in their interior. This greatly enhances the apparent solubility of several hydrophobic substances and forms the basis of detergency.
7. Small-molecule surfactants may undergo specific interactions with macromolecules. Ionic amphiphiles often associate with proteins, thereby materially altering some protein properties (e.g., isoelectric pH, apparent solubility, surface activity). Another example is the interaction of some lipid-like surfactants with amylose.

7.2.8 Summary

- Interfaces are characterized by a contracting force, the surface or interfacial tension γ ($N\ m^{-1}$).
- Adsorption of surfactants leads to a lower γ.
- Two main types of surfactants are polymers (including proteins) and small amphiphilic molecules.
- When two liquids and a solid or three liquids meet, there will be a contact angle between the respective interfaces, which strongly affects wetting and dispersion events.
- Curved interfaces give rise to the so-called Laplace pressure between the concave and convex side of the interface, giving rise to phenomena such as capillary rise, Ostwald ripening, and resistance to deformability of small droplets.
- The rheological properties of an interface containing surfactants can be distinguished as those providing the resistance to shearing deformation (important for several polymeric surfactants including many proteins) and those opposing dilational deformation (important for all surfactants).
- Flow of a liquid along an interface with surfactants leads to surface tension gradient and the other way round. These play an important role in foam stability, preventing coalescence of just formed emulsions droplets, and the Marangoni effect (i.e., retardation of liquid drainage).

7.3 COLLOIDAL INTERACTIONS

In Section 7.1.2, colloidal systems were defined and classified. Generally, forces between colloidal particles originate from material properties of the particles and the interstitial fluid. These colloidal interaction forces act in a direction perpendicular to the particle surface, contrary to the surface forces discussed in Section 7.2, which act in the plane of the surface. These forces could be attractive as well as repulsive.

The net interaction force acting between colloidal particles has important consequences:

1. It determines whether particles will aggregate (Section 7.4.3), which, in turn, may determine further physical instability. For instance, aggregation of particles may lead to increased sedimentation, hence to a rapid formation of a cream layer or sediment. (*Note on terminology*: The terms flocculation and coagulation are also used, often with a more specific connotation; the former would, for instance, refer to reversible aggregation, the latter to irreversible.)
2. In other situations, aggregating particles may form a space-filling network, hence a gel (Section 7.5), and the rheological properties and the stability of systems containing such a network strongly depends on colloidal interaction.
3. The interaction forces greatly affect susceptibility of emulsion droplets and gas bubbles to coalescence, and also partial coalescence of fat globules (Sections 7.6.4 and 7.6.5).

The net effect of colloidal interactions can also depend on external forces, for example, due to gravity or agitation or an electric potential gradient, and on size and shape of the particles. Furthermore, the adsorption of surfactants on the particles may greatly modify the strength of repulsive forces.

We will briefly discuss some aspects of colloidal interactions, generally for the simple cases of identical spheres. Literature on colloid science can be found in textbooks mentioned in the "Further Reading" section.

7.3.1 van der Waals Attraction

van der Waals forces between molecules are ubiquitous, and they also act between larger entities such as colloidal particles. Since these forces are additive, it turns out that, within certain limits, the dependence of the interaction force on interparticle distance (as measured between the outer surfaces) is much weaker between particles than between molecules. For two identical spherical particles the van der Waals interaction free energy is given by

$$V_A \approx \frac{Ar}{12h}, \quad h <\sim 10 \text{ nm} \tag{7.11}$$

where
r is the particle radius
h is the interparticle distance
A is the Hamaker constant

The latter depends on the material of the particles and of the fluid in between, and it increases in magnitude as the differences in the properties of the two materials increase. For most particles in aqueous foods, A is between 1 and 1.5 times kT ($kT \approx 4 \times 10^{-21}$ J at room temperature), but for air bubbles in water, A is much larger, about 10 times kT. Tabulated values are available [33,46,47,85].

If both particles are of the same material and the fluid in between is different, A always is positive and the particles attract each other. If the two particles are of different materials, A may be negative, and there would be van der Waals repulsion, but this is fairly uncommon.

7.3.2 Electric Double Layers

Most particles in an aqueous solution exhibit an electric charge, because of adsorbed ions or ionic surfactants. In most foods, charges predominantly are negative. Since the system must be electroneutral, the particles are accompanied by a cloud of oppositely charged ions, called counterions.

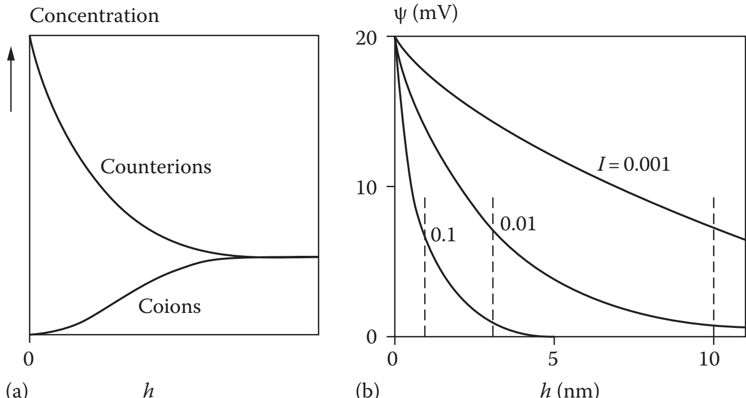


FIGURE 7.10 The electric double layer. (a) The distribution of counterions and coions as a function of the distance *h* from the charged surface. (b) The potential ψ as a function of distance for three values of the ionic strength *I* (mM); the broken lines indicate the Debye length (1/κ).

An example of the distribution of counterions and coions is shown in Figure 7.10a. It is apparent that at a certain distance from the surface, the concentrations of positive and negative charges in the solution become equal. Beyond that distance, the charge on the particle is neutralized, due to an excess of counterions in the electric double layer. The latter is defined as the zone between the particle surface and the plane at which neutralization is achieved. The double layer should not be envisaged as being immobilized, because solvent molecules and ions diffuse in and out of the layer.

The electrical effects are usually expressed in the electric potential ψ (in volts). Its value, as a function of the distance *h* from the surface, is given by

$$\psi = \psi_0 \exp(-kh) \tag{7.12}$$

where ψ_0 is the potential at the surface and the nominal thickness of the electric double layer or Debye length 1/κ is given by

$$\kappa \approx 3.2 I^{0.5}\ (\text{nm}^{-1}) \tag{7.13}$$

for dilute aqueous solutions at room temperature. The ionic strength *I* depends on the total ion concentration and is defined as

$$I \equiv \frac{1}{2} \sum m_i z_i^2 \tag{7.14}$$

where
m is molar concentration
z is valence of each of the ionic species present

Note that for a salt like NaCl, *I* equals the molarity of the solution, but this is not so if ions of higher valence are present. For $CaCl_2$, *I* is three times the molarity.

Calculations of the potential as a function of distance are in Figure 7.10b. Ionic strengths in aqueous foods vary from 1 mM (a typical tap water) to more than 1 M (pickled foods). The *I*-value of milk is about 0.075 M and of blood is about 0.14 M. Consequently, the thickness of the double layer is often only about 1 nm or less.

Electrical interactions depend on the surface potential and this, in turn, is generally dependent on pH. For most food systems, values of $|\psi_0|$ are below 30 mV. At a high concentration of counterions (especially if these are divalent), ion pairs can be formed between counterions and charged groups on the particle surface, thereby lowering $|\psi_0|$.

In a nonaqueous phase, the dielectric constant generally is much smaller than in water, and Equation 7.12 is no longer valid. Moreover, in this situation generally the ionic strength will be negligible. This means that even if there is a charged surface (as may be the case for aqueous droplets floating in oil), electrical interactions forces will generally be unimportant.

7.3.3 Deryagin–Landau, Verwey–Overbeek Theory

If electrically charged particles having the same sign come very close to each other, their electric double layers overlap. This is sensed and the particles repulse each other. The repulsive electric interaction free energy V_E can be calculated. For spheres of equal size, it is in first approximation given by the proportionality

$$V_E \propto r\, \psi_0^2 \exp(-kh) \tag{7.15}$$

The interaction energies V_A (due to van der Waals attraction) and V_E can be added, and this has led to the first useful theory for colloid stability, the Deryagin–Landau, Verwey–Overbeek (DLVO) theory. This theory enables calculation of the total free energy V needed to bring two particles from infinite distance to a distance h. We will not elaborate on this because accurate calculation is generally not possible for food systems. The total interaction energy is usually divided by kT, that is, the average kinetic energy involved in an encounter between two particles by Brownian (heat) motion.

Some trends will be discussed with reference to Figure 7.11. Curve 1 gives an example of van der Waals attraction. It always becomes stronger for a smaller interparticle distance. Curve 2 is an

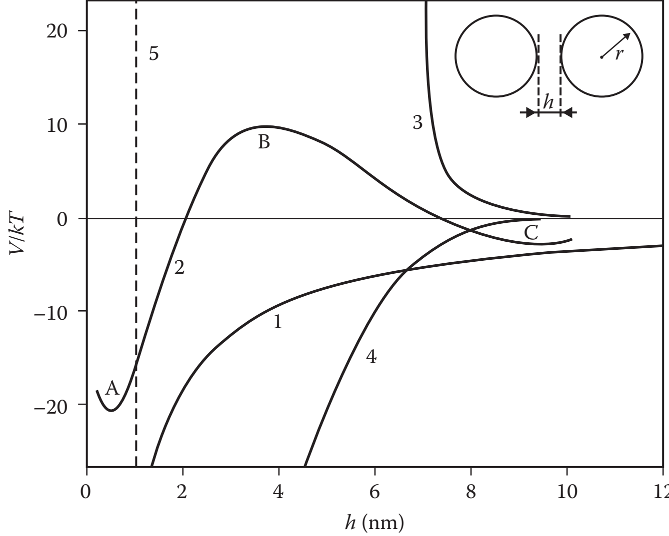


FIGURE 7.11 Calculated examples of the interaction free energy V between two particles as a function of their separation distance h; the insert shows the geometry considered. Curve (1) van der Waals attraction, (2) DLVO interaction, (3) steric repulsion, and (4) depletion interaction. (See the text for further explanation.) (From Walstra, P. et al., *Dairy Science and Technology*, CRC/Taylor & Francis, Boca Raton, FL, 2006.)

example of the sum of van der Waals attraction and electrostatic repulsion. In this case there is a so-called secondary minimum in the curve near C. Brownian motion will then readily cause a pair of particles to reach this point. The value of V is here about $-3kT$, sufficient to cause aggregation of the particles. However, the particles can deaggregate again, because the net attraction is only weak in this example. The particle pair may even occasionally overcome the free energy maximum at point B (about $10kT$), which implies that then they will reach the primary minimum A, which is so deep that the particles become permanently aggregated. Equation 7.11 would even predict that V_A would go to $-\infty$ at $h \rightarrow 0$, but at very small h, hard-core repulsion between the atoms in the surface layer of the two particles will prevent this.

Generally, it is not possible to alter van der Waals attraction, but electrostatic repulsion can be readily modified. Lowering of the ionic strength causes the repulsion to act over a greater distance and will virtually remove the secondary minimum. An increase of the charge on the particles, hence of $|\psi_0|$, either by adding an ionic surfactant or by manipulating the pH, causes especially the maximum in the curve to increase, thereby preventing permanent aggregation. If both $|\psi_0|$ is low and I is high, attraction will prevail over all distances and the particles will rapidly aggregate.

Generally, even though the DLVO theory is very successful for many inorganic systems, it is inadequate for predicting stability of most biogenic systems. Milk fat globules, for example, are stable against aggregation at their isoelectric pH (3.8), where they have zero surface potential, so that the DLVO theory would predict zero repulsion [95]. Consequently, interaction forces other than those considered in this theory must be important.

7.3.4 Steric Repulsion

As depicted in Figure 7.4, some adsorbed molecules (polymers, Tweens, etc.) have flexible molecular chains ("hairs") that protrude into the continuous phase. These may cause steric repulsion. Two mechanisms can be distinguished. First, if the surface of another particle comes close, the hairs are restricted in the conformations they can assume, which implies loss of entropy, hence increase of free energy, and repulsion occurs. This volume-restriction effect can be very large, but it can be of importance only if the surfaces have a very low hair density (number of hairs per unit area). This is because the hairy layers start to overlap on approach of the particles and then a second mechanism will act before the first one comes into play. The overlap causes an increased concentration of protruding hairs and thereby an increased osmotic pressure; this then leads to water moving to the overlap region, which results in repulsion. However, this is true only if the continuous phase is a good solvent for the hairs; if it is not, attraction may result. For example, emulsion droplets covered by casein have protruding hairs, providing stability to the droplets. If ethanol is added to the emulsion, the solvent quality is strongly decreased and the droplets aggregate [19].

In some cases, steric repulsion free energy can be calculated with reasonable accuracy [27]. If these values are added to the van der Waals attraction, curves for total interaction versus interparticle distance are obtained. The solvent quality usually is of overriding importance, and if it is good, repulsion can be very strong (cf. curve 3 in Figure 7.11). In practical food systems, calculation of steric repulsion usually is not possible because the situation is too complex. For instance, the nature of the adsorbing molecules can vary greatly [22,27,89]. An example is proteins that exhibit protruding hairs upon adsorption, such as the caseins; these hairs carry electric charges that can increase repulsion.

On the other hand, adsorbing polymers may cause bridging aggregation, when becoming simultaneously adsorbed onto two particles [27,89]. This may happen if too little polymer is present to fully cover the particle surface area or with certain methods of processing. Moreover, interparticle bonds may be formed between adsorbed proteins, for example, due to –S–S– bridge formation at high temperatures or –Ca– bridges between negative charges on the hairs if sufficient

Ca^{2+} is present. Altogether, subtle changes in composition of an aqueous dispersion may have a profound effect on colloidal stability.

7.3.5 Depletion Interaction

Besides polymer chains protruding from a surface, polymer molecules in solution can affect colloidal interaction. Consider a liquid dispersion, for example, an emulsion, which also contains some (nonadsorbing) dissolved polymer, say, xanthan. The center of a polymer molecule cannot come closer to the surface than a value δ, which is about equal to its radius R_g, as illustrated in Figure 7.12. Hence, a layer of liquid is depleted of polymer. This means that the concentration of polymers in the bulk liquid is increased due to the presence of emulsion droplets. Consequently, the osmotic pressure Π_{osm} of the solution is increased. If now two droplets come close (i.e., become aggregated), part of their depletion layers overlaps, and the concentration of polymer in the bulk liquid decreases. Hence, the osmotic pressure decreases. Since the system will always try to make the osmotic pressure as small as possible, there is a driving force for aggregation of the droplets. The interaction energy is approximately given by

$$V_D \approx -2r\Pi_{osm}(2\delta - h)^2, \quad 0 < h < 2\delta, \quad r \gg \delta \tag{7.16}$$

where
  $\delta = R_g$ is the closest distance of approach of the polymer molecules
  h is the distance between the particles
  r is the radius of the particles

It is, in first approximation, proportional to the molar concentration of the polymer, and it will also depend on the solvent quality.

The result is that polysaccharides can cause depletion aggregation in foods, even at low concentrations; for example, 0.03% xanthan ($R_g \approx 30$ nm) may be sufficient [19]. An example is given in curve 4 of Figure 7.11. Substantially higher concentrations of polymer often lead to the formation of a particle gel, which implies immobilization of the particles (Sections 7.4.2 and 7.5.2).

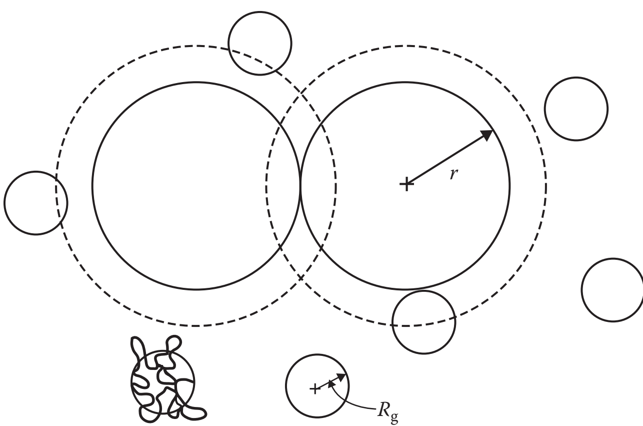


FIGURE 7.12 Schematic of the depletion of nonadsorbing polymer molecules (radius of gyration R_g, depicted by small circles) from the surface of colloidal particles (radius r, depicted by large circles) and of the overlap of the depletion zones (bounded by broken lines) when the particles are aggregated. (Redrawn from Walstra, P., in: *Food Colloids and Polymers: Stability and Mechanical Properties*, Dickinson, E. and Walstra, P., eds., Royal Society of Chemistry, Cambridge, U.K., 1993, pp. 1–15.)

TABLE 7.4
Factors Affecting the Magnitude of Various Contributions to the Interaction Free Energy (*V*) between Particles in Aqueous Systems

Variable	V_A	V_E	V_S
Particle size	+	+	(+)
Particle material	+	–	–
Adsorbed layer	(+)	+	+
pH	–	+	–[a]
Ionic strength	–	+	–[a]
Solvent quality	–	–	+

Subscripts: A, van der Waals attraction; E, electrostatic repulsion; S, steric repulsion. +, effect; –, no effect; (+), effect under some conditions.

[a] In the absence of electrical charges.

7.3.6 Other Aspects

It should now be clear that several kinds of colloidal interactions can occur in foods and that the kind and concentration of surfactants present strongly influence these interactions. Even in the simplest cases, several variables are important (Table 7.4).

Several additional complications may be mentioned. The DLVO theory does not apply at very small distances, nor is the predicted effect of particle size obeyed. The cause may be surface roughness.

At very small distances, *hydrophobic interactions* may occur, and they generally cause attraction. The effect is the result of poor solvent quality. This type of interaction has a strong temperature dependence, being very weak near 0°C and increasing with increasing temperature.

Such hydrophobic interactions may, in principle, occur if a *protein* is the surfactant. However, even in this case, the net result tends to be repulsion. This is due to a combination of steric and electrostatic repulsion, but calculation of the interaction energy generally is not possible. If the pH is near the isoelectric point of the adsorbed protein, electrostatic repulsion may change into electrostatic attraction between negative and positive groups on the surfaces. Moreover, hydrophobic attraction can now occur and protein-covered particles generally aggregate near their isoelectric pH.

7.3.7 Summary

- Colloidal interactions determine stability of particles against aggregation, which in turn affects other physical instabilities.
- van der Waals forces, between similar particles, are always attractive.
- Electric repulsive and attractive forces exist between charged particles; electric double layers form around charged particles.
- DLVO theory describes the sum of attractive van der Waals forces and repulsive forces from overlapping double layers.
- Steric repulsion forces are created by adsorbed polymers.
- Depletion interaction between colloidal particles is facilitated by dissolved polymers in aqueous phase.

7.4 LIQUID DISPERSIONS

7.4.1 Description

Several types of liquid dispersions exist. The discussion here will be limited to suspensions (solid particles in a liquid) and to those aspects of emulsions that follow the same rules. Foods that are suspensions include skim milk (casein micelles in milk serum), fat crystals in oil, many fruit and vegetable juices (cells, cell clusters, and cell fragments in an aqueous solution), and some fabricated foods (e.g., soups). During processing (food fabrication), suspensions are also encountered, for example, starch granules in water, sugar crystals in a saturated solution, and protein aggregates in an aqueous phase.

Dispersions are subject to several kinds of instability, and these are schematically illustrated in Figure 7.13. Changes in particle size and in their arrangement are distinguished. Formation of small aggregates of particles may be considered to belong to both categories. Dissolution and growth of particles depend on the concentration of the material, its solubility, and on diffusion. In a supersaturated solution, nucleation must occur before particles can be formed. Dissolution, nucleation, and growth will not be discussed further. Ostwald ripening is discussed in Sections 7.2.4 and 7.7.2 and coalescence in Section 7.6.4. The other changes are discussed in Figure 7.13.

The various changes may affect each other, as is illustrated in the figure. Moreover, sedimentation is enhanced by any growth in particle size, and sedimentation will enhance the rate of aggregation if the particles tend to aggregate. Agitation of the liquid may enhance the rate of some changes, but it can also disturb sedimentation and disrupt large aggregates.

7.4.2 Sedimentation

If there is a difference in density (ρ) between the dispersed phase (subscript D) and the continuous phase (subscript C), there is a buoyancy force acting on the particles. According to Archimedes, the net force in the direction of sedimentation for spheres is given by $a\pi d^3(\rho_D - \rho_C)/6$, where a

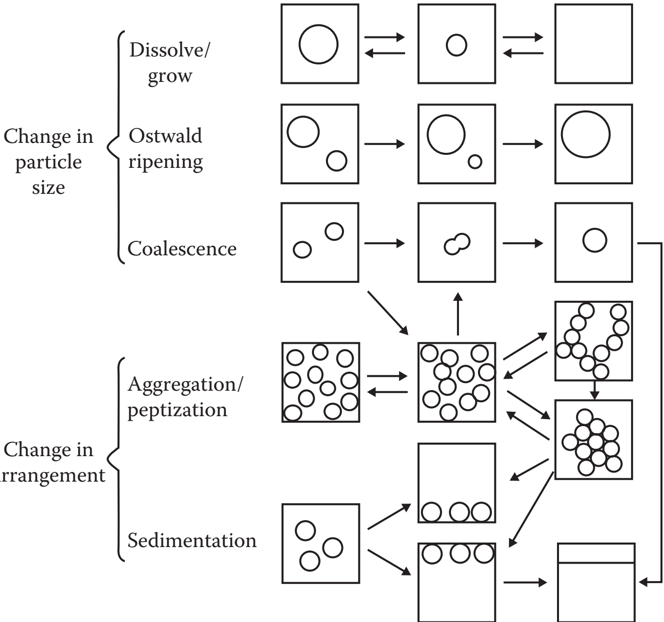


FIGURE 7.13 Illustration of the various changes in dispersity. Highly schematic.

is acceleration. As the sphere accelerates, it encounters a friction force, which equals, according to Stokes, $3\pi d\eta_C v$, where η_C is the viscosity of the continuous phase and v is the instantaneous velocity (with respect to the continuous phase). By putting both forces equal, the equilibrium or Stokes sedimentation velocity is obtained:

$$v_S = \frac{a(\rho_D - \rho_C)d^2}{18\eta_C}. \quad (7.17)$$

If the particles show a size distribution, d^2 should be replaced by $\sum n_i d_i^5 / \sum n_i d_i^3$, where n_i is the number of particles per unit volume in class i with diameter d_i.

For gravity sedimentation, $a = g = 9.81$ m s^{-2}; for centrifugal sedimentation, $a = R\omega^2$, where R is the effective radius of the centrifuge and ω its rotation rate in radians per second. To give an example: if the sphere diameter is 1 μm, the density difference is 100 kg m^{-3} and the viscosity of the continuous phase is 1 mPa s (i.e., water), then the spheres would sediment under gravity at a rate of 55 nm s^{-1} or 4.7 mm per day. Sedimentation greatly depends on particle size, and spheres of 10 μm would move 47 cm in a day. Normally, viscosity decreases and sedimentation rate increases with increasing temperature. If the density difference in Equation 7.17 is negative, sedimentation is upward, and one commonly speaks of creaming; downward sedimentation may be called settling.

The Stokes equation is very useful to predict trends, but it is almost never truly valid. Among the many factors causing deviation from Equation 7.17 [92], the following are the most important for foods:

1. The particles are not homogeneous spheres. An anisometric particle tends to sediment more slowly, because it orients itself during sedimentation in such a way as to maximize friction (i.e., a plate-shaped particle will adopt a "horizontal" orientation). An aggregate of particles, even if spherical, sediments more slowly than a homogeneous sphere of the same size, since the interstitial liquid in the aggregate causes the effective density difference to be smaller.
2. Convection currents in the dispersion caused, for instance, by slight temperature fluctuations, may strongly disturb sedimentation of small particles (<–1 μm).
3. If the volume fraction of particles ϕ is not very small, sedimentation is hindered, roughly according to

   $$v = v_S(1 - f)^8 \quad (7.18)$$

   For $\phi = 0.1$, the sedimentation rate then is already reduced by 57%.
4. If particles aggregate, sedimentation velocity increases: the increase in d^2 is always larger than the decrease in $\Delta\rho$. Moreover, as larger aggregates sediment faster, they overtake smaller ones and thus become even larger, leading to an even greater acceleration of sedimentation rate. This may enhance sedimentation by orders of magnitude. A good example is rapid creaming in cold raw milk, where fat globules aggregate due to the presence of cryoglobulins [95].
5. An assumption implicit in Equation 7.17 is that viscosity is Newtonian, that is, independent of shear rate (or shear stress), and this is not true for many liquid foods. Figure 7.14 gives some examples of the dependence of apparent viscosity η_a on shear stress. The stress caused by a particle is given by the buoyancy force over the particle cross section, that is, about $g\Delta\rho d$ for spheres under gravity. The stress is on the order of 1 mPa for many particles. This then is the stress that the particles sense during sedimentation. Viscosity should be measured at that stress (σ) (or the corresponding shear rate, given by σ/η_a), whereas most viscometers apply stresses of well over 1 Pa. Figure 7.14 shows that the apparent viscosity can differ by orders of magnitude, according to the shear stress applied.

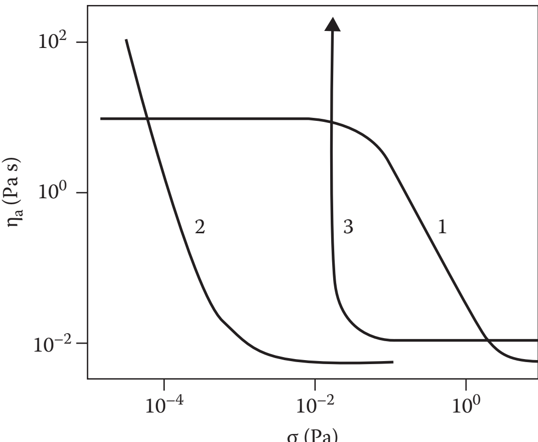


FIGURE 7.14 Schematic examples of non-Newtonian flow behavior of liquids: apparent viscosity η_a as a function of shearing stress σ. Curve 1 is typical for a polymer solution, Curve 2 for a weakly aggregating dispersion of very small particles, and Curve 3 for a system exhibiting a yield stress. (From Walstra, P., Emulsion stability, in: *Encyclopedia of Emulsion Technology*, Vol. 4, Becher, P., ed., Dekker, New York, 1996, pp. 1–62.)

Also shown in Figure 7.14 is an example of a liquid exhibiting a small yield stress. Below that stress the liquid will not flow. This is, however, never noticed during handling, because the yield stress is so very small (a stress of 1 Pa corresponds to a water "column" of 0.1 mm height). Nevertheless, it is often sufficient to prevent sedimentation (or creaming), as well as aggregation. Among liquid foods exhibiting a yield stress are soya milk, many fruit juices, chocolate milk, and several dressings. These aspects are discussed further in Section 7.5.3.

7.4.3 Aggregation

Particles in a liquid exhibit Brownian motion and thereby frequently encounter each other. Such encounters may lead to aggregation, defined as a state in which the particles stay close together for a much longer time than they would do in the absence of attractive colloidal interaction. The rate of aggregation is usually calculated according to Smoluchowski's theory of perikinetic aggregation [66]. The initial aggregation rate in a dilute dispersion of spheres of equal size is

$$-\frac{dN}{dt} = \frac{4kTN^2}{3\eta W} \tag{7.19}$$

where N is the number of particles, that is, unaggregated particles plus aggregates, per unit volume. The stability factor W was assumed to equal unity by Smoluchowski. The time needed to halve the number of particles then is

$$t_{0.5} = \frac{\pi\eta d^3}{8kT\phi} \tag{7.20}$$

where ϕ is the particle volume fraction. This results in $d^3/10\phi$ s for particles in water at room temperature where d is in μm. For $d = 1$ μm and $\phi = 0.1$, this results in 1 s, implying that aggregation would be very rapid.

In most practical situations, aggregation is much slower, because W often has a large value. The magnitude of the stability factor is primarily determined by the colloidal repulsion between the particles (Section 7.3). If it is desired, for example, to increase the halving time from 1 s to 4 months, this would need a W-value of 10^7.

Direct use of Equation 7.19 to predict stability is rarely possible in food systems. There are numerous complications, and some of the more important ones are as follows: (1) it is generally impossible to predict the value of W; (2) the stability factor may change with time (an example is enzymatic hydrolysis of $-COOCH_3$ groups on pectin to $-COO^-$ groups, which can then form bridges with Ca^{2+} ions present); (3) there are other encounter mechanisms, due to streaming (agitation) or sedimentation; and (4) aggregation may take various forms, leading to coalescence (which may occur with oil droplets) or to the formation of aggregates. Nevertheless, application of aggregation theory often is possible and useful, but it is far more intricate than can be discussed here [9,89,92].

According to the nature of the interaction forces between aggregated particles (Section 7.3.3), agents can be added to cause *deaggregation*. This can be done to stabilize a food product and also—in the laboratory—to establish the nature of the forces. It should be realized that often more than one type of force is acting. Diluting with water may cause deaggregation due to (1) lowering of osmotic pressure (if depletion interaction was the main cause of aggregation), (2) lowering ionic strength (which enhances electrostatic repulsion), or (3) enhancing solvent quality (which can increase steric repulsion). Electric forces can also be manipulated by altering pH. Bridging by divalent cations can often be undone by the addition of a chelating agent, say, EDTA. Bridging by adsorbed polymers or proteins can mostly be undone by the addition of a suitable small-molecule surfactant (Section 7.2.2). Reversal of specific interactions (e.g., –S–S– bridges) requires specific reagents. Also, a change in temperature can affect aggregate stability, by altering solvent quality.

If the forces between the particles in an aggregate are not very strong, deaggregation can be achieved by *shear forces*. These exert a stress $\eta \cdot \nabla v$, where ∇v is the velocity gradient (shear rate). In water, $\nabla v = 10^3\ s^{-1}$ would be needed to achieve a shear stress of 1 Pa, which does not seem very large. Nevertheless, shear stresses as occurring during agitation and flow are often sufficient to (partly) break up large aggregates.

Another aspect is that bonds may *strengthen* after aggregation. It may be better to speak of *junctions* between particles, since any such junction may represent many (up to, say, a hundred) separate bonds. Strengthening can occur by several mechanisms [89].

Aggregation of particles in liquid foods often is undesirable. It may lead to inhomogeneity of the product, for example, because aggregation strongly enhances sedimentation, or it may induce coalescence of emulsion droplets. In other cases, weak aggregation may be desirable. It may lead to the formation of a space-filling network of aggregated particles, hence to a (weak) gel. This is further discussed in Section 7.5.2.5. Consequently, the particles are immobilized and do not, or very sluggishly, sediment. Examples are cocoa particles in chocolate milk and cells and tissue fragments in soy bean milk (see Figures 7.17 and 7.20).

7.4.4 Summary

- Particles in liquid dispersions exhibit changes in dispersity as a result of changes in particle size (dissolve/grow, Ostwald ripening, and coalescence) and in arrangement (sedimentation and aggregation).
- Sedimentation/creaming, caused by a difference in density, its speed depending on diameter of particles, density difference, viscosity of continuous phase, and acceleration.
- Aggregation/deaggregation depends on the balance between the interaction forces between the particles as a function of the distance, changes in the dispersion properties due to ionic strength and pH, and the presence of shear forces due to streaming.

7.5 SOFT SOLIDS

Many foods are "soft solids," for example, bread, margarine, peanut butter, tomato ketchup, and cheese. Another term often used is "semisolid." Both terms are ill-defined. They exclude foods that readily flow, as well as true solids, that is, foods that show at most an elastic (i.e., fully recoverable) deformation under a force applied by hand. Virtually, all soft solids are composite materials, which implies that they are inhomogeneous on a mesoscopic, or even on a macroscopic, scale. The main structural classes are as follows:

Gels: These are characterized by a predominance of liquid (*solvent)* and the presence of a continuous matrix of interconnected material. This space-filling network provides the solid character.

Closely packed systems: In these, deformable particles make up by far the largest volume fraction, whereby they deform each other to some extent. The interstitial material is a liquid or, in a few cases, a weak gel. Examples are vegetable purées (e.g., tomato ketchup and apple sauce), concentrated emulsions (e.g., mayonnaise), and polyhedral foams (e.g., beer foam). Concentrated starch gels consisting of highly swollen, partially gelatinized, starch granules also belong to this category; when the granules are destroyed, a highly viscous macromolecular "solution" results.

Cellular materials: Most vegetable and fruit tissues belong to this category. They are characterized by connected, fairly rigid cell walls, enclosing a liquid-like material.

Not all soft solids fit this classification. Meat, for example, has a fibrous structure. Moreover, intermediate types occur. As main classes of soft solids we will primarily discuss gels (Sections 7.5.2 through 7.5.4). These sections will be preceded by a section on phenomena that may occur when various biopolymers are mixed at concentrations (clearly) higher than 1%.

7.5.1 Phase Separation of Mixtures of Biopolymers

Many food products contain mixtures of biopolymers, often mixtures of proteins and polysaccharides. In solution, the nature of the protein–polysaccharide interaction has a large effect on the properties of the mixed system. In general, the following three different situations can be distinguished when a protein and a polysaccharide solution are mixed:

1. The protein and the polysaccharide may mix. However, this result is rare on mixing higher concentrations of both biopolymers, especially if those have a high molar mass.
2. Both biopolymers associate leading to the formation of protein–polysaccharide complexes, *complex coacervation* or associative phase separation.
3. *Thermodynamic incompatibility* or segregative phase separation.

If mixing or phase separation happens, it depends on the sign of the change in Gibbs free energy of mixing ΔF_{mix}, which is given by

$$\Delta F_{\text{mix}} = \Delta H_{\text{mix}} - T\Delta S_{\text{mix}} \tag{7.21}$$

where

ΔH_{mix} is the enthalpy of mixing
ΔS_{mix} the entropy of mixing

If $\Delta F_{\text{mix}} \leq 0$ mixing takes place and when $\Delta F_{\text{mix}} > 0$, the system separates into separate phases. Phase separation may be caused by an increase in ΔH_{mix} or a decrease in ΔS_{mix}, for example, due to

an increase in biopolymer concentration or caused by a change in conditions such as pH and ionic strength. Note that the entropy of mixing in J mol^{-1} K^{-1} will be (much) smaller for polymers than for small molecules and decreases with increasing molecular weight, for example, due to aggregation.

7.5.1.1 Thermodynamic Incompatibility

Thermodynamic incompatibility will be the most usual result when proteins and polysaccharides are mixed at concentrations higher than usually a few percent but may also occur when, for example, two proteins or polysaccharides are mixed (e.g., amylose and amylopectin) in fairly dilute (gelatinized) starch solutions. For a protein–polysaccharide mixture, it leads to a phase rich in protein (and poor in polysaccharide) and a phase rich in polysaccharide and poor in protein [31,71]. This happens, for instance, in mixtures of gelatin and the polysaccharides dextran and maltodextrin.

Thermodynamic incompatibility occurs when the interactions between the macromolecules are repulsive and/or when their affinity toward the solvent differs. Generally, macromolecules prefer to be surrounded by identical molecules or by solvent. The concentration needed for phase separation in biopolymer mixtures is lower when the molar mass of the biopolyers is higher. Important biopolymer properties that impact phase separation are charge density and conformation. Linear polysaccharides are more incompatible with proteins than branched ones. For instance, for gellan gum the coil to helix transition was found to stimulate phase separation. Unfolding of globular proteins also favors phase separation. In addition, aggregation of one of the biopolymers stimulates it.

Proteins and various polysaccharides are polyelectrolytes. If the pH is not close to the isoelectric point and the ionic strength is low, phase separation does not occur. Under these conditions, salt ions might partition between the biopolymers, causing considerable loss of entropy of mixing. The relative difference in salt concentration between the concentrated polyelectrolyte phase and the other phase will decrease with increase of ionic strength and vanished at about 0.1 M, allowing phase separation of protein at pH farther away from the isoelectric point. Close to the isoelectric point, low salt promotes phase separation because the solubility of the protein decreases with decreasing ionic strength.

Figure 7.15a gives a hypothetical phase diagram for a phase separating system. The tie lines indicate how the separation will be. A mixture of composition A will separate into phases of

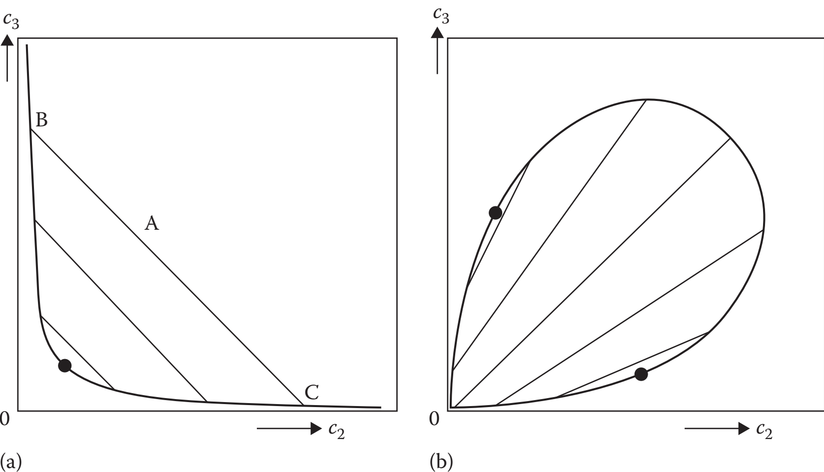


FIGURE 7.15 Idealized cases of phase separation in aqueous mixtures of two macromolecules at concentrations c_2 and c_3. (a) Segregative phase separation or thermodynamic incompatibility. (b) Associative phase separation or complex coacervation. The heavy lines denote the bimodal (solubility limit) and the thin ones the tie lines. The dots indicate critical points. (From Walstra, P., *Physical Chemistry of Foods*, Marcel Dekker, New York, 2003.)

composition B and C. The ratio of the volumes of the two phases B and C is equal to the ratio of the distances AC/AB. The longer the tie line, the stronger the incompatibility. The dot gives the critical point, that is, the composition at which the tie lines vanish. At this point the two "hypothetical" formed phases will have equal composition and volume. It can be determined from the intersection of the line connecting the mid points of the tie lines through the binodal curve. In the region below the bimodal the two biopolymer solutions are completely miscible.

Phase diagrams are often asymmetric; the protein concentration needed for phase separation is usually higher than the polysaccharide concentration. The asymmetry being higher for globular proteins than for more or less unfolded molecules like gelatin and caseins.

Separation rate may vary from very slow to fast depending on the concentrations of both polymers and conditions such as temperature, pH, and ionic strength. Since phase separation often occurs at high concentrations, the diffusion of biopolymer molecules is very slow. At initial stages of phase separation, one of the phases forms droplets, resulting in the formation of a so-called water-in-water emulsion. Which phase becomes the dispersed one depends on the concentration ratio of both polymers and their properties. The interfacial tension between the phases is very small, 10^{-7} to 10^{-4} $N \cdot m^{-1}$. The droplets are easily deformable.

Demixing will be arrested if the continuous phase forms a gel before the system reaches equilibrium in a macroscopic phase separated two-layer system. Some examples of gels formed will be discussed in Section 7.5.4.5.

7.5.1.2 Complex Coacervation

Complex coacervation occurs if the interaction between the different polymers is attractive. A clear example is a mixture of a protein below its isoelectric pH (with positive groups) and a negative-charged polysaccharide at not too high ionic strength I. At high I the charged will be screened strongly. Examples of complex coacervation are, for instance, between an acid gelatin solution and gum arabic and between β-lactoglobulin and gum arabic at pH 2.5–4.5 and low I. As a result, often a two-phase system is formed: One phase containing a concentrated dispersion with the complex of both polymers and the other phase containing mainly water. An idealized phase diagram of this system is shown in Figure 7.15b. Besides coacervates, small soluble complexes may form. If interactions are weak, a homogeneous weak gel will result, and if they are strong, coprecipitation of both polymers will occur.

7.5.2 Gels: Characterization [80]

7.5.2.1 Structure

Types of gels may be distinguished on the basis of various criteria. For food gels, a main division is in polymer and particle networks (see Figure 7.16).

Polymer gels. The matrix consists of long, linear chain molecules, each of which is cross-linked to other molecules at various places along the chain. A subdivision can be made according to the nature of the cross-links: covalent bonds (Figure 7.16a) and physical (noncovalent) cross-links. The latter are predominant in food gels, for instance, salt bridges, microcrystalline regions (Figure 7.16b), or specific kinds of entanglements (see Sections 7.5.4.1 and 7.5.4.2). Another subdivision is that the chains between cross-links can be quite flexible, as in gelatin gels, or rather stiff, as in most polysaccharide gels.

Particle gels are illustrated in Figure 7.16c. As compared to polymer gels, most particle gel networks are much coarser (larger pores). A subdivision can be made into gels made up of hard particles, such as the triacylglycerol crystals in plastic fats, and of deformable particles, such as the casein micelles in various milk gels (e.g., set yoghurt).

It may further be noted that the physical cross-links between polymer molecules, as well as the regions of contact between particles, are better not called "bonds" but "junctions," since

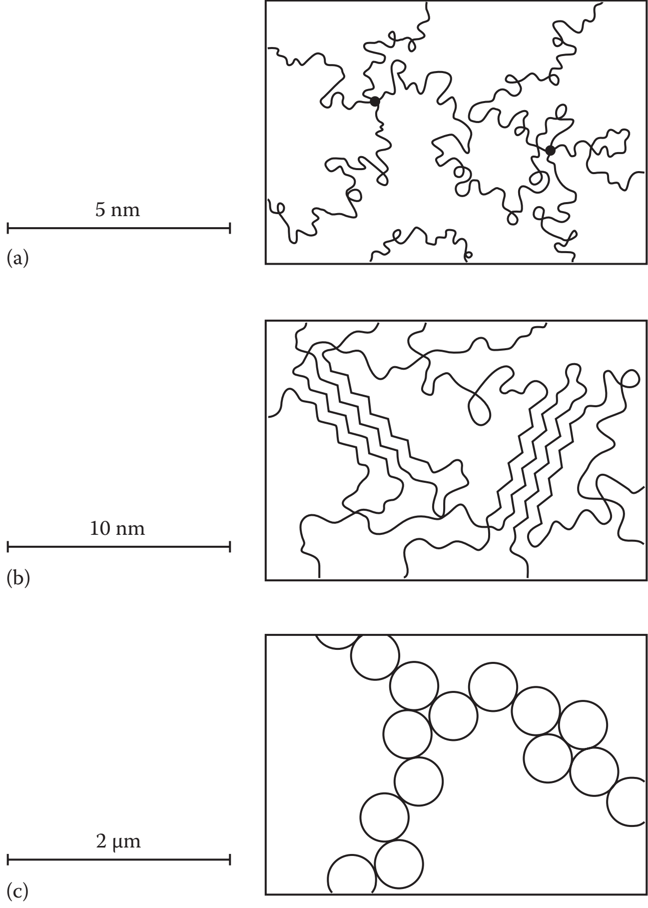


FIGURE 7.16 Highly schematic illustration of three types of gel structure. The dots in (a) denote cross-links. (a) Polymer gel, covalent cross-links, (b) polymer gel microcrystallites, and (c) particle gel. Note the differences in scale. (From Walstra, P., *Physical Chemistry of Foods*, Marcel Dekker, New York, 2003.)

such a junction generally contains many individual bonds, say, 10–100. Moreover, the bonds in one junction may be of various types (e.g., van der Waals, electrostatic, hydrophobic, and hydrogen bonds). Some proteins can also be cross-linked by covalent bonds (e.g., intermolecular –S–S– bonds).

Gelation can be *induced* in various ways, depending on the nature of the gelling material. In general, one may distinguish the following:

Cold-set gels: These are formed after heating to a temperature at which the network-forming material dissolves or forms a dispersion of very small particles. On subsequent cooling, a gel is formed as a result of the formation of physical cross-links. Examples are gelatin, κ-carrageenan, mixtures of locust bean gum and xanthan, and also plastic fats. In the case of polymer gels, cooling often involves a conformational transition of the network-forming molecules, for example, in κ-carrageenan.

Heat-set gels: When a solution of globular proteins is heated above its denaturation temperature, a gel may be formed if the protein concentration is above a critical value c_0; generally, these gels are irreversible and considerably increase in firmness upon cooling. The magnitude of c_0 depends on the nature of the protein, the physicochemical conditions,

and the rate of heating. Examples are egg white, soy protein isolate, whey proteins, and meat proteins. Moreover, some chemically modified polysaccharides can form reversible gels at high temperature. For example, cellulose ethers, such as methyl cellulose, which contains $-OCH_3$ groups, form a gel at high temperature via hydrophobic bonds.

Some gels are formed by *changing conditions* that affect molecular or colloidal interactions, such as pH, ionic strength, specific salts (e.g., Ca^{2+} ions), or enzyme action. Examples are rennet- and acid-induced milk gels and cold gelation by a pH change of a dispersion of thermally denatured globular-protein aggregates (e.g., of β-lactoglobulin or ovalbumin).

7.5.2.2 Rheological and Fracture Parameters

Most usage and eating properties of food gels are largely determined by their mechanical behavior (Section 7.5.3). For a better understanding of these aspects, we will now discuss some basic mechanical properties.

From a rheological point of view, a gel is a material that shows a predominantly *elastic* behavior over the time scale considered and has a modulus that is relatively small ($<10^7$ Pa) as compared to true solids. A modulus is defined as the ratio between a stress ($\sigma \equiv$ force/area) acting on a material and its ensuing relative deformation (or strain, ε); this only applies if σ/ε is independent of ε, which generally means for small deformations. Elastic behavior implies that the material instantaneously deforms under an applied stress to a strain that remains constant in time but returns instantaneously to its original shape as soon as the stress is released (see the broken line in Figure 7.17a).

However, for many gels the deformation is not purely instantaneous: after an initial elastic deformation, the material gradually deforms further during application of the stress (see Figure 7.17a). After removal of the stress, the gel does not return to its original shape, the difference increasing with the time during which the stress was applied. The gel thus exhibits a combination of elastic and viscous (flow) behavior; it is said to behave in a "viscoelastic" manner. Gelatin and κ-carrageenan gels, at temperatures well below their gel points, show almost pure elastic behavior, whereas rennet- and acid-induced milk gels are clearly viscoelastic.

Under a large stress, a gel may fracture or yield, depending on its structure and—in some gels—on the rate at which the stress is increased. *Fracture* implies that the stressed specimen breaks, mostly into several pieces. If the material contains a large proportion of solvent in wide pores, the

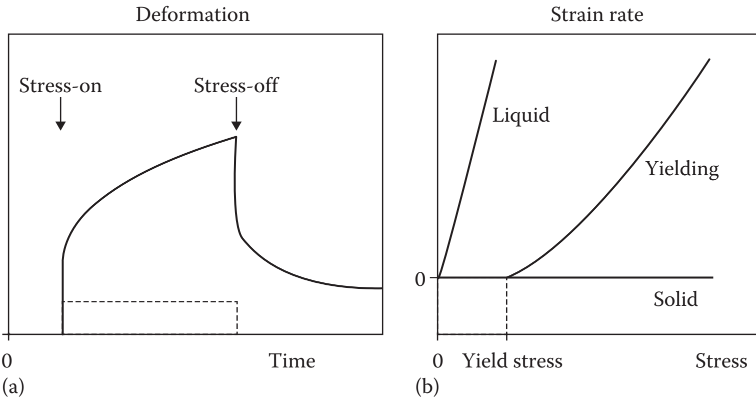


FIGURE 7.17 Viscoelasticity. (a) Example of the relation between deformation (strain) and time when a viscoelastic material is suddenly brought under a given stress, as well as after removal of the stress; the broken line is for a stress below the yield stress of the material. (b) Strain rate as a function of stress for a Newtonian liquid, a soft solid that shows yielding, and an elastic solid.

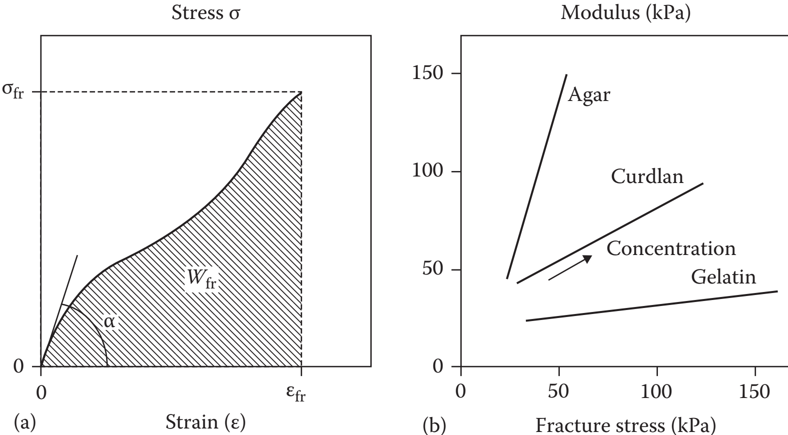


FIGURE 7.18 Large deformation. (a) Hypothetical example of the relation between stress and strain when a soft solid is deformed until it fractures; the modulus equals tan α. W_{fr} denotes the work of fracture. (b) Relation between modulus and fracture stress for gels of various materials (curdlan is a bacterial β-1,3-glucan polymer) at varying concentration. (After Kimura, H. et al., *J. Food Sci*., 38, 668, 1973.)

space between the pieces may be immediately filled with solvent, as is the case when a rennet-induced milk gel is cut during cheese making. *Yielding* implies that the gel starts to flow, while remaining a coherent mass (see Figure 7.17b). Butter, margarine, and most types of jam are examples of gels that yield, while gelatin, agar, and κ-carrageenan gels fracture.

The mechanical properties of gels vary greatly. Figure 7.18a gives a hypothetical stress–strain curve, ending at the point where fracture occurs. The modulus of the material *G*, also called *stiffness*, is the stress divided by the strain, provided that this quotient is constant; generally, the latter is only true for very small strains, often <1%. The *strength* of a material is primarily related to the stress at fracture σ_{fr}, not to the modulus. Terms like firmness, hardness, and strength are often used indiscriminately, but the sensorial attribute generally correlates with the fracture stress. Modulus and fracture stress need not be closely correlated, when comparing gels made at various concentrations (see Figure 7.18b) [36]. It is frequently observed that the addition of inert particles ("fillers") to a gelling material increases the modulus but decreases the fracture stress [44]. Part of the explanation is that a modulus is predominantly determined by number and strength of the bonds in the gel, whereas fracture properties strongly depend on the presence of large-scale inhomogeneities [80].

Properties described as "shortness" and "brittleness" are closely related to the reciprocal of the strain at fracture (ε_{fr}). The latter may vary widely. For gelatin, ε_{fr} equals about 3, and for some polysaccharide gels, a mere 0.1. For gels as depicted in Figure 7.16a and b, ε_{fr} greatly depends on the length and stiffness of the chains between the cross-links.

Another parameter is "toughness," which is related to the work of fracture W_{fr}. This derives from the area under the curve in Figure 7.18a and is expressed in J m^{-3}.

For a more extensive discussion of rheological and fracture properties of foods see [80].

7.5.2.3 Modulus

At small deformations, gels can be characterized by a modulus. A very general expression will be given for the modulus, based on a simplified model of a gel. In this model, the gel is built of strands that are mutually cross-linked. A strand can consist of a polymer chain or a chain of aggregated particles. When a force is applied to such a chain, this will result in a reaction force in the chain. This force is proportional to the deformation Δx times the derivative of the interaction force *f* with

respect to the distance x between the cross-links, df/dx. When multiplying both sides of the equation by the number N of stress-carrying strands per unit cross section, the following expression is obtained:

$$\sigma = -N \frac{df}{dx} \Delta x \tag{7.22}$$

The local change in distance can be recalculated to a macroscopic strain ε by dividing Δx by a characteristic length C, which is determined by the geometry of the network. (The calculation of C is intricate and is not given here.) Since f can generally be expressed as the derivative of the (Gibbs) free energy F with respect to distance x, we obtain

$$\sigma = CN \frac{d^2F}{dx^2} \varepsilon \tag{7.23}$$

Since $G = \sigma/\varepsilon$ and $dF = dH - T\,dS$, where H is enthalpy and S entropy, the following expression for the modulus results:

$$G = CN \frac{d^2F}{dx^2} = CN \frac{d(dH - T\,dS)}{dx^2} \tag{7.24}$$

7.5.2.4 Polymer Gels

Deformation of a gel with long and flexible polymer chains between cross-links leads primarily to a change in conformation of these chains, implying that the entropy of the network decreases. This means that the enthalpy term in Equation 7.24 may be neglected. For gels with stiff polymer chains between cross-links, deformation also implies a change in enthalpy, since chemical bonds in the chains are being bent or stretched. Most polysaccharide gels are in this category, and in some of these the entropy change may even be neglected.

It should further be remarked that in the derivation of Equation 7.24 it is implicitly assumed that the properties of all the strands are identical. This is generally not the case, especially because the cross-links often have the form of junctions, where number and strength of the bonds in a junction may vary substantially. However, in the simple case depicted in Figure 7.16a—chemical cross-links and long flexible chains—Equation 7.24 can be reduced to a very simple expression for the modulus

$$G = \nu kT \tag{7.25}$$

where v is the number of chains between cross-links per unit volume. This equation is well obeyed for very small deformations, provided that the value of v does not change with temperature or other variables (pH, ionic strength, or solvent quality during gelation).

By the application of percolation theories, simple scaling laws for the modulus of polymer gels with the concentration c of gel-forming material have been derived, for instance [69]

$$G \propto (c - c_0)^n \tag{7.26}$$

where the exponent n varies, for the most part between 2 and 4, depending on the structure of the network. There is a minimum concentration c_0 for gel formation, but a physical explanation for its value is not provided. These values depend on the nature of the gel-forming material and on the physicochemical conditions during gelation. Often, relation (7.26) can be fitted well to experimental results.

7.5.2.5 Particle Gels

These gels can form due to aggregation of particles that are made to attract each other, for example, by a change in pH, ionic strength, or solvent quality. The structure of the aggregates formed is generally of a *fractal* nature [100]. When "attractive" particles encounter each other at random, they form small aggregates (at first doublets), and as these encounter other aggregates, larger ones result. This is called cluster–cluster aggregation. A simple relation tends to develop between the (average) number of particles in an aggregate N_p and the radius R of the aggregate:

$$N_p = \left(\frac{R}{r}\right)^D \tag{7.27}$$

where r is the radius of the primary particles. D is a constant <3, which is called the "fractal dimensionality." Because it is smaller than 3, larger aggregates are more tenuous (rarefied) than smaller ones. The average volume fraction of particles in an aggregate is given by

$$\phi_{agg} = \frac{N_p}{N_m} = \frac{(R/r)^D}{(R/r)^3} = (R/r)^{D-3} \tag{7.28}$$

where N_m is the number of primary particles that a sphere of radius R would obtain on close packing. Since $D < 3$, ϕ_{agg} decreases as R increases, until it equals the volume fraction of primary particles in the system ϕ. In principle, all particles will then be included in aggregates, which fill the system. Bonds between aggregates have formed, resulting in a space-filling network (i.e., a gel). The critical radius of the aggregates at the gel point is given by

$$R_g = r\phi^{1/(D-3)} \tag{7.29}$$

This mechanism implies that a gel will be formed at any value of ϕ, however small. However, at very small ϕ, the gel will be too weak to be noticed, and it will readily be broken up by weak agitation. Moreover, sedimentation of aggregates may occur before a gel can form. This disturbance will happen more readily when ϕ is smaller; hence, there will be a critical concentration ϕ_0 for gelation.

An often occurring complication is that soon after small aggregates have formed, particle rearrangement occurs, as illustrated in Figure 7.19. Particles may roll over each other until they have formed bonds with more than one other particle. The fractal nature of the aggregates remains, but instead of r, a larger *effective* radius r_e has to be used in the equations. Moreover, it will imply a higher value of ϕ_0, due to faster sedimentation of small aggregates.

The theory of fractal aggregation allows the derivation of scaling laws for rheological parameters of the gel formed. It is assumed that the modulus of the gel derives exclusively from a change in enthalpy upon deformation. It is further taken into account that fractal structures are (on average) self-similar. This implies that the number of bonds (or junctions) between adjacent aggregates is independent of their radius. The contact area between aggregates in the final gel will scale with R_g^2. This implies that the number of bonds between the aggregates per unit cross section of the gel scales with R_g^{-2}, hence with the number of stress-carrying strands N in Equation 7.24. It causes the modulus to scale with ϕ as

$$G \propto C\frac{d^2H}{dx^2}\phi^{2/(3-D)} \tag{7.30}$$

Often, $D \approx 2.2$, causing G to be proportional to about $\phi^{2.5}$. Since also C may depend on ϕ, both weaker and stronger dependencies of G on ϕ may be observed [51,80].

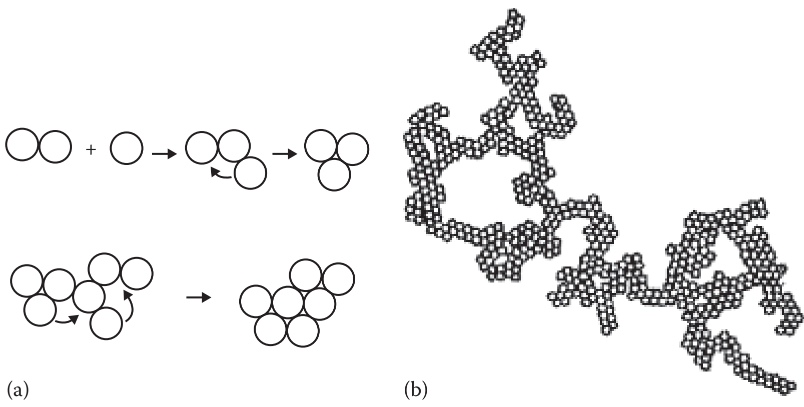


FIGURE 7.19 Short-term rearrangement in fractal aggregates. (a) Examples of particles rolling over each other so that a higher coordination number is attained. (b) Example of a fractal aggregate in two dimensions where some short-term arrangement has occurred. (From Walstra, P., *Physical Chemistry of Foods*, Marcel Dekker, New York, 2003.)

In conclusion, although particle gels seem to be rather disordered structures, simple scaling laws to describe various properties often hold (see also Equation 7.32).

7.5.3 Functional Properties

Food technologists make gels for a purpose, often to obtain a certain consistency or to provide physical stability. The properties desired and the means of achieving them are summarized in Tables 7.5 and 7.6.

Consistency has already been briefly discussed, but the message of Table 7.5 is an important one: according to the purpose in mind, the rheological and fracture measurements must be of a relevant type and conducted at the relevant time scale or strain rate. This need not be difficult. For instance, to evaluate stand-up, which is the propensity of a piece of gel (say, a pudding) to keep its shape under its own weight, measurement of a modulus makes no sense. The proper experiment is simply watching the piece and possibly measuring the height of a specimen that will just start yielding. To ensure stand-up, the yield stress must be greater than $g \times \rho \times H$, where H is specimen height. For a piece 10 cm tall, this would be about $9.8 \times 10^3 \times 0.1 \approx 10^3$ Pa. It should further be realized that the yield stress often is smaller if the time scale is longer.

TABLE 7.5
Consistency of Gels: Desired Mechanical Characteristics of Gels Made for a Given Purpose

Property Desired	Relevant Parameters	Relevant Conditions
Stand-up	Yield stress	Time scale
Firmness	Fracture stress or yield stress	Time scale, strain, strain rate
Shaping[a]	Yield stress + restoration time	Several
Handling, slicing	Fracture stress, work of fracture	Strain rate
Eating properties	Yield and fracture properties; stiffness	Strain rate (strain for stiffness)
Strength (e.g., of film)	Fracture properties	Stress, time scale, strain rate

[a] After making the gel.

TABLE 7.6
Gel Properties Needed to Provide Physical Stability

Prevent or Impede	Gel Property Needed
Motion of *particles*	
Sedimentation	High viscosity or significant yield stress + short restoration time
Aggregation	High viscosity or significant yield stress
Local volume changes	
Ostwald ripening	Very high yield stress
Motion of *solvent*	
Leakage	Small permeability + significant yield stress
Convection	High viscosity or significant yield stress
Motion of *solute*	
Diffusion	Very small permeability, high solvent viscosity

Very *weak gels* were briefly discussed in Section 7.4.3. In daily life, such a system appears to be liquid, as it will readily flow out of a bottle if its yield stress is <10 Pa or so; nevertheless, it has elastic properties at extremely small stresses. This small yield stress may be sufficient to prevent sedimentation. A good example is soya milk (Figure 7.20) [58]. Soya milk contains small particles, consisting of cell fragments and organelles. These particles aggregate, forming a weak reversible gel. If processing conditions are appropriate, the yield stress is sufficient to prevent

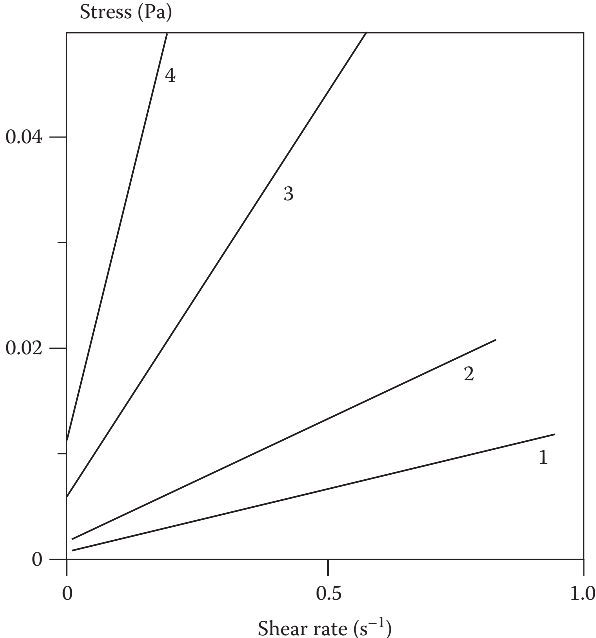


FIGURE 7.20 Flow curves (shear stress vs. shear rate) of soya milk. The yield stresses are given by the *y* intercepts of the curves. The soya milks were made of dehulled (Curves 1 and 2) or whole beans (3 and 4), soaking the beans overnight at room temperature (1 and 3) or 4 hours at 60°C (2 and 4). (After Oguntunde, A.O. et al., Physical characterization of soymilk, in: *Trends in Food Biotechnology*, A.H. Ghee, N.B. Hen, and L.K. Kong, eds., *Proceedings of the Seventh World Congress on Food Science and Technology*, Singapore, 1987, Institute of Food Science and Technology, Singapore 1989, pp. 307–308.)

these particles, and even larger particles, from sedimenting. Also, some mixtures of polysaccharides, for example, solutions of xanthan gum and locust bean gum, even if very dilute, can exhibit a small yield stress (Figure 7.14, curve 3). This yield stress can prevent sedimentation of any particles present [45].

Permeability. Sometimes, it is desired to arrest the motion of liquid, in which case the permeability of the gel is an essential parameter. According to Darcy's law, the superficial velocity v of a liquid through a porous matrix is

$$v \equiv \frac{Q}{A} = \frac{B}{\eta}\frac{\Delta p}{x} \tag{7.31}$$

where

Q is the volume flow rate ($m^3\ s^{-1}$) through a cross-sectional area A

Δp is the pressure difference over distance x

The permeability B (in m^2) is a material constant that varies greatly among gels. A particle gel-like renneted milk (built of paracasein micelles) has a permeability of the order of $10^{-12}\ m^2$, whereas a polymer gel (e.g., gelatin) typically would have a B of 10^{-17}. In the latter case, leakage of liquid from the gel would be negligibly slow.

For fractal particle gels a simple scaling law for the permeability can be derived:

$$B = \frac{r_e^2}{K}\phi^{2/(D-3)} \tag{7.32}$$

where

K is a proportionality constant, often between 50 and 100

r_e is the effective radius of particles

The exponent of ϕ is very well obeyed [100].

Transport of a solute through the liquid in a gel has to occur by *diffusion*, because convection generally is not possible. The diffusion coefficient D of a solute in a concentrated gel is not greatly different from that in solution, at least for small molecules. However, the Stokes relation for diffusivity $D = kT/6\pi\eta r$, where r is molecule radius, cannot be applied here because the macroscopic viscosity of the gel system is irrelevant, since the viscosity as sensed by the diffusing molecules would be the viscosity of the solvent. On the other hand, the solute has to diffuse around the strands of the gel matrix, and the hindrance will be greater for larger molecules in smaller pores between strands in the gel. These aspects are illustrated in Figure 7.21 [55].

Swelling and syneresis are additional properties of gels. Syneresis refers to expulsion of liquid from the gel, and it is the opposite of swelling. There are no general rules governing their occurrence. In a polymer gel, lowering of solvent quality (e.g., by changing temperature), adding salt (in the case of polyelectrolytes), or increasing the number of cross-links or junctions may cause syneresis. However, because both the pressure difference in Equation 7.31 and the value of B are usually very small, syneresis (or swelling) tends to be very slow. In particle gels, syneresis may occur much faster, due to the far greater permeability. It is well known that renneted milk is prone to syneresis, an essential step in cheese making. The combination of variables influencing syneresis is intricate [81].

7.5.4 Some Food Gels

The fairly theoretical points discussed earlier will now be illustrated by discussing some food gels.

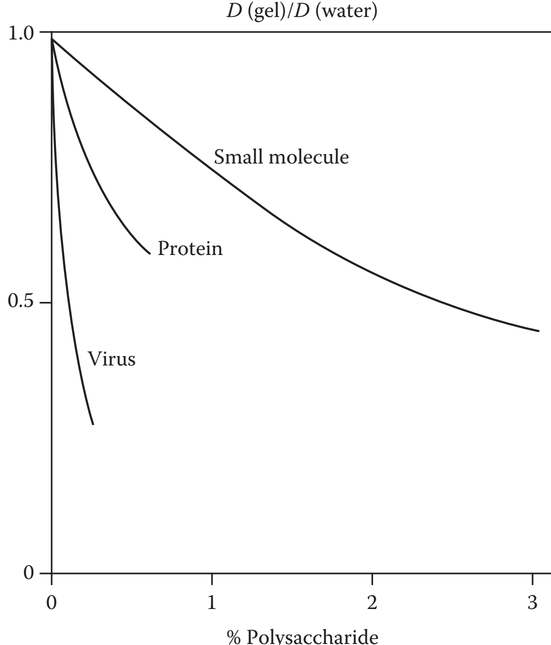


FIGURE 7.21 Diffusion of solutes in polysaccharide gels of various concentrations. *D* is diffusion coefficient. (Highly schematic examples; After Muhr, A.H. and Blanshard, J.M.V., *Polymer*, 23(Suppl.), 1012, 1982.)

7.5.4.1 Polysaccharides [53]

Despite the wide range of polysaccharide types (see Chapter 3), some general rules governing their gel properties exist. Most polysaccharide chains are fairly stiff, one of the causes being that several bulky side groups may be present on the *backbone* chain. Generally, appreciable bending of a chain segment can occur only if its length exceeds about 10 monomers (monosaccharide residues). This characteristic causes polysaccharides to produce highly viscous solutions; for example, 0.1% xanthan will increase the viscosity of water by at least a factor of 10. Some polysaccharides can form gels. Broadly speaking, the gel cross-links are junctions, each containing a great number of (weak) bonds and jointly involving an appreciable portion of the material. This means that the strands between cross-links are not very long. Combined with chain stiffness, this leads to rather short (or even brittle) gels. Actually, they are intermediate between entropic and enthalpic gels (cf. Equation 7.24). Of course, there is considerable variation among polysaccharides in this regard.

Cross-links among polysaccharide molecules can be any of the following three types:

1. *Microcrystallites* (Type 1): The simplest type, local stacking of stretched chain segments, is depicted in Figure 7.16b. This type is not common in gelling polysaccharides (although native cellulose is an example of an almost completely crystallized linear polymer.) Amylose cannot form linear chains, but stacks of single amylose helices can presumably form microcrystalline regions in solutions, and if the amylose concentration is high enough, gelation will occur. With amylopectin, similar behavior is observed. These phenomena are involved in "retrogradation" of gelatinized starch. Microcrystallites can also be formed of other structural elements.
2. *Double helices* (Type 2): Several polysaccharides (e.g., carrageenans, agar, and gellan) can form double helices below a sharply defined temperature, which depends on conditions. Each helix generally involves two molecules, but they can only be formed in so-called

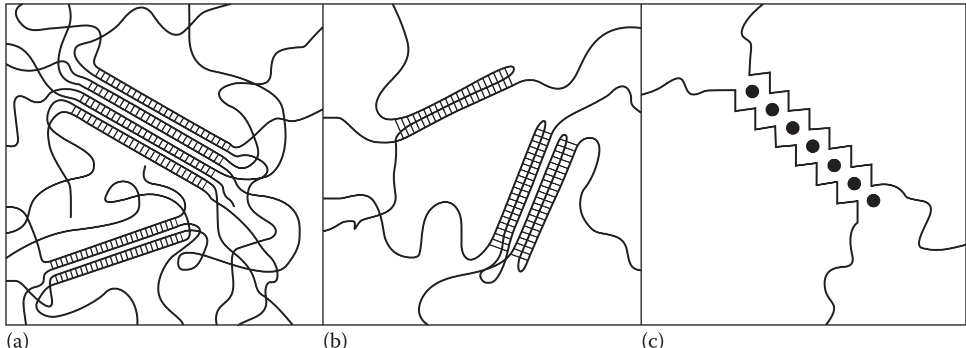


FIGURE 7.22 Various types of junctions in polymer gels. (a) Stacked double helices, for example, in carrageenans. (b) Triple helices in gelatin. (c) *Egg-box junction*, for example, in alginate; the dots denote Ca ions. Highly schematic; helices indicated by hatching.

nonhairy regions of the polymer (i.e., regions that are devoid of bulky side groups). Double helices would thus be cross-links leading to gelation. However, helix formation tends to be very rapid (milliseconds), whereas gelation takes far longer (several seconds). The double helices do form microcrystallites (see Figure 7.22a), at least in κ-carrageenan, which presumably stabilizes them. As soon as the helices *melt*, so does the gel.

3. *Egg-box junctions* (Type 3): These occur with some charged polysaccharides, such as alginate, when divalent cations are present (Figure 7.22c). Alginate has negative charges, often spaced at regular distances, allowing divalent cations, such as Ca^{2+}, to establish bridges between two parallel polymer molecules. In this way, fairly rigid junctions are formed. It is likely that the junctions further arrange themselves in microcrystalline regions. The junctions do not readily *melt* unless the temperature is near 100°C.

Many factors may affect gelation and gel properties of polysaccharides. These include molecular structure, molar mass (Figure 7.23b), concentration (Figure 7.23a and b), temperature (Figure 7.24b), and solvent quality and for polyelectrolytes, pH and ionic strength (Figure 7.24b) [99].

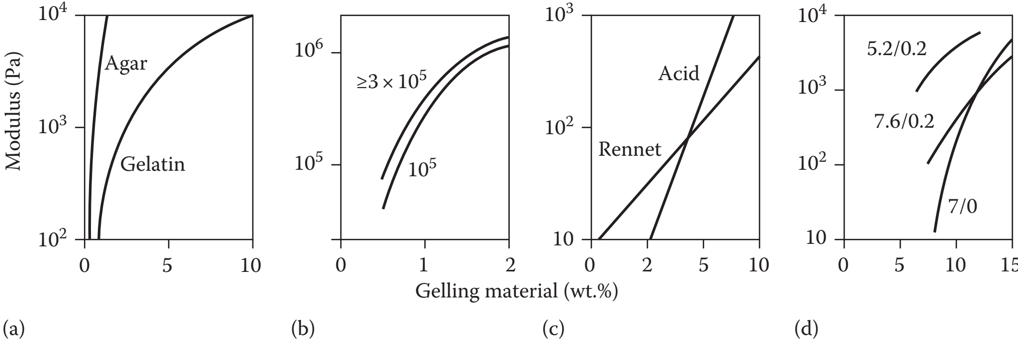


FIGURE 7.23 Effect of concentration of gel-forming material on the shear modulus of the gels. (a) Agar and gelatin. (b) κ-carrageenan of two molar masses (indicated) in 0.1% KCl. (c) Casein gels made by slow acidification or by renneting. (d) Heat-set gels of soya protein isolate; figures near the curves indicate pH/added NaCl (molar).

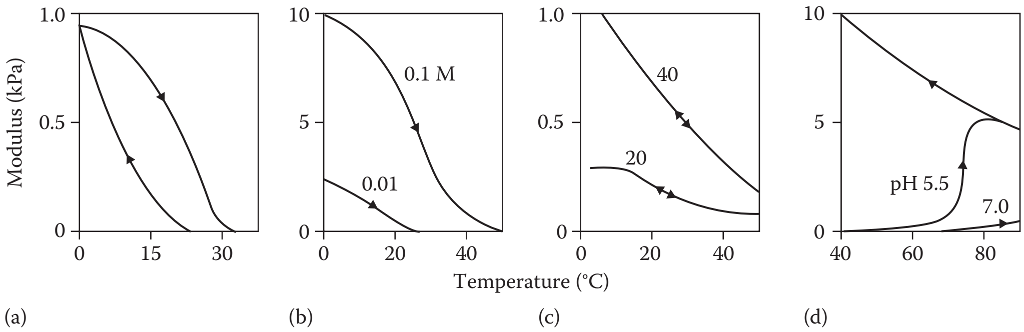


FIGURE 7.24 Effect of measurement temperature on the shear modulus of various gels. Arrows indicate the temperature sequence. (a) Gelatin (2.5%). (b) κ-carrageenan (1%) for two concentrations of $CaCl_2$ (indicated). (c) Acid casein gels (2.5%) made and aged at two temperatures (indicated). (d) β-lactoglobulin (10%) at two pH values (indicated). The results will also depend on heating or cooling rate.

7.5.4.2 Gelatin [11,40]

Of all the food gels, gelatin is closest to an ideal entropic gel. The flexible molecular strands between cross-links are long, and this causes the gel to be very extensible. It is also predominantly elastic, because the cross-links are fairly permanent (at least at low temperature). However, the relation given for the modulus in Equation 7.25 is not well obeyed. The modulus roughly increases with the square of the concentration (Figure 7.23a), and the temperature dependence is very different from the prediction (Figure 7.24a). These discrepancies stem from the mechanism of cross-linking. Despite the severe treatment of the collagen during preparation of gelatin, the molecules may retain much of their length and produce highly viscous aqueous solutions. Upon cooling, the molecules tend to form triple helices, like the proline helices in collagen. This applies to only part of the gelatin and the helical regions are relatively short. A gelatin molecule cannot form an intermolecular double helix, as several polysaccharides can do. This is because peptide bonds cannot rotate over the full 360°, implying that helix formation at one place will cause twisting of other parts of the molecules, which would soon stop due to steric hindrance. Presumably, a gelatin molecule sharply bends at a so-called β-turn and then forms a short double helix. Subsequently, a third strand may wind around this helix, thereby completing it. If the third strand is part of another molecule, a cross-link is formed (see Figure 7.22b). Upon an increase of temperature, triple helices will *melt*, whereby the modulus will decrease.

Actually, the gelation mechanism must be more complicated. As shown in Figure 7.24a, there is considerable hysteresis between cooling and heating curves. Also, when cooling a gelatin solution to a temperature below, say, 25°C, the modulus may keep increasing for days. This goes along with an increase in helical material, up to about 30% of the gelatin, but some structural rearrangement will also occur. It is still a matter of debate to what extent stacking of triple helices occurs.

It may finally be noticed that the temperature dependence of the gel state, which is virtually unique for gelatin, offers several possibilities for food manufacture.

7.5.4.3 Caseinate Gels [83,90,100]

Milk contains casein micelles, proteinaceous aggregates of about 120 nm average diameter, each containing some 10^4 casein molecules (see Chapter 14). The micelles can be made to aggregate by lowering the pH to about 4.6 (thereby decreasing electric repulsion) or by adding a proteolytic enzyme that removes the parts of κ-casein molecules that protrude into the solvent (thereby decreasing steric repulsion). Fractal gels are formed, with a fractal dimensionality of about 2.3. The permeability, which is about 2×10^{-13} m^2 for average casein concentration (c), is strongly dependent on the latter, being about proportional to c^{-3}. For casein gels a linear relation exists between log modulus

and log casein concentration (Figure 7.23c), in accordance with their fractal nature (Equation 7.30). The different slopes imply a difference in structure. The initially tortuous strands formed during rennet gel formation become straightened soon afterward, whereas the strands in acid gels remain tortuous [52].

The building blocks of the gel, that is, the casein micelles, are themselves deformable, and the junctions between them are flexible. Thus, the gel is rather weak and soft. For acid casein gels, fracture stress is about 100 Pa and the fracture strain is about 1.1; for rennet gels, these values are about 10 Pa and 3, respectively. The acid gel is thus shorter. These results apply for slow deformation, say, over 15 min; at faster deformation, the fracture stress is much greater. Applying a stress of slightly over 10 Pa to a rennet gel will cause flow (there is no detectable yield stress), and after considerable time fracture will occur. Applying a stress of 100 Pa leads to fracture within 10 s. A similar behavior is found in some other types of particle gels, but it is by no means universal.

All these values depend on conditions applied, especially temperature. It is seen that the modulus of a casein gel is larger at lower temperature (Figure 7.24c). This may appear strange, since hydrophobic bonds between casein molecules are considered to play a major part in keeping the gel together, and these bonds decrease in strength with decreasing temperature. Presumably, a decrease in hydrophobic bond strength (low T) leads to swelling of the micelles and hence to a larger contact area between adjoining micelles and to a greater number of bonds per junction. Conversely, a higher temperature of gel formation leads to a larger modulus (at least for acid gels; Figure 7.24c), and this is due to a somewhat different network geometry, not to a difference in type of bonds.

At temperatures above about 20°C, rennet gels show syneresis. Syneresis goes along with rearrangement of the network of particles, which implies that some deaggregation occurs. In a region where no liquid can be expelled (i.e., internally in the gel), rearrangement occurs as well, leading to both denser and less dense regions. This is called microsyneresis; it results in an increase in permeability and causes the straightening of the network strands mentioned earlier.

7.5.4.4 Globular Proteins Gels [12,67,68,80]

Many well-soluble globular proteins form a gel upon heating, if the protein concentration is above a critical value c_0 (Figure 7.23d). These heat-set gels form only if at least part of the protein has been heat denatured and does not return to the native state upon cooling (cf. Figure 7.24d). Gel formation is a relatively slow process, taking at least several minutes. It may take far longer to reach the maximum stiffness. Gel formation involves a number of consecutive reactions: (1) protein molecules become denatured, (2) denatured molecules aggregate to roughly spherical or to elongated particles, and (3) these particles form a space-filling network. These reactions partly proceed in parallel.

Details of gel formation and of the gel properties obtained vary widely among proteins, due to their variation in molecular structure and conformational stability. A further complication is that heat-set gels are usually made up of protein mixtures, such as whey protein or soy protein isolates. Bonds involved in gel formation include –S–S– linkages; electrostatic, van der Waals, and hydrophobic interactions; and H-bonds as part of intermolecular junctions between β-strands.

The structure, and thereby the rheological properties, also vary greatly with pH, ionic strength, salt composition, and rate of heating. In general, two types of gel structure can be distinguished by microscopy, namely, fine-stranded and coarse-stranded networks. The former gels are clear (transparent) and consist of relatively thin strands (diameter generally 10–50 nm) that are branched to form a network. They typically form on heating at a pH far from the isoelectric point at low ionic strength. Coarse-stranded or particle gels (some may have a fractal network structure) are turbid and are generally built of roughly spherical particles of 0.1–1 μm. These gels generally form by heating at a pH close to the isoelectric point and/or high ionic strength. Coarse-stranded gels tend to be stiffer than fine-stranded ones. In both types of gel, the tortuosity of the strands may vary considerably, resulting in widely varying fracture strains. Moreover, heat-set protein gels vary substantially in permeability, and thereby in their propensity to lose solvent under pressures acting during further processing or handling.

An alternative process to make gels of globular proteins is by heating a solution at a pH where the molecules denature and form small aggregates, but do not form a gel. After cooling, the pH is brought to a value close to the isoelectric point, and a gel forms; this is known as cold gelation.

Structure formation during extrusion is comparable to heat setting of globular proteins. An important example involves protein-rich products from soya [41].

7.5.4.5 Mixed Gels

It will now be clear that the structure and the properties of gels can vary greatly. The modulus of a 1% gel can vary by almost five orders of magnitude and the strain at fracture by a factor of 100. Nearly every system exhibits specific relations that are often poorly understood.

The situation becomes even more complicated when mixed gels are considered. Relatively simple are gels filled with particles (e.g., emulsion droplets), which gels may have greatly altered properties as compared to unfilled ones [10,44,80]. Often, a mixture of polysaccharides is used. Weak attraction between the polymers at gel-forming conditions may cause gelation, even if both polymers are nongelling [10,54]. For instance, dilute xanthan or locust bean gum solutions do not show an appreciable yield stress, while dilute mixtures do: after heating and subsequent cooling, mixed junctions are formed. Another example is the addition of, say, 0.03% κ-carrageenan to milk, which results in the formation of a weak gel. This is applied (e.g., in chocolate milk) to prevent sedimentation of cocoa particles.

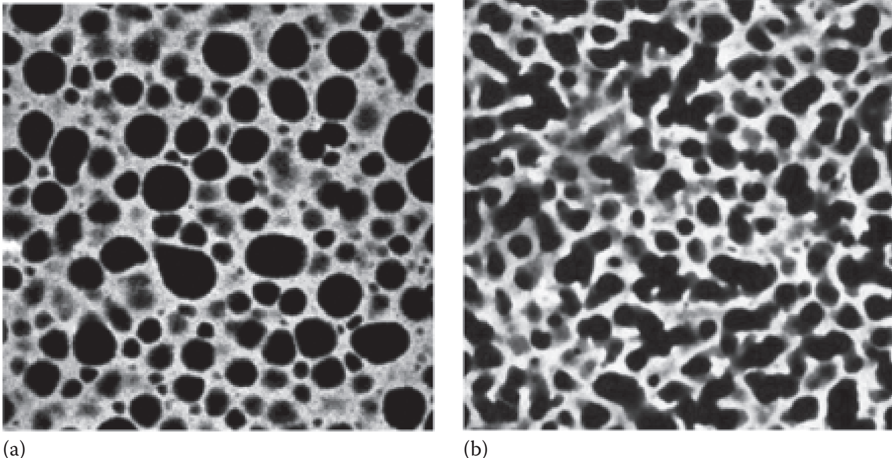
Using mixtures of biopolymers for gel formation often leads to thermodynamic incompatibility during mixing or during gel formation (Section 7.5.1). For instance, in mixtures of gelatin and the nongelling polysaccharide dextran phase, separation occurs directly after mixing at a temperature above the gel point of gelatin; if the gelatin solution is the continuous phase after phase separation, the system becomes frozen after cooling. If both biopolymers may form a gel, the one that gels first will mainly determine the topology of the gel structure. Important factors are the waiting time for gel formation for systems that starts to phase separate directly after mixing. In other systems phase separation is induced by the gel formation process. For instance, for gelatin–maltodextrin mixtures the conformational ordering (helix formation) of the gelatin on cooling induces phase separation [42].

The microstructure of mixed protein–polysaccharide gels will depend on the properties of both biopolymers (Figure 7.25) and their concentrations and conditions during gel formation. For instance, for mixed whey protein–polysaccharide cold-set gels, a slower rate of acidification and with that a slower rate of gel formation resulted in a coarser microstructure of the mixed gels. Neutral polysaccharides as guar and locust bean gum resulted in protein continuous systems and discontinuous serum phases at lower polysaccharide concentrations (Figure 7.25a). With increasing polysaccharide concentration, the area of the protein phase decreased, and, finally, this phase became discontinuous. Mixtures with negatively charged polysaccharides as gellan gum at intermediate concentrations and xanthan gum, κ-carrageenan, carboxymethylcellulose, and pectins at lower polysaccharide concentrations resulted in bicontinuous gels (Figure 7.25b) and phase inversion at higher concentrations. The precise microstructure of the gels formed depended primarily on the charge density of the polysaccharides [16]. The mechanical properties of the protein continuous gels depended primarily on the balance between the increase of the local protein concentration in the protein strands and the decrease in continuity of the protein network due to the polysaccharide.

Complete foods are still more complicated than the systems so far discussed [32]. Nevertheless, knowledge of the principles provided can be of great help in understanding food behavior and for designing experiments to study it.

7.5.5 Mouthfeel of Foods [34,62,79,80]

The eating characteristics of foods form an essential quality attribute. Fabricated foods are often specially designed to optimize these characteristics, which include flavor, texture, and appearance.



(a) (b)

FIGURE 7.25 Examples of microstructures formed on cold gelation to pH 4.8 of a mixture of 3% whey protein isolate with 0.1% locus bean gum (a) and with 0.4% gellan gum (b). With locus bean gum protein continuous gels are formed with roughly spherical droplets containing the locus bean gum, while with 0.4% gellan gum bicontinuous gels are formed. (Based on results by Van den Berg, L. et al., *Food Hydrocoll.*, 22, 1404, 1980b.)

Here, we will focus on texture as perceived in the mouth, which primarily involves consistency and physical inhomogeneity. Actually, the mouth can be considered as a processing unit in which the food is worked, broken down, and transported to the esophagus in a manner depending on its mechanical properties. Moreover, the mouth and the connected nasal cavity contain several sense organs, which are used in the evaluation of eating characteristics of the food.

The manner in which liquids and soft solids are processed in the mouth differs from that of hard solids. Liquids are just transported to the esophagus, mainly by use of the tongue, while the working of hard solid foods involves a few separate stages. In general, we can distinguish (1) ingestion/biting; (2) chewing and moistening, including bolus formation; and (3) swallowing of the bolus and clearance of the mouth. In each stage the food is deformed in different ways at various rates, while it is mixed with saliva. The working of soft solids also involves a pressing and shearing action between the tongue and the palate. During this processing the consumer already starts to evaluate eating characteristics, including several texture attributes, such as thickness, roughness/smoothness, mealiness, and so forth. Several of such characteristics are multicomponent attributes, as they consist of some subattributes that must all be present to a certain extent. *Creaminess*, for example, involves thickness and smoothness, while roughness must be absent. (Probably, flavor can also contribute to the impression of creaminess).

The working in the mouth is best known for liquids and hard solids, although still to a limited extent; nevertheless, some general rules have been established.

The oral evaluation of a liquid depends on its flow characteristics, best expressed as the viscosity as a function of shear rate. As a rule of thumb, for low-viscosity liquids (below 0.1 Pa s) the sensory evaluation of viscosity corresponds with instrumental evaluation at a shear stress of about 10 Pa. For products with a high viscosity (above 10 Pa s), sensory evaluation corresponds with instrumental evaluation at a constant shear rate of 10–20 s^{-1}, although the evaluation in the mouth will often involve a smearing action between the tongue and the palate. For intermediate viscosities, there is a gradual transition of the rheological parameters that are determinant. *Thickness* is often considered as a single sensorial characteristic; however, besides (apparent) viscosity, other rheological properties are probably involved. This is certainly so for soft solids like apple sauce or tomato ketchup.

For low-viscosity liquids, such as water and milk, the flow in the mouth may locally be turbulent. For all liquid and many soft solid foods, the flow rate in the mouth will vary from place to place, and the food is mixed with (diluted by) saliva. Tomato ketchup, for instance, and many products based on hydrocolloid solutions, becomes thinner during flow. The thinning effect is perceived in the mouth and appears to be related to the texture attribute *sliminess*; however, even this simple attribute can probably not be ascribed to one rheological property [79]. For foods that have a yield stress below which they will not flow, as is the case for many soft solids, the perceived thickness will also depend on the magnitude of this yield stress. Finally, the pressing and shearing action between the tongue and the palate will induce elongational flow besides shear flow, and for several, especially polymeric, materials, the elongational viscosity is substantially higher than the *common* simple-shear viscosity.

The effect of temperature on the perception of viscosity and yield stress–related texture attributes is probably relatively small. However, its effect may be much larger when a phase change occurs between the initial temperature of the food and mouth temperature (e.g., melting of fat crystals or of a gelatin gel). Changes can also occur due to enzyme action. Although the residence time for many products in the mouth is only of the order of seconds, significant starch degradation may occur during this period, depending, for instance, on the extent of mixing of food and saliva.

After swallowing, a coating of food remnants is generally left on the tongue and other parts of the oral cavity. The extent of coating depends on the adhesive and cohesive properties of the food as such and of the particulate matter remaining after processing in the mouth. The coating will markedly affect the "after-swallowing" sensory impression. For instance, binding of (macro-) molecules to the mucus layer covering the oral surfaces appears to correlate with an increase of the sensory attribute astringency or roughness.

As mentioned earlier, three stages can be distinguished in the processing of a solid food in the mouth. The biting action can to a great extent be imitated by uniaxial compression between two wedges [84] at a suitable compression rate. The large deformation properties of many food products depend on deformation rate. The biting velocity generally is between 2 and 6 cm s^{-1}. The resistance to biting and the maximum biting force required to fracture the material are related to sensory firmness and hardness, but other properties are also involved. After biting, the food will further be broken down by a grinding and shearing action between the molars. Attributes as crumbly and spreadable do not relate in a simple way to mechanical properties as measured, for instance, by uniaxial compression. For soft solids, these attributes do not only depend on the large deformation properties of the foods up to fracture but to a large extent also on their behavior after that, that is, the way and extent to which they fall apart after fracture [75,76]. Also, attributes, such as crispiness and crumbliness, are complex attributes that combine a wide range of perceptions such as fracture behavior, emitted sound characteristics, density, geometry, and flavor [80].

The tongue plays an important role in transporting the food particles to the molars and in deciding which particles are sufficiently broken down and moistened to be swallowed; the speed and the relative importance of these processes depend on the type of food (see Figure 7.26). The food is then transported to the back of the oral cavity, where it forms a bolus, which is swallowed after some time. Clearance of the oral cavity can be due to mechanical action of the tongue, slow dispersion or dissolution of food remnants in saliva, and enzymatic breakdown.

7.5.6 Summary

- Mixtures of biopolymers will frequently exhibit phase separation at higher concentrations whereby one can distinguish thermodynamic incompatibility and complex coacervation.
- Gels can be distinguished on basis of, for example, structure (polymer and particle gels) and gel formation process (heat-set gels, cold gelation, and formation resulting from changing conditions as pH and enzyme action).

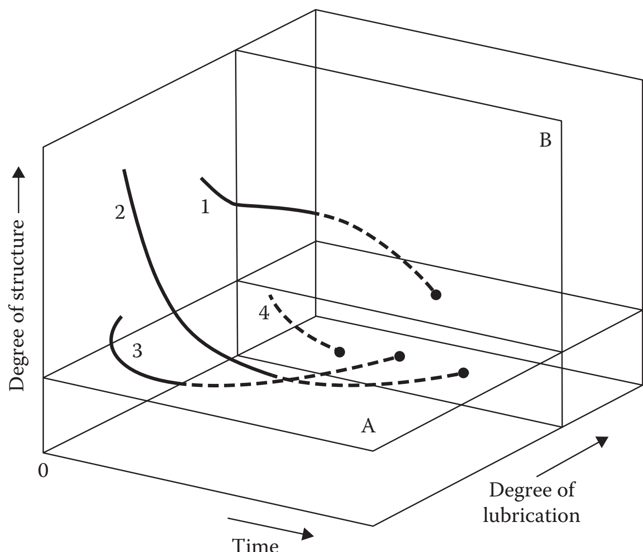


FIGURE 7.26 Schematic of breakdown trajectories in the mouth of some foods after ingestion (at time = 0). The two main processes considered are (a) structure breakdown, which is considered to be sufficient below the horizontal plane A, and (b) degree of lubrication, which would be sufficient beyond the vertical plane B. The processed food (bolus) must fulfill both criteria before it can be swallowed. Examples are (1) tender and juicy meat, (2) tough and dry meat, (3) dry sponge cake (note that the degree of lubrication at first decreases and then increases), and (4) a thick liquid, for example, stirred yoghurt. (The lines are broken when they are below A and/or beyond B.) The points of swallowing are indicated by dots. (Modified after Hutchings, J.B. and Lillford, P.J., *J. Texture Stud.*, 19, 103, 1988.)

- For full characterization of the mechanical properties of gels, a range of parameters are required. Important ones are the modulus, fracture or yield stress and strain, shape of stress versus strain curve, and the way it falls apart after fracture/yielding.
- Polymer gels are characterized by long polymer chains; their flexibility may vary greatly, which might affect the gel properties. Gelatin gels have rather long flexible molecular strands between the cross-links, while (most) polysaccharide gels are characterized by relatively short and fairly stiff strands.
- Particle gels are formed due to aggregation of particles. Structure of aggregates formed has generally a fractal nature. Later this structure may change as a result of rearrangements.
- Before measuring functional properties of gels, both the relevant parameters and conditions that determine the desired property have to be considered.
- Cross-linking of polysaccharide gels can be due to the formation of microcrystallites, double helices, and egg-box junctions.
- Formation of heat-set protein gels is a relatively slow process that is characterized by three consecutive reactions, which partly proceed in parallel. Usually, a series of different interactions play a role in the bond formation between the protein molecules.
- The structure of gels formed by gelation of mixture of biopolymers is clearly related to the occurrence of phase separation before and during gel formation.
- Eating characteristics of foods are directly related to their mechanical properties and differ strongly between liquids and hard solid foods. Liquids are directly transported by the tongue to the esophagus, while processing of hard solids involves several stages. The processing of soft solids will be in between. For liquid foods their viscosity and adherence to the oral coating are important characteristics, while for solids large deformation, fracture, or yielding properties are essential characteristics.

7.6 EMULSIONS

7.6.1 Description

Emulsions are dispersions of one liquid into another. The most important variables determining emulsion properties are as follows:

1. *Type. The type of emulsion, that is, oil-in-water or water-in-oil,* determines, among other things, with what liquid the emulsion can be diluted (Section 7.1.2). Many foods are oil-in-water (o/w) emulsions, examples being milk and milk products, sauces, dressings, and soups. Foods that are true w/o emulsions hardly exist. Butter and margarine contain aqueous drops, but these are embedded in a plastic fat; melting of the crystalline part of the fat produces a w/o emulsion that immediately separates into an oily layer on top of an aqueous layer. The droplets of several o/w emulsions also contain fat crystals, at least at low temperatures, and are thus—strictly speaking—not emulsions.
2. *Droplet-size distribution*. This has an important bearing on physical stability, smaller drops generally giving more stable emulsions. However, the energy and the amount of emulsifier needed to produce an emulsion increase with decreasing droplet size. A typical mean droplet diameter is 1 μm, but it can range between 0.2 and several μm. Because of the great dependence of stability on droplet size, the width of the size distribution is also important. Examples of droplet size distributions are in Figure 7.27a.
3. *Volume fraction* of dispersed phase (ϕ). In most foods, ϕ is between 0.01 and 0.4. For mayonnaise it may be 0.8, that is, above the value for maximum packing of rigid spheres, roughly 0.7; this means that the oil droplets are somewhat distorted. The volume fraction has a large effect on emulsion viscosity, ranging from a thin liquid to a kind of paste, as ϕ increases.

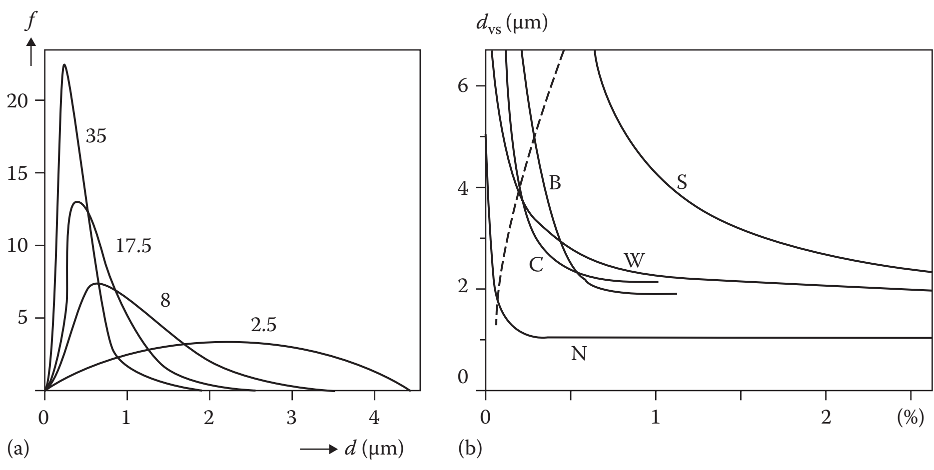


FIGURE 7.27 Emulsification: effects of various conditions on the resulting droplet size. (a) Effect of homogenization pressure (indicated near the curves in MPa) on the volume frequency distribution in % of the oil per 0.1 μm class width versus droplet diameter *d*; 3.5% oil in skim milk. (b) Effect of emulsifier concentration (% w/w) on volume/surface average droplet diameter d_{vs} for various emulsifiers. B, blood protein; C, sodium caseinate; N, nonionic small-molecule surfactant; S, soya protein; W, whey protein. Approximate results for 20% oil and moderate emulsification intensity. (From Walstra, P., *Physical Chemistry of Foods*, Marcel Dekker, New York, 2003.)

4. Composition and thickness of the *surface layer* around the droplets. This determines interfacial properties and colloidal interaction forces (Section 7.2.7); the latter greatly affect physical stability.
5. Composition of the *continuous phase*. This determines solvent conditions for the surfactant, pH, and ionic strength, and thereby colloidal interactions. The viscosity of the continuous phase has a pronounced effect on creaming.

Unlike the solid particles in a suspension, emulsion droplets are spherical (greatly simplifying many predictive calculations) and deformable (allowing droplet disruption and coalescence). Moreover, their interface is fluid, allowing interfacial tension gradients to develop. Nevertheless, in most conditions, emulsion droplets behave like solid particles. From Equation 7.8, the Laplace pressure of a droplet of 1 μm radius and interfacial tension $\gamma = 5$ mN m^{-1} is 10^4 Pa. For a liquid viscosity of $\eta = 10^{-3}$ Pa s (water) and a velocity gradient achieved by agitation or flow ∇v of 10^5 s^{-1} (this is very vigorous), the shear stress $\eta \cdot \nabla v$ acting on the droplet would be 10^2 Pa. This implies that the droplet deformation would be negligible. Moreover, the surfactant at the droplet surface allows this surface to withstand a shear stress (Section 7.2.6). For the conditions mentioned, an interfacial tension difference between two sides of the droplet of 1 mN m^{-1} would more than suffice to prevent lateral motion of the interface, and a difference of this magnitude can be achieved readily. It can be concluded that emulsion droplets behave like solid spheres, unless agitation is extremely vigorous or the droplets are very large.

7.6.2 Emulsion Formation [86,97,98]

In this section the size of the droplets and the surface load obtained during making of emulsions are discussed, especially when protein is used as the surfactant.

7.6.2.1 Droplet Breakup

To make an emulsion, one needs oil, water, an emulsifier (i.e., a suitable surfactant), and energy (generally mechanical energy). Making drops is easy, but to break them up into small droplets generally is difficult. Drops resist deformation and thereby break up because of their Laplace pressure, which becomes larger as droplet size decreases. This necessitates a large input of energy. The energy needed will be reduced if the interfacial tension, hence the Laplace pressure, is reduced by adding an emulsifier, though this is not the latter's main role.

The energy needed to deform and break up droplets is generally provided by intense agitation. Agitation can cause sufficiently strong viscous shear forces if the continuous phase is sufficiently viscous. This is the common situation when making w/o emulsions ($\eta_{oil} \approx 0.05$ Pa s), resulting in droplets with diameters down to a few μm. In o/w emulsions the viscosity of the continuous phase tends to be low, and to break up droplets, inertial forces are needed. These are produced by the rapid, intensive pressure fluctuations occurring in turbulent flow. The machine of choice is the high-pressure homogenizer, which can produce droplets as small as 0.1 μm. The average droplet size obtained is about proportional to the homogenization pressure to the power −0.6 (cf. Figure 7.27a). When using high-speed stirrers, faster stirring, longer stirring, or stirring in a smaller volume results in smaller droplets; however, average droplet diameters below 1 or 2 μm usually cannot be obtained.

There are, however, other factors that affect droplet size. Figure 7.28 depicts the *various processes* that occur during emulsification. Besides disruption of droplets (Figure 7.28a), an emulsifier has to be transported to the newly created interface (Figure 7.28b). The emulsifier is not transported by diffusion, but by convection, and this occurs extremely fast. The intense turbulence (or the high shear rate, if that is the situation) also leads to frequent encounters of droplets (Figure 7.28c and d). If these are as yet insufficiently covered by surfactant, they may recoalesce (Figure 7.28c). All these processes have their own time scales, which depend on several conditions, but a few μs are fairly characteristic. This means that all processes occur numerous times, even during one passage through a homogenizer valve, and that a steady state—where breakup and coalescence balance each other—is more or less attained.

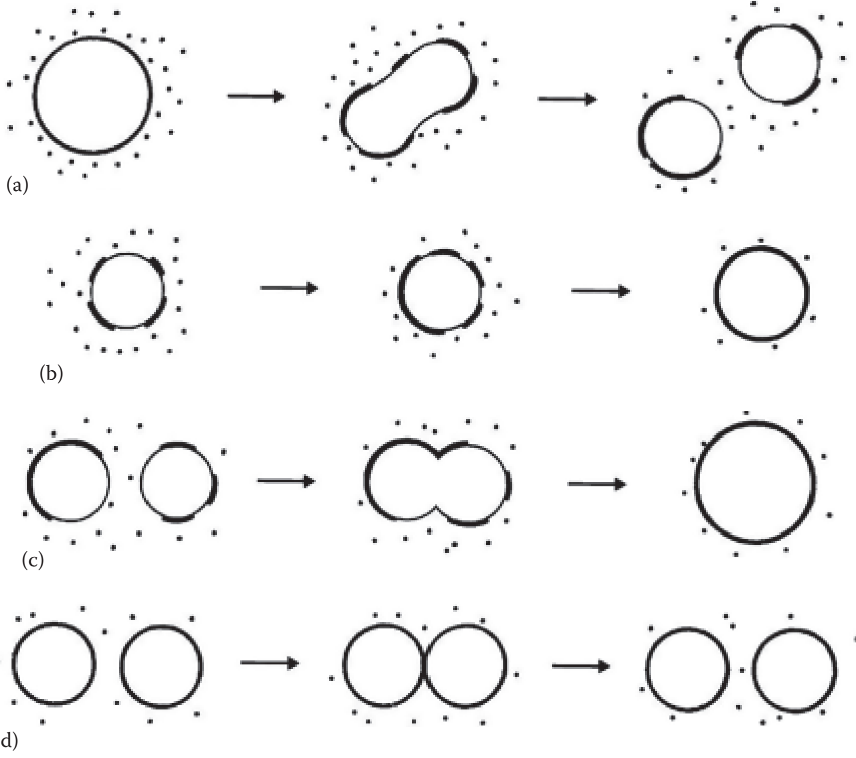


FIGURE 7.28 Important processes occurring during emulsification. The drops are indicated by thin lines and the emulsifier by heavy lines and dots. Highly schematic and not to scale. See the text for further explanation. (From Walstra, P., *Physical Chemistry of Foods*, Marcel Dekker, New York, 2003.)

7.6.2.2 Recoalescence

The main role of the emulsifier is to *prevent recoalescence* of newly formed droplets. It may seem logical to attribute this function to colloidal repulsion between the droplets, caused by the adsorbed surfactant. However, the droplets are repeatedly pressed together due to the agitation, whether in turbulent or in laminar flow. The maximum stress involved is of the order of the stress needed to break up the droplets, which is of the order of their Laplace pressure, which would be, say, 10^4 Pa. Sample calculations show that the "disjoining" pressure between droplets due to colloidal repulsion will generally be far smaller, say, 10^2 Pa or less. Hence, this would be insufficient to prevent the drops coming close and would therefore not prevent their recoalescence. Indeed, experimental work often shows poor correlation between recoalescence during emulsification and coalescence in the finished emulsion.

The mechanism involved in preventing recoalescence is probably as follows. When two droplets that are (partially) covered by surfactant are pressed together, the liquid between them will be squeezed out, causing the formation of an interfacial tension gradient. This is illustrated in Figure 7.29 [91]. The γ-gradient will then cause considerable slowing down of the liquid flow as discussed in relation to Figure 7.9a and b. This can strongly decrease the rate of approach of the droplets. It would not prevent them to come very close, but the stress pushing them together will generally be short-lived or even change sign (i.e., pulling the drops apart) before the droplets can coalesce. Sample calculations confirm that the stresses and the time scales involved are of the right order of magnitude.

The phenomenon is often called the *Gibbs–Marangoni effect*: its magnitude depends on the value of the Gibbs elasticity of the film (i.e., twice the local surface-dilational modulus) and the mechanism is related to the Marangoni effect.

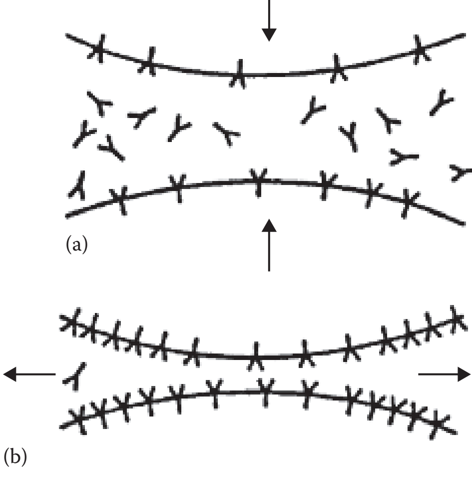


FIGURE 7.29 (a) Two drop approach each other during emulsification. (b) Illustration of the formation of a γ-gradient that slows down the outflow of continuous phase from the gap between the droplets. Surfactant molecules indicated by Y. (Modified after Walstra, P., *Chem. Eng. Sci.*, 48, 333, 1993.)

7.6.2.3 Choice of Emulsifier

Bancroft's rule: This rule states: when making an emulsion of oil, water, and a surfactant (emulsifier), the continuous phase will become the one in which the surfactant is best soluble. In Figure 7.29, the surfactant is present in the continuous phase. If it would be in the droplets, a γ-gradient can hardly develop, since surfactant molecules can readily reach the interface, which will result in an adsorption layer of almost constant composition. If the surfactant is in the continuous phase, the thin film between the approaching droplets will soon be (almost) depleted of surfactant, and a γ-gradient can persist. Hence, when the formation of w/o emulsion is desired, a surfactant with a small HLB number is needed, and for an o/w emulsion, one with a high HLB number.

Proteins are the emulsifiers of choice for o/w food emulsions because they are eatable, surface active, water soluble, and provide superior resistance to coalescence [93]. However, at equal agitation intensity, the droplets obtained are substantially larger than for a suitable small-molecule surfactant at the same mass concentration. The main reason can be derived from Figure 7.3b. It is seen that for a protein, a much higher surface excess concentration (Γ) at the o/w interface is needed to obtain a significant lowering of γ than for SDS. This also means that the possibility to form a significant γ-gradient during emulsification is far smaller than for SDS (and most other small-molecule emulsifiers). This implies, in turn, that the extent of droplet recoalescence will be much stronger than for SDS, as is indeed experimentally observed.

However, droplets can be made smaller by applying more intense emulsification, for example, a higher homogenization pressure, provided that sufficient protein is present. Some examples on average droplet size (d_{vs}) are given in Figure 7.27b. At a high emulsifier concentration, d_{vs} reaches a plateau value. This value is smaller for the nonionic surfactant than for the proteins, for the most part because the former produces a lower interfacial tension.

It is seen that the various proteins give about the same plateau value for d_{vs}. This is not strange because they produce comparable values for the interfacial tension (about 10 mN m^{-1}). But at low concentrations, large differences in d_{vs} are apparent. Several tests have been developed to evaluate the suitability of proteins as emulsifiers. The well-known emulsifying activity index (EAI) involves emulsifying a large quantity of oil in a dilute protein solution [59]. This test corresponds roughly

to the conditions indicated by the broken line in Figure 7.27b. Hence, the test result is not realistic for most practical situations, if only because the protein/oil ratio normally would be much larger. Consequently, EAI values are often irrelevant.

Attempts have been made to explain differences in EAI of various proteins by differences in their surface hydrophobicity [35]. However, the correlation is poor and other workers have refuted this concept (e.g., [63]). In the authors' view, proteins differ primarily in emulsifying efficiency because they differ in molar mass or in solubility. For a larger molar mass and the same mass concentration, the molar concentration is smaller, and the latter is presumably the most important variable in providing a strong Gibbs–Marangoni effect. Proteins of a smaller molar mass would thus be more efficient emulsifiers. It should be realized that several protein preparations, especially industrial products, contain molecular aggregates of various size, thereby greatly increasing effective molar mass and decreasing emulsifying efficiency. By and large, protein preparations that are poorly soluble are poor emulsifiers.

A substantially decreased molar mass, as realized, for instance, by partial hydrolysis of the protein, will indeed lead to the formation of smaller droplets. On the other hand, emulsions obtained with fairly small peptides usually show significant coalescence after preparation [65].

Another important variable is the *surface load* (Γ). If an emulsifier tends to give a high Γ, relatively much of it is needed to produce an emulsion. Compare, for instance, whey protein and soya protein in Figures 7.27b and 7.30. Moreover, a fairly high Γ is usually needed to obtain a stable emulsion.

In the case of small-molecule surfactants, equilibrium is reached between Γ and bulk concentration of surfactant. Consequently, knowledge of total surfactant concentration, o/w interfacial area, and of the adsorption isotherm (e.g., Figure 7.3a) will allow calculation of Γ, irrespective of the manner of formation of the emulsion. This is not the case when a protein (or other polymer) is the emulsifier, because thermodynamic equilibrium is not reached (Section 7.2.2). Thus, the surface load of a protein can depend on the way of making the emulsion, in addition to the variables mentioned.

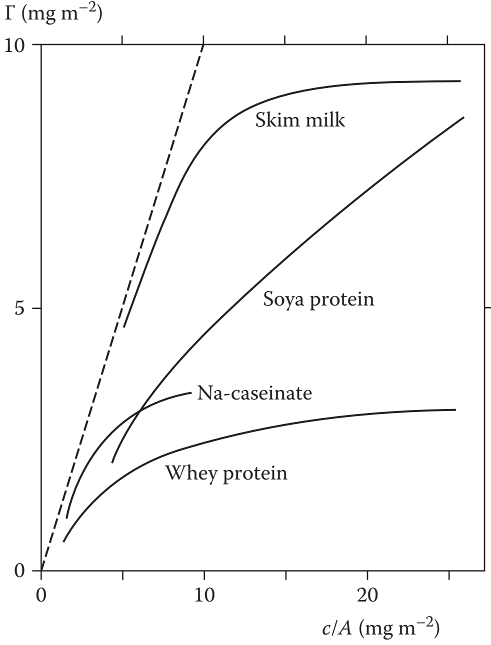


FIGURE 7.30 Protein load (Γ) as a function of the protein concentration (c) per unit oil surface area (A) created by emulsification. The broken line indicates the relation that would be obtained if all of the protein present would adsorb. (From Walstra, P. and de Roos, A.L., *Food Rev. Int.*, 9, 503, 1993.)

It has been observed that plots as given in Figure 7.30 are more suitable to relate Γ to protein concentration. The figure gives some examples of results obtained for various proteins. If *c*/*A* is very small, some proteins presumably almost fully unfold at the o/w interface and form a stretched polypeptide layer, with a Γ of about 1 mg m^{-2}. Several highly soluble proteins give plateau values of about 3 mg m^{-2}. Aggregated proteins can yield much larger values. It may also be noted that any large protein aggregates present tend to become preferentially adsorbed during emulsification, thereby further increasing Γ.

An emulsifier is needed not only for the formation of an emulsion, but also for *providing stability* of the emulsion once made. It is of some importance to clearly distinguish between these main functions, since they often are not related. An emulsifier may be very suitable for making small droplets, but may not provide long-term stability against coalescence, or vice versa. Evaluation of proteins merely for their ability to produce small droplets is, therefore, not very useful. Another, often desirable, feature of a surfactant is to prevent aggregation under a range of conditions (pH near the isoelectric point, high ionic strength, poor solvent quality, high temperature). Types of emulsion instability and means of prevention are discussed next.

7.6.3 Types of Instability [20,92,93]

Emulsions can undergo several types of physical change as illustrated in Figure 7.31. The figure pertains to o/w emulsions, the difference with w/o emulsions is that downward sedimentation rather than creaming would occur.

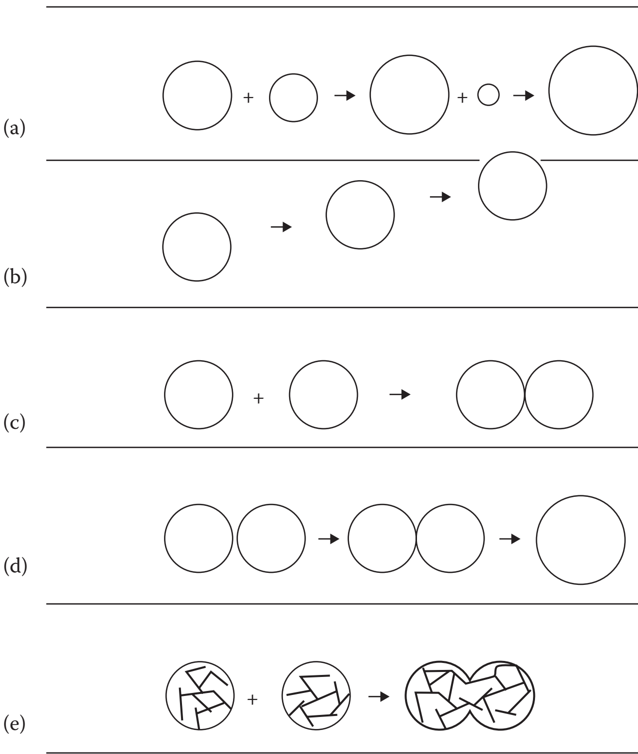


FIGURE 7.31 Types of physical instability for oil-in-water emulsions. Highly schematic. (a) Ostwald ripening, (b) creaming, (c) aggregation, (d) coalescence, and (e) partial coalescence. The size of the contact area in (d) may be greatly exaggerated; the short heavy lines in (e) denote triacylglycerol crystals.

Ostwald ripening (Figure 7.31a) does not normally occur in o/w emulsions, because triacylglycerol oils are commonly used and they are insoluble in water. When essential oils are present (e.g., in citrus juices), some have sufficient solubility so that smaller droplets gradually disappear [24]. w/o emulsions may exhibit Ostwald ripening. Data in Table 7.3 show only a very small solubility excess for a 2 μm droplet, but it would be sufficient to produce marked Ostwald ripening during prolonged storage. This can easily be prevented by adding a suitable solute to the water phase (i.e., one that is insoluble in oil). A low concentration of salt (say, NaCl) will do: as soon as a small droplet shrinks, its salt concentration and osmotic pressure increase, thereby producing a driving force for water transport in the opposite direction. The net result is a stable droplet size distribution.

The other instabilities are discussed in other sections: creaming in Section 7.4.2, aggregation in Section 7.4.3, coalescence in Section 7.6.4, and partial coalescence in Section 7.6.5.

The various changes may affect each other. Aggregation greatly enhances creaming and if this occurs, creaming further enhances aggregation rate, and so on. Coalescence can only occur when the droplets are close to each other (i.e., in an aggregate or in a cream layer). If the cream layer is more compact, which may occur when fairly large separate droplets cream, coalescence will be faster. If partial coalescence occurs in a cream layer, the layer may assume characteristics of a solid plug.

It is often desirable to *establish the kind of instability* that has occurred in an emulsion. Coalescence leads to large drops, not to irregular aggregates or clumps. Clumps due to partial coalescence will coalesce into large droplets when heated sufficiently to melt the fat crystals. A light microscope can be used to establish whether aggregation, coalescence, or partial coalescence has occurred. Section 7.4.3 gives some hints for distinguishing among various causes of aggregation. It is fairly common that coalescence or partial coalescence leads to broad size distributions, and then the larger droplets or clumps cream very rapidly.

Agitation can disturb creaming and may disrupt aggregates of weakly held droplets, but not clumps formed by partial coalescence. Slow agitation tends to counteract true coalescence.

If air is beaten in an o/w emulsion, this may lead to adsorption of droplets onto air bubbles. The droplets may then be disrupted into smaller ones, due to spreading of oil over the a/w interface (Section 7.2.3). If the droplets contain crystalline fat, clumping may occur; beating in of air thus promotes partial coalescence. This is what happens during churning of cream to make butter and also during whipping of cream. In the latter case, the clumped, partially solid droplets form a continuous network that encapsulates and stabilizes the air bubbles and lends stiffness to the foam.

A way to prevent or retard all changes except Ostwald ripening is to *immobilize the drops*, for instance, by causing the continuous phase to gel (Section 7.5.3). Examples are butter and margarine. Here, the water droplets are immobilized by a network of fat crystals. Moreover, some crystals become oriented at the oil–water interface because of the favorable contact angle (Section 7.2.3). In this way, the droplets cannot closely encounter each other. If the product is heated to melt the crystals, the aqueous droplets readily coalesce. Often, a suitable surfactant is added to margarine to prevent rapid coalescence during heating, since this would cause undesirable spattering.

7.6.4 Coalescence [26,92,93]

This discussion will focus on o/w emulsions. The theory is still in a state of some confusion.

7.6.4.1 Film Rupture

Coalescence is induced by rupture of the thin film (lamella) between close droplets (the same applies for a film between gas bubbles). This is illustrated in Figure 7.32a: a small hole can somehow be formed in the film by chance (due to Brownian motion). If the radius of the hole is larger than half the film thickness ($R > \delta/2$), the Laplace pressure near 1 is larger than near 2; hence, liquid in the film will flow from 1 to 2; hence, the hole will expand, which implies that the film ruptures; hence, the droplets will immediately flow together. It also would follow that any film will rupture if it becomes thin enough; colloidal repulsion will oppose this. However, Figure 7.32b illustrates that

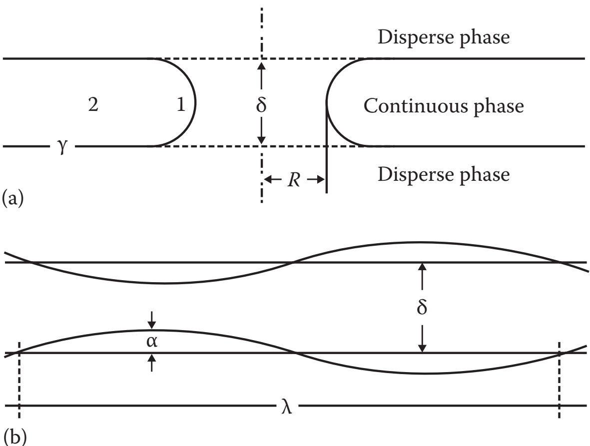


FIGURE 7.32 Cross section of part of a film of (average) thickness δ between drops (or gas bubbles). (a) Illustration of hole formation. (b) Properties of a symmetric wave developing on the film.

the film may become locally thinner because symmetric waves can develop at the film surfaces. The amplitude of the waves can be larger, hence film rupture more likely, when the wavelength of the film is greater (i.e., the film area is larger) and γ is smaller.

We will not further discuss the underlying theory, also because the theory does not explain all observations on droplet coalescence. For instance, it is observed that some macromolecular surfactants cause an additional resistance to coalescence. Presumably, these molecules form coherent monolayers that also have to rupture for the film to rupture. Important examples are proteins that tend to form coherent layers after adsorption, such as β-lactoglobulin; here, formation of intermolecular –S–S– bonds appears to be involved.

7.6.4.2 Factors Affecting Coalescence

Film rupture is thus a chance event and this has important consequences: (1) The probability of coalescence, if it does occur, will be proportional to the time that the droplets are close to each other. Hence, it is especially likely in a cream layer or in aggregates. (2) Coalescence is a first-order rate process with respect to time, unlike aggregation, which is in principle second order. (3) The probability that rupture of a film occurs will be proportional to its area. This implies that flattening of the droplets on approach, leading to the formation of a greater film area, will promote coalescence.

Whether or not a flat film is formed is, therefore, an essential variable. It can be expressed in a *Weber number*, which gives the ratio of the local stress on a droplet pair over the Laplace pressure of the drop. The local stress is the external stress (σ_{ext}) times a stress concentration factor; the latter equals droplet radius over the smallest distance (h) between the drop surfaces. This leads to

$$W_e = \frac{\sigma_{ext} d^2}{8\gamma h} \tag{7.33}$$

If $W_e > 1$, a flat film is formed between the drops, and the larger W_e, the larger the film radius. For $W_e \ll 1$, there is no real film formed and there is virtually no coalescence.

The external stress can be due to colloidal attraction forces, or to hydrodynamic stresses caused by flow or agitation, or to the gravitational or centrifugal stress in the cream layer (or sediment). For small protein-covered emulsion droplets, the condition $W_e \ll 1$ is nearly always fulfilled, unless strong external forces are acting. Even in a cream layer formed under gravity, the external stress is small enough for W_e to be much below unity.

Taking these considerations into account, we can draw the following conclusions. Coalescence is less likely for the following:

1. *Smaller droplets*: (1) They lead to a smaller W_e and hence to a smaller film area between droplets and to a lower probability of rupture of the film. (2) More coalescence events are needed to obtain droplets of a certain size. (3) The rate of creaming is decreased. In practice, average droplet size is often the overriding variable.
2. *A thicker film between droplets*: This implies that strong or far-reaching repulsive forces between droplets (Section 7.3) provide stability against coalescence. For DLVO-type interactions, coalescence will readily occur if the droplets are aggregated in the primary minimum (Figure 7.11). Steric repulsion is often especially effective against coalescence, because it tends to keep the droplets relatively far apart.
3. *A greater interfacial tension*: This may appear strange, because a surfactant is needed to make an emulsion and a surfactant decreases γ. Moreover, a smaller γ implies a smaller surface free energy of the system, hence a smaller driving force for coalescence. However, it is the *activation* free energy for film rupture that counts, which is larger for a larger γ. This is because a larger γ makes it more difficult to form and to deform a film (bulging, development of a wave on it), and local deformation is needed to induce rupture.

Based on these principles, *proteins* appear to be very suitable for preventing coalescence, and this agrees with observation. Proteins do not produce a very small γ, and they often provide considerable repulsion, both electric and steric. Figure 7.33 shows results of experiments in which small droplets in an extremely dilute protein solution were allowed to cream to planar o/w interfaces of a given age, and the time needed for coalescence was observed. A strong effect of droplet size is apparent. The results shown were obtained under conditions (protein concentration and adsorption time) where the surface load of the proteins would have been at most about 0.5 mg m^{-2}, implying very weak repulsion. In cases where a thicker adsorbed layer was allowed to form, the authors observed virtually no coalescence.

Figure 7.33 shows no significant difference between proteins in their ability to prevent coalescence. This is also the general experience in practice, except for gelatin, which is somewhat less

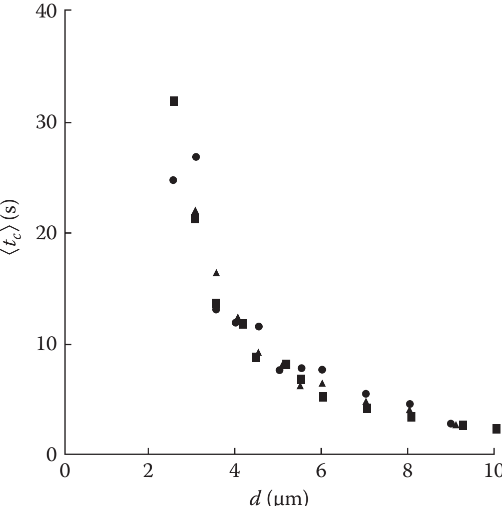


FIGURE 7.33 Average time (⟨t_c⟩) for coalescence of oil droplets of various diameter (*d*) with a plane o/w interface, in 20-min-old 1 ppm protein solutions. ▲, β-casein; ●, κ-casein; ■, lysozyme. (After Dickinson, E. et al., *J. Chem. Soc. Faraday Trans. 1*, 84, 871, 1988.)

effective than most other proteins. Under severe conditions (see next paragraph), differences among proteins may be observed, with caseinates tending to be superior. Partial hydrolysis of proteins can significantly impair their ability to prevent coalescence [65].

Attempts have been made to relate the coalescence-inhibiting ability of proteins (and of other surfactants) to various properties, particularly surface-shear viscosity (Section 7.2.5) of the adsorbed protein layer. In some cases, a positive correlation between (apparent) surface-shear viscosity and coalescence stability is observed, but there are several cases where deviation from this relationship are very large; for example, caseinates give a very low surface-shear viscosity, but quite good coalescence stability. Reasonable correlations are observed for many globular proteins if the drops are relatively large or Γ is fairly small; the cause for the increase in stability is presumably that intermolecular cross-links are formed at the interface, as mentioned earlier.

On the other hand, in a highly concentrated emulsion (e.g., $\phi = 0.8$) that is subjected to strong elongational flow by pressing the emulsion through a small orifice, considerable coalescence can occur if the surface layers consist of cross-linked globular proteins [73]. It has been explained by considering the emulsion as a soft solid that is subject to macroscopic fracture (cf. Section 7.5.2). Apparently, the fracture planes are through the emulsion droplets, leading to strong local coalescence. High-ϕ emulsions stabilized with caseinate, which does not form strong intermolecular cross-links, do not show significant coalescence in elongational flow.

Most *small-molecule surfactants* yield a small interfacial tension. Because a small γ favors coalescence, surfactants that provide considerable steric repulsion, such as the Tweens, are among the most effective. Ionic surfactants are effective against coalescence only at low ionic strength.

Small-molecule surfactants present in (or added to) protein-stabilized emulsions tend to displace protein from the droplet surface (Section 7.2.2; Figure 7.5), and this generally decreases resistance to coalescence. If coalescence is desired, this provides a method to achieve it; for example, add SDS and some salt (to decrease double layer thickness), and rapid coalescence will usually occur.

Food emulsions may exhibit coalescence under *extreme conditions*. For example, during freezing, formation of ice crystals will force the emulsion droplets closer together, often causing copious coalescence on thawing. Something similar happens upon drying and subsequent redispersion; here, coalescence is alleviated by a relatively high concentration of *solids not fat*. In such cases, best stability is obtained by having small droplets and a thick protein layer, for example, of Na-caseinate.

Another extreme condition is centrifugation. This causes a cream layer to form rapidly, pressing the droplets together with sufficient force to cause a high W_e and hence considerable flattening even of small droplets and likely coalescence. This implies that centrifugation tests to predict coalescence stability of emulsions during storage are usually not valid, since the conditions during centrifugation differ so greatly from those during handling of emulsions. (This does not mean that centrifugation tests to predict creaming are useless. They can be quite helpful if the complications discussed in Section 7.4.2 are taken into account.)

Predicting coalescence rate is always very difficult. The best approach is to use a sensitive method to estimate average droplet size (e.g., turbidity at a suitable wavelength) and establish the change over time (say, a few days).

7.6.5 Partial Coalescence [6–8,14,77,92]

In many o/w food emulsions, part of the oil in the droplets can crystallize. The proportion of fat solid, ψ, depends on the composition of the triacylglycerol mixture and on temperature (Chapter 4). In emulsion droplets, ψ can also depend on temperature history, since a finely emulsified oil can show considerable and long-lasting undercooling, the more so for smaller droplets [87]. This is illustrated in Figure 7.34. If an emulsion droplet contains fat crystals, they usually form a continuous network. These phenomena greatly affect emulsion stability. The presence of crystals means that we have no true o/w emulsion and it may be better to speak of fat globules.

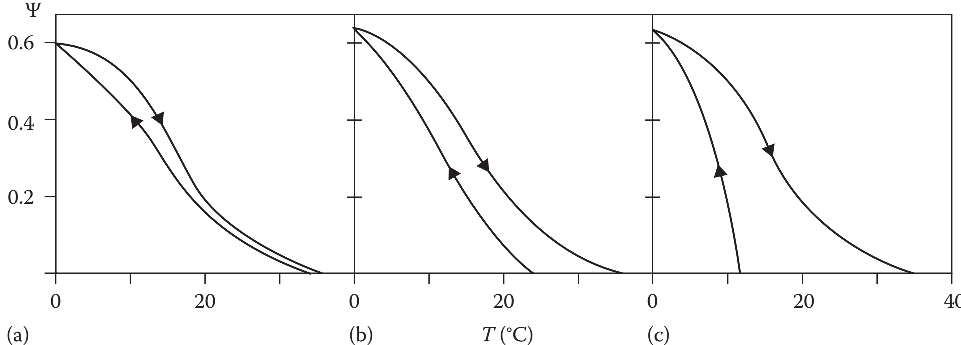


FIGURE 7.34 Proportion of milk fat being solid (ψ) after 24 hours cold storage at temperature *T* and after warming it again (after it had been kept at 0°C). (a) Fat in bulk, (b) the same fat in natural cream (globule size about 4 μm), and (c) the same fat in homogenized cream (globule size about 0.5 μm). (From results by P. Walstra and van Beresteyn, E.C.H., *Neth. Milk Dairy J.*, 29, 35, 1975.)

Fat globules containing a network of fat crystals cannot fully coalesce (Figure 7.31e). If the film between globules ruptures, they form an irregular clump, held together by a "neck" of liquid oil. True and partial coalescence thus have different consequences. Partial coalescence causes an increase in the apparent volume fraction of dispersed material, and if the original volume fraction is about 0.2 or higher and shear rate is fairly small, a solid, or gel-like, network of partially coalesced clumps can form.

Rupture of the film between close globules can be triggered by a crystal that protrudes from the globule surface and pierces the film. This happens particularly during flow or agitation, and then it may occur, say, six orders of magnitude faster than true coalescence (same emulsion, no fat crystals). This implies that partial coalescence is far more important than true coalescence, if an o/w emulsion is subject to fat crystallization.

The kinetics of partial coalescence is complicated and variable, because it is affected by several factors. In many emulsions, large particles (original globules or clumps already formed) are more prone to partial coalescence than small ones, leading to a self-accelerating process, which soon leads to large clumps that cream rapidly. The remaining layer may then exhibit a decreased average particle size. Other emulsions may simply show a gradual increase in average particle size with time.

The most important factors affecting the *rate of partial coalescence* generally are as follows (Figure 7.35):

1. *Shear rate*: This has various effects: (1) The encounter rate between particles is proportional to shear rate (Section 7.4.3). (2) Because of the shear flow, two globules that encounter each other will roll over each other, thereby significantly enhancing the probability that a crystal protruding from one globule attains—for a short while—a position where it is close to the other globule. (3) The shear force tends to press approaching globules closer together against any repulsive force acting between the globules, thereby enhancing the possibility that a protruding crystal at a favorable position can pierce the film. Thus, flow rate has a very large effect on the rate of partial coalescence, and this influence is even stronger when the flow is turbulent rather than laminar.
2. *Volume fraction of droplets*: For a higher ϕ, the rate of partial coalescence is obviously greater, being about second order with respect to ϕ.
3. *Fat crystallization*: If the fraction solid, ψ, is zero, partial coalescence is impossible and it can also not occur in the absence of liquid fat ($\psi = 1$). For fairly low ψ, partial coalescence

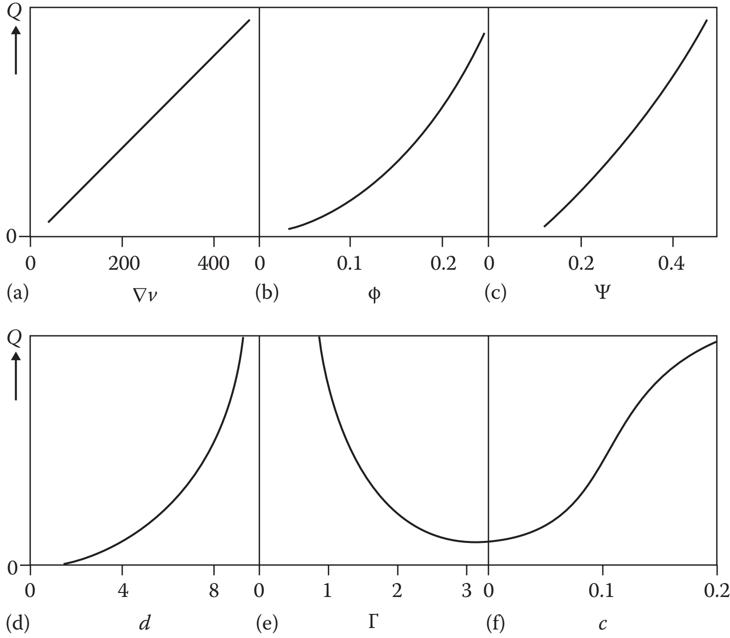


FIGURE 7.35 Approximate results obtained on the rate of partial coalescence (Q) in protein-stabilized emulsions as a function of (a) shear rate ∇v (s^{-1}), (b) volume fraction ϕ, (c) proportion of fat solid ψ, (d) average globule diameter d (μm), (e) protein surface load Γ (mg m^{-2}), and (f) concentration of added small-molecule surfactant c (%). Only meant to illustrate trends. (From Walstra, P., Emulsion stability, in: *Encyclopedia of Emulsion Technology*, Vol. 4, Becher, P., ed., Dekker, New York, 1996, pp. 1–62.)

rate generally increases with increasing ψ, as this causes more crystals to protrude. However, the relation between ψ and rate is variable, largely due to variation in crystal size and arrangement. An important aspect is that the crystals must form a network throughout the globule to support protruding crystals. The minimum ψ needed for such a network often is of the order 0.1. If most of the oil is crystallized and the crystals are very small, the crystal network may tenaciously hold the remaining oil, thereby preventing partial coalescence, even if the film is pierced. Moreover, the protrusion distance may depend on ψ, temperature history, crystal size, and crystal shape.

4. *Globule diameter*: A relation as depicted in Figure 7.35d is usually observed, but the scale of globule size varies substantially among emulsions. The effect of d presumably is due to (1) larger globules sensing a larger shear force and (2) larger globules exhibiting a larger film area between two globules.
5. *Surfactant type and concentration*: Two effects are of major importance. First, these variables will determine the oil–crystal–water contact angle (Section 7.2.3) and thereby affect the distance if a given crystal can protrude. Second, these variables determine repulsion (strength and range) between the globules. The weaker the repulsion, the easier it is for two droplets to closely approach each other, thereby increasing the likelihood that a protruding crystal will pierce the film between them. The repulsion, together with the globule size, will thus determine what minimum shear rate is needed for partial coalescence to occur; values between 5 and 120 s^{-1} have been observed. Some emulsions show no partial coalescence at all at the shear rates studied. The best type of surfactant to achieve this is, again, a protein, if the surface load is high enough (Figure 7.35e). Addition of a small-molecule surfactant generally leads to displacement of the protein from the surface (Section 7.2.2), thereby greatly enhancing partial coalescence (Figure 7.35f).

7.6.5.1 Ice Cream [96]

We will illustrate some consequences of partial coalescence in relation to making and properties of ice cream. This product has an intricate structure. It contains ice crystals, an aqueous phase that consists of concentrated skim milk with added sugar(s), air bubbles, and milk fat globules, which are partially crystalline. The air bubbles and the ice crystals are made in a scraped-surface heat exchanger, during vigorous agitation and rapid cooling. The fat globules are needed for covering the air bubbles to ensure stability against Ostwald ripening and coalescence of the bubbles (Section 7.7.2).

The fat globules show extensive partial coalescence in the heat exchanger, which is desirable. It leads to the formation of a space-filling network of fat globule clumps and fat globule-covered air bubbles. This structure provides a "meltdown resistance," that is, a certain firmness remains after the ice crystals have melted in the mouth. Moreover, it gives the product a *dry* appearance and a *short* consistency; the latter greatly diminishes the stickiness of the product. These properties make the ice cream more attractive for the consumer and allow the use of fast packaging machines.

If ice cream is made of natural (unhomogenized) cream (plus sugar and a number of additives), partial coalescence happens extremely fast. The natural milk fat globules are not very small (for the most part 1.5–6 μm) and have a surface layer that gives a quite low interfacial tension (1–1.5 mN m^{-1}). Moreover, the vigorous agitation causes large clumps of fat globules to form, far too large to be able to fully cover the air bubbles desired. Consequently, the ice cream obtained has a coarse structure, with large air bubbles and large fat clumps. The remedy is to homogenize the cream, which results in far smaller fat globules (say, 0.4–1.2 μm). The surface layers of these globules largely consist of milk plasma proteins. This inhibits partial coalescence (Figure 7.35d and e). The product obtained has small air bubbles and a homogeneous structure, but not the desired meltdown resistance and dryness.

To overcome the latter problem, small-molecule surfactants are added that substantially enhance partial coalescence rate (Figure 7.35f). By varying type and concentration of these surfactants, optimum conditions for obtaining a product of good quality can be established.

7.6.6 Summary

- Two main types of emulsions can be distinguished: o/w and w/o. Main characteristics are droplet size distribution, volume fraction dispersed phase, composition and thickness surface layer, and composition continuous phase.
- To make an emulsion, oil, water, a suitable surfactant, and energy are needed.
- Important processes during emulsification are droplet breakup, transport of emulsifier to the newly created interface, encounters and recoalescence of newly formed droplets, and aggregation and deaggregation.
- Main role of emulsifiers is preventing recoalescence during emulsification by the Gibbs–Marangoni effect and after emulsification by colloidal repulsion.
- For o/w emulsions proteins are mostly the best emulsifiers, primarily because they are eatable and provide superior resistance against coalescence after emulsification.
- Types of physical instabilities are creaming (or sedimentation), Ostwald ripening, aggregation, coalescence, and partial coalescence.
- Ostwald ripening involves growth of larger droplets at the cost of smaller ones and occurs when the discontinuous phase is soluble in the continuous phase.
- Coalescence involves the rupture of the thin film in between two close droplets. It occurs the sooner the film in between is larger and thinner. It will happen less likely for smaller droplets, a thicker film in between the droplets and a greater interfacial tension.
- Extreme conditions as freezing and thawing, drying and redispersion, and centrifugation favor coalescence.

- Partial coalescence may occur, as the oil in o/w emulsions is partially crystallized. The extent to which it happens depends mainly on shear rate, volume fraction dispersed particles, proportion of fat that is solid, diameter droplets, protein surface load, and concentration of (added) small-molecule surfactant.

7.7 FOAMS

In a sense, foams are much like o/w emulsions; both are dispersions of a *hydrophobic* fluid in a hydrophilic liquid. However, because of considerable quantitative differences, their properties are also qualitatively different. Quantitative information is given in Table 7.7. It is evident that bubble diameter is so large that it excludes foam bubbles from the realm of colloids. A large diameter combined with a large density difference causes foam bubbles to cream faster than emulsion droplets by some orders of magnitude. The relatively high solubility of air in water can cause rapid Ostwald ripening (often called disproportionation in foams). If the gas phase is CO_2, as it is in some foods (bread, carbonated beverages), the solubility is even higher, by a factor of about 50. The characteristic time scales during formation are two or three orders of magnitude longer for foams than for most o/w emulsions. Because creaming and Ostwald ripening occur so fast, physical instabilities often occur already during foam formation, which complicates the study of foaming and foam stability.

Several aspects of foams will be briefly discussed. Surface phenomena are of overriding importance to foam formation and properties; background information is provided in Section 7.2. Further sources of literature are [29,60,88,97]. For churning and whipping of cream, see [72]. Also some of the books mentioned in the "Further Reading" section, especially Walstra's and Dickinson's, have chapters on foam.

7.7.1 Formation and Description

In principle, foams can be made in two ways, by supersaturation or mechanically.

7.7.1.1 Via Supersaturation

A gas, usually CO_2 or N_2O, because of their high solubility, is dissolved in an aqueous liquid at high pressure (a few bars). When the pressure is released, gas bubbles form. These do not form

TABLE 7.7
Comparison of Foams and Emulsions: Order of Magnitude of Some Quantities

Property	Foam	Foam	Emulsion w/o	Emulsion o/w	Units
Drop/bubble diameter	10^{-3}	10^{-4}	5×10^{-6}	10^{-6}	m
Volume fraction	0.9	0.8	0.1	0.1	—
Drop/bubble number	10^{9}	10^{11}	10^{15}	10^{17}	m^{-3}
Interfacial tension	0.05	0.05	0.005	0.01	$N\ m^{-1}$
Laplace pressure	2×10^{2}	2×10^{3}	4×10^{3}	4×10^{4}	Pa
Solubility D in C	2.1[a]	2.1[a]	0.15	0	vol.%
Density difference D–C	-10^{3}	-10^{3}	10^{2}	-10^{2}	$kg\ m^{-3}$
Viscosity ratio D/C	10^{-4}	10^{-4}	10^{-2}	10^{2}	—
Time scale[b]	10^{-3}	10^{-4}	10^{-5}	10^{-6}	s

Key: D, disperse phase (air, triacylglycerols, or water); C, continuous phase.
[a] If it concerns CO_2, the solubility is about 100 vol.% at a pressure of 1 bar.
[b] Characteristic times during formation.

by nucleation; a spontaneously formed gas bubble would need to have an initial radius of about 2 nm, which would imply a Laplace pressure (Equation 7.8) of about 10^8 Pa, or 10^3 bar. To achieve this, the gas would have to be brought to this pressure, which is, of course, impractical. Instead, gas bubbles always grow from small air pockets that are already present at the wall of the vessel or on small particles. The contact angle gas/water/solid may be as high as 150° for a fairly hydrophobic solid, and this allows small air pockets to remain in crevices or sharp dents in the solid (Figure 7.7b). For a negative curvature, air can even remain there if less than saturated.

To give an example, if a pressurized vessel of a carbonated liquid is opened, the overpressure is released, CO_2 becomes supersaturated, and it diffuses toward any air pockets present. These grow and become dislodged when large enough, leaving a remnant from which another bubble can grow. The bubbles rise while growing further, and a creamed layer of bubbles is formed (i.e., a foam). These bubbles are always fairly large, say, 1 mm.

Another example is the formation of CO_2 in a leavened dough. Excess CO_2 collects at sites of entrapped small air bubbles, and these sites grow in size. Some of them grow to form visible gas cells, creating a macroscopic foam structure.

7.7.1.2 By Mechanical Forces

A gas stream can be led through narrow openings into the aqueous phase (sparging); this causes bubbles to form, but they are fairly large, generally >1 mm. Smaller bubbles can be made by beating air into the liquid. At first, large bubbles form and these are broken up into progressively smaller ones. Shear forces are typically too weak to obtain small bubbles and the breakup mechanism generally involves pressure fluctuations in a turbulent field, as is the case during the formation of o/w emulsions (Section 7.6.2). Bubbles of about 100 μm can be obtained in this way, the smallest ones being, say, 20 μm.

Beating is the method of choice in industrial processing. If this occurs in an open system, as when whipping egg white in a bowl, the main resultants of the process are the average bubble size and the volume fraction of gas incorporated ϕ. The latter is often expressed as *percent overrun*, which is equal to $100\phi/(1-\phi)$. The factors determining the overrun are insufficiently understood. Therefore, we will not discuss all aspects but give some important variables in Figure 7.36. The same variables affect the resulting bubble size; by and large, a higher beating speed and a higher surfactant concentration result in smaller bubbles. In industrial practice, closed systems are often used, implying that

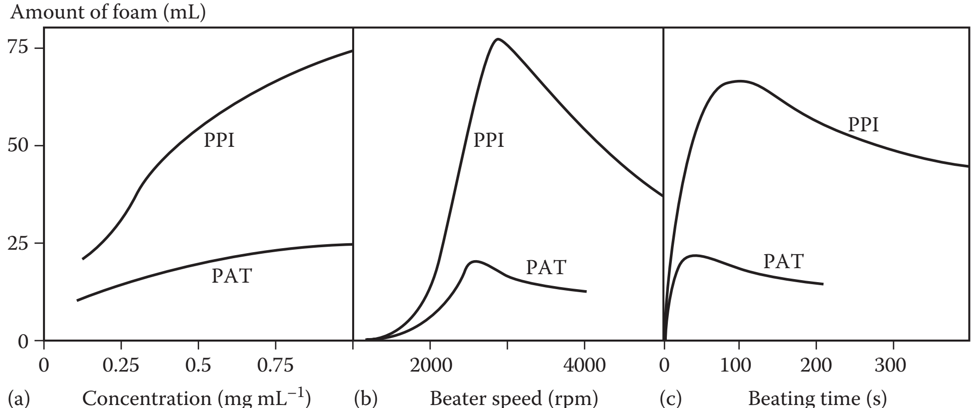


FIGURE 7.36 Amount of foam produced from dilute solutions of potato protein isolate (PPI) and purified patatin (PAT). pH = 7.0, ionic strength = 0.05 molar. (a) Effect of protein concentration. (b) Effect of beater speed (revolutions per minute). (c) Effect of beating time. Results by courtesy of G. van Koningsveld. (From Walstra, P., *Physical Chemistry of Foods*, Marcel Dekker, New York, 2003.)

the amounts of liquid and gas can be metered. This then determines the overrun obtained, provided that enough surfactant is present.

To make foam, a *surfactant* is needed. Almost any type will do, since the only criterion for its functionality is that a certain γ-gradient be created. This does not mean that any surfactant is suitable to make a stable foam, as will be discussed later. Moreover, it is the molar concentration of surfactant that determines the overrun, which means that proteins need higher mass concentrations than small-molecule amphiphiles.

Nevertheless, proteins are the agents of choice in the food industry: they are eatable and tend to give relatively stable foams. As seen in Figure 7.36a, the protein concentration is an important variable. To obtain a high overrun, much higher concentrations are needed. One of the reasons why egg white is a superior foaming agent is that it contains 10% protein. A 5% solution of undenatured whey protein can yield an overrun of, say, 1000%. However, considerable variation exists among proteins in the concentration needed to obtain a given overrun. Some peptides obtained by hydrolysis of a protein can give a higher overrun than the protein itself at the same mass concentration, but the physical stability of the foam often is significantly impaired. As a rule of thumb, mixtures of proteins, or of proteins and peptides, are superior to most pure proteins as foaming agents.

7.7.1.3 Foam Structure Evolution

Figure 7.37 illustrates the stages in foam formation after the creation of initial bubbles. As soon as beating stops, bubbles rise rapidly and form a foam layer (unless liquid viscosity is quite high). The buoyancy force soon is sufficient to cause mutual deformation of bubbles, causing the formation of flat lamellae between them. The stress due to buoyancy is roughly equal to $\rho_{water}gH$, where H is the height in the foam layer (i.e., about 100 Pa for H = 1 cm). However, there is marked stress concentration as spherical bubbles come into contact, and this means that bubbles with a Laplace pressure of 10^3 Pa would become significantly flattened. Further drainage of interstitial liquid causes the bubbles to attain a polyhedral shape. Where three lamellae meet (never >3, because that would be an unstable conformation), a prism-shaped water volume, bounded by cylindrical surfaces, is formed. This structural element is called a Plateau border. Generally, residual small bubbles soon disappear by Ostwald ripening. In this way, a fairly regular polyhedral foam is formed, somewhat resembling a honeycomb structure. In the lower part of a foam layer, bubbles remain more or less spherical.

As the foam keeps draining, its volume fraction of air increases, and a liquid layer forms beneath the foam. The Laplace pressure in the Plateau borders is lower than in the lamellae, and this causes liquid to flow to the Plateau borders. Because the latter are interconnected, they provide pathways through which the liquid can drain. As drainage continues, a ϕ value of 0.95 can readily be reached, which corresponds to an overrun of 1900%. Such a foam is not very substantial as a food.

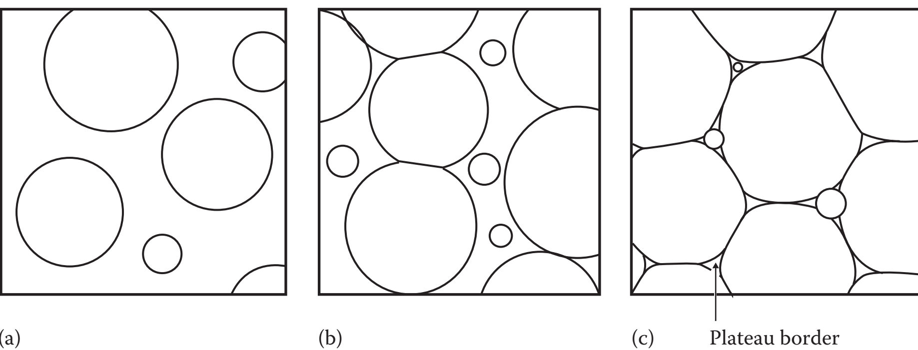


FIGURE 7.37 Subsequent stages (a, b, and c) in the formation of a polyhedral foam, once bubbles have been created. The thicknesses of the lamellae between bubbles are too small to be seen at this scale (bubble diameter <1 mm).

To avoid (excessive) drainage, small filler particles may be incorporated, but they should be hydrophilic; otherwise, considerable coalescence of bubbles can occur (Section 7.7.2.3). Small protein-coated emulsion droplets will function well, and they are incorporated in several whipped toppings. Another approach is gelation of the aqueous phase. This is employed in many aerated food products, such as meringues, foam omelets, bavarois, bread, and cakes. By letting the system gel in an early stage, it is also possible to make a foam with spherical bubbles; in other words a *bubbly* or *wet* foam, rather than a polyhedral or *dry* foam.

A polyhedral foam itself may be considered a gel. Deformation of the foam causes an increase in curvature of bubbles, a corresponding increase in Laplace pressure, and elastic behavior at small deformation. Then, at greater stress, bubbles slip past each other and viscoelastic deformation occurs. There is thus a yield stress (see Section 7.5.2), which is obvious, because even a tall portion of foam may retain its shape under its own weight. The yield stress usually exceeds 100 Pa. For further reading see [80] and references therein.

7.7.2 Stability

Foams are subject to three main types of instabilities:

1. Ostwald ripening (disproportionation), which is the diffusion of gas from small to larger bubbles (or to the atmosphere). This occurs because the pressure in a small bubble is greater than in larger ones.
2. Drainage of liquid from and through the foam layer, due to gravity.
3. Coalescence of bubbles due to instability of the film between them.

These changes are to some extent interdependent: drainage may promote coalescence, and Ostwald ripening or coalescence may enhance drainage.

These instabilities are governed by fundamentally different factors, as will be made clear in the following text. Unfortunately, many studies on foam stability have failed to distinguish between the three types of instability. One reason for this may be the lack of suitable methods to monitor bubble-size distribution.

7.7.2.1 Ostwald Ripening

See Section 7.2 for fundamental aspects. Ostwald ripening is often the most important type of foam instability, especially in foods, where the bubble size is relatively small as compared to many other foams. Within minutes after foam formation, noticeable coarsening of the bubble-size distribution often occurs. Ostwald ripening happens most rapidly at the top of a foam layer, because the air can diffuse directly to the atmosphere and the layer of water between a bubble and the atmosphere is very thin, but also it can takes place inside a foam at a significant rate.

The classical treatment of Ostwald ripening rate, based on Equation 7.9 and the diffusion laws, is by de Vries [18]. He considered a small bubble of radius r_0 surrounded by much larger bubbles at an average distance δ. The change in radius with time t then would be given by

$$r^2(t) = r_0^2 - \left(\frac{RTD\, s_\infty \gamma}{p\delta}\right)t \tag{7.34}$$

where

D is the diffusion coefficient of the gas in water (in $m^2\ s^{-1}$)

s_∞ is solubility for $r = \infty$ (since the solubility of a gas is proportional to its pressure, it is given in mol $m^{-3}\ Pa^{-1}$)

γ is interfacial tension (mostly about 0.05 N m^{-1})

p is ambient pressure (often 10^5 Pa)

It follows from Equation 7.34 that a bubble will shrink ever faster as it becomes smaller. Furthermore, since γ and solubility for most gases in water are high, shrinkage is fast, as is illustrated by the following examples. A nitrogen bubble of radius 0.1 mm at δ = 1 mm in water would disappear in about 3 min, and a similar CO_2 bubble in about 4 s. This is not quite realistic, since the geometric assumptions underlying Equation 7.34 are not fully met in practice and also because the process is somewhat slower if a mixture of gases, like air, is present. Moreover, as the smallest remaining bubbles become larger, the rate of change decreases with time. Nevertheless, Ostwald ripening can occur quite fast.

Can Ostwald ripening be stopped or retarded? If a bubble shrinks, its area decreases and its surface load (Γ) increases, provided the surfactant does not desorb. If no desorption occurs, γ is lowered and thereby the Laplace pressure, which implies that the driving force for Ostwald ripening is decreased. It will even stop when the surface-dilational modulus, E_{SD}, which is a measure of the change in γ with change in area (see Equation 7.10), becomes about equal to γ. However, surfactant normally desorbs and E_{SD} therefore decreases, at a rate that depends on several factors, especially surfactant type. For a foam made with small-molecule surfactants, desorption occurs readily, and retardation of Ostwald ripening tends to be negligible. Proteins, however, tend to desorb sluggishly (see Section 7.2.2), and E_{SD} may remain fairly high (Section 7.2.6), especially if the gas consists of CO_2. Ostwald ripening will then be substantially retarded [61], although shrinking bubbles (and emulsion droplets) may also collapse [23,37,50]. If the gas is air or N_2, implying that Ostwald ripening is much slower, E_{SD} tends to remain low, and Ostwald ripening is not substantially retarded.

Some proteins produce tenacious layers at the a/w interface because of cross-linking reactions between adsorbed molecules. Egg white especially is a good foam stabilizer. During beating, strong surface denaturation occurs, leading to fairly large protein aggregates. These remain irreversibly adsorbed, resulting in strong resistance to Ostwald ripening. Something similar can be achieved with solid particles, if they have a suitable contact angle (Figure 7.6). An example is provided by the partially solid fat globules in whipped cream, which completely coat the air bubbles, and also form a network throughout the system (see Section 7.6.5).

Many complex systems contain at least some solid particles that act in this manner (being small and fairly hydrophilic). Bubble shrinkage occurs until the adsorbed solid particles touch each other. Then a small but stable bubble remains. This is presumably the cause of many undesirable persistent types of foam. Another example is the gas cells in bread dough [82]. They show extensive Ostwald ripening, and the number of visible cells in the final product is less than 1% of those originally present. This does not mean that all the others have disappeared. In fact, many tiny cells remain, presumably stabilized by solid particles. These cells are not visible but scatter light sufficiently to give bread crumb its white appearance.

It should be mentioned that Ostwald ripening can be prevented by a yield stress in the aqueous phase, but it would need to be high, about 10^4 Pa. An example is chocolate containing air bubbles.

7.7.2.2 Drainage

As mentioned in Section 7.2.6, immobilization of the a/w interface by means of a γ-gradient is essential to prevent almost instantaneous drainage (Figure 7.9c and d). The maximum height that a vertical film (lamella) between two bubbles can have while preventing motion of the film surfaces, is given by

$$H_{max} = \frac{2\Delta\gamma}{\rho g \delta} \tag{7.35}$$

The maximum value that $\Delta\gamma$ (between top and bottom of a vertical film) can assume equals the surface pressure Π, which would be about 0.03 N m^{-1}. For an aqueous film of thickness δ = 0.1 mm, H_{max} would be 6 cm, far more than needed in food foams (6 cm is, indeed, about the height of the largest foam bubbles floating on a detergent solution).

The drainage time of a single vertical film with immobilized surfaces is given by

$$t(\delta) \approx \frac{6\eta H}{\rho g \delta^2} \tag{7.36}$$

where $t(\delta)$ is the time needed for the film to drain to a given thickness δ. For a water film of 1 mm height, only 6 s of drainage would be required to achieve a thickness of 10 μm. However, the drainage rate diminishes with decreasing thickness, and it would take 17 days of drainage to achieve $\delta = 20$ nm. The latter is the approximate thickness at which van der Waals attraction forces between the two film surfaces come into play.

Predicting the drainage rate in a real foam is far more difficult, and accurate calculations cannot be made. Equation 7.36 will serve to provide approximate (order of magnitude) values. Drainage can, of course, be slowed down considerably by increasing the viscosity. For this purpose, viscosity should be measured at fairly low shear stress. A yield stress of about $gH\rho_{water}$ (where H is the height of the foam layer) will also arrest drainage.

7.7.2.3 Coalescence

This occurs when a film between bubbles ruptures, but the mechanism differs with circumstances. Three main cases can be distinguished:

1. *Thick films*: This refers to films thick enough so that colloidal interaction between the two surfaces is negligible. In this situation the Gibbs stabilizing mechanism is essential (Section 7.2.6, especially Figure 7.9e). Film rupture, and thereby bubble coalescence, will occur only when surfactant concentration is very low. If a film is extensively stretched, as will always occur during beating, rupture occurs more readily. Indeed, an optimum whipping speed for foam formation is observed (Figure 7.36b), that is, one that achieves greatest air incorporation.
2. *Thin films*: This concerns films thin enough for colloidal interactions to become important. The considerations given in Section 7.6.4 roughly apply (see especially Figure 7.32) and in the absence of strong colloidal repulsion keeping the film thickness relatively large, rupture of the film may readily occur. However, it may take a long time before the film has drained to small thickness. On the other hand, water may evaporate from the film, especially at the top of a foam. Hence, film rupture will especially occur at the top of a foam, leading to a decrease in foam height. Compared to emulsions, foams are far more unstable against coalescence. γ is large (more stable); the films between bubbles are *permanent* (less stable); film area is very large (less stable); moreover, far fewer films have to rupture for coalescence to become significant (see Table 7.7). Again, proteins may yield the most stable films, especially if they form thick adsorbed layers.
3. *Films containing extraneous particles*: It is often observed that the presence of extraneous particles, especially lipids, is very detrimental to foam stability. Such particles can cause rupture of relatively thick films, and several mechanisms have been postulated [29]. Presumably, spreading of oil over an a/w surface of the film plays a dominant role. Protein-covered oil droplets have a hydrophilic surface layer and thus cannot spread oil over the a/w surface. However, if it concerns fat globules, that is, oil droplets containing triacylglycerol crystals, oil can readily reach the a/w surface; cf. the role of such crystals in partial coalescence (Section 7.6.5). Especially large fat globules are quite effective foam breakers. It is well known, for instance, that traces of lipstick are detrimental to beer foam stability. Another example is given by skim milk, which contains less than 0.05% fat and only very small globules that can be made to foam very much better than whole milk.

In relation to this, the number concentration of extraneous particles should be considered. A typical food foam contains, say, 10^{12} lamellae per m^3 of liquid phase. Presumably, 10^{12} particles per m^3 would thus suffice to cause substantial bubble coalescence, provided these particles can induce film rupture. The larger milk fat globules, say, 6 μm in diameter, when containing both liquid and solid fat, would be suitable. 10^{12} of these per m^3 will correspond to about 0.01% of fat. A very small amount can thus induce significant coalescence.

In a typical whipping cream, the number of partially solid fat globules is very large, at least 10^{16} m^{-3}. Many of these globules would be able to induce film rupture. However, their large concentration causes almost simultaneous adsorption of many globules very close to each other. Spreading of liquid oil over any distance then is not possible, film rupture will rarely occur, and a stable and rather firm foam results. However, if whipping goes on, the fat globules undergo extensive partial coalescence, large clumps are formed, and eventually their number becomes so small that film rupture can occur. In other words, overwhipping destroys the foam made at an earlier stage. When churning cream to obtain butter granules, that is, large clumps of fat globules, this occurs on purpose.

7.7.3 Summary

- Foams can be made via supersaturation, which allows small air pockets present in the system to grow out, and mechanically, usually, by beating air into the liquid.
- Volume fraction gas incorporated is often expressed as percent overrun. Main factors determining it are concentration surfactant (often protein), beater speed, and beating time.
- Gas bubbles formed cream rapidly and form a foam layer. Its volume fraction air will increase fast due to drainage and finally it may result in a polyhedral foam.
- Types of physical instability are Ostwald ripening, drainage of liquid, and coalescence of gas bubbles.
- Ostwald ripening is often the most important type of foam instability, faster at top of foam layer than in bulk. Rate is much faster for foam with CO_2 containing gas bubbles than nitrogen bubbles due to the much higher solubility of CO_2 in water. Ostwald ripening can be retarded by adsorbed layers with high surface-dilational modulus and stopped via adsorption of solid particles and via solidifying the continuous phase.
- Drainage can be strongly retarded by immobilization of the a/w interface and by increasing the viscosity of the continuous phase.
- Regarding coalescence, three main cases can be distinguished: (1) thick films, Gibbs stabilizing mechanism essential; (2) thin films, colloidal interactions important; and (3) films containing extraneous particles, for example, oil droplets that may spread over the a/w surface. Since number of gas cells per m^3 is much lower than in emulsions, effects due to coalescence will be noticed much earlier.

FREQUENTLY USED SYMBOLS

A	(Specific) surface area	(m^{-1}, m^2)
	Hamaker constant	(J)
a	Thermodynamic activity	(mole fraction)
	Acceleration	(m s^{-2})
B	Permeability	(m^2)
c	Concentration	(kg m^{-3}; mol m^{-3}; mol L^{-1})
D	Diffusion coefficient	(m^2 s^{-1})
	Fractal dimensionality	(–)
d	Particle diameter	(m)
E_{SD}	Surface-dilational modulus	(N m^{-1})

F	(Gibbs) free energy	(J; J mol^{-1})
f	Force	(N)
G	Elastic shear modulus	(Pa)
g	Acceleration due to gravity	(9.81 m s^{-2})
H	Height	(m)
	Enthalpy	(J; J mol^{-1})
h	Interparticle distance	(m)
I	Ionic strength	(mol L^{-1})
k	Boltzmann constant	(1.38×10^{-23} J K^{-1})
l	Distance, length	(m)
m	Concentration	(mol L^{-1})
N	(Total) number concentration	(m^{-3})
n_i	Number of particles in class i	(m^{-3})
p	Pressure	(Pa)
p_L	Laplace pressure	(Pa)
Q	Volume flow rate	(m^3 s^{-1})
R	Universal gas constant	(8.314 J mol^{-1} K^{-1})
	Radius of aggregate (floc)	(m)
R_{cr}	Critical radius	(m)
R_g	Radius of gyration	(m)
r	Particle radius	(m)
S	Entropy	(J K^{-1}; J mol^{-1} K^{-1})
s	Solubility of gas	(mol m^{-3} Pa^{-1})
T	(Absolute) temperature	(K)
t	Time	(s)
$t_{0.5}$	Halving time	(s)
V	Interaction free energy	(J)
v	Velocity	(m s^{-1})
v_S	Stokes velocity of particle	(m s^{-1})
∇v	Velocity gradient; shear rate	(s^{-1})
W	Stability ratio	(–)
x	Distance	(m)
z	Valence	(–)

Greek Symbols

Γ	Surface excess (load)	(mol m^{-2}, kg m^{-2})
γ	Surface/interfacial tension	(N m^{-1})
δ	Layer (film) thickness	(m)
ε	Strain (relative deformation)	(–)
ε_{fr}	Strain at fracture	(–)
θ	Contact angle	(rad)
κ	Reciprocal Debye length	(m^{-1})
η	Viscosity	(Pa s)
η_a	Apparent viscosity	(Pa s)
Π	Surface pressure	(N m^{-1})
Π_{osm}	Osmotic pressure	(Pa)
ρ	Mass density	(kg m^{-3})
σ	Stress	(Pa)
σ_{fr}	Fracture stress	(Pa)

σ_y	Yield stress	(Pa)
ϕ	Volume fraction	(–)
ψ	Fraction solid	(–)
	Electric potential	(V)

Subscripts

A	Air
C	Continuous phase
D	Dispersed phase
O	Oil
S	Solid
W	Water (aqueous phase)

REFERENCES

1. Adamson, A.W. and A.P. Gast (1997), *Physical Chemistry of Surfaces*, 6th edn., John Wiley, New York.
2. Allen, T. (1981), *Particle Size Measurement*, 3rd edn., Chapman & Hall, London, U.K.
3. Baszkin, A. and W. Norde, eds. (2000), *Physical Chemistry of Biological Interfaces*, Dekker, New York.
4. Benjamins, J. and E.H. Lucassen-Reynders (1988), Surface dilational rheology of proteins adsorbed at air/water and oil/water interfaces, in *Studies in Interface Science* (D. Möbius and R. Miller, eds.), Elsevier, Amsterdam, the Netherlands, pp. 341–384.
5. Benjamins, J. and E.H. Lucassen-Reynders (2003), Static and dynamic properties of proteins adsorbed at three different liquid interfaces, in *Food Colloids, Biopolymers and Materials* (E. Dickinson and T. van Vliet, eds.), Royal Society of Chemistry, Cambridge, U.K., pp. 216–225.
6. Boode, K. and P. Walstra (1993), Kinetics of partial coalescence in oil-in-water emulsions, in *Food Colloids and Polymers: Stability and Mechanical Properties* (E. Dickinson and P. Walstra, eds.), Royal Society Chemistry, Cambridge, U.K., pp. 23–30.
7. Boode, K. and P. Walstra (1993), Partial coalescence in oil-in-water emulsions 1. Nature of the aggregation, *Colloids Surf.* **81**: 121–137.
8. Boode, K., P. Walstra, and A.E.A. de Groot-Mostert (1993), Partial coalescence in oil-in-water emulsions 2. Influence of the properties of the fat, *Colloids Surf.* **81**: 139–151.
9. Bremer, L.G.B., P. Walstra, and T. van Vliet (1995), Estimation of the aggregation time of various colloidal systems, *Colloids Surf. A* **99**: 121–127.
10. Brownsey, G.J. and V.J. Morris (1988), Mixed and filled gels—Models for foods, in *Functional Properties of Food Macromolecules* (J.R. Mitchell and D.A. Ledward, eds.), Elsevier Applied Science, London, U.K., pp. 7–23.
11. Busnell, J.P., S.M. Clegg, and E.R. Morris (1988), Melting behaviour of gelatin: Origin and control, in *Gums and Stabilizers for the Food Industry*, Vol. 4 (G.O. Phillips, D.J. Wedlock, and P.A. Williams, eds.), IRL Press, Oxford, U.K., pp. 105–115.
12. Clark, A.H. and C.D. Lee-Tufnell (1986), Gelation of globular proteins, in *Functional Properties of Food Macromolecules* (J.R. Mitchell and D.A. Ledward, eds.), Elsevier Applied Science, London, U.K., pp. 203–272.
13. Coffey, D.G., D.A. Bell, and A. Henderson (1995), Cellulose and cellulose derivatives, in *Food Polysaccharides and Their Applications* (A.M. Stephen, ed.), Dekker, New York, pp. 123–153.
14. Darling, D.F. (1982), Recent advances in the destabilization of dairy emulsions, *J. Dairy Res.* **49**: 695–712.
15. De Feijter, J.A., J. Benjamins, and M. Tamboer (1987), Adsorption displacement of proteins by surfactants in oil-in-water emulsions, *Colloids Surf.* **27**: 243–266.
16. De Jong, S. and F. van de Velde (2007), Charge density of polysaccharide controls microstructure and large deformation properties of mixed gels, *Food Hydrocoll.* **21**: 1172–1187.
17. De Roos, A.L. and P. Walstra (1996), Loss of enzyme activity due to adsorption onto emulsion droplets, *Colloids Surf. B* **6**: 201–208.
18. de Vries, A.J. (1958), Foam stability. II. Gas diffusion in foams, *Receuil Trav. Chim.* **77**: 209–225.

19. Dickinson, E. (1992), Structure and composition of adsorbed protein layers and the relation to emulsion stability, *J. Chem. Soc. Faraday Trans.* **88**: 2973–2983.
20. Dickinson, E. (1994), Protein-stabilized emulsions, *J. Food Eng.* **22**: 59–74.
21. Dickinson, E. (2003), Hydrocolloids at interfaces and the influence on the properties of dispersed systems, *Food Hydrocoll.* **17**: 25–39.
22. Dickinson, E. and L. Eriksson (1991), Particle flocculation by adsorbing polymers, *Adv. Colloid Interface Sci.* **34**: 1–29.
23. Dickinson, E., R. Ettelaie, B.S. Murray, and Z. Du (2002), Kinetics of disproportionation of air bubbles beneath a planar air–water interface stabilized by food proteins, *J. Colloid Interface Sci.* **252**: 202–213.
24. Dickinson, E., V.B. Galazka, and D.M.W. Anderson (1991), Emulsifying behaviour of gum Arabic. Part 1: Effect of the nature of the oil phase on the emulsion Droplet-size distribution, *Carbohydr. Polym.* **14**: 373–383.
25. Dickinson, E. and Y. Matsumura (1991), Time-dependent polymerization of β-lactoglobulin through disulphide bonds at the oil–water interface in emulsions, *Int. J. Biol. Macromol.* **13**: 26–30.
26. Dickinson, E., B.S. Murray, and G. Stainsby (1988), Coalescence stability of emulsion-sized droplets at a planar oil–water interface and the relation to protein film surface rheology, *J. Chem. Soc. Faraday Trans. 1* **84**: 871–883.
27. Fleer, G.J., M.A. Cohen Stuart, J.M.H.M. Scheutjens, T. Cosgrove, and B. Vincent (1993), *Polymers at Interfaces*, Chapman & Hall, London, U.K.
28. Friberg, S.E., R.F. Goubran, and I.H. Kayali (1990), Emulsion stability, in *Food Emulsions*, 2nd edn. (K. Larsson and S.E. Friberg, eds.), Dekker, New York, pp. 1–40.
29. Garrett, P.R. (1993), Recent developments in the understanding of foam generation and stability, *Chem. Eng. Sci.* **48**: 367–392.
30. Graham, D.E. and M.C. Phillips (1980), Proteins at liquid interfaces IV. Dilational properties, *J. Colloid Interface Sci.* **76**: 227–239.
31. Grinberg, V. and V.B. Tolstoguzov (1997), Thermodynamic incompatibility of proteins and polysaccharides in solutions, *Food Hydrocoll.* **11**: 145–158.
32. Hermansson, A.-M. (1988), Gel structure of food biopolymers, in *Functional Properties of Food Macromolecules* (J.R. Mitchell and D.A. Ledward, eds.), Elsevier Applied Science, London, U.K., pp. 25–40.
33. Hough, D.B. and L.R. White (1980), The calculation of Hamaker constants from Lifshitz theory with application to wetting phenomena, *Adv. Colloid Interface Sci.* **14**: 3–41.
34. Hutchings, J.B. and P.J. Lillford (1988), The perception of food texture: Tthe philosophy of the breakdown path, *J. Texture Stud.* **19**: 103–115.
35. Kato, A. and S. Nakai (1980), Hydrophobicity determined by a fluorescent probe method and its correlation with surface properties of proteins, *Biochim. Biophys. Acta* **624**: 13–20.
36. Kimura, H., S. Morikata, and M. Misaki (1973), Polysaccharide 13140: A new thermo-gelable polysaccharide, *J. Food Sci.* **38**: 668–670.
37. Kloek, W., T. van Vliet, and M. Meinders (2001), Effect of bulk and interfacial rheological properties on bubble dissolution, *J. Colloid Interface Sci.* **237**: 158–166.
38. Krog, N.J. (1990), Food emulsifiers and their chemical and physical properties, in *Food Emulsions*, 2nd edn. (K. Larsson and S.E. Friberg, eds.), Dekker, New York, pp. 127–180.
39. Larsson, K. and P. Dejmek (1990), Crystal and liquid crystal structure of lipids, in *Food Emulsions*, 2nd edn. (K. Larsson and S.E. Friberg, eds.), Dekker, New York, pp. 97–125.
40. Ledward, D.A. (1986), Gelation of gelatin, in *Functional Properties of Food Macromolecules* (J.R. Mitchell and D.A. Ledward, eds.), Elsevier Applied Science, London, U.K., pp. 171–201.
41. Ledward, D.A. and J.R. Mitchell (1988), Protein extrusion—More questions than answers? in *Food Structure—Its Creation and Evaluation* (J.M.V. Blanshard and J.R. Mitchell, eds.), Butterworths, London, U.K., pp. 219–229.
42. Lorén, N., A.-M. Hermansson, M.A.K. Williams, L. Lundin, T.J. Foster, C.D. Hubbard, A.H. Clark, I.T. Norton, E.T. Bergström, and D.M. Goodall (2001), Phase separation induced by conformational ordering of gelatin in gelatin/maltodextrin mixtures, *Macromolecules* **34**: 289–297.
43. Lucassen-Reynders, E.H. (1981), Surface elasticity and viscosity in compression/dilation, in *Anionic Surfactants: Physical Chemistry of Surfactant Action* (E.H. Lucassen-Reynders, ed.), Dekker, New York, pp. 173–216.
44. Luyten, H. and T. van Vliet (1990), Influence of a filler on the rheological and fracture properties of food materials, in *Rheology of Foods, Pharmaceutical and Biological Materials with General Rheology* (R.E. Carter, ed.), Elsevier Applied Science, London, U.K., pp. 43–56.

45. Luyten, H., T. van Vliet, and W. Kloek (1991), Sedimentation in aqueous xanthan + galactomannan mixtures, in *Food Polymers, Gels and Colloids* (E. Dickinson, ed.), Royal Society Chemistry, Cambridge, U.K., pp. 527–530.
46. Lyklema, J. (1991), *Fundamentals of Interface and Colloid Science*, Vol. I, *Fundamentals*, Academic Press, London, U.K., pp. A9.1–A9.7.
47. Lyklema, J., ed. (2005), *Fundamentals of Interface and Colloid Science*, Vol. IV, *Particulate Colloids*, Elsevier Academic Press, London, U.K., pp. A3.1–A3.9.
48. Martin, A., M.A. Bos, M. Cohen Stuart, and T. van Vliet (2002), Stress–strain curves of adsorbed protein layers at the air/water interface measured with surface shear rheology, *Langmuir* **18**: 1238–1243.
49. Martin, A., K. Grolle, M.A. Bos, M. Cohen Stuart, and T. van Vliet (2002), Network forming properties of various proteins adsorbed at the air/water interface in relations to foam stability, *J. Colloid Interface Sci.* **254**: 175–183.
50. Meinders, M.B.J. and T. van Vliet (2004), The role of interfacial rheological properties on Ostwald ripening in emulsions, *Adv. Colloid Interface Sci.* **108–109**: 119–126.
51. Mellema, M., J.H.J. van Opheusden, and T. van Vliet (2002), Categorization of rheological scaling models for particle gels applied to casein gels, *J. Rheol.* **46**: 11–29.
52. Mellema, M., P. Walstra, J.H.J. van Opheusden, and T. van Vliet (2002), Effects of structural rearrangements on the rheology of rennet-induced casein particle gels, *Adv. Colloid Interface Sci.* **98**: 25–50.
53. Morris, V.J. (1986), Gelation of polysaccharides, in *Functional Properties of Food Macromolecules* (J.R. Mitchell and D.A. Ledward, eds.), Elsevier Applied Science, London, U.K., pp. 121–170.
54. Morris, V.J. (1992), Designing polysaccharides for synergistic interactions, in *Gums and Stabilizers for the Food Industry*, Vol. 6 (G.O. Phillips, P.A. Williams, and D.J. Wedlock, eds.), IRL Press, Oxford, U.K., pp. 161–172.
55. Muhr, A.H. and J.M.V. Blanshard (1982), Diffusion in gels, *Polymer* **23**(Suppl.): 1012–1026.
56. Murray, B.S. and E. Dickinson (1996), Interfacial rheology and the dynamic properties of adsorbed films of proteins and surfactants, *Food Sci. Technol. Intern.* **2**: 131.
57. Norde, W. and J. Lyklema (1991), Why proteins prefer interfaces, *J. Biomater. Sci. Polymer Ed.* **2**: 183–202.
58. Oguntunde, A.O., P. Walstra, and T. van Vliet (1989), Physical characterization of soymilk, in *Trends in Food Biotechnology* (A.H. Ghee, N.B. Hen, and L.K. Kong, eds.), *Proceedings of the Seventh World Congress on Food Science and Technology,* Singapore, 1987, Institute of Food Science and Technology, Singapore 1989, pp. 307–308.
59. Pearce, K.N. and J.E. Kinsella (1978), Emulsifying properties of proteins: Evaluation of a turbidimetric technique, *J. Agric. Food Chem.* **26**: 716–723.
60. Prins, A. (1988), Principles of foam stability, in *Advances in Food Emulsions and Foams* (E. Dickinson and G. Stainsby, eds.), Elsevier Applied Science, London, U.K., pp. 91–122.
61. Ronteltap, A.D. and A. Prins (1990), The role of surface viscosity in gas diffusion in aqueous foams. II. Experimental, *Colloids Surf.* **47**: 285–298.
62. Shama, F. and P. Sherman (1973), Identification of stimuli controlling the sensory evaluation of viscosity, *J. Texture Stud.* **4**: 103–118.
63. Shimizu, M., M. Saito, and K. Yamauchi (1986), Hydrophobicity and emulsifying activity of milk proteins, *Agric. Biol. Chem.* **50**: 791–792.
64. Shinoda, K. and H. Kunieda (1983), Phase properties of emulsions: PIT and HLB, in *Encyclopedia of Emulsion Technology*, Vol 1. *Basic Theory* (P. Becher, ed.), Dekker, New York, pp. 337–367.
65. Smulders, P.E.A., P.W.J.R. Caessens, and P. Walstra (1999), Emulsifying properties of β-casein and its hydrolysates in relation to their molecular properties, in *Food Emulsions and Foams* (E. Dickinson and J.M. Rodriguez Patino, eds.), Royal Society of Chemistry, Cambridge, U.K., pp. 61–69.
66. Spielman, L.A. (1978), Hydrodynamic aspects of flocculation, in *The Scientific Basis of Flocculation* (K.J. Ives, ed.), Sijthoff & Noordhoff, Alphen aan den Rijn, the Netherlands, pp. 63–88.
67. Stading, M., M. Langton, and A.-M. Hermansson (1992), Inhomogeneous fine-stranded β-lactoglobulin gels, *Food Hydrocoll.* **6**: 455–470.
68. Stading, M., M. Langton, and A.-M. Hermansson (1993), Microstructure and rheological behaviour of particulate β-lactoglobulin gels, *Food Hydrocoll.* **7**: 195–212.
69. Stauffer, D., A. Coniglio, and M. Adam (1982), Gelation and critical phenomena, *Adv. Polym. Sci.* **44**: 105–107.
70. Stockham, J.D. and E.G. Fochtman (1977), *Particle Size Analysis*, Ann Arbor Science Publication, Ann Arbor, MI.

71. Tolstoguzov, V.B. (1993), Thermodynamic incompatibility of food macromolecules, in *Food Colloids and Polymers: Stability and Mechanical Properties* (E. Dickinson and P. Walstra, eds.), Royal Society of Chemistry, Cambridge, U.K., pp. 94–102.
72. Van Aken, G.A. (2001), Aeration of emulsions by whipping, *Colloids Surf.* **190**: 333–353.
73. Van Aken, G.A. (2002), Flow-induced coalescence in protein-stabilized highly concentrated emulsions, *Langmuir* **18**: 3549–3556.
74. Van Aken, G.A. and M.T.E. Merks (1994), Dynamic surface properties of milk proteins, *Prog. Colloid Polymer Sci.* **97**: 281–284.
75. Van den Berg, L., T. van Vliet, E. van der Linden, M.A.J. van Boekel, and F. van de Velde (1980a), Physical properties giving the sensory perception of whey protein/polysaccharide gels, *Food Biophys.* **3**: 1989–1206.
76. Van den Berg, L., A.L. Carolas, T. van Vliet, E. van der Linden, M.A.J. van Boekel, and F. van de Velde (1980b), Energy storage controls crumbly perception in whey proteins/polysaccharide mixed gels, *Food Hydrocoll.* **22**: 1404–1417.
77. Van Boekel, M.A.J.S. and P. Walstra (1981), Stability of oil-in-water emulsions with crystals in the disperse phase, *Colloids Surf.* **3**: 109–118.
78. Van Kreveld, A. (1974), Studies on the wetting of milk powder, *Neth. Milk Dairy J.* **28**: 23–45.
79. Van Vliet, T. (2002), On the relation between texture perception and fundamental mechanical parameters for liquids and time-dependent solids, *Food Qual. Prefer.* **13**: 111–118.
80. Van Vliet, T. (2013), *Rheology and Fracture Mechanics of Foods*, CRC Press, New York.
81. Van Vliet, T., H.J.M. van Dijk, P. Zoon, and P. Walstra (1991), Relation between syneresis and rheological properties of particle gels, *Colloid Polym. Sci.* **269**: 620–627.
82. Van Vliet, T., A.M. Janssen, A.H. Bloksma, and P. Walstra (1992), Strain hardening of dough as a requirement for gas retention, *J. Texture Stud.* **23**: 439–460.
83. Van Vliet, T., S.P.F.M. Roefs, P. Zoon, and P. Walstra (1989). Rheological properties of casein gels, *J. Dairy Res.* **56**: 529–534.
84. Vincent, J.F.V., G. Jeronimides, A.A. Kahn, and H. Luyten (1991), The wedge fracture test: A new method for measurement of food texture, *J. Texture Stud.* **22**: 45–57.
85. Visser, J. (1972), On Hamaker constants: A comparison between Hamaker constants and Lifshitz–van der Waals constants, *Adv. Colloid Interface Sci.* **3**: 331–363.
86. Walstra, P. (1983), Formation of emulsions, in *Encyclopedia of Emulsion Technology*, Vol. 1 (P. Becher, ed.), Dekker, New York, pp. 57–127.
87. Walstra, P. (1987), Fat crystallization, in *Food Structure and Behaviour* (J.M.V. Blanshard and P. Lillford, eds.), Academic Press, London, U.K., pp. 67–85.
88. Walstra, P. (1989), Principles of foam formation and stability, in *Foams: Physics, Chemistry and Structure* (A.J. Wilson, ed.), Springer, London, U.K., pp. 1–15.
89. Walstra, P. (1993), Introduction to aggregation phenomena in food colloids, in *Food Colloids and Polymers: Stability and Mechanical Properties* (E. Dickinson and P. Walstra, eds.), Royal Society of Chemistry, Cambridge, U.K., pp. 1–15.
90. Walstra, P. (1993), Syneresis of curd, in *Cheese: Chemistry, Physics and Microbiology*, Vol. 1. *General Aspects* (P.F. Fox, ed.), Chapman & Hall, London, U.K., pp. 141–191.
91. Walstra, P. (1993), Principles of emulsion formation, *Chem. Eng. Sci.* **48**: 333–349.
92. Walstra, P. (1996), Emulsion stability, in *Encyclopedia of Emulsion Technology*, Vol. 4 (P. Becher, ed.), Dekker, New York, pp. 1–62.
93. Walstra, P. (2002), The roles of proteins and peptides in formation and stabilisation of emulsions, in *Gums and Stabilizers for the Food Industry*, Vol. 11 (P.A. Williams and G.O. Phillips, eds.), Royal Society of Chemistry, Cambridge, U.K., pp. 237–244.
94. Walstra, P. and A.L. de Roos (1993), Proteins at air–water and oil–water interfaces: Static and dynamic aspects, *Food Rev. Int.* **9**: 503–525.
95. Walstra, P. and R. Jenness (1984), *Dairy Chemistry and Physics*, Wiley, New York.
96. Walstra, P. and M. Jonkman (1998), The role of milkfat and protein in ice cream, in *Ice Cream* (W. Buchheim, ed.), International Dairy Federation, Brussels, Belgium, pp. 17–24.
97. Walstra, P. and I. Smulders (1997), Making emulsions and foams: An overview, in *Food Colloids: Proteins, Lipids and Polysaccharides* (E. Dickinson and B. Bergenståhl, eds.), Royal Society of Chemistry, Cambridge, U.K., pp. 367–381.
98. Walstra, P. and P.E.A. Smulders (1998), Emulsion formation, in *Modern Aspects of Emulsion Science* (B.P. Binks, ed.), Royal Society of Chemistry, Cambridge, U.K., pp. 56–99.

99. Walstra, P. and E.C.H. van Berensteyn (1975), Crystallization of milk fat in the emulsified state, *Neth. Milk Dairy J.* **29**: 35–65.
100. Walstra, P., T. van Vliet, and L.G.B. Bremer (1991), On the fractal nature of particle gels, in *Food Polymers, Gels and Colloids* (E. Dickinson, ed.), Royal Society of Chemistry, Cambridge, U.K., pp. 369–382.
101. Walstra, P., J.M. Wouters, and T.J. Geurts (2006), *Dairy Science and Technology*, CRC/Taylor & Francis, Boca Raton, FL.
102. Wedzicha, B.L. (1988), Distribution of low-molecular-weight food additives in dispersed systems, in *Advances in Food Emulsions and Foams* (E. Dickinson and G. Stainsby, eds.), Elsevier, London, U.K., pp. 329–371.

FURTHER READING

Blanshard, J.M.V. and P. Lillford, eds. (1987), *Food Structure and Behaviour*, Academic Press, London, U.K.
Blanshard, J.M.V. and J.R. Mitchell, eds. (1988), *Food Structure Its Creation and Evaluation*, Butterworth, London, U.K.
Dickinson, E. (1992), *An Introduction into Food Colloids*, Oxford Science, Oxford, U.K.
Friberg, S.E., K. Larsson, and J. Sjöblom, eds. (2004), *Food Emulsions*, 4th edn., Marcel Dekker, New York.
Hill, S.E., D.A. Ledward, and J.R. Mitchell, eds. (1998), *Functional Properties of Food Macromolecules*, 2nd edn., Aspen, Gaithersburg, MD.
Walstra, P. (2003), *Physical Chemistry of Foods*, Marcel Dekker, New York.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Section II

Minor Food Components

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

8 Vitamins

Jesse F. Gregory III

CONTENTS

8.1 Introduction 545
8.1.1 Objectives 545
8.1.2 Summary of Vitamin Stability 545
8.1.3 Toxicity of Vitamins 546
8.1.4 Sources of Vitamins 546
8.2 Addition of Nutrients to Foods 547
8.3 Dietary Recommendations 550
8.4 Analytical Methods and Sources of Data 550
8.5 Bioavailability of Vitamins 553
8.6 General Causes of Variation/Losses of Vitamins in Food 553
8.6.1 Inherent Variation in Vitamin Content 554
8.6.2 Postharvest Changes in Vitamin Content of Foods 555
8.6.3 Preliminary Treatments: Trimming, Washing, and Milling 555
8.6.4 Effects of Blanching and Thermal Processing 556
8.6.5 Losses of Vitamins Following Processing 556
8.6.6 Influence of Processing Chemicals and Other Food Components 558
8.7 Fat-Soluble Vitamins 559
8.7.1 Vitamin A 559
8.7.1.1 Structure and General Properties 559
8.7.1.2 Stability and Modes of Degradation 561
8.7.1.3 Bioavailability 565
8.7.1.4 Analytical Methods 565
8.7.2 Vitamin D 566
8.7.2.1 Structure and General Properties 566
8.7.2.2 Analytical Methods 567
8.7.3 Vitamin E 567
8.7.3.1 Structure and General Properties 567
8.7.3.2 Stability and Mechanism of Degradation 569
8.7.3.3 Bioavailability 570
8.7.3.4 Analytical Methods 572
8.7.4 Vitamin K 572
8.7.4.1 Structure and General Properties 572
8.7.4.2 Analytical Methods 573
8.8 Water-Soluble Vitamins 573
8.8.1 Ascorbic Acid 573
8.8.1.1 Structure and General Properties 573
8.8.1.2 Stability and Modes of Degradation 575

8.8.2 Thiamin 582
8.8.2.1 Structure and General Properties 582
8.8.2.2 Stability and Modes of Degradation 582
8.8.3 Riboflavin 588
8.8.3.1 Structure and General Properties 588
8.8.3.2 Stability and Modes of Degradation 590
8.8.3.3 Bioavailability 590
8.8.3.4 Analytical Methods 591
8.8.4 Niacin 591
8.8.4.1 Structure and General Properties 591
8.8.4.2 Bioavailability 592
8.8.4.3 Analytical Methods 592
8.8.5 Vitamin B6 592
8.8.5.1 Structure and General Properties 592
8.8.5.2 Stability and Modes of Degradation 596
8.8.5.3 Bioavailability of Vitamin B6 601
8.8.5.4 Measurement of Vitamin B6 601
8.8.6 Folate 601
8.8.6.1 Structure and General Properties 601
8.8.6.2 Stability and Modes of Degradation 604
8.8.7 Biotin 610
8.8.7.1 Structure and General Properties 610
8.8.7.2 Stability of Biotin 610
8.8.7.3 Analytical Methods 611
8.8.7.4 Bioavailability 611
8.8.8 Pantothenic Acid 612
8.8.8.1 Structure and General Properties 612
8.8.8.2 Stability and Modes of Degradation 612
8.8.8.3 Bioavailability 613
8.8.8.4 Analytical Methods 613
8.8.9 Vitamin B12 613
8.8.9.1 Structure and General Properties 613
8.8.9.2 Stability and Modes of Degradation 614
8.8.9.3 Bioavailability 615
8.8.9.4 Analytical Methods 615
8.9 Conditionally Essential Vitamin-Like Compounds 616
8.9.1 Choline and Betaine 616
8.9.2 Carnitine 616
8.9.3 Pyrroloquinoline Quinone 617
8.9.4 Coenzyme Q_{10} 617
8.10 Optimization of Vitamin Retention 618
8.10.1 Optimization of Thermal Processing Conditions 618
8.10.2 Prediction of Losses 619
8.10.3 Effects of Packaging 619
8.11 Summary 620
References 620
Further Reading 625

8.1 INTRODUCTION

8.1.1 Objectives

Since the discovery of the basic vitamins and their many forms, a wealth of information has been generated and published on their retention in foods during postharvest handling, commercial processing, distribution, storage, and preparation, and many reviews have been written on this topic. A good summary of older findings regarding this topic is the book *Nutritional Evaluation of Food Processing* [59,60,78], to which the reader is encouraged to refer. There remains a need for an in-depth review of more recent literature and for more systematic studies using contemporary analytical methods.

The major objective of this chapter is to discuss and critically review the chemistry of the individual vitamins and our understanding of the chemical and physical factors that influence vitamin retention and bioavailability in foods. A secondary objective is to indicate gaps in our understanding and to point out factors that affect the quality of data with respect to our understanding of vitamin stability. It should be noted that there is an unfortunate state of inconsistency of nomenclature in the vitamin literature, with many obsolete terms still being used. Throughout this chapter, terminology recommended by the International Union of Pure and Applied Chemistry (IUPAC) and the American Society for Nutrition [1] will be used wherever possible.

8.1.2 Summary of Vitamin Stability

The vitamins comprise a diverse group of organic compounds that are nutritionally essential micronutrients. Vitamins function in vivo in several ways, including (a) as coenzymes or their precursors (niacin, thiamin, riboflavin, biotin, pantothenic acid, vitamin B6, vitamin B12, and folate), (b) as components of the oxidant defense system (ascorbic acid, certain carotenoids, and vitamin E), (c) as factors involved in genetic regulation (vitamins A and D and potentially several others), and (d) in specialized functions such as vitamin A in vision, ascorbate in various hydroxylation reactions, and vitamin K in specific carboxylation reactions.

Vitamins are quantitatively minor constituents of foods. From the viewpoint of food chemistry, we are mainly interested in maximizing vitamin retention by minimizing aqueous extraction (leaching) and chemical changes such as oxidation and reaction with other food constituents. In addition, several of the vitamins influence the chemical nature of food, by functioning as reducing agents, radical scavengers, reactants in browning reactions, and flavor precursors. Although much is known about the stability and properties of vitamins, our knowledge of their behavior in the complex milieu of food is more limited. Many published studies have, sometimes by necessity, involved the use of chemically defined model systems (or even just buffer solutions) to simplify the investigation of vitamin stability. Results of such studies should be interpreted with caution because, in many cases, the degree to which these model systems simulate complex food systems is unclear. While these studies have provided important insight into chemical variables affecting retention, they are sometimes of limited value for predicting the behavior of vitamins in complex food systems. This is so because complex foods often differ markedly from model systems in terms of physical and compositional variables including water activity, ionic strength, pH, enzymatic and trace metallic catalysts, and other reactants (protein, reducing sugars, free radicals, active oxygen species, etc.). Throughout this chapter, emphasis will be placed on the behavior of vitamins under conditions relevant to actual foods.

Most of the vitamins exist as groups of structurally related compounds exhibiting similar nutritional function. Many attempts have been made to summarize the stability of the vitamins,

TABLE 8.1
Summary of Vitamin Stability[a]

Nutrient	Neutral	Acid	Alkaline	Air or Oxygen	Light	Heat	Maximum Cooking Loss (%)
Vitamin A	S	U	S	U	U	U	40
Ascorbic acid	U	S	U	U	U	U	100
Biotin	S	S	S	S	S	U	60
Carotenes	S	U	S	U	U	U	30
Choline	S	S	S	U	S	S	5
Vitamin B12	S	S	S	U	U	S	10
Vitamin D	S	S	U	U	U	U	40
Folate	U	U	U	U	U	U	100
Vitamin K	S	U	U	S	U	S	5
Niacin	S	S	S	S	S	S	75
Pantothenic acid	S	U	U	S	S	U	50
Vitamin B6	S	S	S	S	U	U	40
Riboflavin	S	S	U	S	U	U	75
Thiamin	U	S	U	U	S	U	80
Tocopherols	S	S	S	U	U	U	55

Source: Adapted from Harris, R., General discussion on the stability of nutrients, in: *Nutritional Evaluation of Food Processing*, Harris, R. and von Loesecke, H. (eds.), AVI Publishing Co., Westport, CT, 1971, pp. 1–4. With modifications.

Caution: These conclusions are oversimplifications and may not accurately represent stability under all circumstances.

[a] S, stable (no important destruction); U, unstable (significant destruction).

such as that shown in Table 8.1 [59]. The major limitation of such exercises is the marked variation in stability that can exist among the various forms of each vitamin. Various forms of each vitamin can exhibit vastly different stability (e.g., pH of optimum stability and susceptibility to oxidation) and reactivity. For example, tetrahydrofolic acid and folic acid are two folates that exhibit nearly identical nutritional properties. As described later, tetrahydrofolic acid (a naturally occurring form) is extremely susceptible to oxidative degradation, while folic acid (a synthetic form used in food fortification) is very stable. Thus, attempts to generalize or summarize the properties of the vitamins are at best imprecise and at worst highly misleading.

8.1.3 Toxicity of Vitamins

In addition to the nutritional role of vitamins, it is important to recognize their potential toxicity. Vitamins A, D, and B6 are of particular concern in this respect. Episodes of vitamin toxicity are nearly always associated with overzealous consumption of nutritional supplements. Toxic potential also exists from inadvertent excessive fortification, as once occurred in an isolated incident with vitamin D–fortified milk. This illustrates the need for continued monitoring by regulatory and public health agencies. Instances of intoxication from vitamins occurring endogenously in food are exceedingly rare.

8.1.4 Sources of Vitamins

Although vitamins are consumed in the form of supplements by a growing fraction of the population, in many cases the food supply generally represents the major and most critically important source

of vitamin intake. Foods, in their widely disparate forms, provide vitamins that occur naturally in plant, animal, and microbial sources as well as those added in fortification. In addition, certain dietetic and therapeutic foods, enteric formulas, and intravenous solutions are formulated so that the entire vitamin requirements of the individual are supplied from these sources.

Regardless of whether the vitamins are naturally occurring or added, the potential exists for losses by chemical or physical (leaching or other separations) means. Losses of vitamins are, to some degree, inevitable in the manufacturing, distribution, marketing, home storage, and preparation of processed foods, and losses of vitamins also can occur during the postharvest handling and distribution of fruits and vegetables and during the postslaughter handling and distribution of meat products. Since the modern food supply is increasingly dependent on processed and industrially formulated foods, the nutritional adequacy of the food supply depends, in large measure, on our understanding of how vitamins are lost and on our ability to control these losses.

Although considerable information is available concerning the stability of vitamins in foods, our ability to use such information is frequently limited by a poor understanding of reaction mechanisms, kinetics, and thermodynamics under various circumstances. Thus, it is frequently difficult on the basis of our present knowledge to predict the extent to which given processing, storage, or handling conditions will influence the retention of many vitamins. Without accurate information regarding reaction kinetics and thermodynamics, it is also difficult to select conditions and methods of food processing, storage, and handling to optimize vitamin retention. Thus, there is a great need for more thorough characterization of the basic chemistry of vitamin degradation as it occurs in complex food systems.

8.2 ADDITION OF NUTRIENTS TO FOODS

Throughout the early twentieth century, nutrient deficiency represented a major public health problem in the United States. Pellagra was endemic in much of rural South, while deficiencies of riboflavin, niacin, iron and calcium were widespread. The development of legally defined standards of identity under the authorization of the 1938 Food, Drug, and Cosmetic Act provided for the direct addition of several nutrients to foods, especially certain dairy and cereal grain products. Although technological and historical aspects of fortification are beyond the scope of this chapter, the reader is referred to *Nutrient Additions to Food: Nutritional, Technological, and Regulatory Aspects* [7] for a comprehensive discussion of this topic. The nearly complete eradication of overt vitamin-deficiency disease provides evidence of the exceptional effectiveness of fortification programs and the general improvement in the nutritional quality of the U.S. food supply.

The definitions of terms associated with the addition of nutrients to foods include the following:

1. *Restoration*: Addition to restore the original concentration of key nutrients.
2. *Fortification*: Addition of nutrients in amounts significant enough to render the food a good to superior source of the added nutrients. This may include addition of nutrients not normally associated with the food or addition to levels above that present in the unprocessed food.
3. *Enrichment*: Addition of specific amounts of selected nutrients in accordance with a standard of identity as defined by the U.S. Food and Drug Administration (FDA).
4. *Nutrification*: This is a generic term intended to encompass any addition of nutrients to food.

The addition of vitamins and other nutrients to food, while clearly beneficial in current practice, also carries with it the potential for abuse and, thus, risk to consumers. For these reasons,

important guidelines have been developed that convey a reasonable and prudent approach. These U.S. FDA guidelines [21 CFR Sect. 104.20(g)] state that the nutrient added to a food should be

1. Stable under customary conditions of storage, distribution, and use
2. Physiologically available from the food
3. Present at a level where there is assurance that there will not be excessive intake
4. Suitable for its intended purpose and in compliance with provisions (i.e., regulations) governing safety

Further, it is stated in these guidelines that "the FDA does not encourage the indiscriminant addition of nutrients to foods." Similar recommendations were developed and endorsed jointly by the Council on Foods and Nutrition of the American Medical Association, the Institute of Food Technologists, and the Food and Nutrition Board of the National Academy of Sciences–National Research Council [4].

Additionally, AMA-IFT-FNB guidelines recommend that the following prerequisites be met to justify fortification: (1) the intake of the particular nutrient is inadequate for a substantial portion of the population, (2) the food (or category) is consumed by most individuals in the target population, (3) there is reasonable assurance that excessive intake will not occur, and (4) the cost is reasonable for the intended population. The joint statement also included the following endorsement of enrichment programs:

> Specifically the following practices in the United States continue to be endorsed: The enrichment of flour, bread, degerminated and white rice (with thiamin, riboflavin, niacin, and iron); the retention or restoration of thiamin, riboflavin, niacin, and iron in processed food cereals; the addition of vitamin D to milk, fluid skimmed milk, and nonfat dry milk, the addition of vitamin A to margarine, fluid skim milk, and nonfat dry milk, and the addition of iodine to table salt. The protective action of fluoride against dental caries is recognized and the standardized addition of fluoride is endorsed in areas in which the water supply has a low fluoride content.

The most recent change in fortification policy concerns folic acid. As of January 1, 1998, the inclusion of folic acid is required in enriched cereal grain products (i.e., those with standards of identity, including most wheat flours, rice, corn meals, breads, and pastas). This has proven to be a viable approach to providing supplemental folic acid for the purpose of reducing the risk of certain birth defects (spina bifida and anencephaly), and it has improved the folate nutritional status of the population. The level of addition of folic acid was chosen to minimize the risk of excessive intake (>1 mg folic acid/day) to reduce the risk of masking the diagnosis of vitamin B12 deficiency. Most risk of excessive folic acid exposure comes from supplement usage rather than consumption of fortified foods. Internationally, fortification policies vary widely, but over 70 countries now allow or require folic acid addition to food.

The stability of vitamins in fortified and enriched foods has been fairly well evaluated. As shown in Table 8.2, the stability of added vitamins in enriched cereal grain products under conditions of accelerated shelf life testing is excellent [3,22]. Similar results have been reported with fortified breakfast cereals (Table 8.3). This excellent retention is due, in part, to the stability of the chemical forms of these vitamins used, as well as the favorable environment with respect to water activity and temperature. The stability of vitamins A and D in fortified milk products also has been shown to be satisfactory.

TABLE 8.2
Stability of Vitamins Added to Cereal Grain Products

Vitamin	Claim	Found	Storage Time (Months at 23°C) 2	4	6
In 1 lb of white flour					
Vitamin A (IU)	7500	8200	8200	8020	7950
Vitamin E (IU)[a]	15.0	15.9	15.9	15.9	15.9
Pyridoxine (mg)	2.0	2.3	2.2	2.3	2.2
Folate (mg)	0.30	0.37	0.30	0.35	0.3
Thiamin (mg)	2.9	3.4	—	—	3.4
In 1 lb of yellow corn meal					
Vitamin A (IU)	—	7500	7500	—	6800
Vitamin E (IU)[a]	—	15.8	15.8	—	15.9
Pyridoxine (mg)	—	2.8	2.8	—	2.8
Folate (mg)	—	0.30	0.30	—	0.29
Thiamin (mg)	—	3.5	—	—	3.6

		After baking	5 days of storage (23°C)
In 740 g of bread			
Vitamin A (IU)	7500	8280	8300
Vitamin E (IU)[a]	15	16.4	16.7
Pyridoxine (mg)	2	2.4	2.5
Folate (mg)	0.3	0.34	0.36

Source: Cort, W.M. et al., *Food Technol.*, 30, 52, 1976.

[a] Vitamin E is expressed as DL,α-tocopherol acetate.

TABLE 8.3
Stability of Vitamins Added to Breakfast Cereal Products

Vitamin Content (Per Gram of Product)	Initial Value	Storage Time 3 Months (40°C)	6 Months (23°C)
Vitamin A (IU)	193	168	195
Ascorbic acid (mg)	2.6	2.4	2.5
Thiamin (mg)	0.060	0.060	0.064
Riboflavin (mg)	0.071	0.074	0.67
Niacin (mg)	0.92	0.85	0.88
Vitamin D	17.0	15.5	16.6
Vitamin E (IU)	0.49	0.49	0.46
Pyridoxine (mg)	0.085	0.088	0.081
Folate (mg)	0.018	0.014	0.018
Vitamin B12 (μg)	0.22	0.21	0.21
Pantothenic acid (mg)	0.42	0.39	0.39

Source: Anderson, R.H., *Food Technol.*, 30, 110, 1976.

8.3 DIETARY RECOMMENDATIONS

To assess the impact of food composition and intake patterns on the nutritional status of individuals and populations and to determine the nutritional effects of particular food processing and handling practices, a nutritional reference standard is essential. In the United States, the recommended dietary allowances (RDAs) have been developed for these purposes. The RDA values have been defined by the Committee on Dietary Allowances of the Institute of Medicine's Food and Nutrition Board as "the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97%–98%) healthy individuals in a particular stage of life and gender group" [71]. To the extent possible, the RDA values are formulated to include allowances for variability within the population with respect to nutrient requirements as well as the potential for incomplete bioavailability of nutrients. However, limitations in our current knowledge of the bioavailability of vitamins in foods render such allowances somewhat uncertain. Many other countries and several international organizations such as the FAO/WHO have developed reference values similar to the RDAs, and these sometimes differ quantitatively because of differences in scientific judgment or philosophy.

For food labeling to be meaningful to consumers, the concentration of micronutrients is best expressed relative to reference values. In the United States, nutrition labeling data for micronutrients have been traditionally expressed as a percentage of a "U.S. RDA" value, a practice that was originated at the onset of nutrition labeling in the early 1970s. The U.S. RDAs currently used for nutrition labeling were derived from the 1968 RDA values and differ somewhat from the current RDA values reported by the Food and Nutrition Board (Table 8.4). These differences, although not readily evident to the consumer, should be recognized and understood. Federal regulations permit modification of U.S. RDAs by the FDA "from time to time as more information on human nutrition becomes available" [21 CFR § 101.9(c)(7)(b)(ii)], although few changes have been implemented. Under the revised labeling regulation implemented by the FDA in 1994, the U.S. RDA term was replaced by the "reference daily intake (RDI)," which is currently equivalent to the previous U.S. RDAs. In the current nutrition labeling format, vitamin content is expressed as a percentage of the RDI and is listed on labels as "% daily value." Current FDA policy states:

> There are two sets of reference values for reporting nutrients in nutrition labeling: 1) Daily Reference Values (DRVs) and 2) Reference Daily Intakes (RDIs). These values assist consumers in interpreting information about the amount of a nutrient that is present in a food and in comparing nutritional values of food products. DRVs are established for adults and children four or more years of age, as are RDIs, with the exception of protein. DRVs are provided for total fat, saturated fat, cholesterol, total carbohydrate, dietary fiber, sodium, potassium, and protein. RDIs are provided for vitamins and minerals and for protein for children less than four years of age and for pregnant and lactating women. In order to limit consumer confusion, however, the label includes a single term (i.e., Daily Value (DV)), to designate both the DRVs and RDIs. Specifically, the label includes the % DV, except that the % DV for protein is not required unless a protein claim is made for the product or if the product is to be used by infants or children under four years of age. (http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm064928.htm, accessed September 28, 2016.)

8.4 ANALYTICAL METHODS AND SOURCES OF DATA

The major source of information regarding the content of vitamins in U.S. foods is the U.S. Department of Agriculture's National Nutrient Database for Standard Reference that provides online searchable data for over 8000 foods (USDA Food Composition Databases, 2015. http://ndb.nal.usda.gov/, accessed September 29, 2016.). There is a continued need for the improvement and validation of methods. A summary of the analytical methods used, sampling approaches, and statistical approaches for the National Nutrient Database for Standard Reference is now publically available (http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/SR26/sr26_doc.pdf, accessed September 29, 2016). Issues regarding the development and use of nutrient databases have been discussed by Holden et al. [65].

TABLE 8.4
Comparison of Recommended Dietary Allowances for Vitamins and "Reference Daily Intake" Currently Used in Nutritional Labeling in the United States

Category	Age (Year) or Condition	Vitamin A (µg RAE)[a]	Vitamin D (µg)	Vitamin E (mg as α-tocopherol)	Vitamin K (µg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg NE)	Vitamin B6 (mg)	Folate (µg DFE)	Vitamin B12 (µg)	Pantothenic Acid (mg)	Biotin (µg)	Choline (mg)
Infants	0.0–0.5	400	5	4	2.0	40	0.2	0.3	2	0.1	65	0.4	1.7	5	125
	0.5–1.0	500	5	5	2.5	50	0.3	0.4	4	0.3	80	0.5	1.8	6	150
Children	1–3	300	5	6	30	15	0.5	0.5	6	0.5	150	0.9	2	8	200
	4–8	400	5	7	55	25	0.6	0.6	8	0.6	200	1.2	3	12	200
Males	9–13	600	5	11	60	45	0.9	0.9	12	1.0	300	1.8	4	20	375
	14–18	900	5	15	75	75	1.2	1.3	16	1.3	400	2.4	5	25	550
	19–30	900	5	15	120	90	1.2	1.3	16	1.3	400	2.4	5	30	550
	31–50	900	5	15	120	90	1.2	1.3	16	1.3	400	2.4	5	30	550
	51–70	900	10	15	120	90	1.2	1.3	16	1.7	400	2.4	5	30	550
	>70	900	10	15	120	90	1.2	1.3	16	1.0	400	2.4	5	30	550
Females	9–13	600	5	11	60	90	0.9	0.9	12	1.2	300	1.8	4	20	375
	14–18	700	5	15	75	90	1.0	1.0	14	1.3	400	2.4	5	25	400
	19–30	700	5	15	90	90	1.1	1.1	14	1.3	400	2.4	5	30	425
	31–50	700	5	15	90	90	1.1	1.1	14	1.3	400	2.4	5	30	425
	51–70	700	10	15	90	90	1.1	1.1	14	1.5	400	2.4	5	30	425
	>70	700	10	15	90	90	1.1	1.1	14	1.5	400	2.4	5	30	425

(*Continued*)

TABLE 8.4 (*Continued*)
Comparison of Recommended Dietary Allowances for Vitamins and "Reference Daily Intake" Currently Used in Nutritional Labeling in the United States

Category	Age (Year) or Condition	Vitamin A (μg RAE)[a]	Vitamin D (μg)	Vitamin E (mg as α-tocopherol)	Vitamin K (μg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg NE)	Vitamin B6 (mg)	Folate (μg DFE)	Vitamin B12 (μg)	Pantothenic Acid (mg)	Biotin (μg)	Choline (mg)
Pregnant	<18	750	5	15	75	80	1.4	1.4	18	1.9	600	2.6	6	30	450
	19–30	770	5	15	90	85	1.4	1.4	18	1.9	600	2.6	6	30	450
	31–50	770	5	15	90	85	1.4	1.4	18	1.9	600	2.6	6	30	450
Lactating	<18	1200	5	19	75	115	1.4	1.6	17	2.0	500	2.8	7	35	550
	19–30	1300	5	19	90	120	1.4	1.6	17	2.0	500	2.8	7	35	550
	31–50	1300	5	19	90	120	1.4	1.6	17	2.0	500	2.8	7	35	550
RDI[b] (used in food labeling)		1000 (5000 IU)	10 (400 IU)	20 (30 IU)	No RDI	60	1.5	1.7	20	2.0	400	6.0	No RDI	No RDI	No RDI

Sources: Institute of Medicine, *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*, National Academy Press, Washington, DC, 2000; Institute of Medicine. Food and Nutrition Board, *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc—Institute of Medicine*, National Academy Press, Washington, DC, 2001; Institute of Medicine. Food and Nutrition Board, *Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*, National Academy Press, Washington, DC, 1998.

[a] Units (per day): RE, retinol equivalent (1 μg RAE = 1 μg retinol or 12 μg β-carotene, 24 μg a-carotene, 24 μg cryptoxanthin); vitamin E as α-tocopherol equivalent; NE, niacin equivalent (1 mg NE = 1 mg niacin or 60 mg tryptophan); DFE, dietary folate equivalent (μg DFE = μg naturally occurring food folate + 1.7 × μg synthetic folic acid).

[b] Reference daily intake is the reference unit used in U.S. nutrition labeling of foods, formerly termed the U.S. RDA.

The adequacy of analytical methods is a serious problem with respect to many vitamins for food analysis. While current analytical methods are generally acceptable for some vitamins (e.g., ascorbic acid, thiamin, riboflavin, niacin, vitamin B6, vitamin A, and vitamin E), they are less uniformly adequate for others (e.g., folate, pantothenic acid, biotin, carotenoids, vitamin B12, vitamin D, and vitamin K). Factors that limit the suitability of analytical methods may involve a lack of specificity of traditional chemical methods, interferences in microbiological assays, incomplete extraction of the analyte(s) from the food matrix, lack of measurement of certain nutritionally active vitamers in chromatographic methods, and incomplete measurement of complexed forms of a vitamin. Improvement of analytical data for vitamins will require additional support for methods development research, improved training of analysts, development of quality control protocols (i.e., validation and standardization of procedures), and development of standard reference materials for vitamin analysis. The strengths and limitations of analytical methods for each vitamin will be briefly addressed in this chapter.

8.5 BIOAVAILABILITY OF VITAMINS

The term "bioavailability" refers to the degree to which an ingested nutrient undergoes intestinal absorption and metabolic function or utilization within the body. In the broad sense, bioavailability involves both absorption and utilization of the nutrient *as consumed*. This concept does not refer to losses that may occur prior to consumption. For a complete description of the nutritional adequacy of a food, three factors must be known: (1) the concentration of the vitamin *at the time of consumption*, (2) the identity of various chemical species of the vitamin present, (3) and the bioavailability of these forms of the vitamin *as they exist in the meal consumed*.

Factors that influence the bioavailability of vitamins include (1) composition of the diet, which could influence intestinal transit time, viscosity, emulsion characteristics, and pH; (2) form of the vitamin (forms may differ in rate or extent of absorption, stability in the stomach and intestine prior to digestion, ease of conversion to metabolically active or coenzymic form, or metabolic functionality); (3) interactions between a vitamin and components of the diet (e.g., proteins, starches, dietary fiber, lipids) that interfere with intestinal absorption of the vitamin. Although our understanding of the relative bioavailability of the various species of each vitamin is rapidly improving, the complex influences of food composition on vitamin bioavailability remain poorly understood. In addition, the effects of processing and storage on vitamin bioavailability have been only partially determined.

The application of information regarding bioavailability of vitamins is limited at this time. Bioavailability is generally considered in the development of dietary recommendations (e.g., RDA values), but this involves only the use of estimated mean bioavailability values. Knowledge is too fragmentary and variability too great to permit vitamin bioavailability data to be included in food composition tables. However, even if our understanding of the bioavailability of vitamins in individual foods were much more complete, such data regarding *individual foods* may be of little use. A far greater need is for a better understanding of vitamin bioavailability in the *diet as a whole* (including interactive effects of individual foods) and the sources of variation in this respect among individual people.

8.6 GENERAL CAUSES OF VARIATION/LOSSES OF VITAMINS IN FOOD

Beginning at the time of harvesting, all foods inevitably undergo some loss of vitamins. The nutritional significance of partial loss of vitamins depends on the nutritional status of the individual (or population) for the vitamin of interest, the importance of the particular food as a source of that vitamin, and the bioavailability of the vitamin. Many processing, storage, and handling methods are intended to minimize vitamin losses. The following is a summary of the various factors responsible for variation in the vitamin content of foods.

8.6.1 Inherent Variation in Vitamin Content

The concentration of vitamins in fruits and vegetables often varies with the genetic characteristics of the cultivar, stage of maturity, site of growth, and climate. During maturation of fruits and vegetables, vitamin concentration is determined by the rates of synthesis and degradation. Information on the time course of vitamin concentration in most fruits and vegetables is not available except for ascorbic acid and β-carotene in a few products. In the example shown in Table 8.5, the maximum concentration of ascorbic acid in tomatoes occurred prior to full maturity. A similar phenomenon has been seen in studies of folate in tomatoes, with a 35% reduction observed during ripening. A study of carrots showed that carotenoid concentration varied markedly with variety but was not influenced significantly by stage of maturity.

Little is known about developmental changes in vitamin content of cereal grains and legumes. In contrast to fruits and vegetables, cereal grains and legumes are harvested at a fairly uniform stage of maturity.

Agricultural practices and environmental conditions undoubtedly influence the content of vitamins in plant-derived foods, but few data are available on this subject. Klein and Perry [85] determined the content of ascorbic acid and vitamin A activity (from carotenoids) in selected fruits and vegetables sampled from six different locations across the United States. In their study, wide variation was found among sampling sites, possibly as a result of geographic/climatic effects, varietal differences, and effects of local agricultural practices. Interactions among agricultural practices including type and amount of fertilizer and irrigation regimen, environment, and genetics would certainly influence vitamin content of plant-derived foods, but these relationships would be very difficult to characterize in a systematic fashion. The technology exists for food plants to be genetically engineered to produce increased amounts of certain vitamins (e.g., folate, tocopherols) or vitamin-active compounds (e.g., β-carotene) to achieve "biofortification" [27,30] or by selective breeding approaches.

The vitamin content of animal products is governed both by biological control mechanisms and by the diet of the animal. In the case of many B vitamins, the concentration of the vitamin in tissues is limited by the capacity of the tissues to take up the vitamin from the blood and to convert it to the coenzymic form(s). A nutritionally inadequate animal diet can yield reduced tissue concentrations of both water-soluble and fat-soluble vitamin(s) involved. In contrast to the situation with water-soluble vitamins, dietary supplementation with fat-soluble vitamins can more readily increase tissue concentrations. This has been examined as a means of increasing the vitamin E concentration of certain animal products to improve oxidative stability and color retention.

TABLE 8.5
Influence of Degree of Maturity on Ascorbic Acid Content of Tomatoes

Weeks from Anthesis	Mean Weight (g)	Color	Ascorbic Acid (mg/100 g)
2	33.4	Green	10.7
3	57.2	Green	7.6
4	102	Green-yellow	10.9
5	146	Yellow-red	20.7
6	160	Red	14.6
7	168	Red	10.1

Source: Malewski, W. and Markakis, P., *J. Food Sci.*, 36, 537, 1971.

8.6.2 Postharvest Changes in Vitamin Content of Foods

Fruits, vegetables, and animal tissues often retain enzymatic activities that contribute to postharvest changes in the vitamin content of foods. The release of oxidative and hydrolytic enzymes, as a result of deterioration of cellular integrity and enzymatic compartmentation, can cause changes in the distribution of chemical forms and activity of vitamins. For example, dephosphorylation of vitamin B6, thiamin or flavin coenzymes, deglycosylation of vitamin B6 glucosides, and the deconjugation of polyglutamyl folates can cause differences between post-harvest distributions and those occurring naturally in the plant or animal prior to harvest or slaughter. The extent of such changes will depend on physical damage encountered during handling, possible temperature abuse, and the length of time between harvest and processing. Such changes will have little influence on the net concentration of a vitamin but may influence its bioavailability. In contrast, oxidative changes such as those caused by lipoxygenases can reduce the concentration of many vitamins, while ascorbic acid oxidase can specifically reduce the concentration of ascorbic acid.

Postharvest changes in vitamin concentration are inevitable but can be minimized when proper procedures are followed during postharvest handling of fruits and vegetables. The mishandling of plant products through prolonged holding or shipment at ambient temperatures can contribute to major losses of labile vitamins. Continued metabolism of plant tissues postharvest can be responsible for changes in total concentration as well as distribution of chemical forms of certain vitamins depending on the storage conditions. Postharvest losses of vitamins in meat products are usually minimal under typical conditions of refrigerated storage.

8.6.3 Preliminary Treatments: Trimming, Washing, and Milling

The peeling and trimming of fruits and vegetables can cause losses of vitamins to the extent that they are concentrated in the discarded stem, skin, or peel fractions. Although this can be a source of significant loss relative to the intact fruit or vegetable, in most cases this must be considered to be an inevitable loss regardless of whether it occurs in industrial processing or home preparation.

Alkaline treatments to enhance peeling can cause increased losses of labile vitamins such as folate, ascorbic acid, and thiamin at the surface of the product. However, losses of this kind tend to be small compared to the total vitamin content of the product.

Any exposure of cut or otherwise damaged tissues of plant or animal products to water or aqueous solutions causes the loss of water-soluble vitamins by extraction (leaching). This can occur during washing, transportation via flumes, and exposure to brines during cooking. The extent of such losses depends on factors that influence the diffusion and solubility of the vitamin, including pH (which can affect the solubility and dissociation of vitamins from binding sites within the tissue), ionic strength of the extractant, temperature, the volume ratio of food to aqueous solution, and the surface-to-volume ratio of the food particles. Extractant properties that affect destruction of the vitamin once extracted include dissolved oxygen, ionic strength, concentration and type of catalytic trace metals, and the presence of solutes that are destructive (e.g., chlorine) or protective (e.g., certain reducing agents).

The milling of cereal grains involves grinding and fractionation to remove the bran (seed coat) and embryo. Because many vitamins are concentrated in the embryo and bran, major losses of vitamins can occur during milling (Figure 8.1). Such losses, as well as the prevalence of vitamin-deficiency diseases, contributed to the rationale for the initiating the enrichment of cereal grain products with several added nutrients (riboflavin, niacin, thiamin, iron, and calcium) and, more recently, folic acid. The beneficial impact of this enrichment program on public health has been enormous.

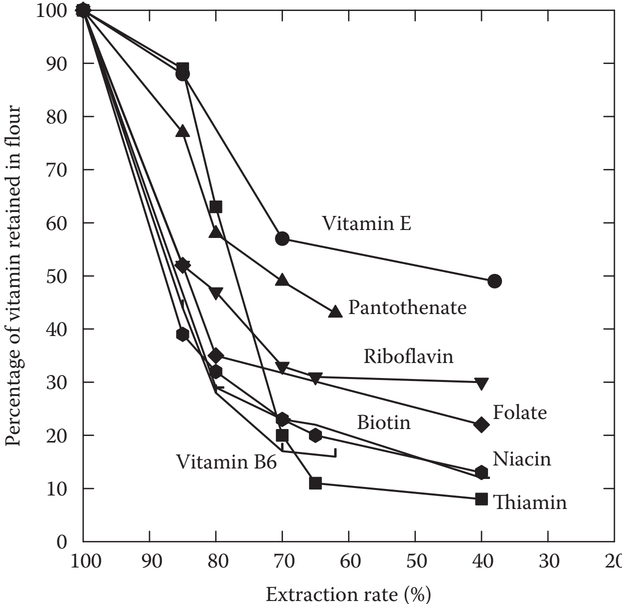


FIGURE 8.1 Retention of selected nutrients as a function of degree of refining in the production of wheat flour. Extraction rate refers to the percentage recovery of flour from whole grain during milling. (Redrawn from Moran, T., *Nutr. Abstr. Rev. Ser. Hum. Exp.*, 29, 1, 1959.)

8.6.4 Effects of Blanching and Thermal Processing

Blanching, a mild heat treatment, is an essential step in the processing of fruits and vegetables. The primary purposes are to inactivate potentially deleterious enzymes, reduce microbial loads, and decrease interstitial gasses prior to further processing. Inactivation of enzymes often has a beneficial effect on the stability of many vitamins during subsequent food storage.

Blanching can be accomplished in hot water, flowing steam, hot air, or with microwaves. Losses of vitamins occur primarily by oxidation and aqueous extraction (leaching), with heat being a factor of secondary importance. Blanching in hot water can cause large losses of water-soluble vitamins by leaching (e.g., Figure 8.2). It has been well documented that, as a result of this difference, high-temperature, short-time (HTST) treatments improve retention of labile nutrients during blanching and other thermal processes. Specific effects of blanching have been reviewed [128].

Changes in the vitamin content of foods during processing have been addressed in extensive study and thorough review [59,60,78,124]. The elevated temperature of thermal processing accelerates reactions that would otherwise occur more slowly at ambient temperature. Thermally induced losses of vitamins depend on the chemical nature of the food, its chemical environment (pH, relative humidity, transition metals, other reactive compounds, concentration of dissolved oxygen, etc.), the stabilities of the individual forms of vitamins present, and the opportunity for leaching. The nutritional significance of such losses depends on the degree of loss and the importance of the food as a source of the vitamin in typical diets. Although subject to considerable variation, representative data for losses of vitamins during the canning of vegetables are shown in Table 8.6.

8.6.5 Losses of Vitamins Following Processing

Compared to loss of vitamins during thermal processing, subsequent storage often has a small but significant effect on vitamin content. Several factors contribute to small postprocessing losses: (a) reaction rates are relatively slow at ambient or reduced temperature, (b) dissolved oxygen may be

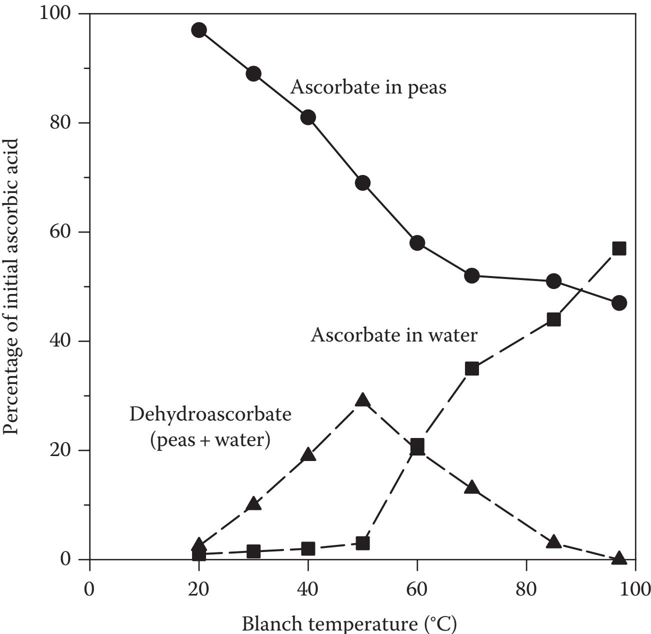


FIGURE 8.2 Retention of ascorbic acid in peas during experimental water blanching for 10 min at various temperatures. (Redrawn from Selman, J., *Food Chem.*, 49, 137, 1994.)

TABLE 8.6
Typical Losses of Vitamins during Canning[a,b]

Product	Biotin	Folate	B6	Pantothenic Acid	A	Thiamin	Riboflavin	Niacin	C
Asparagus	0	75	64	—	43	67	55	47	54
Lima beans	—	62	47	72	55	83	67	64	76
Green beans	—	57	50	60	52	62	64	40	79
Beets	—	80	9	33	50	67	60	75	70
Carrots	40	59	80	54	9	67	60	33	75
Corn	63	72	0	59	32	80	58	47	58
Mushrooms	54	84	—	54	—	80	46	52	33
Green peas	78	59	69	80	30	74	64	69	67
Spinach	67	35	75	78	32	80	50	50	72
Tomatoes	55	54	—	30	0	17	25	0	26

[a] Includes blanching.
[b] From various sources, compiled by Lund [93,94].

depleted, and (c) pH may change during processing (pH usually declines) because of thermal effects or concentrative effects (drying or freezing), and this can have a favorable effect on the stability of vitamins such as thiamin and ascorbic acid. For example, Figure 8.3 illustrates how vitamin C retention in potatoes can be affected by thermal processing. The relative importance of leaching, chemical degradation, and the type of container (cans or pouches) is apparent from these data.

In reduced moisture foods, vitamin stability is strongly influenced by water activity (i.e., relative vapor pressure) in addition to the other factors to be discussed. In the absence of oxidizing lipids, water-soluble vitamins generally exhibit little degradation at water activity less than or equal to

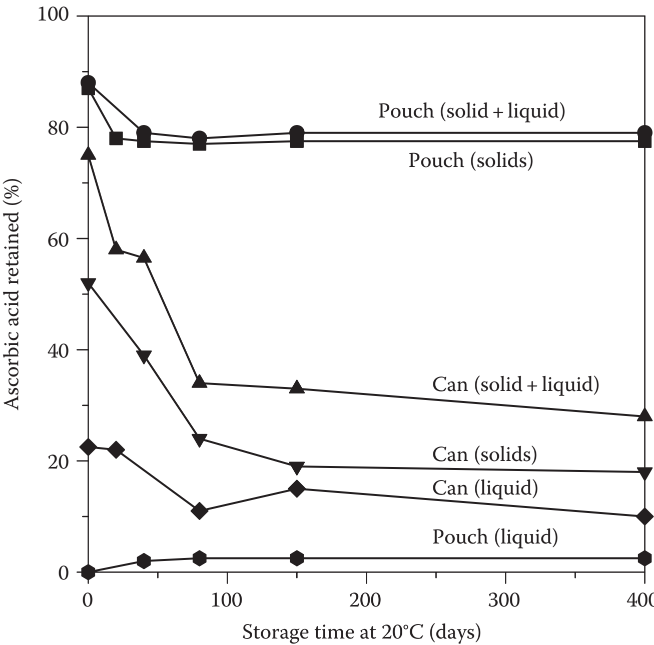


FIGURE 8.3 Retention and distribution of ascorbic acid in potatoes thermally processed in cans or flexible pouches. Values show the content of ascorbic acid, relative to that present prior to processing, in the potatoes and liquid of the containers. Lethality values (F_o) were not provided. (Redrawn from Ryley, J. and Kajda, P., *Food Chem.*, 49, 119, 1994.)

monolayer hydration (~0.2–0.3 a_w). Degradation rates increase in proportion to water activity in regions of multilayer hydration, which reflects greater solubility of the vitamin, potential reactants, and catalysts. In contrast, the influence of water activity on the stability of fat-soluble vitamins and carotenoids parallels the pattern for unsaturated fats, that is, a minimum rate at monolayer hydration and increased rates above or below this value (see Chapter 2). Substantial losses of oxidation-sensitive vitamins can occur if foods are over dried.

8.6.6 Influence of Processing Chemicals and Other Food Components

The chemical composition of food can strongly influence the stability of vitamins. Oxidizing agents directly degrade ascorbic acid, folate, vitamin A, carotenoids, and vitamin E and may indirectly affect other vitamins. The extent of their impact is dictated by concentration of the oxidant and its oxidation potential. In contrast, reducing agents such as ascorbic and isoascorbic acids and various thiols would increase the stability of oxidizable vitamins by their reducing action and as scavengers of oxygen and free radicals. The following is a brief discussion of the influence of several other processing chemicals on vitamins. See later sections for vitamin-specific details.

Chlorine may be applied to foods as hypochlorous acid (HOCl), hypochlorite anion (OCl^-), sodium chlorite ($NaClO_2$), molecular chlorine (Cl_2), or chlorine dioxide (ClO_2). These compounds can interact with vitamins by electrophilic substitution, by oxidation, or by chlorination of double bonds. The extent of vitamin loss caused by treatments of food with chlorinated water has not been thoroughly studied; however, one would predict relatively minor effects if the application is confined to the product surface. Chlorination of cake flour presumably has little influence on vitamins in other ingredients used in baking because residual chlorine would be negligible. Reaction products of various forms of chlorine with vitamins are, for the most part, unknown.

Sulfite and other sulfiting agents (SO_2, bisulfite, metabisulfite), as used in wines for antimicrobial effects and in dried food to inhibit enzymatic browning, have a protective effect on ascorbic acid and a deleterious effect on several other vitamins. Sulfite ions directly react with thiamin causing its inactivation. Sulfite also reacts with carbonyl groups and is known to convert vitamin B6 aldehydes (pyridoxal and pyridoxal phosphate) to their presumably inactive sulfonated derivatives. The extent to which sulfiting agents affect other vitamins has not been extensively studied.

Nitrite is used in the preservation and curing of meats and may develop through microbial reduction of naturally occurring nitrate. Ascorbic acid or isoascorbic acid is added to nitrite-containing meats to prevent formation of *N*-nitrosamines. This is accomplished by forming NO and preventing formation of undesirable nitrous anhydride (N_2O_3, the primary nitrosating agent). The proposed reactions are shown here [91]:

$$\text{Ascorbic acid} + HNO_2 \rightarrow \text{2-Nitrite ester of ascorbic acid} \rightarrow \text{Semidehydroascorbate radical} + NO$$

The formation of NO is desirable because it is the desired ligand for binding to myoglobin to form the cured meat color. The residual semidehydroascorbate radical retains partial vitamin C activity.

Chemical sterilants are used in highly specific applications such as treating spices with ethylene and propylene oxides for deinfestation. The biocidal function of these compounds occurs by alkylation of proteins and nucleic acids. Similar effects have been observed to occur with some vitamins, although loss of vitamin activity by this means is insignificant in the overall food supply.

Chemicals and food ingredients that influence pH will directly affect the stability of vitamins such as thiamin and ascorbic acid, particularly in the neutral to mildly acidic pH range. Acidulation increases the stability of ascorbic acid and thiamin. In contrast, alkalizing compounds reduce the stability of ascorbic acid, thiamin, pantothenic acid, and certain folates.

8.7 FAT-SOLUBLE VITAMINS

8.7.1 Vitamin A

8.7.1.1 Structure and General Properties

Vitamin A refers to a group of nutritionally active unsaturated hydrocarbons, including retinol and related compounds (Figure 8.4) and certain carotenoids (Figure 8.5). Vitamin A activity in animal tissues is predominantly in the form of retinol or its esters, retinal, and, to a lesser extent, retinoic acid. The concentration of vitamin A is greatest in liver, the major body pool, in which retinol and retinol esters are the primary forms present. The term “retinoids” refers to the class of compounds including retinol and its chemical derivatives having four isoprenoid units. Several retinoids that are analogues of the nutritionally active forms of vitamin A exhibit useful pharmacological properties. In addition, synthetic retinyl acetate and retinyl palmitate are used widely in synthetic form for food fortification.

Carotenoids contribute significant vitamin A activity to foods of both plant and animal origin. Of approximately 600 known carotenoids, ~50 exhibit some provitamin A activity (i.e., they are partially converted to vitamin A in vivo). Preformed vitamin A does not exist in plants and fungi; their vitamin A activity is associated with certain carotenoids. The structures of selected carotenoids, along with their vitamin A activities determined by rat bioassay, are presented in Figure 8.5. The reader is referred to Chapter 10 for further discussion of the properties of the carotenoids in the context of their role as food pigments.

For a compound to have vitamin A or provitamin A activity, it must exhibit certain structural similarities to retinol, including (a) at least one intact nonoxygenated β-ionone ring and (b) an isoprenoid side chain terminating in an alcohol, aldehyde, or carboxyl function (Figure 8.4). The vitamin A–active carotenoids such as β-carotene (Figure 8.5) are considered to have provitamin A activity until they undergo oxidative enzymatic cleavage of the central $C^{15}–C^{15'}$ bond in the

FIGURE 8.4 Structures of common retinoids.

intestinal mucosa to release two molecules of active retinal. Among the carotenoids, β-carotene exhibits the greatest provitamin A activity. Carotenoids with ring hydroxylation or the presence of carbonyl groups exhibit less provitamin A activity than β-carotene if only one ring is affected, and they have no activity if both rings are oxygenated. Although potentially two molecules of vitamin A are produced from each molecule of dietary β-carotene, the inefficiency of the process accounts for the fact that β-carotene exhibits only ~50% of the vitamin A activity exhibited by retinol, on a mass basis. This was the basis of the initial belief that the relative vitamin A activity of retinol and β-carotene was 1:2 on a mass basis. Considerable variation exists among various animal species and humans with respect to the efficiency of utilization of carotenoids and the extent of absorption of carotenoid molecules in intact form from food sources (see the discussion in Section 8.7.1.3), and some scientific disagreement exists regarding the vitamin A equivalence of β-carotene. Revised assessment of the issues of bioavailability and bioconversion (i.e., conversion of carotenoids to vitamin A) by the U.S. Institute of Medicine led to the recommendation that data be expressed in units of retinol activity equivalents [70]. In this system, the retinol activity equivalents for retinol, β-carotene, and other vitamin A–active carotenoids are 1:12:24 on a mass basis. For example, 1 μg retinol activity equivalents = 12 μg β-carotene from a typical diet. The in vivo antioxidative function attributed to dietary carotenoids requires absorption of the intact molecule [15].

The retinoids and provitamin A carotenoids are very lipophilic compounds. Consequently, they associate with lipid components, specific organelles, or carrier proteins in foods and living cells. In many food systems, retinoids and carotenoids are found associated with lipid droplets or micelles dispersed in an aqueous environment. For example, both retinoids and carotenoids are present in the fat globules of milk, while in orange juice the carotenoids associate with dispersed oils. The conjugated double bond system of retinoids gives strong and characteristic ultraviolet absorption spectra, while the additional conjugated double bond system of carotenoids causes absorption in the visible

Compound	Relative activity	Retinol activity equivalents
β-Carotene	50	12
α-Carotene	25	24
α-Apo-8′-carotenal	25–30	
Cryptoxanthin	25	24
Canthaxanthin	0	
Astacene	0	
Lycopene	0	

FIGURE 8.5 Structures and provitamin A activities of selected carotenoids. Relative activity values are based on an assumption of 50% for β-carotene, relative to retinol, and should be viewed as maximal estimates.

spectrum and the yellow-orange color of these compounds. All-trans isomers exhibit the greatest vitamin A activity and are the predominant naturally occurring forms of retinoids and carotenoids in foods (Tables 8.7 and 8.8). Conversion to cis isomers, which can occur during thermal processing, causes a loss of vitamin A activity.

The carotenoids that do not have vitamin A activity may still serve important functions in maintaining health. Analysis of tissues reveals that several carotenoids concentrate in certain tissues, which may reflect specific antioxidative functions. Of particular interest are the roles of lycopene in the prostate and zeaxanthin and lutein in the retina. Epidemiological studies support such relationships.

8.7.1.2 Stability and Modes of Degradation

The degradation of vitamin A (retinoids and vitamin A–active carotenoids) generally parallels the oxidative degradation of unsaturated lipids. Factors that promote oxidation of unsaturated lipids enhance degradation of vitamin A, either by direct oxidation or by indirect effects of free radicals. Changes in the β-carotene content of cooked dehydrated carrots illustrate typical extents of degradation during processing and typical exposure to oxygen during associated handling (Table 8.9).

TABLE 8.7
Relative Vitamin A Activity of Stereoisomeric Forms of Retinol Derivatives

	Relative Vitamin A Activity[a]	
Isomer	**Retinyl Acetate**	**Retinal**
All-trans	100	91
13-cis	75	93
11-cis	23	47
9-cis	24	19
9,13-di-cis	24	17
11,13-di-cis	15	31

Source: Ames, S.R., *Fed. Proc.*, 24, 917, 1965.
[a] Molar vitamin A activity relative to all-trans retinyl acetate in rat bioassays.

TABLE 8.8
Relative Vitamin A Activity of Stereoisomeric Forms of Carotenes

Compound and Isomer	Relative Vitamin A Activity[a]
β-Carotene	
All-trans	100
9-cis (neo-U)	38
13-cis (neo-B)	53
α-Carotene	
All-trans	53
9-cis (neo-U)	13
13-cis (neo-B)	16

Source: Zechmeister, L., *Vitam. Horm.*, 7, 57, 1949.
[a] Activity relative to that of all-trans β-carotene in rat bioassays.

TABLE 8.9
Concentration of β-Carotene in Cooked Dehydrated Carrots

Sample	β-Carotene Concentration (μg/g Solids)
Fresh	980–1860
Explosive puff-dried	805–1060
Vacuum freeze-dried	870–1125
Conventional air-dried	636–987

Source: Dellamonica, E. and McDowell, P., *Food Technol.*, 19, 1597, 1965.

TABLE 8.10
Distribution of β-Carotene Isomers in Selected Fresh and Processed Fruits and Vegetables

Product	Status	Percentage of Total β-Carotene		
		13-cis	All-trans	9-cis
Sweet potato	Fresh	0.0	100.0	0.0
Sweet potato	Canned	15.7	75.4	8.9
Carrot	Fresh	0.0	100.0	0.0
Carrot	Canned	19.1	72.8	8.1
Squash	Fresh	15.3	75.0	9.7
Squash	Canned	22.0	66.6	11.4
Spinach	Fresh	8.8	80.4	10.8
Spinach	Canned	15.3	58.4	26.3
Collard	Fresh	16.6	71.8	11.7
Collard	Canned	26.6	46.0	27.4
Cucumber	Fresh	10.5	74.9	14.5
Pickle	Pasteurized	7.3	72.9	19.8
Tomato	Fresh	0.0	100.0	0.0
Tomato	Canned	38.8	53.0	8.2
Peach	Fresh	9.4	83.7	6.9
Peach	Canned	6.8	79.9	13.3
Apricot	Dehydrated	9.9	75.9	14.2
Apricot	Canned	17.7	65.1	17.2
Nectarine	Fresh	13.5	76.6	10.0
Plum	Fresh	15.4	76.7	8.0

Source: Chandler, L. and Schwartz, S., *J. Food Sci.*, 52, 669, 1987.

It should be noted, however, that extended storage of vitamin A in foods such as fortified breakfast cereal products, infant formulas, fluid milk, fortified sucrose, and condiments is usually not highly detrimental to the retention of added vitamin A.

Losses of vitamin A activity of retinoids and carotenoids in foods occur mainly through reactions involving the unsaturated isoprenoid side chain, either by autoxidation or geometric isomerization. Retinoid and carotenoid molecules largely remain chemically intact during thermal processing, although they do undergo some isomerization. HPLC analysis has revealed that many foods contain a mixture of all-trans and cis isomers of retinoids and carotenoids. As summarized in Table 8.10, conventional canning of fruits and vegetables is sufficient to induce isomerization and ensuing losses of vitamin A activity. In addition to thermal isomerization, the conversion of all-trans forms of retinoids and carotenoids to various cis isomers can be induced by exposure to light, acid, chlorinated solvents (e.g., chloroform), and dilute iodine. Chlorinated solvents often used in lipid analysis enhance the photochemical isomerization of retinyl palmitate and, presumably, other retinoids and carotenoids.

The occurrence of cis isomers of carotenoids has been known for many years (Figure 8.6). Previous nomenclature for β-carotene isomers was derived from chromatographic separations and included neo-β-carotene U (9-cis-β-carotene) and neo-β-carotene B (13-cis-β-carotene). Confusion exists in the literature because neo-β-carotene B was originally identified incorrectly as 9,13′-di-cis-β-carotene [143]. Analogous isomerization occurs with other carotenoids. The maximum extent of

FIGURE 8.6 Structures of selected cis isomers of β-carotene: (a) all-trans, (b) 11,15-di-cis, (c) 9-cis, (d) 13-cis, and (e) 15-cis.

thermal isomerization generally observed in canned fruits and vegetables is about 40% 13-cis-β-carotene and 30% 9-cis-β-carotene (Table 8.10). The values observed for cis isomers of β-carotene in processed foods are similar to the equilibrium values observed in the iodine-catalyzed isomerization of β-carotene, which suggests that the extent and specificity of isomerization is similar regardless of the mechanism.

Photochemical isomerization of vitamin A compounds occurs both directly and indirectly via a photosensitizer. The ratios and quantities of cis isomers produced differ with the means of photoisomerization. Photoisomerization of all-trans-β-carotene involves a series of reversible reactions, and each isomerization is accompanied by photochemical degradation (Figure 8.7). Similar rates of photoisomerization and photodegradation have been observed in aqueous dispersions of β-carotene and in carrot juice. These photochemical reactions also have been observed when retinoids in foods are exposed to light (e.g., milk). The type of packaging material can have a substantial effect on net retention of vitamin A activity in food exposed to light during storage.

Oxidative degradation of vitamin A and carotenoids in foods can occur by direct peroxidation or by indirect action of free radicals produced during oxidation of fatty acids. β-Carotene, and probably other carotenoids, has the ability to act as an antioxidant under conditions of reduced oxygen

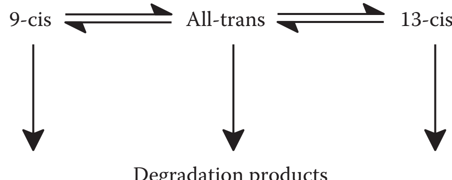


FIGURE 8.7 Model of photochemically induced reactions of β-carotene. (From Pesek, C. and Warthesen, J., *J. Agric. Food Chem.*, 38, 1313, 1990.)

concentration (<150 torr O_2), although it may act as a prooxidant at higher oxygen concentrations [15,16]. β-Carotene can act as an antioxidant by scavenging singlet oxygen, hydroxyl, and superoxide radicals and by reacting with peroxyl radicals (ROO•). Peroxyl radicals attack β-carotene to form an adduct postulated to be ROO-β-carotene•, in which the peroxyl radical moiety is bonded to the C^7 position of β-carotene, while the unpaired electron is delocalized across the conjugated double bond system. β-Carotene apparently does not act as a chain-breaking radical (donating H•) as do phenolic antioxidants. This antioxidant behavior of β-carotene, and presumably other carotenoids, causes a reduction or total loss of vitamin A activity regardless of the mechanism by which free radical initiation occurs. For retinol and retinyl esters, however, the attack of free radicals occurs mainly at the C^{14} and C^{15} positions.

Oxidation of β-carotene involves the formation of the 5,6-epoxide, which may isomerize to the 5,8-epoxide (mutachrome). Photochemically induced oxidation yields mutachrome as the primary degradation product. Fragmentation of β-carotene to many lower-molecular-weight compounds can occur especially during high-temperature treatments. Resulting volatiles can have a significant effect on flavor. Such fragmentation also occurs during oxidation of retinoids. An overview of these reactions and other aspects of the chemical behavior of vitamin A is shown in Figure 8.8.

8.7.1.3 Bioavailability

Retinoids are absorbed effectively except under conditions in which malabsorption of fat occurs. Retinyl acetate and palmitate are as effectively utilized as nonesterified retinol. Diets containing nonabsorbable hydrophobic materials such as certain fat substitutes may contribute to malabsorption of vitamin A. The bioavailability of vitamin A added to rice has been demonstrated in human subjects.

Aside from the inherent difference in utilization between retinol and the provitamin A carotenoids, carotenoids in many foods undergo markedly less intestinal absorption. Absorption may be impaired by the specific binding of carotenoids as carotenoproteins or by entrapment in poorly digestible vegetable matrices. In studies with human subjects, β-carotene from carrots yielded only ~21% of the plasma β-carotene response obtained from an equivalent dose of pure β-carotene, while β-carotene in broccoli exhibited a similarly low bioavailability [12].

8.7.1.4 Analytical Methods

Early methods of vitamin A analysis centered on the reactions of retinoids with Lewis acids such as antimony trichloride and trifluoroacetic acid to yield a blue color. In addition, fluorometric methods have been used to measure vitamin A [142]. Interferences often occur when these methods are applied to foods. Furthermore, these methods do not detect trans–cis isomerization that may occur during processing or storage of foods. Because the cis isomers exhibit less nutritional activity than the all-trans compound, it is inaccurate to regard *total* vitamin A or provitamin A activity simply as the sum of all isomeric forms. HPLC is the method of choice because it enables individual retinoids to be determined with considerable accuracy. Liquid chromatography–mass spectrometry (LCMS) approaches also have gained extensive application. Accurate measurement of carotenoids is a very complex task in view of the many naturally occurring chemical forms present in foods [12,20,79].

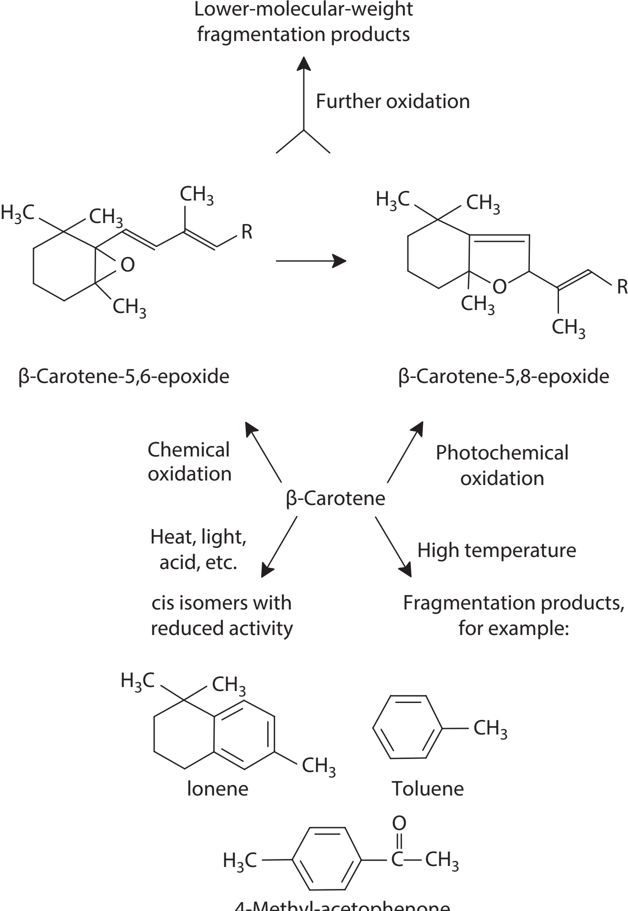


FIGURE 8.8 Overview of carotenoid degradation.

8.7.2 Vitamin D

8.7.2.1 Structure and General Properties

Vitamin D activity in foods is associated with several lipid-soluble sterol analogues including cholecalciferol (vitamin D3) from animal sources and ergocalciferol (vitamin D2) produced synthetically (Figure 8.9). Both of these compounds are used in synthetic form for food fortification. Recent evidence suggests that the vitamin D activity of cholecalciferol exceeds that of ergocalciferol [49,67]. Cholecalciferol forms in human skin upon exposure to sunlight, and this is a multistep process involving photochemical modification of 7-dehydrocholesterol followed by nonenzymatic isomerization. Because of this in vivo synthesis, the requirement for dietary vitamin D will depend on the extent of exposure to sunlight. Ergocalciferol is an exclusively synthetic form of vitamin D that is formed by commercial irradiation of phytosterol (a plant sterol) with UV light. Several hydroxylated metabolites of vitamin D2 and D3 form in vivo. The 1,25-dihydroxy derivative of cholecalciferol is the main physiologically active form, and it is involved in the regulation of calcium absorption and metabolism. 25-Hydroxycholecalciferol, in addition to cholecalciferol, comprises a significant amount of the naturally occurring vitamin D activity in meat and milk products.

Fortification of most fluid milk products with either ergocalciferol or cholecalciferol makes a significant contribution to dietary needs. Vitamin D is susceptible to degradation by light, and this may occur in glass-packaged milk during retail storage. For example, approximately 50% of

FIGURE 8.9 Structure of ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3).

cholecalciferol added to skim milk is lost during 12 days of continual exposure to fluorescent light at 4°C. It is not known whether this degradation involves direct photochemical degradation, a mechanism involving a photosensitizer yielding an active oxygen species (e.g., $^1O_2$), or an indirect effect of light-induced lipid oxidation. Like other unsaturated fat-soluble components of foods, vitamin D compounds are susceptible to oxidative degradation. Overall, however, the stability of vitamin D in foods, especially under anaerobic conditions, is not a major concern.

8.7.2.2 Analytical Methods

Measurement of vitamin D is performed primarily by HPLC and LCMS methods [68]. Alkaline conditions yield rapid degradation of vitamin D; thus, saponification as used widely in the analysis of lipid-soluble materials cannot be employed. Various preparative chromatographic methods have been developed for the purification of food extracts prior to HPLC analysis.

8.7.3 Vitamin E

8.7.3.1 Structure and General Properties

Vitamin E is the generic term for tocols and tocotrienols that exhibit vitamin activity similar to that of α-tocopherol. Tocols are 2-methyl-2(4′,8′,12′-trimethyltridecyl)chroman-6-ols, while tocotrienols are identical except for the presence of double bonds at positions 3′, 7′, and 11′ of the side chain (Figure 8.10). Tocopherols, which are typically the main compounds having vitamin E activity in foods, are derivatives of the parent compound tocol and have one or more methyl groups at positions 5, 7, or 8 of the ring structure (chromanol ring) (Figure 8.10). The α, β, γ, and δ forms of tocopherol and tocotrienol differ according to the number and position of the methyl groups and thus differ significantly in vitamin E activity (Table 8.10). The data presented in Table 8.10 represent the traditional views of the relative activities of these compounds, with α-tocopherol being the highest vitamin E activity. In a new system of reporting vitamin E activity [69], α-tocopherol is viewed as the sole form exhibiting specific vitamin E activity, while α-tocopherol *and* all other tocopherols and tocotrienols provide a general antioxidant function. This remains an area of controversy among some researchers.

There are three asymmetric carbons (2, 4′, and 8′) in the tocopherol molecule, and the stereochemical configuration at these positions influences the vitamin E activity of the compound. Early nomenclature for vitamin E compounds is confusing with regard to the vitamin activity of the stereoisomers. The naturally occurring configuration of α-tocopherol exhibits the greatest vitamin E activity and is now designated RRR-α-tocopherol; other terminology, such as the term D-α-tocopherol, should be discontinued. Synthetic forms of α-tocopheryl acetate are used widely in food fortification. The presence of the acetate ester greatly improves the stability of the compound by blocking the phenolic hydroxyl group and, thus, eliminating its radical-quenching activity. Synthetic forms that are racemic mixtures consisting of eight possible combinations of geometric isomers involving

α-Tocopherol

α-Tocopheryl acetate

	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

FIGURE 8.10 Structures of tocopherols. The structures of tocotrienols are identical to the corresponding tocopherols, except for the presence of double bonds at positions 3′, 7′, and 11′.

positions 2, 4′, and 8′ should be designated all-rac-α-tocopheryl acetate rather than the previously used term DL-α-tocopheryl-acetate. Vitamin E activity of tocopherols and tocotrienols varies according to the particular form present (α, β, γ, or δ) (Table 8.11), in addition to the chiral nature of the tocopherol side chain (Table 8.12). The lower vitamin E activity of all-rac-α-tocopheryl acetate, relative to naturally occurring RRR isomers of the vitamin, should be recognized and compensated for when using these compounds for food fortification. α-Tocopherol is the major form of vitamin E in most animal products, and other tocopherols and tocotrienols occur in varying proportions in plant products (Table 8.13). A novel principle of increasing vitamin E content and vitamin activity in plants has been demonstrated, which involved experimental genetic engineering of plants to increase γ-tocopherol synthesis along with increased conversion of γ-tocopherol to α-tocopherol [131].

The tocopherols and tocotrienols are very nonpolar and exist mainly in the lipid phase of foods. All tocopherols and tocotrienols, when not esterified, have the ability to act as antioxidants; they quench free radicals by donating the phenolic H and an electron. Tocopherols are natural constituents of biological membranes and are thought to contribute to membrane stability through their antioxidant activity. Naturally occurring tocopherols and tocotrienols also contribute to the stability of highly unsaturated vegetable oils through this antioxidant action. In contrast, α-tocopheryl acetate added in food fortification has no antioxidant activity because the acetate ester has replaced the phenolic hydrogen atom. α-Tocopheryl acetate does exhibit vitamin E activity and in vivo antioxidant effects as a result of enzymatic cleavage of the ester. The concentration of dietary vitamin E in animals has been shown to influence the oxidative stability of meats after slaughter. For example, the susceptibility of pork muscle products to oxidation of cholesterol and other lipids is inversely related to α-tocopheryl acetate intake of the pigs.

TABLE 8.11
Traditional Views of Relative Vitamin E Activity of Tocopherols and Tocotrienols

	Bioassay Method			
Compound	**Rat Fetal Resorption**	**Rat Erythrocyte Hemolysis**	**Muscular Dystrophy (Chicken)**	**Muscular Dystrophy (Rat)**
α-Tocopherol	100	100	100	100
β-Tocopherol	25–40	15–27	12	
γ-Tocopherol	1–11	3–20	5	11
δ-Tocopherol	1	0.3–2		
α-Tocotrienol	27–29	17–25		28
β-Tocotrienol	5	1–5		

Source: Sies, H. et al., *Ann N Y Acad Sci*., 669, 7, 1992.

TABLE 8.12
Vitamin E Activity of Isomeric Forms of α-Tocopheryl Acetate

Form of α-Tocopheryl Acetate[a]	Relative Vitamin E Activity (%)
RRR	100
All-rac	77
RRS	90
RSS	73
SSS	60
RSR	57
SRS	37
SRR	31
SSR	21

Source: Weiser, H. and Vecchi, M., *Int. J. Vitam. Nutr. Res.*, 52, 351, 1982.
[a] R and S refer to the chiral configuration of the 2, 4′, and 8′ positions, respectively. R is the naturally occurring chiral form. All-rac signifies fully racemic.

8.7.3.2 Stability and Mechanism of Degradation

Vitamin E compounds exhibit reasonably good stability in the absence of oxygen and oxidizing lipids. Anaerobic treatments in food processing, such as retorting of canned foods, have little effect on vitamin E activity. In contrast, the rate of vitamin E degradation increases in the presence of molecular oxygen and can be especially rapid when free radicals are also present. Oxidative degradation of vitamin E is strongly influenced by the same factors that influence oxidation of unsaturated lipids. The a_w dependence of α-tocopherol degradation is similar to that of unsaturated lipids, with a rate minimum occurring at the monolayer moisture value and greater rates at either higher or lower a_w values (see Chapter 2). The use of intentional oxidative treatments, such as the bleaching of flour, can lead to large losses of vitamin E.

An interesting nonnutritional use of α-tocopherol in foods is in the curing of bacon to reduce formation of nitrosamines. It is thought that α-tocopherol serves as a lipid-soluble phenolic compound to quench nitrogen free radicals ($NO^\bullet$, $NO_2^\bullet$) in a radical-mediated nitrosation process.

TABLE 8.13
Concentration of Tocopherols and Tocotrienols in Selected Vegetable Oils and Foods

Food	α-T	α-T3	β-T	β-T3	γ-T	γ-T3	δ-T	δ-T3
Vegetable oils (mg/100 g)								
Sunflower	56.4	0.013	2.45	0.207	0.43	0.023	0.087	
Peanut	14.1	0.007	0.396	0.394	13.1	0.03	0.922	
Soybean	17.9	0.021	2.80	0.437	60.4	0.078	37.1	
Cottonseed	40.3	0.002	0.196	0.87	38.3	0.089	0.457	
Corn	27.2	5.37	0.214	1.1	56.6	6.17	2.52	
Olive	9.0	0.008	0.16	0.417	0.471	0.026	0.043	
Palm	9.1	5.19	0.153	0.4	0.84	13.2	0.002	
Other foods (μg/mL or g)								
Infant formula (saponified)	12.4		0.24		14.6		7.41	
Spinach	26.05	9.14						
Beef	2.24							
Wheat flour	8.2	1.7	4.0	16.4				
Barley	0.02	7.0		6.9		2.8		

Sources: Thompson, J. and Hatina G., *J. Liquid Chromatogr.*, 2, 327, 1979; van Niekerk, P. and Burger, A., *J. Am. Oil Chem. Soc.*, 62, 531, 1985.

Abbreviations: T, tocopherol; T3, tocotrienol.

Reactions of vitamin E compounds, especially α-tocopherol, in foods have been studied extensively. As summarized in Figure 8.11, α-tocopherol can react with a peroxyl radical (or other free radicals) to form a hydroperoxide and an α-tocopheryl radical. As with other phenolic radicals, this is relatively unreactive because the unpaired electron resonates across the phenolic ring system. Radical termination reactions can occur to form covalently linked tocopheryl dimers and trimers, while additional oxidation and rearrangement can yield tocopheroxide, tocopheryl hydroquinone, and tocopheryl quinone (Figure 8.11). Rearrangement and further oxidation can yield many other products. Although α-tocopheryl acetate and other vitamin E esters do not participate in radical quenching, they are subject to oxidative degradation but at a lower rate than that of nonesterified compounds. The degradation products of vitamin E exhibit little or no vitamin activity. Through their ability to act as phenolic antioxidants, nonesterified vitamin E compounds contribute to the oxidative stability of food lipids.

Vitamin E compounds also can contribute indirectly to oxidative stability of other compounds by scavenging singlet oxygen while being concurrently degraded. As shown in Figure 8.12, singlet oxygen directly attacks the tocopherol molecule ring system to form a transient hydroperoxydieneone derivative. This can rearrange to form both the tocopheryl quinone and the tocopheryl quinone 2,3-oxide that have little vitamin E activity. The order of reactivity toward singlet oxygen is $\alpha > \beta > \gamma > \delta$, and antioxidative potency is in the reverse order. Tocopherols also can physically quench singlet oxygen, which involves deactivation of the singlet state oxygen without oxidation of the tocopherol. These attributes of tocopherols are consistent with the fact that tocopherols are potent inhibitors of photosensitized, singlet oxygen–mediated oxidation of soybean oil.

8.7.3.3 Bioavailability

The bioavailability of vitamin E compounds is usually quite high in individuals who digest and absorb fat normally. On a molar basis, bioavailability of α-tocopheryl acetate is nearly equivalent to that of α-tocopherol [16] except at high doses where the enzymatic de-esterification of α-tocopheryl

α-Tocopherol

Peroxyl radical (ROO•)

Hydroperoxide (ROOH)

α-Tocopheryl radical

α-Tocopheroxide α-Tocopheryl quinone α-Tocopheryl hydroquinone

FIGURE 8.11 Overview of the oxidative degradation of vitamin E. In addition to the initial oxidation products shown, many other compounds are formed as a result of further oxidation and rearrangement.

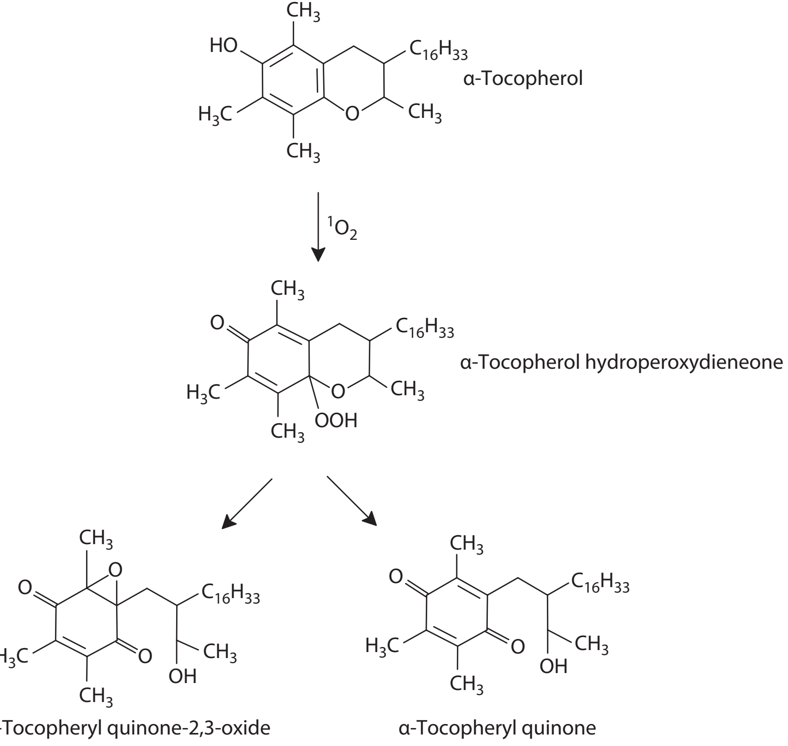


FIGURE 8.12 Reaction of singlet oxygen and α-tocopherol.

acetate can be limiting. Previous studies indicating that α-tocopheryl acetate was more potent than α-tocopherol on a molar basis may have been biased by the oxidative instability of α-tocopherol.

8.7.3.4 Analytical Methods

HPLC methods for determination of vitamin E have largely superseded previous spectrophotometric and direct fluorometric procedures. The use of HPLC permits the measurement of specific forms of vitamin E (e.g., α-, β-, γ-, δ-tocopherols and tocotrienols) and, thus, estimation of total vitamin E activity in a product based on relative potencies of the specific compounds. Detection can be accomplished using either UV absorbance or fluorescence. When saponification is used to aid in separation of lipids from vitamin E, any vitamin E ester will be hydrolyzed to free α-tocopherol. Care must be taken to prevent oxidation during extraction, saponification, and other preliminary treatments.

8.7.4 Vitamin K

8.7.4.1 Structure and General Properties

Vitamin K consists of a group of naphthoquinones that exist with or without a terpenoid side chain in the 3 position (Figure 8.13). The unsubstituted form of vitamin K is menadione, and it is of primary significance as a synthetic form of the vitamin that is used in vitamin supplements and food fortification. Phylloquinone (vitamin K1) is a product of plant origin, while menaquinones (vitamin K2) of varying chain length are products of bacterial synthesis, mainly by intestinal microflora. Phylloquinones occur in relatively large quantities in leafy vegetables including spinach, kale, cauliflower, and cabbage, and they are present, but less abundant, in tomatoes and certain vegetable oils. Vitamin K deficiency is rare in healthy individuals because of the widespread presence of phylloquinones in the diet and because microbial menoquinones are absorbed from the lower intestine. Vitamin K deficiency is ordinarily associated with malabsorption syndromes or the use of pharmacological anticoagulants. Although the use of certain fat substitutes has been reported to impair vitamin K absorption, moderate intakes of these substitutes have no significant effect on vitamin K utilization.

O
CH3
Menadione
H
O

O
CH3 Phylloquinone (vitamin K1)
CH3 CH3
CH2—CH=C—CH2—(CH2—CH2—CH—CH2)3—H
O

O
CH3 Menaquinones (vitamin K2)
CH3
(CH2—CH=C—CH2)n—H
O

FIGURE 8.13 Structure of various forms of vitamin K.

The quinone structure of vitamin K compounds can be reduced to the hydroquinone form by certain reducing agents, but vitamin K activity is retained. Photochemical degradation can occur, but the vitamin is quite stable to heat. Hydrogenation of oils causes a reduction of vitamin K activity by conversion of vitamin K1 to dihydro-vitamin K1 [10].

8.7.4.2 Analytical Methods

Spectrophotometric and chemical assays based on the measurement of oxidation–reduction properties of vitamin K lack the specificity required for food analysis. Various HPLC and LCMS methods exist that provide satisfactory specificity and permit individual forms of vitamin K to be measured.

8.8 WATER-SOLUBLE VITAMINS

8.8.1 Ascorbic Acid

8.8.1.1 Structure and General Properties

L-Ascorbic acid (AA) (Figure 8.14) is a carbohydrate-like compound whose acidic and reducing properties are contributed by the 2,3-enediol moiety. This compound is highly polar; thus, it is readily soluble in aqueous solution and insoluble in less nonpolar solvents. AA is acidic in character as a result of ionization of the C-3 hydroxyl group (pK_{a1} = 4.04 at 25°C). A second ionization, dissociation of the C-2 hydroxyl, is much less favorable (pK_{a2} = 11.4). Two-electron oxidation with hydrogen dissociation converts AA to L-dehydroascorbic acid (DHAA). DHAA exhibits approximately the same vitamin activity as AA because of its facile reduction to AA in the body.

L-Isoascorbic acid, the C-5 optical isomer, and D-ascorbic acid, the C-4 optical isomer (Figure 8.14), behave in a chemically similar manner to AA, but these compounds have essentially no vitamin C activity. L-Isoascorbic acid and AA are widely used as food ingredients for their reducing and antioxidative activity (e.g., in the curing of meats and for inhibiting enzymatic browning in fruits and vegetables), but isoascorbic acid (or D-ascorbic acid) has no nutritional value.

L-Ascorbic acid* L-Dehyroascorbic acid*

L-Isoascorbic acid L-Isodehydroascorbic acid

D-Ascorbic acid D-Dehydroascorbic acid

FIGURE 8.14 Structures of L-ascorbic acid and L-dehydroascorbic acid and their isomeric forms. (* Indicates vitamin C activity.)

AA occurs naturally in fruits and vegetables and, to a lesser extent, in animal tissues and animal-derived products. It occurs naturally almost exclusively in the reduced AA form. The concentration of DHAA found in foods is almost always substantially lower than AA and is a function of the rates of ascorbate oxidation and DHAA hydrolysis to 2,3-diketogulonic acid. Dehydroascorbate reductase and ascorbate free radical reductase activity exist in certain animal tissues. These enzymes are believed to conserve the vitamin through recycling and contribute to low DHAA concentrations. A significant but currently unknown fraction of the DHAA in foods and biological materials likely is an analytical artifact that arises from oxidation of AA to DHAA during sample preparation and analysis. The instability of DHAA further complicates this analysis.

AA may be added to foods as the undissociated acid or as the neutralized sodium salt (sodium ascorbate). Conjugation of AA with hydrophobic compounds confers lipid solubility to the ascorbic acid moiety. Fatty acid esters such as ascorbyl palmitate and ascorbic acid acetals (Figure 8.15) are lipid soluble and can provide a direct antioxidative effect in lipid environments.

Oxidation of AA takes place as either two sequential one-electron transfer processes or as a single two-electron reaction without detection of the semidehydroascorbate intermediate (Figure 8.16). In one-electron oxidations, the first step involves transfer of an electron to form the free radical semidehydroascorbic acid. Loss of an additional electron yields dehydroascorbic acid, which is highly unstable because of the susceptibility to hydrolysis of the lactone bridge. Such hydrolysis, which irreversibly forms 2,3-diketogulonic acid (Figure 8.16), is responsible for loss of vitamin C activity.

FIGURE 8.15 Structures of ascorbyl palmitate and acetals.

FIGURE 8.16 Sequential one-electron oxidations of L-ascorbic acid. All have vitamin C activity except 2,3-diketogulonic acid.

AA is highly susceptible to oxidation, especially when catalyzed by metal ions such as Cu^{2+} and Fe^{3+}. Heat and light also accelerate the process, while factors such as pH, oxygen concentration, and water activity strongly influence the rate of reaction. Since hydrolysis of DHAA occurs very readily, oxidation to DHAA represents an essential and frequently rate-limiting aspect of the oxidative degradation of vitamin C.

A frequently overlooked property of AA is its ability, at low concentrations, to act as a pro-oxidant with high oxygen tension. Presumably this occurs by ascorbate-mediated generation of hydroxyl radicals ($OH^{\bullet}$) or other reactive species. This appears to be of minor importance in most aspects of food chemistry.

8.8.1.2 Stability and Modes of Degradation

8.8.1.2.1 Overview

Because of the high solubility of AA aqueous solutions, the potential exists for significant losses by leaching from freshly cut or bruised surfaces of fruits and vegetables. Chemical degradation primarily involves oxidations to DHAA, followed by hydrolysis to 2,3-diketogulonic acid and further oxidation, dehydration, and polymerization to form a wide array of other nutritionally inactive products. The oxidation and dehydration processes closely parallel dehydration reactions of sugars that lead to many unsaturated products and polymers. The primary factors affecting the rate, mechanism, and qualitative nature of the AA degradation products include pH, oxygen concentration, and the presence of trace metal catalysts.

Foods can undergo large losses of AA during routine storage and handling, including frozen storage. For example, commercially packed frozen green peas, spinach, green beans, and okra experienced losses of AA that could be described by first-order kinetics, with temperature dependence according to the Arrhenius equation [42]. In this study, the stability of AA was least in spinach ($t_{½}$ = 8–155 days at a temperature ranging from −5°C to 20°C) and greatest in okra ($t_{½}$ = 40–660 days at a temperature ranging from −5°C to 20°C). These findings illustrate that AA stability is dependent on food composition in addition to storage conditions; thus, the rate of AA degradation determined for one type of food cannot necessarily be used to predict the kinetics of AA degradation in another food system even if only subtle differences in composition exist.

The rate of oxidative degradation of the vitamin is a nonlinear function of pH because the various ionic forms of the AA differ in their susceptibility to oxidation: fully protonated (AH_2) < ascorbate monoanion (AH^-) < ascorbate dianion (A^{2-}). Under conditions relevant to most foods, the pH dependence of oxidation is governed mainly by the relative concentrations of AH_2 and AH^- species, and this, in turn, is governed by pH (pK_{a1} 4.04). The presence of significant concentrations of the A^{2-} form, as controlled by pK_{a2} of 11.4, yields an increase in rate at pH ≥ 8. Studies of these relationships are complicated by oxygen and trace metal concentration.

8.8.1.2.2 Catalytic Effects of Metal Ions

The overall scheme of AA degradation depicted in Figure 8.17 is an integrated view of the effects of metal ions and the presence or absence of oxygen on the mechanism of ascorbic acid degradation. The rate of oxidative degradation of AA is generally observed to be first order with respect to the concentration of the ascorbate monoanion (HA^-), molecular oxygen, and the metal ion. It was once believed that oxidative degradation of AA at neutral pH and in the absence of metal ions (i.e., the “uncatalyzed” reaction) occurred at a rate that was slow but significant. For example, a first-order rate constant of 5.87×10^{-4} s^{-1} has been reported for the assumed spontaneous uncatalyzed oxidation of ascorbate at neutral pH. However, later evidence indicates a much smaller rate constant of 6×10^{-7} s^{-1} for AA oxidation in an air-saturated solution at pH 7.0 [13]. This difference suggests that uncatalyzed oxidation is essentially negligible and that trace metals in foods or experimental solutions are responsible for much of the oxidative degradation. Rate constants obtained in the presence of metal ions at concentrations of several ppm are several orders of magnitude greater than those obtained in solutions nearly devoid of metal ions.

The rate of metal-catalyzed oxidation of AA is proportional to the partial pressure of dissolved oxygen over the range of 1.0–0.4 atm and is independent of oxygen concentration at partial pressures <0.20 atm [80]. In contrast, the oxidation of AA catalyzed by metal chelates is independent of oxygen concentration [75].

The potency of metal ions in catalyzing ascorbate degradation depends on the metal involved, its oxidation state, and the presence of chelators. Catalytic potency is as follows: Cu(II) is about 80 times more potent than Fe(III), and the chelate of Fe(III) and ethylenediaminetetraacetic acid (EDTA) is ~4 times more catalytic than free Fe(III) [13]. When the rate expression of ascorbate oxidation is presented as

$$-\frac{d[TA]}{dt} = k_{cat} \times [AH^{-}] \times [Cu(II) \text{ or } Fe(III)],$$

the metal ion concentration and the k_{cat} for metal ions can be used to estimate the rate AA degradation (where [TA] is the concentration of total ascorbic acid). In pH 7.0 phosphate buffers (20°C), k_{cat} values for Cu(II), Fe(III), and Fe(III)-EDTA are 880, 42, and 10 (M^{-1} s^{-1}), respectively. It should be noted that the relative and absolute values of these catalytic rate constants in simple solutions may differ from those of actual food systems. This is likely because trace metals may associate with other constituents (e.g., amino acids) or may participate in other reactions, some of which may generate reactive free radicals or active oxygen species that may hasten oxidation of ascorbic acid.

In contrast to the enhanced catalytic potency of Fe(III) when chelated by EDTA, Cu(II)-catalyzed oxidation of ascorbate is largely inhibited in the presence of EDTA [13]. Thus, the influence of EDTA or other chelators (e.g., citrate and polyphosphates) on the oxidation of ascorbic acid in foods is not fully predictable.

8.8.1.2.2.1 Mechanisms of AA Degradation Oxidation of AA can be initiated by formation of a ternary complex (ascorbate monoanion, metal ion, and O_2), as described earlier, or by a variety of one-electron oxidations. As reviewed by Buettner [14], there are many ways in which the one-electron oxidation of AH^- to $A^{-\bullet}$ and $A^{-\bullet}$ to form DHAA can occur. A ranking of the oxidation–reduction potential, that is, reactivity, of relevant oxidants is summarized in Table 8.14. This illustrates the interrelationships in antioxidative function of several vitamins including AA, α-tocopherol, and riboflavin and illustrates how the reducing power of AA (as the monoanion) can regenerate oxidized food components such as free radicals of unsaturated fatty acids, other lipid-derived free radicals, and the radical form of vitamin E (α-tocopheroxyl$^\bullet$ radical).

The mechanism of AA degradation may differ depending on the nature of the food system or reaction medium. Metal-catalyzed degradation of AA has been proposed to occur through formation of a ternary complex of ascorbate monoanion, O_2, and a metal ion (Figure 8.17). The ternary complex of ascorbate, oxygen, and metal catalyst appears to yield directly DHAA as the product, without detectable formation of the product of one-electron oxidation, semidehydroascorbate radical.

The loss of vitamin C activity during oxidative degradation of AA occurs with the hydrolysis of the DHAA lactone to yield 2,3-diketogulonic acid (DKG). This hydrolysis is favored by alkaline conditions, and DHAA is most stable at pH 2.5–5.5. The stability of DHAA at pH > 5.5 is very poor and becomes more so as pH increases. For example, half-time values for DHAA hydrolysis at 23°C are 100 and 230 min at pH 7.2 and 6.6, respectively [9]. The rate of DHAA hydrolysis markedly increases with increasing temperature but is unaffected by the presence or absence of oxygen. In view of the labile nature of DHAA at neutral pH, analytical data showing significant quantities of DHAA in foods should be viewed with caution because elevated DHAA concentrations may also reflect uncontrolled oxidation during the analysis.

Although the ternary complex, as proposed by Khan and Martell [80], is apparently an accurate model of AA oxidation, later findings have expanded our knowledge of the mechanism.

TABLE 8.14
Reduction Potential of Selected Free Radicals and Antioxidants Arranged from the Most Highly Oxidizing (Top) to the Most Highly Reducing

Couple[a]		
Oxidized	**Reduced**	**$\Delta E°'$ (mV)**
$HO^\bullet, H^+$	H_2O	2310
$RO^\bullet, H^+$	ROH	1600
$HO_2^\bullet, H^+$	H_2O_2	1060
$O_2^{-\bullet}, 2H^+$	H_2O_2	940
$RS^\bullet$	RS^-	920
$O_2\ (^1\Delta_g)$	$O_2^{-\bullet}$	650
$PUFA^\bullet, H^+$	PUFA-H	600
α-Tocopheroxyl$^\bullet$, H^+	α-Tocopherol	500
H_2O_2, H^+	$H_2O, OH^\bullet$	320
Ascorbate$^{-\bullet}$, H^+	Ascorbate monoanion	282
Fe(III)EDTA	Fe(II)EDTA	120
Fe(III)aq	Fe(II)aq	110
Fe(III)citrate	Fe(II)citrate	~100
Dehydroascorbate	Ascorbate$^{-\bullet}$	~100
Riboflavin	Riboflavin$^{-\bullet}$	–317
O_2	$O_2^{-\bullet}$	–330
O_2, H^+	$HO_2^\bullet$	–460

Source: Buettner, G.R., *Arch. Biochem. Biophys.*, 300, 535, 1993.
Each oxidized species in an oxidation–reduction couple is capable of abstracting an electron or H atom from any reduced species below it.
[a] *Nomenclature*: ascorbate$^{-\bullet}$, semidehydroascorbate radical; PUFA$^\bullet$, polyunsaturated fatty acid radical; PUFA-H, polyunsaturated fatty acid, bis-allylic H; RO$^\bullet$, aliphatic alkoxy radical. $\Delta E°'$ is the standard one-electron reduction potential (mV).

Scarpa et al. [127] observed that metal-catalyzed oxidation of the ascorbate monoanion (AH^-) forms superoxide ($O_2^{-\bullet}$) in the rate-determining step:

$$AH^- + O_2 \rightarrow AH^\bullet + O_2^{-\bullet}$$

Subsequent steps of the reaction involve superoxide as a rate enhancer, effectively doubling the overall rate of ascorbate oxidation to form dehydroascorbate (A) through

$$AH^- + O_2^{-\bullet} + 2H^+ \rightarrow AH^\bullet + H_2O_2$$

$$AH^\bullet + O_2^{-\bullet} + H^+ \rightarrow A + H_2O_2$$

A termination-like reaction also can occur, which involves two ascorbate radicals as

$$2AH^\bullet \rightarrow A + AH^-$$

FIGURE 8.17 Overview of mechanisms for the oxidative and anaerobic degradation of ascorbic acid. Structures with bold lines are primary sources of vitamin C activity. *Abbreviations*: AH_2, fully protonated ascorbic acid; AH^-, ascorbate monoanion; AH•, semidehydroascorbate radical; A, dehydroascorbic acid; FA, 2-furoic acid; F, 2-furaldehyde; DKG, diketogulonic acid; DP, 3-deoxypentosone; X, xylosone; Mn^+, metal catalyst; HO_2•, perhydroxyl radical. (Based on Buettner, G.R., *J. Biochem. Biophys. Methods*, 16, 27, 1988; Buettner, G.R., *Arch. Biochem. Biophys.*, 300, 535, 1993; Khan, M. and Martell, A., *J. Am. Chem. Soc.*, 89, 4176, 1967; Khan, M. and Martell, A., *J. Am. Chem. Soc.*, 89, 7104, 1969; Liao, M.-L. and Seib, P., *Food Technol.*, 31, 104, 1987; Tannenbaum, S. et al., Vitamins and minerals, in: *Food Chemistry*, Fennema, O., ed., Marcel Dekker, New York, 1985, pp. 477–544.)

Anaerobic degradation of AA (Figure 8.17) is relatively insignificant as a mode of loss of the vitamin in most foods. The anaerobic pathway becomes most significant in canned products, for example, vegetables, tomatoes, and fruit juices after depletion of residual oxygen, but even in these products loss of AA through anaerobic means typically occurs very slowly. Surprisingly, the anaerobic pathway has been identified as the predominant mechanism for loss of AA during storage of dehydrated tomato juice in the presence or absence of oxygen. Trace metal catalysis of anaerobic degradation has been demonstrated, with the rate increasing in proportion to copper concentration.

The mechanism of anaerobic degradation of AA has not been fully established. Direct cleavage of the 1,4-lactone bridge *without* prior oxidation to DHAA appears to be involved, perhaps following an enol–keto tautomerization as shown in Figure 8.17. Unlike degradation of AA under oxidative conditions, anaerobic degradation exhibits a maximum rate at pH ~3–4. This maximum rate in the mildly acidic range may reflect the effects of pH on the opening of the lactone ring and on the concentration of the monoanionic ascorbate species.

The complexity of the anaerobic degradation mechanism, and the influence of food composition, is suggested by the significant change in activation energy at 28°C for the loss of total vitamin C from single strength orange juice during storage. In contrast, the Arrhenius plot for the degradation of total vitamin C during the storage of canned grapefruit juice is linear over the same range (~4°C–50°C), which suggests that a single mechanism predominates [106]. The reason for this kinetic and/or mechanistic difference in such similar products is not known.

In view of the existence of residual oxygen present in many food packages, degradation of ascorbic acid in sealed containers, especially cans and bottles, would typically occur by both oxidative and anaerobic pathways. In most cases, rate constants for anaerobic degradation of ascorbic acid will be two to three orders of magnitude less than those for the oxidative reaction.

8.8.1.2.2.2 Products of AA Degradation Regardless of the mechanism of degradation, opening of the lactone ring irreversibly destroys vitamin C activity. Although lacking in nutritional relevance, the many reactions involved in the terminal phases of ascorbate degradation are important because of their involvement in producing flavor compounds or precursors and through their participation in nonenzymatic browning.

Over 50 low-molecular-weight products of ascorbic acid degradation have been identified. The kinds and concentrations of such compounds and the mechanisms involved are strongly influenced by factors such as the temperature, pH, water activity, concentrations of oxygen and metal catalysts, and presence of active oxygen species. Three general types of decomposition products have been identified: (1) polymerized intermediates, (2) unsaturated carboxylic acids of 5- and 6-carbon chain length, and (3) fragmentation products having five or fewer carbons. Generation of formaldehyde during thermal degradation of ascorbate at neutral pH also has been reported. Some of these compounds are likely contributors to the changes in flavor and odor that occur in citrus juices during storage or excessive processing.

The degradation of sugars and ascorbic acid is strikingly similar, and in some cases mechanistically identical. Qualitative differences between aerobic and anaerobic conditions occur in the pattern of AA degradation, and pH exerts an influence in all circumstances. The major AA breakdown products in neutral and acidic solution include L-xylosone, oxalic acid, L-threonic acid, tartaric acid, 2-furaldehyde (furfural), and furoic acid, as well as a wide variety of carbonyls and other unsaturated compounds. As with sugar degradation, the extent of fragmentation increases under alkaline conditions.

AA degradation is associated with discoloration reactions in both the presence and absence of amines. DHAA as well as the dicarbonyls formed during its degradation can participate in Strecker degradation with amino acids. Following Strecker degradation of DHAA with an amino acid, the sorbamic acid product (Figure 8.18) can form dimers, trimers, and tetramers, several of which are reddish or yellowish in color. In addition, 3,4-dihydroxy-5-methyl-2(5H)-furanone, an intermediate product of dehydration following decarboxylation during anaerobic degradation of AA, has a brownish color. Further polymerization of these or other unsaturated products forms either melanoidins (nitrogenous polymers) or nonnitrogenous caramel-like pigments. Although the nonenzymatic browning of citrus juices and related beverages is a complex process, the contribution of AA to browning has been clearly demonstrated [74].

8.8.1.2.2.3 Other Environmental Variables Aside from the factors affecting ascorbate stability as discussed previously (e.g., oxygen, catalysts, pH), many other variables influence retention of

CH_2OH, HCOH, H, O, O, O — L-Dehydroascorbic acid + R—C($COOH$)(NH_2)—H → (−R—CHO, $-CO_2$) CH_2OH, HCOH, H, O, O, NH_2 — Sorbamic acid

FIGURE 8.18 Participation of dehydroascorbic acid in the Strecker degradation reaction.

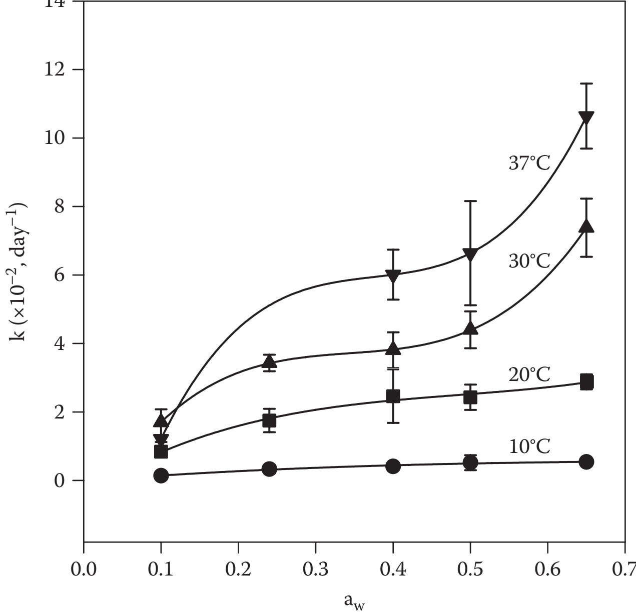


FIGURE 8.19 Degradation of ascorbic acid as a function of storage temperature and water activity (a_w) in dehydrated model food systems simulating breakfast cereal products. Data (means ± SD) are expressed as apparent first-order rate constants (k) for the loss of total ascorbic acid (AA + DHAA). (From Kirk, J. et al., *J. Food Sci.*, 42, 1274, 1977.)

this vitamin in foods. As with many other water-soluble compounds, the rate of oxidation of AA in low-moisture food systems simulating breakfast cereals has been found to increase progressively over the range of ~0.10–0.65 water activity [76,83,86] (Figure 8.19). This apparently is associated with increased availability of water to act as a solvent for reactants and catalysts. The presence of certain sugars (ketoses) can increase the rate of anaerobic degradation. Sucrose has a similar effect at low pH, which is consistent with its pH-dependent generation of fructose. In contrast, some sugars and sugar alcohols exert a protective effect against the oxidative degradation of AA, possibly by binding metal ions and reducing their catalytic potency. Adverse effects of photosensitizing agents on ascorbic acid retention occur through the generation of singlet oxygen. The significance of these observations to actual foods remains to be determined.

8.8.1.2.2.4 Functions of Ascorbic Acid in Foods In addition to its function as an essential nutrient, AA is widely used as a food ingredient/additive because of its reducing and antioxidative properties. As discussed elsewhere in this book, AA effectively inhibits enzymatic browning primarily by reducing orthoquinone products. Other functions include (1) reductive action in dough conditioners; (2) protection of certain oxidizable compounds (e.g., folates) by reductive effects,

free radical scavenging, and oxygen scavenging; (3) inhibition of nitrosamine formation in cured meats; and (4) reduction of metal ions.

The antioxidative role of ascorbic acid is multifunctional, with ascorbate inhibiting lipid autoxidation by several mechanisms [14,91,132]. These include (1) scavenging singlet oxygen; (2) reduction of oxygen- and carbon-centered radicals, with formation of a less reactive semidehydroascorbate radical or DHAA; (3) preferential oxidation of ascorbate, with concurrent depletion of oxygen; and (4) regeneration of other antioxidants, for example, through reduction of the tocopherol radical.

AA is a very polar compound and therefore, essentially insoluble in oils. However, AA is surprisingly effective as an antioxidant when dispersed in oils as well as in emulsions [40]. Combinations of ascorbic acid and α-tocopherol are especially effective in oil-based systems, while the combination of α-tocopherol and the lipophilic ascorbyl palmitate is more effective in oil-in-water emulsions. Similarly, ascorbyl palmitate has been shown to act synergistically with α-tocopherol and other phenolic antioxidants.

8.8.1.2.2.5 Bioavailability of Ascorbic Acid in Foods The principal dietary sources of AA are fruits, vegetables, juices, and fortified foods (e.g., breakfast cereals). The bioavailability of AA in cooked broccoli, orange sections, and orange juice has been shown to be equivalent to that of vitamin–mineral tablets for human subjects [96]. Bioavailability of AA in raw broccoli is 20% lower than that in cooked broccoli. This may be caused by incomplete disruption of cells during chewing and/or digestion. The relatively small difference in AA bioavailability in raw broccoli and potentially other raw vegetables, relative to their cooked forms, may have little nutritional significance. Overall, it is clear that AA in most fruits and vegetables is highly available to humans [44,69].

8.8.1.2.2.6 Analytical Methods Many procedures exist for the measurement of AA in foods, and selection of a suitable analytical method is essential to obtain accurate results [109]. Ascorbic acid absorbs UV light strongly (λ_{max} ~ 245 nm), although direct spectrophotometric analysis is precluded by the many other chromophores found in most foods. DHAA absorbs only weakly at its λ_{max} ~ 300 nm. Traditional analytical procedures involve redox titration of the sample with a dye such as 2,6-dichlorophenolindophenol, during which oxidation of AA accompanies reduction of the redox dye to its colorless form. A limitation of this approach is the interference by other reducing agents and the lack of a response to DHAA. Sequential analysis of the sample before and after saturation with H_2S gas or treatment with a thiol reagent to reduce DHAA to AA permits the measurement of total ascorbic acid. The measurement of DHAA by difference lacks the precision of direct analysis, however.

An alternative approach involves condensation of DHAA (formed by controlled oxidation of AA in the sample) with various carbonyl reagents. Direct treatment with phenylhydrazine to form the spectrophotometrically detectable ascorbyl-bis-phenylhydrazone derivative permits the simple measurement of L-AA in pure solution. Many carbonyl compounds in foods will interfere with this procedure. A similar method involves the reaction of DHAA with o-phenylenediamine, which forms a tricyclic highly fluorescent condensation product. Although more specific and sensitive than the phenylhydrazine method, the o-phenylenediamine procedure is also subject to interference by certain dicarbonyls in foods. Foods containing isoascorbic acid cannot be analyzed for vitamin C by redox titration or condensation with carbonyl reagents because these methods respond to this nutritionally inactive compound.

Many HPLC methods permit accurate and sensitive measurement of total ascorbic acid (before and after treatment with a reducing agent), and certain methods permit direct measurement of AA and DHAA. The coupling of chromatographic separation with spectrophotometric, fluorometric, or electrochemical detection makes HPLC analysis far more specific than traditional redox methods. Many HPLC methods have been reported, including those that permit the simultaneous determination of ascorbic and isoascorbic acids as well as their dehydro forms [145]. A method based on gas chromatography–mass spectrometry has been reported, but extensive sample preparation is a disadvantage of the procedure [29].

8.8.2 Thiamin

8.8.2.1 Structure and General Properties

Thiamin (vitamin B1) is a substituted pyrimidine linked through a methylene bridge ($-CH_2-$) to a substituted thiazole (Figure 8.20). Thiamin is widely distributed in plant and animal tissues. Most naturally occurring thiamin exists as thiamin pyrophosphate (Figure 8.20), with lesser amounts of nonphosphorylated thiamin, thiamin monophosphate, and thiamin triphosphate. Thiamin pyrophosphate functions as a coenzyme of various α-keto acid dehydrogenases, α-keto acid decarboxylases, phosphoketolases, and transketolases. Thiamin is commercially available as the hydrochloride and mononitrate salts, and these forms are widely used for food fortification and as nutritional supplements (Figure 8.20).

The thiamin molecule exhibits unusual acid–base behavior. The first pK_a (~4.8) involves dissociation of the protonated pyrimidine N^1 to yield the uncharged pyrimidyl moiety of thiamin free base (Figure 8.21). In the alkaline pH range another transition is observed (apparent pK_a 9.2) that corresponds to the uptake of two equivalents of base to yield the thiamin pseudobase. The pseudobase can undergo opening of the thiazole ring to yield the thiol form of thiamin, accompanied by dissociation of a single proton. Another characteristic of thiamin is the quaternary N of the thiazole ring, which remains cationic at all pH values. The marked pH dependence of thiamin degradation corresponds to the pH-dependent changes in ionic form. Protonated thiamin is far more stable than free base, pseudobase, and thiol forms, which accounts for the greater stability observed in acidic media (Table 8.15). Although thiamin is relatively stable to oxidation and light, it is among the least stable of the vitamins when in solution at neutral or alkaline pH.

8.8.2.2 Stability and Modes of Degradation

8.8.2.2.1 Stability Properties

A wealth of published data exists concerning the stability of thiamin in foods [37,98]. Representative studies, as summarized previously by Tannenbaum et al. [138], illustrate the potential for large losses under certain conditions (Table 8.16). Losses of thiamin from foods are favored when (1) conditions favor leaching of the vitamin into surrounding aqueous media, (2) the pH is approximately neutral or greater, and/or (3) exposure to a sulfiting agent occurs. Losses of thiamin also can occur in fully hydrated foods during storage at moderate temperatures, although at predictably lower rates than those observed during thermal processing (Table 8.17). Thiamin degradation in foods almost always follows first-order kinetics. Because degradation of thiamin can occur by several possible mechanisms, multiple mechanisms sometimes occur simultaneously. The occurrence of nonlinear Arrhenius plots for thermal losses of thiamin in certain foods is evidence of multiple degradation mechanisms that have different temperature dependences.

Thiamin

Thiamin pyrophosphate

Thiamin hydrochloride

Thiamin mononitrate

FIGURE 8.20 Structures of various forms of thiamin. All have thiamin (vitamin B1) activity.

FIGURE 8.21 Summary of the major pathways for ionization and degradation of thiamin. (Adapted in modified form from Tannenbaum, S. et al., Vitamins and minerals, in: *Food Chemistry*, Fennema, O., ed., Marcel Dekker, New York, 1985, pp. 477–544; Dwivedi, B.K. and Arnold, R.G., *J. Agric. Food Chem.*, 21, 54, 1973.)

TABLE 8.15
Comparison of Thermal Stability of Thiamin and Thiamin Pyrophosphate in 0.1 M Phosphate Buffer at 265°C

	Thiamin		Thiamin Pyrophosphate	
Solution pH	k[a] (min^{-1})	t½ (min)	k[a] (min^{-1})	t½ (min)
4.5	0.0230	30.1	0.0260	26.6
5.0	0.0215	32.2	0.0236	29.4
5.5	0.0214	32.4	0.0358	19.4
6.0	0.0303	22.9	0.0831	8.33
6.5	0.0640	10.8	0.1985	3.49

Source: Adapted from Mulley, E.A. et al., *J. Food Sci.*, 40, 989, 1975.

[a] k is the first-order rate constant and t½ is the time for 50% thermal degradation.

TABLE 8.16
Representative Rates of Degradation (Half-Life at Reference Temperature of 100°C) and Energy of Activation for Losses of Thiamin from Foods during Thermal Processing

Food System	PH	Temperature Range Studied (°C)	Half-Life (h)	Energy of Activation (kJ/mol)
Beef heart puree	6.10	109–149	4	120
Beef liver puree	6.18	109–149	4	120
Lamb puree	6.18	109–149	4	120
Pork puree	6.18	109–149	5	110
Ground meat product	Not reported	109–149	4	110
Beef puree	Not reported	70–98	9	110
Whole milk	Not reported	121–138	5	110
Carrot puree	6.13	120–150	6	120
Green bean puree	5.83	109–149	6	120
Pea puree	6.75	109–149	6	120
Spinach puree	6.70	109–149	4	120
Pea puree	Not reported	121–138	9	110
Peas in brine puree	Not reported	121–138	8	110
Peas in brine	Not reported	104–133	6	84

Sources: Mauri, L. et al., *Int. J. Food Sci. Technol.*, 24, 1, 1989; Data compiled from multiple sources.

Water activity estimated to be 0.98–0.99. Half-life and energy of activation values rounded to 1 and 2 significant figures, respectively.

TABLE 8.17
Typical Losses of Thiamin from Canned Foods during Storage

	Retention after 12 Months of Storage (%)	
Food	**38°C**	**1.5°C**
Apricots	35	72
Green beans	8	76
Lima beans	48	92
Tomato juice	60	100
Peas	68	100
Orange juice	78	100

Source: Freed, M. et al., *Food Technol.*, 3, 148, 1948.

Thiamin exhibits excellent stability under conditions of low water activity at ambient temperature. Thiamin in dehydrated model systems simulating breakfast cereals underwent little or no loss at temperatures less than 37°C at a_w 0.1–0.65 (Figure 8.22). In contrast, thiamin degradation was markedly accelerated at 45°C, especially at a_w 0.4 or greater (i.e., above the apparent monomolecular moisture value of $a_w \sim 0.24$). In these model systems, the maximum rate of thiamin degradation occurred at water activities of 0.5–0.65 (Figure 8.23). In similar model systems, the rate of thiamin degradation declined as the a_w was increased from 0.65 to 0.85 [5].

Thiamin is somewhat unstable in many fish and crustaceans postharvest, and this has been attributed to the presence of thiaminases. However, at least part of this thiamin-degradation activity is caused by heme proteins (myoglobin and hemoglobin) that are nonenzymatic catalysts of thiamin degradation [113]. The presence of thiamin-degrading heme proteins in tuna, pork, and beef muscle suggests that denatured myoglobin may be involved in the degradation of thiamin during food processing and storage. This nonenzymatic, thiamin-modifying activity apparently does not cause

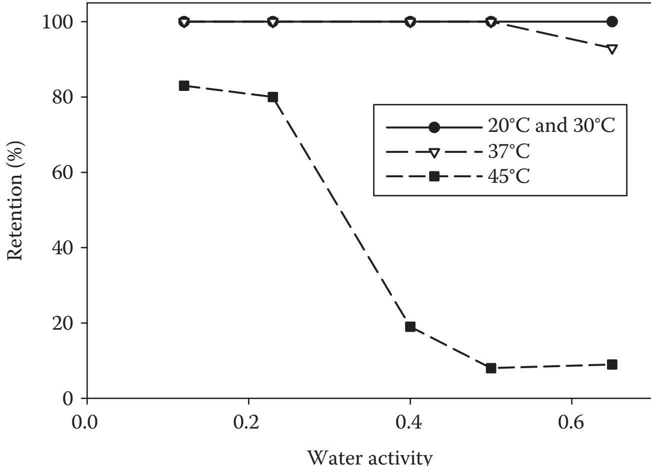


FIGURE 8.22 Influence of water activity and temperature on the retention of thiamin in a dehydrated model food system simulating a breakfast cereal product. Percentage retention values apply to an 8-month storage period. (From Dennison, D. et al., *J. Food Process. Preserv.*, 1, 43, 1977.)

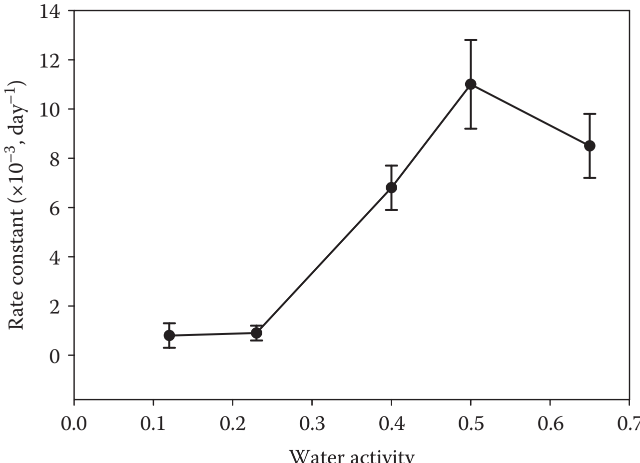


FIGURE 8.23 Influence of water activity on the first-order rate constant of thiamin degradation in a dehydrated model food system simulating a breakfast cereal product stored at 45°C. (From Dennison, D. et al., *J. Food Process. Preserv.*, 1, 43, 1977.)

cleavage of the thiamin molecule, as is common in thiamin degradation. The antithiamin component of fish viscera, previously reported to be a thiaminase, is now believed to be a thermostable and probably nonenzymatic catalyst.

Other components of food can influence degradation of thiamin in foods. Tannins can inactivate thiamin apparently by the formation of several biologically inactive adducts. Various flavonoids may alter the thiamin molecule, but the apparent product of flavonoid oxidation in the presence of thiamin is thiamin disulfide, a compound that has thiamin activity. Proteins and carbohydrates can reduce the rate of thiamin degradation during heating or in the presence of bisulfite, although the extent of this effect is difficult to predict in complex food systems. A part of the stabilizing effect of protein may occur through the formation of mixed disulfides with the thiol form of thiamin, a reaction that appears to retard further modes of degradation. Chlorine (as hypochlorite ion), at levels present in water used in food formulation and processing, can cause rapid degradation of thiamin by a cleavage process that is apparently identical to the thermal cleavage of thiamin under acidic conditions.

Another complicating factor in the assessment and prediction of thiamin stability is the inherent difference in stability and pH dependence between free thiamin and the major naturally occurring form, thiamin pyrophosphate. Although thiamin and thiamin pyrophosphate exhibit nearly equivalent rates of thermal degradation at pH 4.5, thiamin pyrophosphate degrades almost three times faster at pH 6.5 (Table 8.15).

Significant differences exist in the stability of hydrochloride and mononitrate forms of synthetic thiamin. Thiamin HCl is more soluble than the mononitrate, and this is advantageous for fortification of liquid products. Because of differing energies of activation, thiamin mononitrate is more stable at temperatures less than 95°C, while the hydrochloride exhibits greater stability at temperatures >95°C–110°C (Table 8.18).

8.8.2.2.2 Mechanisms of Degradation

The rate and mechanism of thermal degradation of thiamin are strongly influenced by pH of the reaction medium, but degradation usually involves cleavage of the molecule at the central methylene bridge.

In acidic conditions (i.e., pH ≤ 6), thermal degradation of thiamin occurs slowly and involves cleavage of the methylene bridge to release the pyrimidine and thiazole moieties largely in

TABLE 8.18
Kinetic Values for Thiamin Loss in Semolina Dough Subjected to High Temperatures

a_w	Temperature (°C)	k (× 10^4, min^{-1}) ± 95% CI[a]	Half-Life (min)	Energy of Activation (kcal/mol)
Hydrochloride				
0.58	75	3.72 ± 0.01	1863	95.4
	85	11.41 ± 3.64	607	
	95	22.45 ± 2.57	309	
0.86	75	5.35 ± 2.57	1295	92.1
	85	12.20 ± 4.45	568	
	95	30.45 ± 8.91	228	
Mononitrate				
0.58	75	2.88 ± 0.01	2406	109
	85	7.91 ± 0.01	876	
	95	22.69 ± 2.57	305	
0.86	75	2.94 ± 0.01	2357	111
	85	8.31 ± 0.01	834	
	95	23.89 ± 0.01	290	

Source: Labuza, T. and Kamman, J., *J. Food Sci.*, 47, 664, 1982.
[a] First-order rate constant ± 95% confidence interval.

unchanged form. Between pH 6 and 7, thiamin degradation accelerates along with a large increase in the extent of fragmentation of the thiazole ring, and at pH 8, intact thiazole rings are not found among the products. Thiamin degradation is known to yield a large number of sulfur-containing compounds that presumably arise from fragmentation and rearrangement of the thiazole ring. These compounds have been shown to contribute to meat flavor. Products from thiazole fragmentation are thought to arise from the small amounts of thiamin that exist in the thiol or pseudobase forms at pH > 6.

Thiamin degrades rapidly in the presence of bisulfite ions, a phenomenon that stimulated federal regulations prohibiting the use of sulfiting agents in foods that are significant sources of dietary thiamin. The cleavage of thiamin by bisulfite is similar to that occurring at pH ≤ 6, although the pyrimidine product is sulfonated (Figure 8.21). This reaction is described as a base exchange or nucleophilic displacement at the methylene carbon, by which the bisulfite ion displaces the thiazole moiety. It is unclear whether other nucleophiles relevant to foods can have a similar effect. Cleavage of thiamin by bisulfite occurs over a broad pH range, with a maximum rate occurring at pH ~ 6 [156]. A bell-shaped pH profile of this reaction occurs because the sulfite ion primarily reacts with the protonated form of thiamin.

Several researchers have noted a correspondence of the conditions (e.g., pH and water activity) favoring degradation of thiamin and progress of the Maillard reaction. Specifically, thiamin has a primary amino group on its pyrimidyl moiety, shows a maximum rate of degradation at an intermediate water activity, and exhibits greatly increased reaction rates at neutral and alkaline pH values. Early studies demonstrated the ability of thiamin to react with sugars under certain conditions; however, sugars often tend to increase the stability of thiamin. Despite the similarity of conditions favoring thiamin degradation and Maillard browning, there appears to be little or no direct interaction of thiamin with the reactants or intermediates of the Maillard reaction in foods.

8.8.2.2.3 Bioavailability

The bioavailability of thiamin appears to be nearly complete in most foods examined [52,71]. As mentioned previously, formation of thiamin disulfide and mixed thiamin disulfides during food

processing apparently has little effect on thiamin bioavailability. Thiamin disulfide exhibits 90% of the activity of thiamin in animal bioassays.

8.8.2.2.4 Analytical Methods

Although microbiological growth methods exist for measurement of thiamin in foods, they are rarely used because of the availability of fluorometric and HPLC procedures [41]. Thiamin is generally extracted from the food by heating (e.g., autoclaving) a homogenate in dilute acid. For analysis of total thiamin, treatment of the buffered extract with a phosphatase hydrolyzes phosphorylated forms of the vitamin. Following chromatographic removal of nonthiamin fluorophores, treatment with an oxidizing agent converts thiamin to the highly fluorescent thiochrome that is easily measured (Figure 8.21).

Total thiamin can be determined by HPLC following phosphatase treatment or as the sum of free thiamin and its several phosphorylated forms. Fluorometric HPLC analysis can be used following conversion of thiamin to thiochrome or, alternatively, postcolumn oxidation to thiochrome can permit fluorometric detection.

8.8.3 Riboflavin

8.8.3.1 Structure and General Properties

Riboflavin, also known as vitamin B2, is the generic term for the group of compounds that exhibit the biological activity of riboflavin (Figure 8.24). The parent compound of the riboflavin family is 7,8-dimethyl-10(1′-ribityl)isoalloxazine, and all derivatives of riboflavin are given the generic name flavins. Phosphorylation of the 5′-position of the ribityl side chain yields flavin mononucleotide (FMN), whereas flavin adenine dinucleotide (FAD) has an additional 5′-adenosyl monophosphate moiety (Figure 8.24). FMN and FAD function as coenzymes in a large number of flavin-dependent enzymes that catalyze various oxidation–reduction processes. Both forms are readily convertible to riboflavin by action of phosphatases that are present in foods and those of the digestive system. A relatively

FIGURE 8.24 Structures of riboflavin, flavin mononucleotide, and flavin adenine dinucleotide.

FIGURE 8.25 Oxidation–reduction behavior of flavins.

minor fraction (<10%) of the FAD in biological materials exists in a covalently bound coenzyme form in which position 8α is covalently linked to an amino acid residue of the enzyme protein.

The chemical behavior of riboflavin and other flavins is complex, with each form able to exist in several oxidation states as well as multiple ionic forms. Riboflavin, as the free vitamin and in its coenzymic function, undergoes redox cycling among three chemical species. These include the native (fully oxidized) yellow flavoquinone (Figure 8.25), the flavosemiquinone (red or blue depending on pH), and the colorless flavohydroquinone. Each conversion in this sequence involves a one-electron reduction and H^+ uptake. The flavosemiquinone N^5 has a pK_a of ~8.4, while the flavohydroquinone N^1 has a pK_a of ~6.2.

Several minor forms of riboflavin also exist in foods, although their chemical origin and quantitative significance in human nutrition have not been fully determined. As shown in Table 8.19, FAD and free riboflavin account for over 80% of the total flavins in cow's and human milk [120,121]. Of the minor forms present, the most interesting is 10-hydroxyethylflavin, a product of bacterial flavin metabolism. 10-Hydroxyethylflavin is a known inhibitor

TABLE 8.19
Distribution of Riboflavin Compounds in Fresh Human and Cow's Milk

Compound	Human Milk (%)	Cow's Milk (%)
FAD	38–62	23–46[a]
Riboflavin	31–51	35–59
10-Hydroxyethylflavin	2–10	11–19
10-Formylmethyflavin	Trace	Trace
7α-Hydroxyriboflavin	Trace–0.4	0.1–0.7
8α-Hydroxyriboflavin	Trace	Trace–0.4

Sources: Adapted from Roughead, Z.K. and McCormick, D.B., *Am. J. Clin. Nutr.*, 52, 854, 1990; Roughead, Z.K. and McCormick, D.B., *J. Nutr.*, 120, 382, 1990.

[a] Following pasteurization, FAD in bulk raw milk decreases from 26% to 13%, with a corresponding increase in the percentage of riboflavin.

of mammalian flavokinase and may inhibit the uptake of riboflavin into tissues. Other minor derivatives (such as lumiflavin) may also act as antagonists. Thus, foods contain flavins such as riboflavin, FAD, and FMN that exhibit vitamin activity, but in addition they may contain compounds that act as antagonists of riboflavin transport and metabolism. This illustrates the need for a thorough analysis of the forms of riboflavin and other vitamins in order to assess accurately the nutritional properties of foods.

8.8.3.2 Stability and Modes of Degradation

Riboflavin exhibits its greatest stability in acidic medium, is somewhat less stable at neutral pH, and rapidly degrades in alkaline environments. Retention of riboflavin in most foods is moderate to very good during conventional thermal processing, handling, and preparation. Losses during storage of riboflavin in various dehydrated food systems (breakfast cereals and model systems) are usually negligible. Rates of degradation increase measurably at a_w values above the monolayer value when temperatures are above ambient [28].

The typical mechanism of degradation of riboflavin is photochemical that yields two biologically inactive products, lumiflavin and lumichrome (Figure 8.26), and an array of free radicals [152]. Exposure of solutions of riboflavin to visible light has been used for many years as an experimental technique to generate free radicals. Photolysis of riboflavin yields superoxide and riboflavin radicals ($R^{\bullet}$), and the reaction of O_2 with $R^{\bullet}$ provides peroxy radicals and a wide range of other products. The extent to which photochemical degradation of riboflavin is responsible for photosensitized oxidation reactions in foods has not been quantitatively determined, although this process assuredly contributes significantly. Riboflavin is involved in the photosensitized degradation of ascorbic acid and presumably other labile vitamins. Sunlight-induced off-flavor in milk, which is no longer common, is a riboflavin-mediated photochemical process. Although the mechanism of off-flavor formation has not been fully determined, light-induced (probably radical-mediated) decarboxylation and deamination of methionine to form methional ($CH_3{-}S{-}CH_2{-}CH_2{-}CH{=}O$) is at least partially responsible. Concurrent mild oxidation of milk lipids also occurs. Changes in packaging and commercial distribution have minimized this problem.

8.8.3.3 Bioavailability

Relatively little is known regarding the bioavailability of naturally occurring forms of riboflavin; however, there is little evidence of problems associated with incomplete bioavailability.

FIGURE 8.26 Photochemical conversion of riboflavin to lumichrome and lumiflavin.

The covalently bound forms of FAD coenzymes have been shown to exhibit very low availability when administered to rats, although these are minor forms of the vitamin. The nutritional significance of dietary riboflavin derivatives that have potential antivitamin activity has not yet been determined in animals or humans.

8.8.3.4 Analytical Methods

Flavins are highly fluorescent compounds in their fully oxidized flavoquinone form (Figure 8.25), and this property serves as the basis for most analytical methods. The traditional assay procedure for the measurement of total riboflavin in foods involves measurement of fluorescence before and after chemical reduction to the nonfluorescent flavohydroquinone [129]. Fluorescence is a linear function of concentration in dilute solution, although certain food components can interfere with accurate measurement. A number of contemporary HPLC and LCMS methods are also suitable for measurement of total riboflavin in food extracts based on principles outlined previously [38]. Common HPLC procedures require extraction by autoclaving in dilute acid followed either by a direct analysis of riboflavin, FMN, and FAD [121] or else by a phosphatase treatment to release riboflavin from FMN and FAD.

8.8.4 Niacin

8.8.4.1 Structure and General Properties

Niacin is the generic term for pyridine 3-carboxylic acid (nicotinic acid) and derivatives that exhibit similar vitamin activity (Figure 8.27). Nicotinic acid and the corresponding amide (nicotinamide; pyridine 3-carboxamide) are probably the most stable of the vitamins. The coenzyme forms of niacin are nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), either of which can exist in oxidized or reduced form. NAD and NADP function as coenzymes (in the transfer of reducing equivalents) in many dehydrogenase reactions. Heat, especially under acid or alkaline conditions, converts nicotinamide to nicotinic acid without loss of vitamin activity. Niacin is not affected by light, and no thermal losses occur under conditions relevant to food processing. As with other water-soluble nutrients, losses can occur by leaching during washing, blanching, and processing/preparation and by exudation of fluids from tissues (i.e., drip).

NH$_2$ N N N N O $^-$O—P—O—CH$_2$ O O O=P—O$^-$ O HO OR (H or PO$_3^-$) O CNH$_2$ CH$_2$ N+ O HO OH O COH N O CNH$_2$ N

(a) (b) (c)

FIGURE 8.27 Structures of (a) nicotinic acid, (b) nicotinamide, and (c) nicotinamide adenine dinucleotide (phosphate).

Niacin is widely distributed in vegetables and foods of animal origin. Niacin deficiency is rare in the United States partially as a result of programs to enrich cereal grain products with this nutrient. Diets high in protein reduce the requirement for dietary niacin because of the metabolic conversion of tryptophan to nicotinamide.

In certain cereal grain products, niacin exists in several chemical forms that, unless hydrolyzed, exhibit no niacin activity. These inactive niacin forms include poorly characterized complexes involving carbohydrates, peptides, and phenols. Analysis of these nutritionally unavailable, chemically bound forms of niacin has revealed chromatographic heterogeneity and variation in chemical composition, indicating that many bound forms of niacin exist naturally. Alkaline treatments release niacin from these complex derivatives, which permits measurement of total niacin. Several esterified forms of nicotinic acid exist naturally in cereal grains, and these compounds contribute little to niacin activity in foods.

Trigonelline, or N-methyl-nicotinic acid, is a naturally occurring alkaloid found at relatively high concentrations in coffee and at lower concentrations in cereal grains and legumes. Under the mildly acidic conditions that prevail during roasting of coffee, trigonelline is demethylated to form nicotinic acid, yielding a 30-fold increase in the niacin concentration and activity of coffee. Cooking also changes the relative concentration of certain niacin compounds through interconversion reactions [147,148]. For example, heating releases free nicotinamide from NAD and NADP during the boiling of corn. In addition, the distribution of niacin compounds within a product varies as a function of variety (e.g., sweet corn versus field corn) and stage of maturity.

8.8.4.2 Bioavailability

The existence of nutritionally unavailable forms of niacin in many foods of plant origin has been known for many years, although the chemical identities of the unavailable forms of the vitamin are poorly characterized. In addition to the chemically bound forms discussed earlier, several other forms of niacin contribute to its incomplete availability in foods of plant origin [148]. NADH, the reduced form of NAD, and presumably NADPH, exhibits very low bioavailability because of their instability in the gastric acid environment. This may be of little nutritional significance because of the low concentration of these reduced forms in many foods. The primary factor affecting niacin bioavailability is the proportion of the total niacin that is chemically bound. As shown in Table 8.20, there is often much more niacin measurable following alkaline extraction than there is by rat bioassays (biological available niacin) or by direct analysis (free niacin).

8.8.4.3 Analytical Methods

Niacin can be measured by microbiological assay. The principal traditional chemical assay involves a reaction of niacin with cyanogen bromide to yield an N-substituted pyridine that is then coupled to an aromatic amine to form a chromophore [35]. A number of HPLC and LCMS methods are available for measurement of nicotinic acid, nicotinamide, NAD, NADP, and other niacin derivatives in foods and biological materials [38,84], and HPLC has been used to determine individual free and bound forms of niacin in cereal grains [147,148].

8.8.5 Vitamin B6

8.8.5.1 Structure and General Properties

Vitamin B6 is a generic term for the group of 2-methyl, 3-hydroxy, 5-hydroxymethyl-pyridines having the vitamin activity of pyridoxine. The various forms of vitamin B6 differ according to the nature of the one-carbon substituent at the 4 position, as shown in Figure 8.28. For pyridoxine (PN) the substituent is an alcohol, for pyridoxal (PL) it is an aldehyde, and for pyridoxamine

TABLE 8.20
Concentration of Niacin in Selected Foods as Determined by Chemical Assay (Acidic or Alkaline Extraction Methods) or Rat Bioassay

	Type of Chemical Assay		
Food	**Free Niacin (μg/g)**[a]	**Total Niacin (Alkaline Extraction) (μg/g)**[a]	**Rat Bioassay (μg/g)**[a]
Corn	0.4	25.7	0.4
Boiled corn	3.8	23.8	6.8
Corn after alkaline heating (liquid retained)	24.6	24.6	22.3
Tortillas	11.7	12.6	14
Sweet corn (raw)	—	54.5	40
Steamed sweet corn	45	56.4	48
Boiled sorghum grain	1.1	45.5	16
Boiled rice	17	70.7	29
Boiled wheat	—	57.3	18
Baked potatoes	12	51	32
Baked liver	297	306	321
Baked beans	19	24	28

Sources: Adapted from Carpenter, K.J. et al., *J. Nutr.*, 118, 165, 1988; Wall, J. and Carpenter, K., *Food Technol.*, 42, 198, 1988.

Analysis of HCl extract yields a measure of "free niacin," assay of the alkaline extract provides a measure of total niacin, and the rat bioassay is a measure of biologically available niacin.

[a] Wet weight basis.

FIGURE 8.28 Structures of vitamin B6 compounds.

(PM) it is an amine. These three basic forms can also be phosphorylated at the 5′-hydroxymethyl group, yielding pyridoxine 5′-phosphate (PNP), pyridoxal 5′-phosphate (PLP), or pyridoxamine 5′-phosphate (PMP). Vitamin B6, in the form of PLP and, to a lesser extent, PMP, functions as a coenzyme in over 140 enzymatic reactions involved in the metabolism of amino acids, carbohydrates, neurotransmitters, and lipids. All of the mentioned forms of vitamin B6 possess vitamin activity because they can be converted in vivo to these coenzymes. The use of "pyridoxine" as a generic term for vitamin B6 has been discontinued. Similarly, the term "pyridoxol" has been discontinued in favor of pyridoxine.

TABLE 8.21
pK_a Values of Vitamin B6 Compounds

	pK_a				
Ionization[a]	**PN**	**PL**	**PM**	**PLP**	**PMP**
3-OH	5.00	4.20–4.23	3.31–3.54	4.14	3.25–3.69
Pyridinium N	8.96–8.97	8.66–8.70	7.90–8.21	8.69	8.61
4′-Amino group			10.4–10.63		ND
5′-Phosphate ester					
pK_{a1}				<2.5	<2.5
pK_{a2}				6.20	5.76

Source: Snell, E., *Compr. Biochem.*, 2, 48, 1963.

[a] *Abbreviations*: PN, pyridoxine; PL, pyridoxal; PM, pyridoxamine; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; ND, not determined.

Glycosylated forms of vitamin B6 are present in most fruits, vegetables, and cereal grains, generally as pyridoxine-5′-β-D-glucoside (Figure 8.28; [55]). These comprise 5%–75% of the total vitamin B6 and account for 15%–20% of the vitamin B6 in typical mixed diets. Pyridoxine glucoside becomes nutritionally active only after hydrolysis of the glucoside by the action of β-glucosidases in the intestine or other organs. Several other glycosylated forms of vitamin B6 are also found in certain plant products.

Vitamin B6 compounds exhibit complex ionization that involves several ionic sites (Table 8.21). Because of the basic character of the pyridinium N ($pK_a \sim 8$) and the acidic nature of the 3-OH ($pK_a \sim 3.5$–5.0), the pyridine ring system of vitamin B6 molecules mainly exists in zwitterionic form at neutral pH. The net charge on vitamin B6 compounds varies widely as a function of pH. The 4′-amino group of PM and PMP ($pK_a \sim 10.5$) and the 5′-phosphate ester of PLP and PMP ($pK_a < 2.5$, ~6, and ~12) also contribute to the charge of these forms of the vitamin.

All chemical forms of vitamin B6 exist in foods, although the distribution varies markedly. PN-glucoside exists only in plant products, although most plant products also contain all other forms of the vitamin. Vitamin B6 in muscle and organ meats is predominantly (>80%) PLP and PMP, with minor amounts of the nonphosphorylated species. Disruption of raw plant tissues by freeze-thaw cycling or homogenization releases phosphatases and β-glucosidases that can alter the forms of vitamin B6 compounds by catalyzing dephosphorylation and deglycosylation reactions. Similarly, disruption of animal tissues prior to cooking can cause extensive dephosphorylation of PLP and PMP. PNP is a transient intermediate in vitamin B6 metabolism and is usually a negligible component of the total vitamin B6 content. Pyridoxine (as the HCl salt) is the form of vitamin B6 used for food fortification and in nutritional supplements because of its good stability. Intakes of supplemental vitamin B6 above 100 mg/day should be avoided because of its potential neurotoxicity. Supplements containing any other form of vitamin B6, such as pyridoxal phosphate, generally offer no added nutritional benefit beyond that of pyridoxine. Aside from the HCl form, pyridoxine has been marketed in the form of the pyridoxine-α-ketoglutarate (PAK) complex. The claims regarding PAK are poorly substantiated, and doses reported border on the range now known to be hazardous. Claims that high-dose PM supplements have antidiabetic properties are not strongly supported by experimental evidence.

The aldehyde and amine forms of vitamin B6 readily participate in carbonyl-amine reactions: PLP or PL with amines or PMP or PM with aldehydes or ketones (Figure 8.29). The coenzymic action of PLP in most B6-dependent enzymes occurs by an enzymatic mechanism involving carbonyl-amine condensation. In foods and other nonenzymatic systems, PLP and PL readily

FIGURE 8.29 Formation of Schiff base structures from PL and PM. Analogous reactions occur with PLP and PMP.

form Schiff bases with the neutral amino groups of amino acids, peptides, or proteins. Coordinate covalent bonding to a metal ion increases the stability of the Schiff base in nonenzymatic systems, although Schiff bases can exist in solutions devoid of metal ions. PLP forms Schiff bases much more readily than PL because the phosphate group of PLP blocks the formation of an internal hemi-acetal and maintains the carbonyl in reactive form (Figure 8.30; [151]). As with other carbonyl-amine reactions, the nonenzymatic formation of a vitamin B6 Schiff base is strongly pH dependent and exhibits an alkaline pH optimum. The stability of the Schiff base complexes is also strongly pH dependent, with dissociation occurring in acidic environments. Thus, Schiff base forms of vitamin B6 would be expected to dissociate fully in acidic media such as the postprandial gastric contents. In addition to the Schiff base in Figure 8.29, several other tautomeric and ionic forms exist in equilibrium.

Depending on the chemical nature of the amino compound condensing with PLP or PL in the Schiff base, further rearrangement to various cyclic structures can occur. For example, cysteine condenses with PL or PLP to form a Schiff base, and then the SH group attacks the Schiff base 4′-C to form the cyclic thiazolidine derivative (Figure 8.31). Histidine, tryptophan, and several related

FIGURE 8.30 Formation of pyridoxal hemiacetal.

FIGURE 8.31 Formation of the Schiff base and thiazolidine complexes of PL and cysteine.

compounds (e.g., histamine and tryptamine) can form similar cyclic complexes with PL or PLP bases through reactions of the imidazolium and indolyl side chains, respectively.

8.8.5.2 Stability and Modes of Degradation

Thermal processing and storage of foods can influence the vitamin B6 content in several ways. As with other water-soluble vitamins, exposure to water can cause leaching and consequent losses. Chemical changes can involve interconversion of chemical forms of vitamin B6, thermal or photochemical degradation, as well as irreversible complexation with proteins, peptides, or amino acids.

The interconversion of vitamin B6 compounds occurs mainly by nonenzymatic transamination, which involves formation of a Schiff base and migration of the Schiff base double bond, followed by hydrolysis and dissociation. Such nonenzymatic transamination has been studied extensively as a model of PLP-mediated enzymatic transaminations. This process occurs extensively during the thermal processing of foods that contain the aldehyde or amine forms of vitamin B6. For example, increases in the proportion of PM and PMP are frequently observed in the cooking or thermal processing of meat and dairy products [11,47] and in studies of protein-based liquid model systems [46]. The occurrence of such transamination has no adverse nutritional effect. Similar transamination has been shown to occur during the storage of intermediate-moisture, model food systems ($a_w \sim 0.6$). PL-mediated nonenzymatic elimination of H_2S and methyl mercaptan from sulfur-containing amino acids can also occur during food processing. This can be a significant source of flavor and can cause discoloration in canned foods through formation of black FeS [56].

All vitamin B6 compounds are susceptible to light-induced degradation, which can cause losses during food processing, preparation, and storage and during analysis. The mechanism of vitamin B6 degradation by light is not well understood and the relationship between reaction rate and wavelength is not known. Light-mediated oxidation appears to be involved, presumably with a free radical intermediate. Exposure of vitamin B6 to light causes formation of the nutritionally inactive derivatives 4-pyridoxic acid (from PL and PM) and 4-pyridoxic acid 5′-phosphate (from PLP and PMP), providing evidence of susceptibility to photochemical oxidation [117,125]. However, the rates of photochemical degradation of PLP, PMP, and PM and the amount of degradation products obtained differ only slightly in the presence or absence of air, suggesting that initiation of oxidation does not require direct attack by O_2. Photochemical degradation of PL in low-moisture model food systems occurs at a greater rate than that of PL and PN. The reactions are first order in PL concentration, are strongly influenced by temperature, but are affected only slightly by water activity (Table 8.22).

The rate of nonphotochemical degradation of vitamin B6 is strongly dependent on the form of the vitamin, temperature, pH of the solution, and the presence of other reactive compounds (e.g., proteins, amino acids, and reducing sugars). All forms of vitamin B6 exhibit excellent stability

TABLE 8.22
Influence of Temperature, Water Activity, and Light Intensity on Degradation of Pyridoxal in a Dehydrated Model Food System

Light Intensity (Lumens/m²)	a_w	Temperature (°C)	k[a] (Day⁻¹)	t½[a] (Days)
4300	0.32	5	0.092	7.4
		28	0.1085	6.4
		37	0.2144	3.2
		55	0.3284	2.1
4300	0.44	5	0.0880	7.9
		28	0.1044	6.6
		55	0.3453	2.0
2150	0.32	27	0.0675	10.3

Source: Adapted from Saidi, B. and Warthesen, J., *J. Agric. Food Chem.*, 31, 876, 1983.

[a] First-order rate constant and time for 50% degradation, respectively.

at very low pH (e.g., 0.1 M HCl), a condition used during traditional extraction methods for vitamin B6 analysis. During incubation of vitamin B6 compounds at 40°C or 60°C in aqueous solutions buffered at pH 4–7 for up to 140 days, PN exhibited no loss, PM exhibited the greatest loss at pH 7, and PL showed its greatest loss at pH 5 (Table 8.23; [124]). Degradation of PL and PM followed first-order kinetics in these studies. On the contrary, in similar studies of the degradation of PL, PN, and PM at 110°C–145°C in aqueous solution buffered at pH 7.2, these compounds exhibited kinetics best described as second order, 1.5 order, and pseudo-first order, respectively [107]. In parallel studies, thermal degradation of vitamin B6 in cauliflower puree also did not conform to first-order kinetics. The reasons responsible for the kinetic differences of these studies are not clear. Under conditions of dry heat simulating toasting processes, degradation of PN in dehydrated model systems exhibited consistent first-order kinetics [36].

Studies of the thermal stability of vitamin B6 in foods are complicated because the multiple forms of the vitamin can undergo various degradation reactions and interconversions can also occur among the various forms of the vitamin. Losses of total vitamin B6 in food processing or storage are similar to those observed with other water-soluble vitamins. For example, garbanzo and lima beans exhibit approximately 20%–25% loss of total vitamin B6 during blanching and canning process.

The development of HPLC methods has facilitated studies of the chemical behavior of vitamin B6 compounds during processing and storage. The simultaneous interconversion of PL and PM, along with first-order loss of total vitamin B6, has been observed in studies of thermal processing and storage of intermediate-moisture model food systems and in liquid model systems simulating infant formula (Figure 8.32 and Table 8.24) [46]. PN exhibited greater stability than PL or PM, although the magnitude of the difference varied with temperature (Table 8.23). Although differences in energy of activation suggest a difference in degradation mechanism for these three forms of the vitamin, thermodynamic calculations provide evidence of a common rate-limiting step for loss of PL, PM, and PN [45]. There is a need for further studies to assess more fully the behavior of naturally occurring vitamin B6 in various foods.

Extensive examination of the thermal stability of vitamin B6 in milk products was prompted by an unfortunate incident involving infant formulas. In the early 1950s, over 50 cases of convulsive seizures occurred in infants that had consumed a commercially available milk-based

TABLE 8.23
Influence of pH and Temperature on the Degradation of Pyridoxal and Pyridoxamine in Aqueous Solution

Compound	Temperature (°C)	pH	k[a] (Day^{-1})	t½[a] (Days)
Pyridoxal	40	4	0.0002	3466
		5	0.0017	407
		6	0.0011	630
		7	0.0009	770
Pyridoxal	60	4	0.0011	630
		5	0.0225	31
		6	0.0047	147
		7	0.0044	157
Pyridoxamine	40	4	0.0017	467
		5	0.0024	289
		6	0.0063	110
		7	0.0042	165
Pyridoxamine	60	4	0.0021	330
		5	0.0044	157
		6	0.0110	63
		7	0.0108	64

Source: Adapted from Saidi, B. and Warthesen, J., *J. Agric. Food Chem.*, 31, 876, 1983.

No significant degradation of pyridoxine was found at pH 4–7 at 40°C or 60°C for up to 140 days.

[a] First-order rate constant and time for 50% degradation, respectively.

infant formula [23], whereas thousands of infants consumed the same formula without ill effects. These convulsive disorders were corrected by administration of PN to the infants. The problem of inadequate vitamin B6 content in the processed formulas was corrected by fortification with PN, which is much more stable than PL, the major naturally occurring form of the vitamin in milk [61]. Commercial sterilization of evaporated milk or unfortified infant formula causes 40%–60% loss of the naturally occurring vitamin B6. Little or no loss of added PN was found during a comparable thermal process. This incident highlights the need for complete and thorough assessment of the nutritional quality of foods, especially when new formulations and processing methods are employed.

The occurrence of vitamin B6 deficiency in the case of these unfortified infant formulas has been attributed, at least in part, to the interaction of PL with milk proteins during processing to form a sulfur-containing derivative, bis-4-pyridoxyl-disulfide (Figure 8.33). Bis-4-pyridoxyl-disulfide has been reported to form slowly after heating a concentrated solution of PL and cysteine [150], and it exhibits only partial (~20%) vitamin activity in rat bioassays. Evidence of an involvement of sulfhydryl groups in the interaction of PL with milk proteins also has been reported [134]. However, HPLC analysis of thermal processing of milk containing radiolabeled PL and PLP revealed no evidence of the formation of bis-4-pyridoxyl-disulfide [47]. Alternatively, PL and PLP were found to undergo extensive binding to lysyl ε-amino groups of milk proteins by reduction of the Schiff base −C=N− linkage (Figure 8.34). The formation of such pyridoxyllysyl residues also has been detected in thermally processed muscle and liver and during storage of intermediate-moisture model food systems. The mechanism by which the reduction of the Schiff base linkage occurs has not been determined.

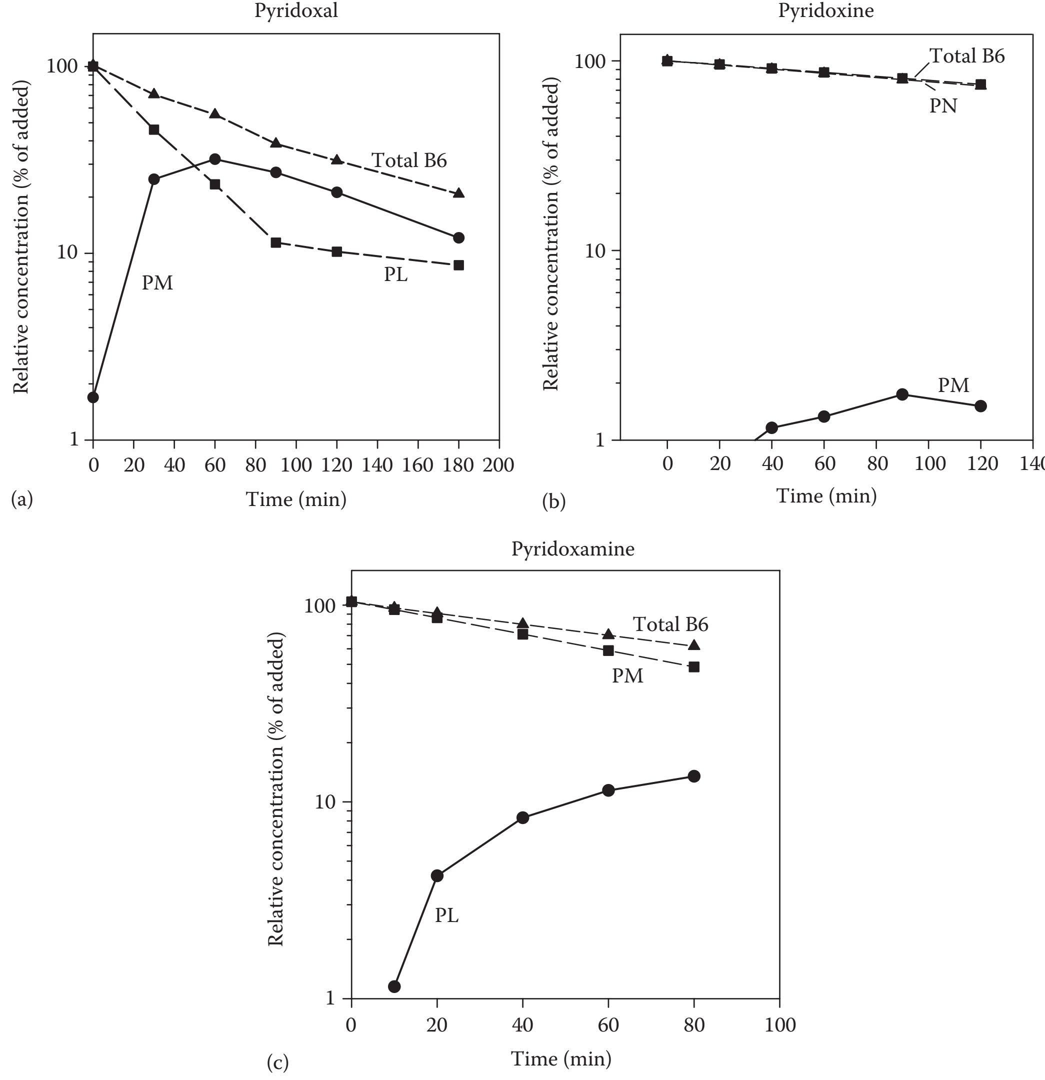


FIGURE 8.32 Degradation and interconversion of vitamin B6 compounds during thermal processing at 118°C of a liquid model food system simulating infant formula. (From Gregory, J. and Hiner, M., *J. Food Sci.*, 48, 2434, 1983.) Orginal vitamin B6 content 100% for pyridoxal (PL) (a), 100% pyridoxine (PN) (b), and 100% pyridoxamine (PM) (c).

Pyridoxyllysyl residues associated with food proteins have been shown to exhibit approximately 50% of the vitamin B6 nutritional activity of PN [54]. When administered to vitamin B6-deficient rats, this compound exacerbates the deficiency. This effect may have been involved in the deficiencies associated with consumption of an unfortified thermally processed infant formula mentioned earlier.

The role of protein sulfhydryl groups in the interaction of PL with protein has not been fully resolved. Sulfhydryl groups, as is true of ε-amino or imidazolium groups of amino acid side chains, can reversibly interact with the Schiff base linkage of protein-bound PL to form a substituted aldimine in a manner analogous to that shown in Figure 8.31.

Vitamin B6 also can be converted to biologically inactive compounds by reactions with free radicals. Hydroxyl radicals generated during degradation of ascorbic acid can directly attack the

TABLE 8.24
Rate Constants and Energies of Activation for the Thermal Loss of Total Vitamin B6 in Liquid Model System Simulating Infant Formula

Form of Vitamin B6 Added	Temperature (°C)	k[a] (min^{-1})	t½[a] (min)	Energy of Activation (kJ/mol)
Pyridoxine	105	0.0006	1120	114
	118	0.0025	289	
	133	0.0083	62	
Pyridoxamine	105	0.0021	340	99.2
	118	0.0064	113	
	133	0.0187	35	
Pyridoxal	105	0.0040	179	87.0
	118	0.0092	75	
	133	0.0266	24	

Source: Gregory, J. and Hiner, M., *J. Food Sci.*, 48, 2434, 1983.
[a] First-order rate constant and time for 50% degradation, respectively.

FIGURE 8.33 Structure of bis-4-pyridoxyl-disulfide.

FIGURE 8.34 Interaction of PL with the ε-amino group of a lysyl residue of a food protein to form a Schiff base, followed by reduction to a pyridoxylamino complex.

C^6 position of PN to form the 6-hydroxy-derivative [136]. Presumably this reaction could occur with all other forms of vitamin B6. 6-Hydroxypyridoxine totally lacks vitamin B6 activity.

8.8.5.3 Bioavailability of Vitamin B6

Many factors influence the bioavailability of vitamin B6 [53]. The bioavailability of the total vitamin B6 content of a typical mixed diet has been estimated to be approximately 75% for adult humans [139]. Dietary PL, PN, PM, PLP, PMP, and PNP appear to be efficiently absorbed and effectively function in vitamin B6 metabolism. Schiff base forms of PL, PLP, PM, and PMP dissociate in the acidic environment of the stomach and exhibit high bioavailability.

PN-glucoside and other glycosylated forms of vitamin B6 are partially utilized in human nutrition. The mean bioavailability of PN-glucoside is 50%–60% relative to PN, although wide variation is observed among individuals. The importance of the incomplete bioavailability of PN-glucoside in human diets depends largely on the total quantity of vitamin B6 consumed and the selection of foods. However, even foods with high percentages of PN-glucoside can be quite effective sources of dietary vitamin B6 because of this partial bioavailability. PN-glucoside bioavailability varies markedly among animal species. It is essential that analytical methods for vitamin B6 are able to detect the glycosylated forms and provide a specific measurement of their quantities.

Determining the bioavailability of PL or PLP in the form of a pyridoxylamino compound (e.g., pyridoxyllysine, Figure 8.34) is not simple. In contrast to the Schiff base forms that are readily dissociable, pyridoxylamino forms have a very stable reduced linkage between PL or PLP and the lysine ε-amino group. Because of this stable covalent linkage, there is little or no dissociation of the B6 moiety of pyridoxylamino compounds during extraction conditions typically used in vitamin B6 analysis. Thus, the reductive binding of PL or PLP to protein appears in most food analysis to be a mode of vitamin B6 degradation and is seen as a loss of measurable vitamin B6. However, as stated earlier, studies of the bioavailability of PLP bound reductively (pyridoxyllysyl residues) to dietary protein have indicated a bioavailability of approximately 50% [54]. Mammals can partially utilize pyridoxyllysine released in protein digestion through in vivo enzymatic phosphorylation and oxidative cleavage that frees the PLP moiety. Pyridoxyllysine can exert a weak antivitamin B6 activity, although this contributes to vitamin B6 deficiency only when the diet is marginal with respect to total vitamin B6 content [54].

8.8.5.4 Measurement of Vitamin B6

Vitamin B6 can be measured by microbiological assay methods or by HPLC [51]. Microbiological assays for total vitamin B6 can be performed using the yeasts *Saccharomyces uvarum* (formerly *S. carlsbergensis*) or *Kloeckera brevis*. Yeast growth assays involve a prior acid hydrolysis to extract vitamin B6 from food and to hydrolyze phosphate esters and β-glucosides. Care must be taken when using microbiological assays because the organisms used may underestimate PM. HPLC methods are based mainly on reverse phase or ion exchange separation with fluorometric detection or by recent LCMS methods. HPLC methods that involve chemically or enzymatically converting the B6 vitamers to a single species (e.g., PN) are prone to error arising from incomplete interconversions.

8.8.6 Folate

8.8.6.1 Structure and General Properties

The generic term "folate" refers to the class of pteridine derivatives having chemical structure and nutritional activity similar to that of folic acid (pteroyl-L-glutamic acid). The various components of this class are designated as "folates." The use of "folacin" and "folic acid" as generic terms is no longer recommended. Folic acid consists of L-glutamic acid that is coupled through its α-amino group to the carboxyl group of para-aminobenzoic acid that, in turn, is linked to a 2-amino-4-hydroxypteridine (Figure 8.35). In folic acid, the pteridine moiety is fully oxidized; that is, it exists as a fully double-bonded conjugated system. All folates contain an amide-like structure involving N^3 and C^4 that resonates between the two forms shown in Figure 8.36.

Folic (pteroyl-L-glutamic) acid

Polyglutamyl tetrahydrofolates

Substituent (R)		Position
—CH_3	(methyl)	5
—CHO	(formyl)	5 or 10
—CH=NH	(formimino)	5
—CH_2—	(methylene)	5 and 10
—CH=	(methenyl)	5 and 10

FIGURE 8.35 Structures of folates.

FIGURE 8.36 Resonance of the 3,4-amide site of folate pteridine ring system. The fully oxidized pteridine system of folic acid is shown; H_4folates and H_2folates exhibit identical behavior.

Folic acid (pteroyl-L-glutamic acid) exists naturally only in trace quantities. The major naturally occurring forms of folate in materials of plant, animal, and microbial sources are polyglutamyl species of 5,6,7,8-tetrahydrofolates (H_4folates) (Figure 8.35), in which two double bonds of the pteridine ring system are reduced. Small amounts of 7,8-dihydrofolates (H_2folates) also exist naturally (Figure 8.35). H_4folates are mediators of the metabolic one-carbon acquisition, transport, and transformation, that is, the transfer, oxidation, and reduction of one-carbon units, which account for the presence of folates having various one-carbon substituted forms in living cells. One-carbon substituents can exist on either the N^5 or N^{10} positions (predominantly as methyl or formyl groups) or as methylene ($-CH_2-$) or methenyl ($-CH=$) units bridging between N^5 and N^{10} (Figure 8.35). Many of the naturally occurring folates in plant and animal tissues and in foods derived from plant and animal sources have a side chain of 5–7 glutamate residues with γ-peptide linkages. It is generally assumed that approximately 50%–80% of naturally occurring dietary folate exists in polyglutamyl form, depending on the pattern of food selection. Beginning in 1998, the federally mandated addition of folic acid to most cereal grain foods (e.g., flours, enriched bread, rolls, pasta, rice) changed this pattern so that 25%–50% of folate intake often is obtained as synthetic folic acid (pteroyl-L-glutamate) in typical diets. However, limited data exist concerning the distribution of the various folates in individual foods or whole diets. All folates, regardless of oxidation state of the pteridine ring system, N^5 or N^{10} one-carbon substituent, or polyglutamyl chain length, exhibit vitamin activity in mammals, including humans. Many structural analogues of folates, such as those with a 4-amino group, are potent antagonists used in chemotherapy for cancer and autoimmune disease.

TABLE 8.25
pK_a Values for Ionizable Groups of Folates

Folate Compound	Amide[a]	N^1	N^5	N^{10}	α-COOH	γ-COOH
5,6,7,8-H_4folate	10.5	1.24	4.82	−1.25	3.5	4.8
7,8-H_2folate	9.54	1.38	3.84	0.28	ND[b]	ND[b]
Folic acid	8.38	2.35	≤1.5	0.20	ND[b]	ND[b]

Source: Adapted from Poe, M., *J. Biol. Chem.*, 252, 3724, 1977.

[a] Amide refers to dissociation of the N^3–C^4 amide-like site.

[b] ND, not determined due to insufficient solubility. It is assumed that the pK_a values of these carboxyl groups are similar for all folates.

Folates undergo changes in ionic form as a function of pH (Table 8.25). Changes in the charge of the pteridine ring system account, in part, for the pH dependence of folate stability, UV absorption spectra, and the pH-dependent behavior of folates during chromatographic separation.

Asymmetric carbons (glutamyl α-carbon of all folates and pteridine C^6 of H_4folates) can each exist in either of two configurations; thus, folates are termed diastereoisomers. The glutamic acid moiety must be in the L isomeric form for vitamin activity, while C^6 must be in the correct chiral form for tetrahydrofolates to exhibit vitamin activity. Tetrahydrofolates synthesized by nonspecific chemical reduction of folic acid contain a mixture of 6R and 6S diastereoisomers, only one of which is nutritionally active. Formal nomenclature dictates that the natural, nutritionally active form of H_4folate, 5-methyl, 5-formyl, and 5-formimino H_4folates should be designated as 6S, while those with a substituent at the C^{10} position (10-formyl, 5,10-methylene, and 5,10-methenyl H_4folates) should be designated 6R. Care is needed in using this nomenclature. A shorthand nomenclature designates all natural, nutritionally active forms of tetrahydrofolate as L (e.g., L-5-methyltetrahydrofolate). Since the natural configuration is required for vitamin activity in animals (including humans) and in microbiological assays for folate, diastereoisomeric mixture (6R + 6S) forms of H_4folates only exhibit 50% nutritional activity.

The 5-formyl and 10-formyl H_4folate have an aldehyde group as the one-carbon substituent. Formyl forms of H_4folate are interconvertible through the 5,10-methenyl intermediate. Formation of the methenyl species from either 5-formyl or 10-formyl-H_4folate is favored only at pH < 2; thus, this form is a minor constituent of folates in most foods. The transient existence of 5,10-methenyl-H_4folate at pH > 2 accounts for the conversion of 10-formyl-H_4folate to the more stable 5-formyl-H_4folate when heated in weak acid and for the pH-dependent formation of 10-formyl-H_4folate from 5-formyl-H_4folate [119].

Large differences in stability exist among the various H_4folates as a result of the influence of the one-carbon substituent on susceptibility to oxidative degradation. In most cases, folic acid (with the fully oxidized pteridine ring system) exhibits substantially greater stability than the H_4folates or H_2folates. The order of stability of the H_4folates is 5-formyl-H_4folate > 5-methyl-H_4folate > 10-formyl-H_4folate ≥ H_4folate. Stability of each folate is dictated only by the chemical nature of the pteridine ring system, with no influence of polyglutamyl chain length. The inherent differences in stability among folates, as well as chemical and environmental variables influencing folate stability, will be discussed further in the next section.

All folates are subject to oxidative degradation, although the mechanism and the nature of the products vary among the various chemical species of the vitamin. Reducing agents such as ascorbic acid and thiols exert multiple protective effects on folates through their actions as oxygen scavengers, reducing agents, and free radical scavengers.

Aside from molecular oxygen, other oxidizing agents found in foods can have deleterious effects on folate stability. For example, at concentrations similar to those used for antimicrobial

treatments, hypochlorite causes oxidative cleavage of folic acid, H_2folate, and H_4folate to nutritionally inactive products. Under the same oxidizing conditions, certain other folates (e.g., 5-methyl-H_4folate) are converted to forms that may retain at least partial nutritional activity. Light is also known to promote the degradation of folates, although the mechanism overall is poorly understood. Prior to the initiation of fortification with folic acid in the United States, folate was frequently one of the most limiting of the vitamins in human diets. This remains the case in most other countries that do not practice the addition of folic acid to food. The frequent insufficiency of naturally occurring dietary folate is mainly due to (a) poor diet selection, especially with respect to foods rich in folate (e.g., fruits, especially citrus, green leafy vegetables, and organ meats); (b) losses of folate during food processing and/or home preparation by oxidation, leaching, or both; and (c) incomplete bioavailability of many naturally occurring forms of folate in many human diets [48,51].

Folic acid, because of its excellent stability, is the sole form of folate added to foods, and it is also used in vitamin pills. In clinical situations requiring use of a reduced folate, 5-formyl-H_4folate is employed because of its stability (similar to folic acid), and 5-methyl-H_4folate also is available in a few nutritional supplements.

8.8.6.2 Stability and Modes of Degradation

8.8.6.2.1 Folate Stability

Folic acid exhibits excellent retention during the processing and storage of fortified foods and premixes [48,50]. As shown in Tables 8.2 and 8.3, little degradation of this form of the vitamin occurs during extended low-moisture storage. Similar good retention of added folic acid has been observed during the retorting of fortified infant formulas and medical formulas.

Many studies have shown the potential for extensive losses of folate during processing and home preparation of foods. In addition to susceptibility to oxidative degradation, folates are readily extracted from foods by aqueous media (Table 8.26). By either means, large losses of naturally occurring folate can occur during food processing and preparation. The overall loss of folate from a food depends on the extent of extraction, forms of folate present, and the nature of the chemical environment (catalysts, oxidants, pH, buffer ions, etc.). Thus, folate retention is difficult to predict for a given food.

TABLE 8.26
Effect of Cooking on Folate Content of Selected Vegetables

Vegetable (Boiled 10 min in Water)	Total Folate[a] (μg/100 g Fresh wt.)		
	Raw	Cooked	Folate in Cooking Water
Asparagus	175 ± 25	146 ± 16	39 ± 10
Broccoli	169 ± 24	65 ± 7	116 ± 35
Brussels sprouts	88 ± 15	16 ± 4	17 ± 4
Cabbage	30 ± 12	16 ± 8	17 ± 4
Cauliflower	56 ± 18	42 ± 7	47 ± 20
Spinach	143 ± 50	31 ± 10	92 ± 12

Source: Adapted from Leichter, J. et al., *Nutr. Rep. Int.*, 18, 475, 1978.
[a] Mean ± SD, n = 4.

8.8.6.2.2 Degradation Mechanisms

The mechanism of folate degradation depends on the form of the vitamin and the chemical environment. As mentioned previously, folate degradation generally involves changes at the C^9–N^{10} bond, the pteridine ring system, or both. Folic acid, H_4folate, and H_2folate can undergo C^9–N^{10} cleavage and resulting inactivation in the presence of either oxidants or reductants [97]. Dissolved SO_2 has been found to cause cleavage of certain folates, but few other reducing agents relevant to foods can induce such cleavage. There is only slight direct oxidative conversion of H_4folate to H_2folate or folic acid.

It is well known that oxidative cleavage of H_4folates, H_2folate, and, to a lesser extent, folic acid yields nutritionally inactive products (*p*-aminobenzoylglutamate and a pterin). The mechanism of oxidation and the nature of the pterin produced during oxidative cleavage of H_4folate vary with pH, as shown in Figure 8.37.

The major naturally occurring form of folate in many foods is 5-methyl-H_4folate. The degradation of 5-methyl-H_4folate can occur by conversion to at least two products (Figure 8.38). The first has been identified tentatively as 5-methyl-5,6-dihydrofolate (5-methyl-H_2folate), which retains vitamin activity since it can be readily reduced back to 5-methyl-H_4folate by weak reductants such as thiols or ascorbate. 5-Methyl-H_2folate undergoes cleavage of the C^9–N^{10} bond in acidic medium, which causes loss of vitamin activity. Some data suggest that a rearrangement of the pteridine can occur to form a pyrazino-s-triazine (Figure 8.38; [73]). An alternate product of 5-methyl-H_4folate degradation appears to be 4a-hydroxy-5-methyl-H_4folate, which actually may be the predominant degradation product in some foods and other biological systems. Many aspects of chemical mechanisms of

FIGURE 8.37 One of two proposed mechanisms for the oxidation of tetrahydrofolate to 7,8-dihydropterin, formaldehyde, and p-aminobenzoylglutamate via the quinoid dihydrofolate intermediate. (Adapted from Reed, L. and Archer, M., *J. Agric. Food Chem.*, 28, 801, 1980.) The alternative proposed mechanism yields 6-formyl-pterin and p-aminobenzoylglutamate (not shown).

FIGURE 8.38 Proposed mechanisms for oxidative degradation 5-methyl-H_4folate.

processes involved in 5-methyl-H_4folate degradation remain to be determined. 5-Methyl-H_4folate has been reported to undergo photodegradation at low O_2 concentration by a photosensitizer-mediated mechanism, whereas light-derived singlet oxygen readily decomposes 5-methyl-H_4folate at higher O_2 concentration [108].

Blair et al. [8] reported that the pH dependence of 5-methyl-H_4folate oxidation is pronounced. Stability (as monitored by oxygen uptake) increases as pH is reduced from 6 to 4; this range corresponds to the range of protonation of the N^5 position. Contrary results have been reported [100], and factors responsible for this contradiction have not been determined.

In certain foods, including various animal and plant tissues, 10-formyl-H_4folate and/or 5,10-methenyl-H_4folate may account for as much as 1/3 of the total folate. Oxidative degradation of 10-formyl-H_4folate can occur either by oxidation of the pteridine moiety to yield 10-formyl-H_2folate or 10-formyl-folate or by oxidative cleavage to form a pterin and N-formyl-p-aminobenzoylglutamate (Figure 8.39). Both 10-formyl-H_2folate or 10-formyl-folate exhibit nutritional activity while the cleavage products do not. The detection of 10-formyl-H_2folate or 10-formyl-folate in a variety of foods [111] suggests that oxidation of 10-formyl-H_4folate occurs readily during food storage preparation and processing. Factors that influence the relative importance of these oxidative pathways in foods have not been determined. In contrast to 10-formyl-H_4folate, 5-formyl-H_4folate exhibits excellent thermal and oxidative stability. HPLC and LCMS methods of folate analysis that do not allow quantification of 10-formyl-H_4folate, 5,10-methenyl-H_4folate, 10-formyl-H_2folate, and 10-formyl-folate may seriously underestimate total folate content in many foods.

8.8.6.2.3 Factors Affecting Folate Stability

Many studies have been conducted to compare the relative stability of folates in buffered solution as a function of pH, oxygen concentration, and temperature. Stability of folates in complex foods is less well understood.

Folic acid is generally the most stable form. It is resistant to oxidation, although reduced stability occurs in acidic media. H_4folate is the least stable form of the vitamin. Maximal stability of H_4folate is observed between pH 8–12 and 1–2, while the stability is minimal between pH 4 and 6.

FIGURE 8.39 Proposed mechanisms for oxidative degradation of 10-formyl-H_4folate.

However, even in the favorable pH zones, H_4folate is extremely unstable. H_4folates having a substituent at the N^5 position exhibit much greater stability than does unsubstituted H_4folate. This suggests that the stabilizing effect of the N^5 methyl group is due, at least in part, to steric hindrance in restricting access of oxygen or other oxidants to the pteridine ring. The stabilizing effect of the N^5-substituent is more pronounced with 5-formyl-H_4folate than with 5-methyl-H_4folate, and both exhibit much greater stability than H_4folate or 10-formyl-H_4folate. Under conditions of low oxygen concentration, 5-methyl-H_4folate and folic acid exhibit similar stability during thermal processing.

The influence of oxygen concentration on the stability of folates in foods, buffer solutions, and model food systems has been widely studied. As mentioned previously, the rate of oxidation of 5-methyl-H_4folate is dependent on the concentration of dissolved oxygen in accord with a second-order or pseudo-first-order relationship. In relatively anaerobic conditions, the presence of added components such as ascorbate, ferrous iron, and reducing sugar all tends to improve the oxidative stability of folic acid and 5-methyl-H_4folate. These components apparently function by reducing the concentration of dissolved oxygen through their own oxidation reactions (Figure 8.40). These findings indicate that complex foods can contain components that influence folate stability by consuming oxygen, acting as reducing agents, or both.

Barrett and Lund [6] studied thermal degradation of 5-methyl-H_4folate in neutral buffer solution and observed both aerobic and anaerobic degradation. Surprisingly, rate constants for aerobic and anaerobic degradation reactions are of similar magnitude (Table 8.27). The extent to which other folates conform to this behavior has not been determined. Clearly the loss of 5-methyl-H_4folate during food processing is minimized but not eliminated by minimizing oxygen availability [146].

The ionic composition of the medium also significantly influences the stability of most folates. Phosphate buffers have been reported to accelerate oxidative degradation of folates, while this effect can be overcome by the addition of citrate ions. The frequent presence of Cu(II) as a contaminant in phosphate buffer salts may explain this effect because metal catalysts are known to accelerate folate oxidations. For example, in aerobic solutions of 5-methyl-H_4folate in water, addition of 0.1 mM Cu(II) causes nearly a 20-fold acceleration in oxidation rate, although Fe(III) causes only a 2-fold increase [8]. Under anaerobic conditions, Fe(III) catalyzes oxidation of H_4pteridines

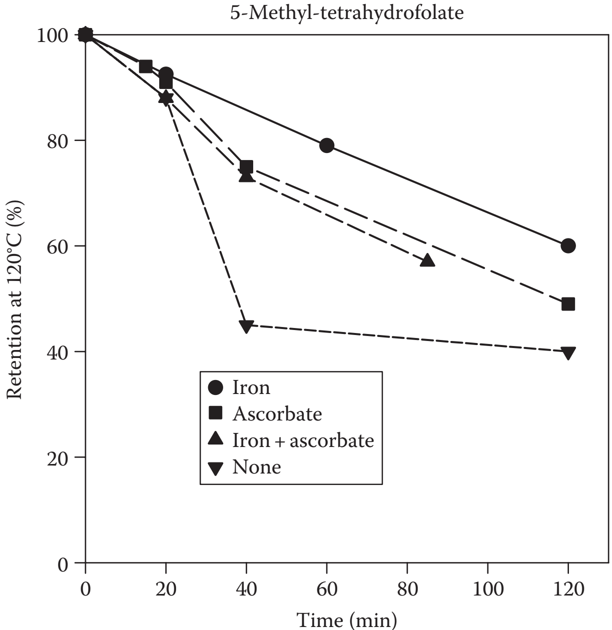


FIGURE 8.40 Thermal processing effects on 5-methyl-H_4folate in liquid model food systems simulating infant formula. The model system consisted of 1.5% (w/v) potassium caseinate and 7% (w/v) lactose in 0.1 M phosphate buffer, pH 7.0. When present, iron was added at 6.65 mg/100 mL ferrous sulfate heptahydrate and ascorbate was added as 6.38 mg/100 mL sodium ascorbate. Initial concentration of folates was 10 μg/mL. (From Day, B.P.F. and Gregory, J.F., *J. Food Sci.*, 48, 581, 1983.)

TABLE 8.27

Reaction Rate Constants for the Degradation of 5-Methyl-H_4folate by Oxidative and Nonoxidative Processes in 0.1 M Phosphate Buffer, pH 7.0[a,b]

Temperature (°C)	$k_{(O2+N2)}$ (Combined Oxidative + Nonoxidative, min^{-1})	k_{N2} (Nonoxidative, min^{-1})	k_{O2} (Oxidative, min^{-1})
40	0.004 ± 0.0002	0.0005 ± 0.00001	0.004 ± 0.00005
60	0.020 ± 0.0005	0.009 ± 0.0004	0.011 ± 0.0001
80	0.081 ± 0.010	0.046 ± 0.003	0.035 ± 0.009
92	0.249 ± 0.050	0.094 ± 0.009	0.155 ± 0.044

Source: Barrett, D.M. and Lund, D.B., *J. Food Sci.*, 54, 146, 1989.

[a] Values are means ± 95% confidence intervals.

[b] Values for apparent first-order rate constants: k_{N2}, degradation by nonoxidative process (in N_2-saturated environment); k_{O2}, degradation by oxidative process (in O_2-saturated environment); $k_{(O2+N2)}$, degradation by both oxidative and nonoxidative processes.

(e.g., H_4folate) → H_2pteridines (e.g., H_2folate) → fully oxidized pteridines (e.g., folic acid). The reason for the differences in the catalytic efficiency of these metals is not known. Folates can undergo degradation by reaction with superoxide radicals [130,137], but the extent of such radical-mediated losses of folates in foods has not been determined.

Several reactive components of foods may accelerate degradation of folates. Dissolved SO_2 can cause reductive cleavage of folates, as stated previously. Exposure to nitrite ions contributes to the

oxidative cleavage of 5-methyl-H_4folate and H_4folate. In contrast, nitrite reacts with folic acid to yield 10-nitroso-folic acid, a weak carcinogen. However, it is reassuring to note that foods containing nitrite do not generally contain folic acid and have low concentrations of other folates. The significance of the latter reaction in foods is minimal because folic acid does not occur significantly in foods containing nitrite. Oxidative degradation of folates by exposure to hypochlorite may yield significant losses of folates in certain foods.

8.8.6.2.4 Bioavailability of Folate in Foods

The absorption of folates takes place mainly in the jejunum and requires hydrolysis of the polyglutamyl chain by a specific peptidase (pteroylpolyglutamate hydrolase), followed by absorption via a carrier-mediated transport process [50,155]. Bioavailability of naturally occurring folate in foods is incomplete, often averaging 50% or less [48,71]. Moreover, the bioavailability of *naturally occurring* folates in most foods has not been fully determined under conditions of actual consumption, including the consequences of interactions among various foods. The mean bioavailability of polyglutamyl folates varies widely and is typically 70% *relative to the monoglutamyl species*, which suggests a rate-limiting nature of intestinal deconjugation. Although it has been reported based on early studies that the bioavailability of folic acid added to cereal grain products is only 30%–60% [21], later investigations showed that folic acid is highly bioavailable in fortified cereal grain food products [48,71].

Factors responsible for incomplete bioavailability include (1) effects of the food matrix, presumably through noncovalent binding of folates or entrapment in cellular structure; (2) possible degradation of labile H_4folates in the acidic gastric environment; and (3) incomplete intestinal enzymatic conversion of polyglutamyl folates to the absorbable monoglutamyl forms. Many foods contain compounds that inhibit intestinal pteroylpolyglutamate hydrolase when studied in vitro; however, the significance of these effects with respect to in vivo folate bioavailability is unclear. Many raw fruits, vegetables, and meats also contain active conjugases capable of deconjugating polyglutamyl folates. Homogenization, freezing and thawing, and other procedures that disrupt cells may release these enzymes and promote the deconjugation process. The extent to which this would improve bioavailability of dietary folates has not been determined. Little or no deconjugation of polyglutamyl folates occurs during food preparation and processing unless cells are disrupted.

8.8.6.2.5 Analytical Methods

Techniques potentially suitable for the measurement of folate in foods include microbiological growth methods, HPLC and LCMS methods, and competitive-binding radioassay procedures [114]. Measurement of folate is complicated by the need to account for all forms of the vitamin, which could easily include several dozen compounds if each form of the folate nucleus existed in all possible combinations with several different polyglutamate chain lengths. Prior to the early 1960s, folate assays often yielded grossly inaccurate results because a necessary reducing agent in the extraction buffer and in the microbiological assay medium was not included. Either ascorbate, a thiol reagent such as mercaptoethanol, or a combination of ascorbate and thiol are needed to stabilize folates during extraction and analysis.

Extraction of folate from food samples involves (1) disruption of the food matrix and cellular structure by homogenization in a buffer solution; (2) heat (typically 100°C) to release folate from folate-binding proteins, to inactivate enzymes capable of catalyzing interconversion of folates, and to deproteinate the sample; (3) centrifugation to yield a clarified extract; and (4) treatment with a pteroylpolyglutamate hydrolase ("conjugase"), if the assay responds only to monoglutamyl or other short-chain folates. Other enzymes such as a protease and/or amylase may be useful in improving extraction of folate from certain foods (e.g., cereal grains). Needs remain for standardization extraction and enzymatic pretreatment methods so interlaboratory precision and accuracy of folate assays can be improved.

Microbiological growth assays serve as the traditional method of folate analysis and are based on the nutritional requirements of microorganisms (*Lactobacillus rhamnosus,* formerly

Lactobacillus casei, *Pediococcus cerevisiae*, and *Streptococcus faecium*). *P. cerevisiae* and *S. faecium* (used in an AOAC official method) have little use in food analysis because they do not respond to all forms of the vitamin. In contrast, *L. rhamnosus* responds to all forms of folate and serves as the most appropriate test organism for microbiological assays of total folate in food. With appropriate control of pH in the growth medium, *L. rhamnosus* yields equivalent response to all forms of folate. Since foods typically contain several folates, verification of equivalent response in microbiological assays is essential.

Competitive-binding assays involve competition between folate in the sample or standard with radiolabeled folate for the binding site of a folate-binding protein, typically from milk. In spite of the speed and convenience of these assays, their application to food analysis is limited because of varying affinity for different forms of folate. Comparisons of competitive-binding assays with the *L. rhamnosus* method have yielded poor agreement, presumably for this reason.

8.8.7 Biotin

8.8.7.1 Structure and General Properties

Biotin is a bicyclic, water-soluble vitamin that functions coenzymatically in carboxylation and transcarboxylation reactions. The two naturally occurring forms are free D-biotin and biocytin (ε-*N*-biotinyl-L-lysine) (Figure 8.41). Biocytin functions as the coenzyme form and actually consists of a biotinylated lysyl residue, formed by posttranslational biotinylation of various carboxylases. The ring system of biotin can exist in eight possible stereoisomers, only one of which (D-biotin) is the natural, biologically active form. Both free biotin and protein-bound biocytin exhibit biotin activity when consumed in the diet, whereas the naturally occurring catabolic products of biotin in animal tissues (bisnorbiotin and biotin sulfoxide) do not exhibit vitamin activity. Biotin is widely distributed in plant and animal products, and biotin deficiency is rare in normal humans.

8.8.7.2 Stability of Biotin

Biotin is very stable to heat, light, and oxygen. Extremes of high or low pH can cause degradation, possibly because they promote hydrolysis of the −N−C=O (amide) bond of the biotin ring system. Oxidizing conditions such as exposure to hydrogen peroxide can oxidize the sulfur to form biologically inactive biotin sulfoxide or sulfone. Reaction of the biotin ring carbonyl with amines also may occur, although this has not been examined. Losses of biotin during food processing and storage have been documented and summarized [66,94]. Such losses may occur by chemical degradation processes as mentioned earlier and by leaching of free biotin. Little degradation of biotin occurs during low-moisture storage of fortified cereal products. Overall, biotin is quite well retained in foods.

The stability of biotin during storage of human milk also has been examined [101,102]. The biotin concentration of the milk samples did not change over 1 week at ambient temperature, 1 month at 5°C, or at −20°C or lower for 1.5 years.

FIGURE 8.41 Structures of biotin and biocytin.

8.8.7.3 Analytical Methods

Measurement of biotin in foods is performed by microbiological assay (usually with *Lactobacillus plantarum*) or by various ligand-binding procedures involving avidin as the binding protein. Several HPLC methods also have been developed. Most of these involve the use of an avidin-binding procedure to provide sensitivity and increased sensitivity. The microbiological, HPLC, and ligand-binding assays respond to free biotin and biocytin, but biocytin cannot be determined unless it is first released from the protein by cleavage of the peptide bond by enzymatic or acid hydrolysis [101,102]. Care should be taken because acid hydrolysis can degrade a substantial proportion of the biotin. The existence of nutritionally inactive biotin analogues, such as bisnorbiotin and biotin sulfoxide detected in some animal tissues and in human urine, may complicate analyses. Such analogues may respond in avidin-binding procedures and certain microbiological assays. Separation of the biotin derivatives by HPLC prior to the avidin-binding assay of alleviates such problems by allowing their individual measurement.

8.8.7.4 Bioavailability

Relatively little is known about the bioavailability of biotin in foods. There appears to be sufficient biotin in normal diets that incomplete bioavailability usually has little adverse nutritional impact. Bacterial synthesis of biotin in the lower intestine provides an additional source of partially available biotin for humans. The majority of naturally occurring biotin in many foods exists as protein-bound biocytin. This is released by biotinidase in pancreatic juice and in the intestinal mucosa to convert the bound biotin to the functionally active free form; however, some absorption of biotinyl peptides also may occur.

Absorption of biotin is almost totally prevented by the consumption of raw egg albumen that contains the biotin-binding protein avidin. Avidin is a tetrameric glycoprotein in egg albumen that is capable of binding one biotin per subunit. This protein binds biotin very tightly (dissociation constant ~10^{-15} M) and resists digestion. Little or no avidin-bound biotin is absorbed. Chronic consumption of raw eggs or raw egg albumen will, thus, impair biotin absorption and can lead to deficiency. Small amounts of avidin in the diet have no nutritional consequence. The use of dietary avidin (or egg albumen) permits the experimental development of biotin deficiency in laboratory animals. Cooking denatures avidin and eliminates its biotin-binding properties.

While little information exists regarding the bioavailability of biotin in humans, much more is known about its bioavailability in animal feedstuffs. As shown in Table 8.28, the bioavailability of biotin is low in some materials.

TABLE 8.28
Bioavailability of Biotin in Feedstuffs for Pigs and Turkeys

	Biotin Bioavailability (%)	
Material	**Pigs (Sauer et al. [126])**	**Turkeys (Misir and Blair [99])**
Soybean meal	55.4	76.8
Meat and bone meal	2.7	ND[a]
Canola meal	3.9	65.4
Barley	4.8	19.2
Corn	4.0	95.2
Wheat	21.6	17.0
Supplemental biotin	93.5	ND
Sorghum	ND	29.5

[a] ND, not determined.

8.8.8 Pantothenic Acid

8.8.8.1 Structure and General Properties

Pantothenic acid, or D-*N*-(2,4-dihydroxy-3,3-dimethyl-butyryl-β-alanine), is a water-soluble vitamin comprised of β-alanine in amide-linkage to 2,4-dihydroxy-3,3-dimethyl-butyric (pantoic) acid (Figure 8.42). Pantothenic acid functions metabolically as a component of coenzyme A (Figure 8.42) and as a covalently bound prosthetic group (without the adenosyl moiety of coenzyme A) of acyl carrier protein in fatty acid synthesis. Formation of a thioester derivative of coenzyme A with organic acids facilitates a wide variety of metabolic processes that mainly involve addition or removal of acyl groups, in an array of biosynthetic and catabolic reactions. Pantothenic acid is essential for all living things and is distributed widely among meats, cereal grains, eggs, milk, and many fresh vegetables.

Pantothenic acid in many foods and most biological materials is mainly in the form of coenzyme A, the majority of which exists as thioester derivatives of a wide variety of organic acids. Although analytical data are quite limited with respect to the free and coenzyme A forms of pantothenic acid in foods, free pantothenic acid has been found to account for only half of the total content of this vitamin in beef muscle and peas [57]. Coenzyme A is fully available as a source of pantothenic acid because it is converted to free pantothenic acid in the small intestine by the action of alkaline phosphatase and an amidase. Intestinal absorption occurs through a carrier-mediated absorption process.

Synthetic pantothenic acid is used in food fortification and in vitamin supplements in the form of calcium pantothenate. This compound is a white crystalline material that exhibits greater stability and is less hygroscopic than the free acid. Panthenol, the corresponding alcohol, also has been used as a feed supplement for animals. Panthenol also is used as an ingredient in certain shampoos for apparent physical (i.e., lubricating) effects, rather than nutritional effects, when applied to hair.

8.8.8.2 Stability and Modes of Degradation

In solution, pantothenic acid is most stable at pH 5–7. Pantothenic acid exhibits relatively good stability during food storage, especially at reduced water activity. Losses occur in cooking and thermal processing in proportion to the severity of the treatment and extent of leaching, and these range from 30% to 80%. Leaching of pantothenic acid or its loss in tissue fluids can be very significant. Although the mechanism of thermal loss of pantothenic acid has not been fully determined, an acid- or base-catalyzed hydrolysis of the linkage between β-alanine and the 1,4-dihydroxy,3,3-butyryl-carboxylic acid group appears likely. The pantothenic acid molecule is otherwise quite unreactive and interacts little with other food components. Coenzyme A is susceptible to the formation of mixed disulfides with other thiols in foods; however, this exerts little effect on the net quantity of available pantothenic acid.

Degradation of pantothenic acid during thermal processing conforms to first-order kinetics [57]. Rate constants for degradation of free pantothenic acid in buffered solutions increase with decreasing pH over the range of pH 6.0–4.0, while the energy of activation decreases over this range. The rates of degradation reported for pantothenic acid are substantially less than those for other labile nutrients (e.g., thiamin). These findings suggest that losses of pantothenic acid reported in other studies of food processing may be predominantly due to leaching rather than actual destruction. The net result would be the same, however.

$$HOOC-CH_2-CH_2-N(H)-C(=O)-CH(OH)-C(CH_3)_2-CH_2-OH$$

FIGURE 8.42 Structure of various forms of pantothenic acid.

8.8.8.3 Bioavailability

The mean bioavailability of pantothenate in a mixed diet has been reported to be ~50% [139]. There is little concern regarding any adverse consequences of this incomplete bioavailability because pantothenic acid intake is generally adequate. No evidence of nutritionally significant problems of incomplete bioavailability has been reported, and the complexed coenzymic forms of the vitamin are readily digested and absorbed.

8.8.8.4 Analytical Methods

Pantothenic acid in foods may be measured primarily by microbiological assay using *L. plantarum*, radioimmunoassay, or GCMS [43,122]. A key factor that affects the validity of pantothenic acid analysis is the pretreatment needed to release bound forms of the vitamin [43]. Various combinations of proteases and phosphatases have been used to release pantothenic acid from the many coenzyme A derivatives and protein-bound forms.

8.8.9 Vitamin B12

8.8.9.1 Structure and General Properties

Vitamin B12 is the generic term for the group of compounds (cobalamins) having vitamin activity similar to that of cyanocobalamin. These compounds are corrinoids, which are tetrapyrrole structures in which a cobalt ion is coordinately covalently bonded to the four pyrrole nitrogens. The fifth coordinate covalent bond to Co is with a nitrogen atom of the dimethylbenzimidazole moiety, while the sixth position may be occupied by cyanide, a 5′-deoxyadenosyl group, a methyl group, glutathione, water, a hydroxyl ion, or other ligands such as nitrite, ammonia, or sulfite (Figure 8.43). All forms of vitamin B12 shown in Figure 8.43 exhibit vitamin B12 activity. Cyanocobalamin, a synthetic form of vitamin B12 used in food fortification and nutrient supplements, exhibits

Ligand (R)	B12 Form
–CN	Cyanocobalamin
–OH	Hydroxocobalamin
$–H_2O$	Aquocobalamin
–Glutathione	Glutathionylcobalamin
$–CH_3$	Methylcobalamin
–5′-Deoxyadenosine	5′-Deoxyadenosyl-cobalamin

FIGURE 8.43 Structure of various forms of vitamin B12.

TABLE 8.29
Classification of Foods according to Their Vitamin B12 Concentration

Food	Vitamin B12 (μg/100 g Wet wt.)
Rich sources: organ meats (liver, kidney, heart), bivalves (clams and oysters)	>10
Moderately rich sources: nonfat dry milk, some fish and crabs, egg yolks	3–10
Moderate sources: muscle meats, some fish, fermenting cheeses	1–3
Others: fluid milk, cheddar cheese, cottage cheese	<1

Source: Adapted from Herbert, V., Vitamin B-12, in: *Present Knowledge in Nutrition*, Brown, M., ed., International Life Sciences Institute, Nutrition Foundation, Washington, DC, 1990, pp. 170–178.

superior stability and is readily available commercially. The coenzyme forms of vitamin B12 are methylcobalamin and 5′-deoxyadenosylcobalamin. Methylcobalamin functions coenzymatically in the transfer of a methyl group (from 5-methyltetrahydrofolate) in methionine synthase, while 5′-deoxyadenosylcobalamin serves as the coenzyme in an enzymatic rearrangement reaction catalyzed by methylmalonyl-CoA mutase. Little or no naturally occurring cyanocobalamin exists in foods; in fact, the original identification of vitamin B12 as cyanocobalamin involved its formation as an artifact of the isolation procedure. Cyanocobalamin has a reddish color in the crystalline state and in solution. This coloration may pose a limitation in the possible addition of cyanocobalamin to certain foods, especially lightly colored products (e.g., white bread).

Unlike other vitamins that are synthesized primarily in plants, only microorganisms produce cobalamins. Certain legumes have been reported to absorb small amounts of vitamin B12 produced by bacteria associated with root nodules, but little enters the seeds [104]. Most plant-derived foods are devoid of vitamin B12 unless contaminated by fecal material, for example, from fertilizer [62]. The vitamin B12 in most animal tissues consists mainly of the coenzyme forms, methylcobalamin and 5′-deoxyadenosylcobalamin, in addition to aquocobalamin. Herbert [63] has classified foods according to their vitamin B12 content, as shown in Table 8.29.

Approximately 20 naturally occurring analogues of vitamin B12 have been identified. Some of these have no biological activity in mammals, some may be vitamin B12 antagonists, and others exhibit at least partial vitamin activity but are often poorly absorbed.

8.8.9.2 Stability and Modes of Degradation

Under most conditions of food processing, preservation, and storage, there is little nutritionally significant loss of vitamin B12. Cyanocobalamin added to breakfast cereal products has been reported to undergo an average loss of 17% during processing, with an additional 17% loss during storage for 12 months at ambient temperature [135]. In studies of the processing of fluid milk, 96% mean retention has been observed during HTST pasteurization and similar retention (>90%) was found in milk processed using various modes of ultrahigh-temperature (UHT) processing [39]. Although refrigerated storage of milk has little impact on vitamin B12 retention, storage of UHT-processed milk at ambient temperature for up to 90 days causes progressive losses that can approach 50% of the initial vitamin B12 concentration [17]. Sterilization of milk for 13 min at 120°C has been reported to cause only 23% retention of vitamin B12 [77], but prior concentration (as in production of evaporated milk) contributes to more severe losses. This indicates the potential for substantial losses of vitamin B12 during prolonged heating of foods at or near neutral pH. Typical oven heating of commercially prepared convenience dinners has been shown to yield 79%–100% retention of vitamin B12.

Ascorbic acid has long been known to accelerate the degradation of vitamin B12, although this may be of little practical significance because foods containing vitamin B12 usually do not contain significant amounts of ascorbic acid. The use of ascorbate or erythorbate in curing solutions for ham

has no influence on vitamin B12 retention [35]. Thiamin and nicotinamide in solution can accelerate degradation of vitamin B12, but the relevance of this phenomenon to foods is probably minimal.

The mechanism of vitamin B12 degradation has not been fully determined, in part because of the complexity of the molecule and the very low concentration in foods. Photochemical degradation of vitamin B12 coenzymes yields aquocobalamin. Such a reaction interferes with experimental studies of B12 metabolism and function, but this conversion has no influence on the total vitamin B12 activity of foods because aquocobalamin retains vitamin B12 activity. The overall stability of vitamin B12 is greatest at pH 4–7. Exposure to acid causes the hydrolytic removal of the nucleotide moiety, and additional fragmentation occurs as the severity of the acidic conditions increases. Exposure to acid or alkaline conditions causes hydrolysis of amides, yielding biologically inactive carboxylic acid derivatives of vitamin B12.

Interconversions among cobalamins can occur through exchange of the ligand bonded to the Co atom. For example, bisulfite ion causes conversion of aquocobalamin to sulfitocobalamin, while similar reactions can occur to form cobalamins substituted with ammonia, nitrite, or hydroxyl ions. These reactions have little influence on the net vitamin B12 activity of foods.

8.8.9.3 Bioavailability

The bioavailability of vitamin B12 has been examined mainly in the context of the diagnosis of vitamin B12 deficiency associated with malabsorption. Little is known about the influence of food composition on the bioavailability of vitamin B12. Several studies have shown that pectin and, presumably, similar gums reduce vitamin B12 bioavailability in rats. The significance of this effect in humans remains unclear. Although little or no vitamin B12 is present in most plants, certain forms of algae do contain significant quantities of the vitamin. Forms of algae are not recommended as a source of vitamin B12 because of its very low bioavailability [24].

In normal human beings, absorption of vitamin B12 from eggs has been shown to be less than half that of cyanocobalamin administered in the absence of food [32]. Similar results have been obtained regarding vitamin B12 bioavailability in studies with fish and various meats [31,33]. Certain individuals are marginally deficient in vitamin B12 because of poor protein digestion and incomplete release of cobalamins from the food matrix even though they absorb the pure compound normally [18]. Such malabsorption of vitamin B12 from food is most prevalent in the elderly. Recent studies show that cyanocobalamin added to bread or milk is well absorbed by elderly people, which suggests that fortification of these products is technically feasible [123].

8.8.9.4 Analytical Methods

The concentration of vitamin B12 in foods is determined primarily by microbiological growth assays using *Lactobacillus leichmannii* or by radioligand binding and similar procedures. Although the various forms of vitamin B12 can be separated chromatographically, HPLC methods are not readily suitable for food analysis because of the very low concentrations typically found except in fortified products. Early types of radioligand-binding assays for vitamin B12 in clinical specimens and foods were often inaccurate because the binding protein employed could bind to active forms of vitamin B12 as well as biologically inactive analogues. The specificity of such assays has been greatly improved through the use of a vitamin B12–binding protein (generally porcine intrinsic factor) that is specific for the biologically active forms of the vitamin. Microbiological assays with *L. leichmannii* may be subject to interference if samples contain high concentrations of deoxyribonucleosides.

Food samples are generally prepared by homogenization in a buffered solution, followed by incubation at elevated temperature (~60°C) in the presence of papain and sodium cyanide. This treatment releases protein-bound forms of vitamin B12 and converts all cobalamins to the more stable cyanocobalamin form. Conversion to cyanocobalamin also improves the performance of assays that may differ in response to the various forms of the vitamin.

8.9 CONDITIONALLY ESSENTIAL VITAMIN-LIKE COMPOUNDS

8.9.1 Choline and Betaine

Choline (Figure 8.44) exists in all living things both in free form and as a constituent of a number of cellular components including phosphatidylcholine (the most prevalent dietary source of choline), sphingomyelin, and acetylcholine. Although choline synthesis occurs in humans and other mammals, there is a growing body of evidence that an adequate supply of dietary choline is also required [72] and a nutritional requirement recently has been established for choline [71]. However, healthy individuals consuming a varied diet rarely have inadequate choline intakes because choline exists in abundance (as choline, phosphocholine, and the membrane constituents sphingomyelin and phosphatidylcholine) in many food sources. Choline is used as chloride and bitartrate salts in fortification of infant formulas. It is not ordinarily added to other foods except as an ingredient, for example, phosphatidylcholine as an emulsifier. Choline is a very stable compound. No significant loss of choline occurs during food storage, handling, processing, or preparation.

Betaine (N-trimethylglycine, Figure 8.44) is a component in the metabolic breakdown of choline. It occurs naturally in the diet and is especially high in beets, wheat, spinach, shrimp, and related food sources [154]. Betaine serves metabolically as an alternative to 5-methyl-H_4folate in a reaction that converts homocysteine to methionine for protein synthesis and, after formation of S-adenosylmethionine (SAM), many cellular methylation reactions. This process helps conserve methionine, control homocysteine levels, and facilitate SAM-dependent methylation processes in a manner that does not depend on a steady supply of folate. Because betaine is obtained from common foods and is generated in vivo from the generally ubiquitous choline, there is rarely a metabolic limitation in betaine. In situations in which plasma homocysteine is elevated for nutritional or genetic reasons, supplemental betaine is occasionally administered, along with vitamin supplements (B6, B12, and folic acid), in an effort to maximize the conversion of homocysteine to methionine.

8.9.2 Carnitine

Carnitine (Figure 8.45) can be synthesized by the human body; however, certain individuals appear to benefit from additional dietary carnitine [115]. No nutritional requirements have been established for carnitine. Although little or no carnitine is found in plants and plant products, it is widely distributed in foods of animal origin. Carnitine functions metabolically in the transport of organic acids across biological membranes and, thus, facilitates their metabolic utilization and/or disposal. Carnitine also facilitates transport of certain organic acids to lessen the potential for toxicity in

$$HO{-}CH_2{-}CH_2{-}\overset{+}{N}(CH_3)_3$$

FIGURE 8.44 Structure of choline.

$$(CH_3)_3\overset{+}{N}{-}CH_2{-}CH(OH){-}CH_2{-}COO^-$$

FIGURE 8.45 Structure of carnitine.

certain cells. In animal-derived foods, carnitine exists in free and acylated form. The acyl carnitines occur with various organic acids esterified to the carnitine C^3 hydroxyl group. Carnitine is highly stable and undergoes little or no degradation in foods.

Synthetic carnitine is used in certain clinical applications as the biologically active L-isomer. D-Carnitine has no biological activity. L-Carnitine is added to infant formulas to raise their carnitine concentration to that of human milk.

8.9.3 Pyrroloquinoline Quinone

Pyrroloquinoline quinone (PQQ) is a tricylic quinone (Figure 8.46) that functions as a coenzyme in several bacterial oxidoreductases and has been reported to be a coenzyme in mammalian lysyl oxidase and amine oxidases [82]. However, later findings indicate that the coenzyme originally designated as PQQ in these mammalian enzymes was misidentified and is probably 6-hydroxy-dihydroxyphenylalanine quinone [58]. Although no function of PQQ is currently known in mammals, several studies have shown a very small nutritional requirement for rats and mice that appears to be associated with the formation of connective tissue and normal reproduction [82]. Thus, the function of PQQ in mammalian species remains an enigma. Because of the ubiquitous nature of PQQ and its synthesis by intestinal bacteria, the development of spontaneous deficiency of PQQ in rodents or humans is unlikely.

8.9.4 Coenzyme Q_{10}

Coenzyme Q_{10} (also known as ubiquinone) is a substituted quinone whose primary biochemical function involves its action as a coenzyme in the mitochondrial electron transport system [24]. The substituted quinone moiety of coenzyme Q_{10} facilitates its redox function by accommodating two sequential one-electron reductions in vivo (Figure 8.47). The long isoprenoid side chain provides lipid solubility and appears to serve as a membrane anchor during its redox function in mitochondria. The ubiquinol form is a potent antioxidant and serves as a component of the oxidative defense system protecting membrane lipids and, as such, it may have relevance to certain food systems. Coenzyme Q_{10} is not an essential nutrient because it is synthesized in ample quantities by the human body; however, dietary sources (both plant and animal) do appear to contribute at least partially bioavailable coenzyme Q_{10} for utilization by humans. At the present time, there is little evidence that supplemental coenzyme Q_{10} is necessary or beneficial for the maintenance of health. The therapeutic administration of coenzyme Q_{10} may be useful in nutritional support in certain forms of cancer, heart disease, and Parkinson's disease, to counteract antagonistic effects of certain drugs, and in certain inherited disorders of mitochondrial metabolism and for general antioxidant function.

FIGURE 8.46 Structure of pyrroloquinoline quinone.

FIGURE 8.47 Structure of coenzyme Q_{10}.

8.10 OPTIMIZATION OF VITAMIN RETENTION

To varying degrees, inevitable losses of nutritional value occur during the course of the postharvest handling, cooking, processing, and storage of foods. Such losses occur in the food processing industry, in food service establishments, and in the home. Optimization of nutrient retention is a responsibility of food manufacturers and processors and is in the mutual interest of the industry and the public. Likewise, maximization of nutrient retention in the home and in institutional and retail food services is an opportunity that should not be overlooked.

Many approaches to optimization of vitamin retention are based on the chemical and physical properties of the particular nutrients involved. For example, the use of acidulants, if compatible with the product, would promote the stability of thiamin and ascorbic acid. Reduction in pH would decrease the stability of certain folates, however, which illustrates the complexity of this approach. Cooking or commercial processing under conditions that minimize exposure to oxygen and excess liquid lessens the oxidation of many vitamins and the extraction (i.e., leaching) of vitamins and minerals. HTST conditions will, in many instances, cause less vitamin degradation than will conventional thermal processes of equal thermal severity (based on microbial inactivation). In addition, certain combinations of ingredients can enhance retention in of several nutrients (e.g., the presence of natural antioxidants would favor retention of many vitamins).

Several examples of nutrient optimization follow. The reader is referred to additional discussions of this topic [76,92].

8.10.1 Optimization of Thermal Processing Conditions

Losses of nutrients frequently occur during thermal processing procedures intended to provide a shelf-stable product. Such losses often involve both chemical degradation and leaching. The kinetics

and thermodynamics of chemical changes involving the destruction of microorganisms and vitamins differ markedly. Thermal inactivation of microorganisms occurs largely by denaturation of essential macromolecules and involves large energies of activation (typically 200–600 kJ/mol). In contrast, reactions associated with the degradation of vitamins generally exhibit activation energies of 20–100 kJ/mol. Thus, rates of microbial inactivation and rates of vitamin degradation have temperature dependencies that differ significantly. Consequently, the rate of microbial inactivation increases as a function of temperature much more rapidly than does the rate of vitamin degradation. These principles of reaction kinetics and thermodynamics form the basis of enhancement of nutrient retention when HTST conditions are used. Classical studies by Teixeira et al. [140] involved a variety of thermal processing conditions, all of which provided equivalent microbial lethality. These authors showed that thiamin retention during thermal processing of pea puree could be enhanced at least 1.5-fold through selection of the proper time–temperature combination. Although many other vitamins are less labile than thiamin during the processing of low-acid foods, a similar enhancement of their retention would be predicted.

8.10.2 Prediction of Losses

Predicting the magnitude of losses of vitamins requires accurate knowledge of degradation kinetics and temperature dependence of the particular form(s) of the vitamin(s) considered in the chemical milieu of the food(s) of interest. Different chemical forms of vitamins react differently to various food compositions and to specific processing conditions. One must first determine whether kinetic studies of *total content* (i.e., sum of all forms) of the vitamin of interest yield useful information or whether more specific information on the various forms of the vitamin is needed. Processing studies must be conducted under conditions identical to those prevailing during the actual commercial processing or storage condition being modeled because of the sensitivity of many nutrients to their chemical and physical environments. As summarized previously [64,90], reaction kinetics should be obtained at several temperatures to permit calculation of rate constants and an energy of activation. In addition, the experimental conditions should be selected to provide sufficient loss of the vitamin being studied so that the rate constant can be determined with appropriate precision [64]. Accelerated storage studies may be performed if the kinetics and mechanisms at elevated temperature are consistent with those occurring under the actual storage conditions. Because temperatures fluctuate during actual storage and transportation of foods, models of vitamin stability should include provisions for assessing the effects of temperature fluctuation [42,88].

8.10.3 Effects of Packaging

Packaging influences vitamin stability in several ways. In canning, foods that transmit heat energy primarily by conduction (solids or semi-solids) will undergo greater overall loss of nutrients than will foods that transmit heat by convection, especially if large containers are used. This difference is caused by the requirement that the thermal process must be based on the "slowest to heat" portion of the product, which, for conduction-heating foods, is the geometric center of the container. Such losses are minimized by using containers with a large surface-to-mass ratio, that is, small cans and noncylindrical containers such as retortable pouches [118]. Pouches also offer the advantage of requiring less liquid for filling; thus, leaching of nutrients during the processing of particulate foods can be minimized.

The permeability of the packaging material also can have a substantial effect on the retention of vitamins during food storage. Ascorbic acid in juices and fruit beverages have been shown to exhibit much greater stability when packages with low permeability to oxygen are used [74]. In addition, use of opaque packaging materials prevents the photochemical degradation of photolabile vitamins such as vitamin A and riboflavin and of other nutrients that are susceptible to photosensitized modes of degradation.

8.11 SUMMARY

As discussed in this chapter, vitamins are organic chemicals that exhibit a wide range of properties with respect to stability, reactivity, susceptibility to environmental variables, and influence on other constituents of foods. Prediction of net vitamin retention or mechanisms of degradation under a given set of circumstances is often fraught with difficulty because of the multiple forms of most vitamins. With that caveat, the reader is referred to Table 8.1 for an overview of the general characteristics of each vitamin.

REFERENCES

1. American Society for Nutrition. Nomenclature policy: Generic descriptors and trivial. Names for vitamins and related compounds. *J Nutr* 120: 12–19, 1990.
2. Ames SR. Bioassay of vitamin A compounds. *Fed Proc* 24: 917–923, 1965.
3. Anderson RH, Maxwell DL, Mulley AE, and Fritsch CW. Effects of processing and storage on micronutrients in breakfast cereals. *Food Technol* 30: 110–114, 1976.
4. Anonymous. The nutritive quality of processed food. General policies for nutrient addition. *Nutr Rev* 40: 93–96, 1982.
5. Arabshahi A and Lund D. Thiamin stability in simulated intermediate moisture food. *J Food Sci* 53: 199–203.
6. Barrett DM and Lund DB. Effect of oxygen on thermal degradation of 5-methyl-5,6,7,8-tetrahydrofolic acid. *J Food Sci* 54: 146–149, 1989.
7. Bauernfeind J and LaChance P. *Nutrient Additions to Food. Nutritional, Technological and Regulatory Aspects*. Trumbull, CT: Food and Nutrition Press, Inc., 1992.
8. Blair JA, Pearson AJ, and Robb AJ. Autoxidation of 5-methyl-5,6,7,8-tetrahydrofolic acid. *J Chem Soc Perkin Transactions*. II: 18, 1975.
9. Bode AM, Cunningham L, and Rose RC. Spontaneous decay of oxidized ascorbic acid (dehydro-L-ascorbic acid) evaluated by high-pressure liquid chromatography. *Clin Chem* 36: 1807–1809, 1990.
10. Booth SL, Pennington JA, and Sadowski JA. Dihydro-vitamin K1: Primary food sources and estimated dietary intakes in the American diet. *Lipids* 31: 715–720, 1996.
11. Bowers J and Craig J. Components of vitamin B6 in turkey breast muscle. *J Food Sci* 43: 1619–1621, 1978.
12. Brown ED, Micozzi MS, Craft NE, Bieri JG, Beecher G, Edwards BK, Rose A, Taylor PR, and Smith JC, Jr. Plasma carotenoids in normal men after a single ingestion of vegetables or purified beta-carotene. *Am J Clin Nutr* 49: 1258–1265, 1989.
13. Buettner GR. In the absence of catalytic metals ascorbate does not autoxidize at pH 7: Ascorbate as a test for catalytic metals. *J Biochem Biophys Methods* 16: 27–40, 1988.
14. Buettner GR. The pecking order of free radicals and antioxidants: Lipid peroxidation, alpha-tocopherol, and ascorbate. *Arch Biochem Biophys* 300: 535–543, 1993.
15. Burton GW and Ingold KU. beta-Carotene: An unusual type of lipid antioxidant. *Science* 224: 569–573, 1984.
16. Burton GW and Traber MG. Vitamin E: Antioxidant activity, biokinetics, and bioavailability. *Annu Rev Nutr* 10: 357–382, 1990.
17. Burton H, Ford JE, Franklin JG, and Porter J. Effect of repeated heat treatments on the levels of some vitamins of the B-complex in milk. *J Dairy Res* 34: 193–197, 1967.
18. Carmel R, Sinow RM, Siegel ME, and Samloff IM. Food cobalamin malabsorption occurs frequently in patients with unexplained low serum cobalamin levels. *Arch Intern Med* 148: 1715–1719, 1988.
19. Carpenter KJ, Schelstraete M, Vilicich VC, and Wall JS. Immature corn as a source of niacin for rats. *J Nutr* 118: 165–169, 1988.
20. Chandler L and Schwartz S. HPLC separation of *cis-trans* carotene isomers in fresh and processed fruits and vegetables. *J Food Sci* 52: 669–672, 1987.
21. Colman N, Green R, and Metz J. Prevention of folate deficiency by food fortification. II. Absorption of folic acid from fortified staple foods. *Am J Clin Nutr* 28: 459–464, 1975.
22. Cort WM, Borenstein B, Harley J, Osadca M, and Scheiner J. Nutrient stability of fortified cereal products. *Food Technol* 30: 52–62, 1976.
23. Coursin DB. Convulsive seizures in infants with pyridoxine-deficient diet. *J Am Med Assoc* 154: 406–408, 1954.
24. Dagnelie PC, van Staveren WA, and van den Berg H. Vitamin B-12 from algae appears not to be bioavailable. *Am J Clin Nutr* 53: 695–697, 1991.

25. Day BPF and Gregory JF. Thermal stability of folic acid and 5-methyltetrahydrofolic acid in liquid model food systems. *J Food Sci* 48: 581–587, 1983.
26. Dellamonica E and McDowell P. Comparison of beta-carotene content of dried carrots prepared by three dehydrated processes. *Food Technol* 19: 1597–1599, 1965.
27. DellaPenna D. Nutritional genomics: Manipulating plant micronutrients to improve human health. *Science* 285: 375–379, 1999.
28. Dennison D, Kirk J, Bach J, Kokoczka P, and Heldman D. Storage stability of thiamin and riboflavin in a dehydrated food system. *J Food Process Preserv* 1: 43–54, 1977.
29. Deutsch JC and Kolhouse JF. Ascorbate and dehydroascorbate measurements in aqueous solutions and plasma determined by gas chromatography-mass spectrometry. *Anal Chem* 65: 321–326, 1993.
30. Díaz de la Garza R, Gregory J, and Hanson A. Folate biofortification of tomato fruit. *Proc Natl Acad Sci USA* 104: 4218–4222, 2007.
31. Doscherholmen A, McMahon J, and Economon P. Vitamin B12 absorption from fish. *Proc Soc Exp Biol Med* 167: 480–484, 1981.
32. Doscherholmen A, McMahon J, and Ripley D. Vitamin B12 absorption from eggs. *Proc Soc Exp Biol Med* 149: 987–990, 1975.
33. Doscherholmen A, McMahon J, and Ripley D. Vitamin B12 assimilation from chicken meat. *Am J Clin Nutr* 31: 825–830, 1978.
34. Dwivedi BK and Arnold RG. Chemistry of thiamine degradation in food products and model systems: A review. *J Agric Food Chem* 21: 54–60, 1973.
35. Eitenmiller RR and de Souza S. Niacin. In: *Methods of Vitamin Assay*, Augustin J, Klein B, Becker D, and Venugopal P, eds., 1985, John Wiley & Sons, New York, pp. 385–398.
36. Evans S, Gregory J, and Kirk J. Thermal degradation kinetics of pyridoxine hydrochloride in dehydrated model food systems. *J Food Sci* 48: 555–558, 1981.
37. Farrer K. The thermal destruction of vitamin B1 in foods. *Adv Food Res* 6: 257–311, 1955.
38. Finglas P and Falks R. Critical review of HPLC methods for the determination of thiamin, riboflavin and niacin in food. *J Micronutr Anal* 3: 555–558, 1987.
39. Ford JE, Porter J, Thompson S, Toothill J, and Edwards-Webb J. Effects of ultra-high-temperature (UHT) processing and of subsequent storage on the vitamin content of milk. *J Dairy Res* 36: 447–454, 1969.
40. Frankel EN, Huang S-W, Kanner J, and German J. Interfacial phenomena in the evaluation of antioxidants: Bulk oils vs. emulsions. *J Agric Food Chem* 42: 1054–1059, 1994.
41. Freed M, Brenner S, and Wodicka V. Prediction of thiamine and ascorbic acid stability in canned stored foods. *Food Technol* 3: 148–151, 1948.
42. Giannakourou MC and Taoukis, P. Kinetic modelling of vitamin C loss in frozen green vegetables under variable storage conditions. 83: 33–41, 2003.
43. Gonthier A, Fayol V, Viollet J, and Hartmann D. Determination of pantothenic acid in foods: Influence of the extraction method. *Food Chem* 63: 287–294, 1998.
44. Gregory J. Ascorbic acid bioavailability in foods and supplements. *Nutr Rev* 51: 301–303, 1993.
45. Gregory J. Chemical reactions of vitamins during food processing. In: *Chemical Changes in Food Processing*, Richardson T and Finley J, eds., Westport, CT: AVI Publishing Co., 1985, pp. 373–408.
46. Gregory J and Hiner M. Thermal stability of vitamin B6 compounds in liquid model food systems. *J Food Sci* 48: 2434–2437, 1983.
47. Gregory J, Ink S, and Sartain D. Degradation and binding to food proteins of vitamin B-6 compounds during thermal processing. *J Food Sci* 51: 1345–1351, 1986.
48. Gregory J, Quinlivan E, and Davis S. Integrating the issues of folate bioavailability, intake and metabolism in the era of fortification. *Trends Food Sci Technol* 16: 229–240, 2005.
49. Gregory JF. Accounting for differences in the bioactivity and bioavailability of vitamers. *Food Nutr Res* 56: 5809, 2012.
50. Gregory JF, III. Chemical and nutritional aspects of folate research: Analytical procedures, methods of folate synthesis, stability, and bioavailability of dietary folates. *Adv Food Nutr Res* 33: 1–101, 1989.
51. Gregory JF. Bioavailability of folate. *Eur J Clin Nutr* 51(Suppl 1): S54–S59, 1997.
52. Gregory JF. Bioavailability of thiamin. *Eur J Clin Nutr* 51(Suppl 1): S34–S37, 1997.
53. Gregory JF. Bioavailability of vitamin B-6. *Eur J Clin Nutr* 51(Suppl 1): S43–S48, 1997.
54. Gregory JF. Effects of epsilon-pyridoxyllysine bound to dietary protein on the vitamin B-6 status of rats. *J Nutr* 110: 995–1005, 1980.
55. Gregory JF and Ink SL. Identification and quantification of pyridoxine-beta-glucoside as a major form of vitamin B6 in plant-derived foods. *J Agric Food Chem* 35: 76–82, 1987.

56. Gruenwedel D and Patnaik R. Release of hydrogen sulfide and methyl mercaptan from sulfur-containing amino acids. *J Agric Food Chem* 19: 775–779, 1971.
57. Hamm DJ and Lund DB. Kinetic parameters for thermal inactivation of pantothenic acid. *J Food Sci* 43: 631–633, 1978.
58. Harris ED. The pyrroloquinoline quinone (PQQ) coenzymes: A case of mistaken identity. *Nutr Rev* 50: 263–267, 1992.
59. Harris R. General discussion on the stability of nutrients. In: *Nutritional Evaluation of Food Processing*, Harris R and von Loesecke H, eds. Westport, CT: AVI Publishing Co., 1971, pp. 1–4.
60. Harris R and Karmas E. *Nutritional Evaluation of Food Processing*. Westport, CT: AVI Publishing Co., 1975.
61. Hassinen JB, Durbin GT, and Bernhart FW. The vitamin B6 content of milk products. *J Nutr* 53: 249–257, 1954.
62. Herbert V. Vitamin B-12: Plant sources, requirements, and assay. *Am J Clin Nutr* 48: 852–858, 1988.
63. Herbert V. Vitamin B-12. In: *Present Knowledge in Nutrition*, Brown M, ed. Washington, DC: International Life Sciences Institute, Nutrition Foundation, 1990, pp. 170–178.
64. Hill MK and Grieger-Block R. Kinetic data: Generation, interpretation, and use. *Food Technol* 34: 56–66, 1980.
65. Holden J, Harnly J, and Beecher G. Food composition. In: *Present Knowledge in Nutrition*, Bowman B and Russell R, eds. Washington, DC: International Life Science Institute, 2006, pp. 781–794.
66. Hoppner K and Lampi B. Pantothenic acid and biotin retention in cooked legumes. *J Food Sci* 58: 1084–1085, 1089, 1993.
67. Houghton LA and Vieth R. The case against ergocalciferol (vitamin D2) as a vitamin supplement. *Am J Clin Nutr* 84: 694–697, 2006.
68. Huang M, LaLuzerne P, Winters D, and Sullivan D. Measurement of vitamin D in foods and nutritional supplements by liquid chromatography/tandem mass spectrometry. *J AOAC Int* 92: 1327–1335, 2009.
69. Institute of Medicine. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington, DC: National Academy Press, 2000.
70. Institute of Medicine. Food and Nutrition Board. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc—Institute of Medicine*. Washington, DC: National Academy Press, 2001.
71. Institute of Medicine. Food and Nutrition Board. *Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. Washington, DC: National Academy Press, 1998.
72. Jiang X, Yan J, and Caudill M. Choline. In: *Handbook of Vitamins*, Zempleni J, Suttie J, Gregory J, and Stover P, eds. Boca Raton, FL: CRC Press, 2014, pp. 491–513.
73. Jongehan JA, Mager H, and Berends W. Autoxidation of 5-alkyl-tetrahydropteridines. The oxidation product of 5-methyl-THF. In: *The Chemistry and Biology of Pteridines*, Kisliuk R, ed. Elsevier North Holland, Inc., Amsterdam, the Netherlands, 1979, pp. 241–246.
74. Kacem B, Cornell J, Marshall M, Shireman, and Matthews R. Nonenzymatic browning in aseptically packaged orange drinks: Effect of ascorbic acid, amino acids and oxygen. *J Food Sci* 52: 1668–1672, 1987.
75. Kahn M and Martell A. Metal ion and metal chelate catalyzed oxidation of ascorbic acid by molecular oxygen. II. Cupric and ferric chelate catalyzed oxidation. *J Am Chem Soc* 89: 7104–7111, 1969.
76. Karel M. Prediction of nutrient losses and optimization of processing conditions. In: *Nutritional and Safety Aspects of Food Processing*, Tannenbaum SR, ed. Marcel Dekker, New York, 1979, pp. 233–263.
77. Karlin R. Folate content of large mixture of milks. Effect of different thermic treatments on the amount of folates, B12 and B6 of these milks. *Int Z Vitaminforsch* 39: 359–371, 1969.
78. Karmas E and Harris R. *Nutritional Evaluation of Food Processing*. Van Nostrand Reinhold Co., 1988.
79. Khachik F, Beecher GR, and Lusby WR. Separation, identification, and quantification of the major carotenoids in extracts of apricots, peaches, cantaloupe, and pink grapefruit by liquid chromatography. *J Agric Food Chem* 37: 1465–1473, 1989.
80. Khan M and Martell A. Metal ion and metal chelate catalyzed oxidation of ascorbic acid by molecular oxygen. I. Cupric and ferric ion catalyzed oxidation. *J Am Chem Soc* 89: 4176–4185, 1967.
81. Khan M and Martell A. Metal ion and metal chelate catalyzed oxidation of ascorbic acid by molecular oxygen. II. Cupric and ferric chelate catalyzed oxidation. *J Am Chem Soc* 89: 7104–7111, 1969.
82. Killgore J, Smidt C, Duich L, Romero-Chapman N, Tinker D, Reiser K, Melko M, Hyde D, and Rucker RB. Nutritional importance of pyrroloquinoline quinone. *Science* 245: 850–852, 1989.

83. Kirk J, Dennison D, Kokoczka P, and Heldman D. Degradation of ascorbic acid in a dehydrated food system. *J Food Sci* 42: 1274–1279, 1977.
84. Kirkland J. Niacin. In: *Handbook of Vitamins*, Zempleni J, Suttie J, Gregory J, and Stover P, eds. Boca Raton, FL: CRC Press, 2014.
85. Klein B and Perry A. Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. *J Food Sci* 47: 941–945, 1982.
86. Labuza T. The effect of water activity on reaction kinetics of food deterioration. *Food Technol* 34: 36–41, 59, 1980.
87. Labuza T and Kamman J. A research note. Comparison of stability of thiamin salts at high temperature and water activity. *J Food Sci* 47: 664–665, 1982.
88. Labuza TP. A theoretical comparison of losses in foods under fluctuating temperature sequences. *J Food Sci* 44: 1162–1168, 1979.
89. Leichter J, Switzer V, and Landymore A. Effect of cooking on folate content of vegetables. *Nutr Rep Int* 18: 475–479, 1978.
90. Lenz MK and Lund D. Experimental procedures for determining destruction kinetics of food components. *Food Technol* 34: 51–55, 1978.
91. Liao M-L and Seib P. Selected reactions of L-ascorbic acid related to foods. *Food Technol* 31: 104–107, 1987.
92. Lund D. Designing thermal processes for maximizing nutrient retention. *Food Technol* 31: 71–78, 1977.
93. Lund D. Effects of commercial processing on nutrients. *Food Technol* 33: 28–34, 1979.
94. Lund D. Effects of heat processing on nutrients. In: *Nutritional Evaluation of Food Processing*, Karmas E and Harris R, eds. New York: Van Nostrand Reinhold Co., 1988, pp. 319–354.
95. Malewski W and Markakis P. Ascorbic acid content of developing tomato fruit. *J Food Sci* 36: 537–539, 1971.
96. Mangels AR, Block G, Frey CM, Patterson BH, Taylor PR, Norkus EP, and Levander OA. The bioavailability to humans of ascorbic acid from oranges, orange juice and cooked broccoli is similar to that of synthetic ascorbic acid. *J Nutr* 123: 1054–1061, 1993.
97. Maruyama T, Shiota T, and Krumdieck CL. The oxidative cleavage of folates. A critical study. *Anal Biochem* 84: 277–295, 1978.
98. Mauri L, Alzamora S, Chirife J, and Tomio M. Review: Kinetic parameters for thiamine degradation in foods and model solutions of high water activity. *Int J Food Sci Technol* 24: 1–9, 1989.
99. Misir R and Blair R. Biotin bioavailability of protein supplements and cereal grains for starting turkey poults. *Poult Sci* 67: 1274–1280, 1988.
100. Mnkeni AP and Beveridge T. Thermal destruction of 5-methyltetrahydrofolic acid in buffer and model food systems. *J Food Sci* 48: 595–599.
101. Mock D. Biotin. In: *Handbook of Vitamins*, Zempleni J, Suttie J, Gregory J, and Stover P, eds. Boca Raton, FL: CRC Press, 2014, pp. 397–419.
102. Mock DM, Mock NI, and Langbehn SE. Biotin in human milk: Methods, location, and chemical form. *J Nutr* 122: 535–545, 1992.
103. Moran T. Nutritional significance of recent work on wheat, flour and bread. *Nutr Abstr Rev Ser Hum Exp* 29: 1–16, 1959.
104. Mozafar A, Zentrum E, Oertli JJ, and Zentrum E. Uptake of a microbially-produced vitamin (B12) by soybean roots. *Plant Soil* 139: 23–30, 2014.
105. Mulley EA, Strumbo C, and Hunting W. Kinetics of thiamine degradation by heat. Effect of pH and form of the vitamin on its rate of destruction. *J Food Sci* 40: 989–992, 1975.
106. Nagy S. Vitamin C contents of citrus fruit and their products: A review. *J Agric Food Chem* 28: 8–18, 1980.
107. Navankasattusas S and Lund DB. Thermal destruction of vitamin B6 vitamers in buffer solution and cauliflower puree. *J Food Sci* 47: 1512–1518, 1982.
108. Offer T, Ames BN, Bailey SW, Sabens EA, Nozawa M, and Ayling JE. 5-Methyltetrahydrofolate inhibits photosensitization reactions and strand breaks in DNA. *FASEB J* 21: 2101–2107, 2007.
109. Pelletier O. Vitamin C. In: *Methods of Vitamin Assay*, Augustin J, Klein B, Becker D, and Venugopal P, eds. New York: John Wiley & Sons, 1985, pp. 303–347.
110. Pesek C and Warthesen J. Kinetic model for photoisomerization and concomitant photodegradation of .beta.-carotenes. *J Agric Food Chem* 38: 1313–1315, 1990.
111. Pfeiffer C, Rogers L, and Gregory J. Determination of folate in cereal-grain food products using trienzyme extraction and combined affinity and reversed-phase liquid chromatography. *J Agric Food Chem* 45: 407–413, 1997.

112. Poe M. Acidic dissociation constants of folic acid, dihydrofolic acid, and methotrexate. *J Biol Chem* 252: 3724–3728, 1977.
113. Porzio MA, Tang N, and Hilker DM. Thiamine modifying properties of heme proteins from Skipjack tuna, pork, and beef. *J Agric Food Chem* 21: 308–310, 1973.
114. Quinlivan E, Hanson A, and Gregory J. The analysis of folate and its metabolic precursors in biological samples. *Anal Biochem* 348: 163–184, 2006.
115. Rebouche C. Carnitine. In: *Present Knowledge in Nutrition*, Bowman B and Russell R, eds. Washington, DC: International Life Science Institute, 2006, pp. 340–351.
116. Reed L and Archer M. Oxidation of tetrahydrofolic acid by air. *J Agric Food Chem* 28: 801–805, 1980.
117. Reiber H. Photochemical reactions of vitamin B 6 compounds, isolation and properties of products. *Biochim Biophys Acta* 279: 310–315, 1972.
118. Rizvi S and Acton J. Nutrient enhancement of thermostabilized foods in retort pouches. *Food Technol* 36: 105–109, 1982.
119. Robinson D. The nonenzymatic hydrolysis of N5,N10-methenyltetrahydrofolic acid and related reactions. In: *Methods in Enzymology*, Chytyl F, ed. San Diego, CA: Academic Press, 1971, pp. 716–725.
120. Roughead ZK and McCormick DB. Flavin composition of human milk. *Am J Clin Nutr* 52: 854–857, 1990.
121. Roughead ZK and McCormick DB. Qualitative and quantitative assessment of flavins in cow's milk. *J Nutr* 120: 382–388, 1990.
122. Rucker R and Bauerly K. Pantothenic acid. In: *Handbook of Vitamins*, Zempleni J, Suttie J, Gregory J, and Stover P, eds. Boca Raton, FL: CRC Press, 2014, pp. 325–350.
123. Russell RM, Baik H, and Kehayias JJ. Older men and women efficiently absorb vitamin B-12 from milk and fortified bread. *J Nutr* 131: 291–293, 2001.
124. Ryley J and Kajda P. Vitamins in thermal processing. *Food Chem* 49: 119–129, 1994.
125. Saidi B and Warthesen J. Influence of pH and light on the kinetics of vitamin B6 degradation. *J Agric Food Chem* 31: 876–880, 1983.
126. Sauer WC, Mosenthin R, and Ozimek L. The digestibility of biotin in protein supplements and cereal grains for growing pigs. *J Anim Sci* 66: 2583–2589, 1988.
127. Scarpa M, Stevanato R, Viglino P, and Rigo A. Superoxide ion as active intermediate in the autoxidation of ascorbate by molecular oxygen. Effect of superoxide dismutase. *J Biol Chem* 258: 6695–6697, 1983.
128. Selman J. Vitamin retention during blanching of vegetables. *Food Chem* 49: 137–147, 1994.
129. Shah J. Riboflavin. In: *Methods of Vitamin Assay*, Augustin J, Klein B, Becker D, and Venugopal P, eds. New York: John Wiley & Sons, 1985, pp. 365–383.
130. Shaw S, Jayatilleke E, Herbert V, and Colman N. Cleavage of folates during ethanol metabolism. Role of acetaldehyde/xanthine oxidase-generated superoxide. *Biochem J* 257: 277–280, 1989.
131. Shintani D and DellaPenna D. Elevating the vitamin E content of plants through metabolic engineering. *Science* 282: 2098–2100, 1998.
132. Sies H, Stahl W, and Sundquist AR. Antioxidant functions of vitamins. Vitamins E and C, beta-carotene, and other carotenoids. *Ann N Y Acad Sci* 669: 7–20, 1992.
133. Snell E. Vitamin B6. *Compr Biochem* 2: 48–58, 1963.
134. Srncova V and Davidek J. Reaction of pyridoxal and pyridoxal-5-phosphate with proteins. Reaction of pyridoxal with milk serum proteins. *J Food Sci* 37: 310–312, 1972.
135. Steele C. Cereal fortification—Technological problems. *Cereal Foods World* 21: 538–540, 1976.
136. Tadera K, Arima M, and Yagi F. Participation of hydroxyl radical in hydroxylation of pyridoxine by ascorbic acid. *Agric Biol Chem* 52: 2359–2360, 1988.
137. Taher MM and Lakshmaiah N. Hydroperoxide-dependent folic acid degradation by cytochrome *c*. *J Inorg Biochem* 31: 133–141, 1987.
138. Tannenbaum S, Young V, and Archer M. Vitamins and minerals. In: *Food Chemistry*, Fennema O, ed. New York: Marcel Dekker, 1985, pp. 477–544.
139. Tarr JB, Tamura T, and Stokstad EL. Availability of vitamin B6 and pantothenate in an average American diet in man. *Am J Clin Nutr* 34: 1328–1337, 1981.
140. Teixeira A, Dixon J, Zahradnik J, and Zinsmeister G. Computer optimization of nutrient retention in the thermal processing of conduction-heating foods. *Food Technol* 23: 845, 1969.
141. Thompson J and Hatina G. Determination of tocopherols and tocotrienols in foods and tissues by high performance liquid chromatography. *J Liquid Chromatogr* 2: 327–344, 1979.
142. Thompson JN. Problems of official methods and new techniques for analysis of foods and feeds for vitamin A. *J Assoc Off Anal Chem* 69: 727–738, 1986.

143. Tsukida K, Saiki K, and Sugiura M. Structural elucidation of the main *cis* beta-carotenes. *J Nutr Sci Vitaminol* (*Tokyo*) 27: 551–561, 1981.
144. van Niekerk P and Burger A. The estimation of the composition of edible oil mixtures. *J Am Oil Chem Soc* 62: 531–538, 1985.
145. Vanderslice J and Higgs D. Chromatographic separation of ascorbic acid, isoascorbic acid, dehydroascorbic acid and dehydro-isoascorbic acid and their quantitation in food products. *J Micronutr Anal* 4: 109–118, 1988.
146. Viberg U, Jagestad M, Oste R, and Sjöholm I. Thermal processing of 5-methyltetrahydrofolic acid in the UHT region in the presence of oxygen. *Food Chem* 59: 381–386, 1997.
147. Wall J and Carpenter K. Variation in availability of niacin in grain products. Changes in chemical composition during grain development and processing affect the nutritional availability of niacin. *Food Technol* 42: 198–204, 1988.
148. Wall J, Young M, and KJ C. Transformation of niacin-containing compounds in corn during grain development: Relationship to niacin nutritional availability. *J Agric Food Chem* 35: 752–758, 1987.
149. Weiser H and Vecchi M. Stereoisomers of alpha-tocopheryl acetate. II. Biopotencies of all eight stereoisomers, individually or in mixtures, as determined by rat resorption-gestation tests. *Int J Vitam Nutr Res* 52: 351–370, 1982.
150. Wendt G and Bernhart FW. The structure of a sulfur-containing compound with vitamin B6 activity. *Arch Biochem Biophys* 88: 270–272, 1960.
151. Wiesinger H and Hinz HJ. Kinetic and thermodynamic parameters for Schiff base formation of vitamin B6 derivatives with amino acids. *Arch Biochem Biophys* 235: 34–40, 1984.
152. Woodcock E, Warthesen J, and Labuza T. Riboflavin photochemical degradation in pasta measured by high performance liquid chromatography. *J Food Sci* 47: 545–549, 1982.
153. Zechmeister L. Stereoisomeric provitamins A. *Vitam Horm* 7: 57–81, 1949.
154. Zeisel SH, Mar MH, Howe JC, and Holden JM. Concentrations of choline-containing compounds and betaine in12 common foods. *J Nutr* 133: 1302–1307, 2003.
155. Zhao R, Diop-Bove N, Visentin M, and Goldman ID. Mechanisms of membrane transport of folates into cells and across epithelia. *Annu Rev Nutr* 31: 177–201, 2011.
156. Zoltewicz JA and Kauffmann GM. Kinetics and mechanism of the cleavage of thiamin, 2-(1-hydroxyethyl)thiamin, and a derivative by bisulfite ion in aqueous solution. Evidence for an intermediate. *J Am Chem Soc* 99: 3134–3142, 1977.

FURTHER READING

Augustin J, Klein BP, Becker DA, and Venugopal PB (eds.). *Methods of Vitamin Assay*, 4th edn. John Wiley & Sons, New York, 1985.

Bauernfeind JC and Lachance PA. *Nutrient Additions to Food. Nutritional, Technological and Regulatory Aspects*. Trumbull, CT: Food and Nutrition Press, Inc., 1992.

Caudill MA, Miller JW, Gregory JF, and Shane B. Folate, choline, vitamin B12, and vitamin B6. In: *Biochemical, Physiological, and Molecular Aspects of Human Nutrition*, 3rd edn., Stipanuk MH and Caudill MA, eds. St. Louis, MO: Elsevier, 2012, pp. 565–609.

Chytyl F and McCormick DB (eds.). *Methods in Enzymology, Vol. 122 and 123, Parts G and H* (*Respectively*). *Vitamins and Coenzymes*. San Diego, CA: Academic Press, 1986.

Davidek J, Velisek J, and Polorny J (eds.). Vitamins. In: *Chemical Changes during Food Processing*. Amsterdam, the Netherlands: Elsevier, 1990, pp. 230–301.

Eitenmiller RR and Landen WO Jr. *Vitamin Analysis for the Health and Food Sciences*, Weimar, TX: Culinary and Hospitality Industry Publications Services, 1998.

Erdman JW, MacDonald IA, and Zeisel SH (eds.). Vitamin B6. In: *Present Knowledge in Nutrition*, 10th edn. New York: Wiley-Blackwell, 2012.

Gregory JF, Quinlivan EP, and Davis SR. Integrating the issues of folate bioavailability, Intake and metabolism in the era of fortification. *Trends Food Sci Technol* 16: 229–240, 2005.

Harris RS and Karmas E. *Nutritional Evaluation of Food Processing*, 2nd edn. Westport, CT: AVI Publishing Co, 1975.

Harris RS and von Loesecke H. *Nutritional Evaluation of Food Processing*. Westport, CT: AVI Publishing Co., 1971.

Institute of Medicine. *Nutrition Labeling. Issues and Directions for the 1990s*, Porter DV and Earl RO, eds. Washington, DC: National Academy Press, 1990.

Karmas E and Harris RS. *Nutritional Evaluation of Food Processing*, 3rd edn. New York: Van Nostrand Reinhold Co., 1988.

McCormick DB. Coenzymes, Biochemistry. In: *Encyclopedia of Human Biology*, R. Dulbecco, ed., Vol. 2. San Diego, CA: Academic Press, 1991, pp. 527–545.

McCormick DB, Suttie JW, and Wagner C. *Methods in Enzymology, Vols. 280 and 281, Parts K and J* (*respectively*)*, Vitamins and Coenzymes*. San Diego, CA: Academic Press, 1997.

Stipanuk MH and Caudill MA (eds.). *Biochemical, Physiological, and Molecular Aspects of Human Nutrition*, 3rd edn. St. Louis, MO: Elsevier, 2012.

Zempleni J, Suttie JW, Gregory JF, and Stover PJ (eds.). *Handbook of Vitamins*, 5th edn. Boca Raton, FL: CRC Press, 2014.

9 Minerals

Dennis D. Miller

CONTENTS

9.1 Introduction ... 628
9.2 Principles of Mineral Chemistry ... 629
9.2.1 Solubility of Minerals in Aqueous Systems ... 629
9.2.2 Minerals and Acid/Base Chemistry ... 629
9.2.2.1 Bronsted Theory of Acids and Bases ... 629
9.2.2.2 Lewis Theory of Acids and Bases ... 630
9.2.3 Chelate Effect ... 632
9.3 Nutritional Aspects of Minerals ... 634
9.3.1 Essential Mineral Elements ... 634
9.3.2 Dietary Reference Intakes for Mineral Nutrients (the United States and Canada) ... 636
9.3.3 Bioavailability of Minerals ... 637
9.3.3.1 Bioavailability Enhancers ... 637
9.3.3.2 Bioavailability Antagonists ... 640
9.3.4 Nutritional Aspects of Essential Minerals: Overview ... 643
9.3.5 Nutritional Aspects of Essential Minerals: Individual Minerals ... 643
9.3.5.1 Calcium ... 644
9.3.5.2 Phosphorus ... 646
9.3.5.3 Sodium, Potassium, and Chloride ... 646
9.3.5.4 Iron ... 647
9.3.5.5 Zinc ... 649
9.3.5.6 Iodine ... 650
9.3.5.7 Selenium ... 651
9.3.6 Toxicology of Food-Borne Heavy Metals ... 653
9.3.6.1 Lead ... 654
9.3.6.2 Mercury ... 654
9.3.6.3 Cadmium ... 656
9.4 Mineral Composition of Foods ... 657
9.4.1 Ash: Definition and Significance in Food Analysis ... 657
9.4.2 Individual Minerals ... 657
9.4.3 Factors Affecting the Mineral Composition of Foods ... 657
9.4.3.1 Factors Affecting the Mineral Composition of Plant Foods ... 658
9.4.3.2 Adequacy of Plant Foods for Supplying the Mineral Needs of Humans ... 659
9.4.3.3 Factors Affecting the Mineral Composition of Animal Foods ... 660
9.4.3.4 Adequacy of Animal Foods for Supplying Mineral Needs of Humans ... 660
9.4.4 Fortification and Enrichment of Foods with Minerals ... 660
9.4.4.1 Iron ... 661
9.4.4.2 Zinc ... 664
9.4.4.3 Iodine ... 664
9.4.5 Effects of Processing ... 665

9.5 Chemical and Functional Properties of Minerals in Foods ... 666
9.5.1 Calcium ... 666
9.5.2 Phosphates ... 666
9.5.3 Sodium Chloride ... 669
9.5.4 Iron ... 670
9.5.5 Nickel ... 670
9.5.6 Copper ... 670
9.6 Glossary of Terms ... 671
References ... 673

9.1 INTRODUCTION

Ninety chemical elements occur naturally in the earth's crust. About 25 are known to be essential to life and therefore are present in living cells (Figure 9.1). Since our food is ultimately derived from living plants or animals, we can expect to find these 25 elements in foods as well. Foods also contain other elements because living systems can accumulate nonessential as well as essential elements from their environment. Furthermore, elements may enter foods as contaminants during harvesting, processing, and storage or they may be present in intentional food additives.

While there is no universally accepted definition of *mineral* as it applies to food and nutrition, the term usually refers to elements other than C, H, O, and N that are present in foods. These four nonmineral elements are present primarily in organic molecules and water and constitute about 99% of the total number of atoms in living systems [29]. Thus, mineral elements are present in relatively low concentrations in foods. Nonetheless, they play key functional roles in both living systems and foods.

Historically, minerals have been classified as either major or trace, depending on their concentrations in plants and animals. This classification arose at a time when analytical methods were not capable of measuring small concentrations of elements with much precision. Thus, the term "trace" was used to indicate the presence of an element that could not be measured accurately. Today, modern methods and instruments allow for extremely precise and accurate measurement of virtually all of the elements in the periodic table [86]. Nevertheless, the terms major and trace continue to be used to describe mineral elements in biological systems. Major minerals include calcium, phosphorus, magnesium, sodium, potassium, and chloride. Trace elements include iron, iodine, zinc, selenium, chromium, copper, fluorine, and tin.

I-A	II-A	III-B	IV-B	V-B	VI-B	VII-B	VIII	VIII	VIII	I-B	II-B	III-A	IV-A	V-A	VI-A	VII-A	O
H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	Ln	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	Th	Pa	U												

FIGURE 9.1 Periodic table of the naturally occurring elements. Shaded elements are believed to be essential nutrients for animals and humans.

9.2 PRINCIPLES OF MINERAL CHEMISTRY

Mineral elements are present in foods in many different chemical forms. These forms are commonly referred to as "species" and include compounds, complexes, and free ions [126]. Given the diversity of chemical properties among the mineral elements, the number and diversity of nonmineral compounds in foods that can bind mineral elements, and the chemical changes that occur in foods during processing and storage, it is not surprising that the number of different mineral species in foods is very large. Since foods are so complex and since many mineral species are transient, it is very difficult to isolate and characterize mineral species in foods. Thus, our understanding of the exact chemical forms of minerals in foods remains limited. Fortunately, principles and concepts from the vast literature in inorganic and organic chemistry and biochemistry can be very useful in guiding predictions about the behavior of mineral elements in foods.

9.2.1 Solubility of Minerals in Aqueous Systems

Most nutrients are delivered to and metabolized by organisms in an aqueous environment. Thus, the availabilities and reactivities of minerals depend, in large part, on their solubility in water. This excludes the elemental form of nearly all elements (dioxygen and nitrogen are exceptions) from physiological activity in living systems since these forms, for example, elemental iron, are insoluble in water and therefore unavailable for incorporation into organisms or biological molecules.

The species (forms) of elements present in food vary considerably depending on the chemical properties of the element. Elements in groups IA and VIIA (Figure 9.1) exist in foods predominantly as free ionic species (Na^+, K^+, Cl^-, and F^-). These ions are highly water soluble and have low affinities for most ligands; thus, they exist primarily as free ions in aqueous systems. Most other minerals are present as weak coordinate complexes, chelates, or oxygen-containing anions (see below for a discussion of complexes and chelates, Section 9.2.3).

The solubilities of mineral complexes and chelates may be very different from the solubilities of inorganic salts. For example, if ferric chloride is dissolved in water, the iron will soon precipitate as ferric hydroxide. On the other hand, ferric iron chelated with citrate is quite soluble. Conversely, calcium as calcium chloride is very soluble, while calcium chelated with oxalate ion is insoluble.

9.2.2 Minerals and Acid/Base Chemistry

Much of the chemistry of the mineral elements can be understood by applying the concepts of acid/base chemistry. Moreover, acids and bases may profoundly influence functional properties and stabilities of other food components by altering the pH of the food. Thus, acid/base chemistry is critically important in food science. A brief review of acid/base chemistry follows. For a more complete treatment of this topic, see Shriver et al. [116] or other textbooks on inorganic chemistry.

9.2.2.1 Bronsted Theory of Acids and Bases

A Bronsted acid is any substance capable of donating a proton.
A Bronsted base is any substance capable of accepting a proton.

Many acids and bases occur naturally in foods, and they may be used as food additives or processing aids. Common organic acids include acetic, lactic, and citric acids. Phosphoric acid is an

example of a mineral acid present in foods. It is used as an acidulant and flavoring agent in some carbonated soft drinks. It is a tribasic acid (contains three available protons).

$$H_3PO_4 \rightleftarrows H_2PO_4^- + H^+ \quad pK_1 = 2.12$$

$$H_2PO_4^- \rightleftarrows HPO_4^{-2} + H^+ \quad pK_2 = 7.1$$

$$HPO_4^{-2} \rightleftarrows PO_4^{-3} + H^+ \quad pK_3 = 12.4$$

Other common mineral acids include HCl and H_2SO_4. They are rarely added to foods directly although they may be generated in foods during processing or cooking. For example, H_2SO_4 is produced when the common leavening acid sodium aluminum sulfate is heated in the presence of water:

$$Na_2SO_4 \cdot Al_2(SO_4)_3 + 6H_2O \rightarrow Na_2SO_4 + 2Al(OH)_3 + 3H_2SO_4$$

9.2.2.2 Lewis Theory of Acids and Bases

An alternative, and more general, definition of an acid and a base was developed by G.N. Lewis in the 1930s [116]:

A Lewis acid is an electron pair acceptor
A Lewis base is an electron pair donor

By convention, Lewis acids are often represented as A and Lewis bases as :B. The reaction between a Lewis acid and a Lewis base then becomes

$$A + :B \rightarrow A - B$$

It is important to remember that this reaction does not involve a change in the oxidation state of either A or B, that is, it is not a redox reaction. Thus, A must possess a vacant low-energy orbital, and B must possess an unshared pair of electrons. The bonding results from the interaction of orbitals from the acid and the base to form new molecular orbitals. The stability of the complex depends in large part on the reduction of electronic energy that occurs when orbitals from A and :B interact to form bonding molecular orbitals. The electronic structures of these complexes are very intricate since multiple atomic orbitals may be involved. The d-block metals, for example, can contribute up to nine atomic orbitals (1s, 3p, and 5d orbitals) to the formation of molecular orbitals. The product of the reaction between a Lewis acid and a Lewis base is commonly referred to as a complex where A and :B are bonded together through the sharing of the electron pair donated by :B.

The Lewis acid/base concept is key to understanding the chemistry of minerals in foods because metal cations are Lewis acids and they bind to Lewis bases. The complexes resulting from reactions between metal cations and food molecules range from metal hydrates, to metal-containing pigments such as hemoglobin and chlorophyll, to metalloenzymes.

The number of Lewis base molecules that may bind to a single metal ion is more or less independent of the charge on the metal ion. This number, usually referred to as the coordination number, may range from 1 to 12 but is most commonly 6. For example, Fe^{+3} binds six water molecules to form hexaaquairon, which takes on an octahedral geometry (Figure 9.2).

FIGURE 9.2 Ferric iron with six coordinated water molecules. This is the predominant form of Fe^{3+} in acidic (pH < 1) aqueous solutions.

FIGURE 9.3 Examples of ligands coordinated with a metal ion (M^+).

The electron donating species in these complexes are commonly referred to as "ligands." The principal electron donating atoms in ligands are oxygen, nitrogen, and sulfur. Thus, many food molecules including proteins, carbohydrates, phospholipids, and organic acids are ligands for mineral ions. Ligands may be classified according to the number of bonds they can form with a metal ion. Those that form one bond are monodentate ligands, those that form two bonds are bidentate, and so on. Ligands that form two or more bonds are referred to collectively as multidentate ligands. Some common examples of ligands are shown in Figure 9.3.

Stabilities of metal complexes may be expressed as the equilibrium constant for the reaction representing the formation of the complex. The terms "stability constant" (k) and "formation constant" are often used interchangeably. The generalized reaction for formation of a complex between a metal ion (M) and a ligand (L) is [116]

$$M + L \leftrightarrows ML \quad k = \frac{[ML]}{[M][L]}$$

$$ML + L \leftrightarrows ML_2 \quad k = \frac{[ML_2]}{[ML][L]}$$

$$\downarrow \quad \downarrow \quad \downarrow$$

$$\downarrow \quad \downarrow \quad \downarrow$$

$$ML_{n-1} + L \leftrightarrows ML_n \quad k_n = \frac{[ML_n]}{[ML_{n-1}][L]}$$

When more than one ligand is bound to one metal ion, the overall formation constant may be expressed as

$$K = \beta_n = \frac{[ML_n]}{[M][L]^n}$$

where $K = \beta_n = k_1 k_2 \ldots k_n$ and n is the number of ligands bound per metal ion.

Some stability constants for Cu^{2+} and Fe^{3+} are shown in Table 9.1.

TABLE 9.1
Stability Constants (log K) for Selected Metal Complexes and Chelates

Ligand	Cu^{2+}	Fe^{3+}
OH^-	6.3	11.8
Oxalate	4.8	4.8
Histidine	10.3	10.0
Ethylenediaminetetraacetate	18.7	25.1

Source: Adapted from Shriver, D.F. et al., *Inorganic Chemistry*, 2nd edn., W.H. Freeman, New York, 1994.
Note: Values are corrected to a constant ionic strength.

9.2.3 Chelate Effect

A chelate is a complex resulting from the combination of a metal ion and a multidentate ligand such that the ligand forms two or more bonds with the metal resulting in a ring structure that includes the metal ion. The term chelate is derived from "chele," the Greek word for claw. Thus, a chelating ligand (also called a chelating agent) must contain at least two functional groups capable of donating electrons. In addition, these functional groups must be spatially arranged so that a ring containing the metal ion can form. Chelates have greater thermodynamic stabilities than similar complexes that are not chelates, a phenomenon referred to as the "chelate effect." Several factors interact to affect the stability of a chelate. Kratzer and Vohra [67] summarized these factors as follows:

1. *Ring size*: Five-membered unsaturated rings and six-membered saturated rings tend to be more stable than larger or smaller rings.
2. *Number of rings*: The greater the number of rings in the chelate, the greater the stability.
3. *Lewis base strength*: Stronger Lewis bases tend to form stronger chelates.
4. *Charge of ligand*: Charged ligands form more stable chelates than uncharged ligands. For example, citrate forms more stable chelates than citric acid.
5. *Chemical environment of the donating atom*: Relative strengths of metal–ligand bonds are shown here in decreasing order:
   Oxygen as donor: $H_2O > ROH > R_2O$
   Nitrogen as donor: $H_3N > RNH_2 > R_3N$
   Sulfur as donor: $R_2S > RSH > H_2S$
6. *Resonance in chelate ring*: Enhanced resonance tends to increase stability.
7. *Steric hindrance*: Large bulky ligands tend to form less stable chelates.

Thus, chelate stabilities are affected by many factors and are difficult to predict. However, the concept of Gibbs free energy ($\Delta G = \Delta H - T\Delta S$) is useful for explaining the chelate effect. Consider the following example of Cu^{2+} complexing with either ammonia or ethylenediamine [116]:

$$Cu(H_2O)_6{}^{2+} + 2NH_3 \rightarrow [Cu(H_2O)_4(NH_3)_2]^{2+} + 2H_2O$$
$$(\Delta H = -46\ \text{kJ/mol};\ \Delta S = -8.4\ \text{J/K/mol};\ \text{and}\ \log\ \beta = 7.7)$$
$$Cu(H_2O)_6{}^{2+} + NH_2CH_2CH_2NH_2 \rightarrow [Cu(H_2O)_4(NH_2CH_2CH_2NH_2)]^{2+} + 2H_2O$$
$$(\Delta H = -54\ \text{kJ/mol};\ \Delta S = +23\ \text{J/K/mol};\ \text{and}\ \log\ K = 10.1)$$

FIGURE 9.4 Cu^{2+} complexed with ammonia and ethylenediamine.

Both complexes have two nitrogens bound to a single copper ion (Figure 9.4), and yet the stability of the ethylenediamine complex is much greater than that of the ammonia complex (log of formation constants are 10.1 and 7.7, respectively). Both enthalpy and entropy contribute to the difference in stabilities, but the entropy change is a major factor in the chelate effect. Ammonia, a monodentate ligand, forms one bond to copper while ethylenediamine, a bidentate ligand, forms two. The difference in entropy change is due to the change in the number of independent molecules in solution. In the first reaction (i.e., with NH_3), the number of molecules is equal on both sides of the equation so the entropy change is small. The chelation reaction (with ethylenediamine), on the other hand, results in a net increase in the number of independent molecules in solution and, thus, an increase in entropy.

Ethylenediaminetetraacetate (EDTA) ion provides an even more dramatic illustration of the chelate effect [97]. EDTA is a hexadentate ligand. When it forms a chelate with a metal ion in solution, it displaces six water molecules from the metal, and this has a large effect on the entropy of the system (Figure 9.5):

$$Ca(H_2O)_6^{2+} + EDTA^{4-} \rightarrow Ca(EDTA)^{2-} + 6H_2O \ (\Delta S = +118 \text{ J/K/mol})$$

Moreover, EDTA chelates contain five rings, which also enhances stability. EDTA forms stable chelates with many metal ions.

Chelates are very important in foods and in all biological systems. Chelating agents may be added to foods to sequester mineral ions, such as iron or copper, to prevent them from acting as prooxidants. Preformed chelates, such as ferric sodium EDTA, may be added to foods as fortificants [10]. Furthermore, most complexes resulting from interactions between metal ions and food molecules are chelates.

(a) EDTA (b) $[Ca(EDTA)]^{2-}$

FIGURE 9.5 (a) Ethylenediaminetetraacetic acid (EDTA) and (b) a Ca^{2+}–EDTA chelate. Note that in the chelate, the carboxyl groups on EDTA are ionized; thus, the net charge on the chelate is –2.

9.3 NUTRITIONAL ASPECTS OF MINERALS

9.3.1 Essential Mineral Elements

Several definitions of an essential mineral element have been proposed. A widely accepted definition is the following: an element is essential for life if its removal from the diet or other route of exposure to an organism "results in a consistent and reproducible impairment of a physiological function" [122]. Thus, essentiality can be demonstrated by feeding diets low in a particular element to humans or experimental animals and watching for signs of impaired function.

Human requirements for essential minerals vary from a few micrograms per day up to about 1 g/day. If intakes are low for some period of time, deficiency signs will develop. Conversely, excessively high intakes can result in toxicity. Fortunately, the range of safe and adequate intake for most minerals is fairly wide, so deficiency or toxicity is relatively rare provided a varied diet is consumed.

This broad range of safe and adequate intakes is possible because organisms have homeostatic mechanisms for dealing with both low and high exposures to essential nutrients. Homeostasis may be defined as the processes whereby an organism maintains tissue levels of nutrients within a narrow and constant range. In higher organisms, homeostasis is a complex set of processes involving regulation of absorption, excretion, metabolism, and storage of nutrients. Without homeostatic mechanisms, intakes of nutrients would have to be very tightly controlled to prevent deficiency or toxicity (Figure 9.6). Homeostasis can be overridden when dietary levels are excessively low or high for extended periods of time. Persistently low intakes of mineral nutrients are not uncommon, especially in poor populations where access to a variety of foods is often limited. Toxicities caused by high dietary intakes of essential minerals are less common, although high sodium intakes appear to be a major factor in hypertension (high blood pressure) [79].

Minerals are essential for hundreds of enzymatic reactions in the body, they are key players in the regulation of metabolism, they are essential for the strength and rigidity of bones and teeth, they facilitate the transport of oxygen and carbon dioxide in the blood, and they are necessary for cell adhesion and cell division. Minerals can also be toxic, and there are many documented cases of severe injury and even death from exposure to minerals. Table 9.2 summarizes some of the key nutritional and toxicological aspects of minerals.

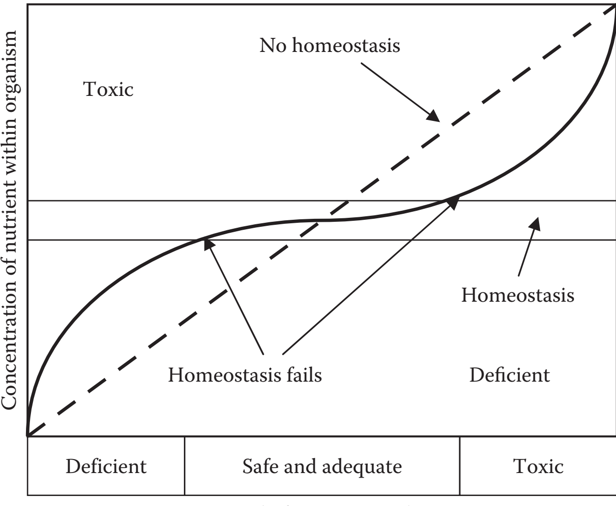


FIGURE 9.6 Homeostasis in living organisms. Without homeostasis (dashed line), the range of safe and adequate intakes of nutrients would be very narrow. With homeostasis (solid line), the range of safe and adequate intakes is much wider. Homeostatic mechanisms fail when intakes are very low or very high producing deficiency or toxicity, respectively. (Redrawn from Mertz, W., *Nutr. Today*, 19(1), 22, 1984.)

TABLE 9.2
Nutritional and Toxicological Aspects of Minerals

Mineral	Function	Deficiency Effects	Adverse Effects from Excessive Intake	Food Sources
Calcium	Bone and tooth mineralization, blood clotting, hormone secretion, nerve transmission	Increased risk for osteoporosis, hypertension, and some cancers.	Excessive intakes rare; may cause kidney stones and milk alkali syndrome.	Milk, yogurt, cheese, fortified juices, tofu, kale, broccoli, fish bones.
Phosphorus	Bone mineralization; DNA and RNA synthesis; phospholipid synthesis, energy metabolism, cell signaling	Deficiency rare due to ubiquitous distribution in foods; low intakes may impair bone mineralization.	Impaired bone formation, kidney stones, decreased Ca and Fe absorption, iron and zinc deficiency due to high phytate intakes.	Present in virtually all foods. High-protein foods (meats, dairy, etc.), cereal products, and cola beverages (as H_3PO_4) are especially rich sources.
Magnesium	Cofactor for numerous enzymes	Deficiency is rare except in certain clinical situations; patients recovering from cardiac surgery are often hypomagnesemic.	Rarely occurs except from overconsumption of Mg supplements; causes intestinal distress, diarrhea, cramping, and nausea.	Green leafy vegetables, milk, whole grains.
Sodium	Predominant cation in extracellular fluid; controls extracellular fluid volume and blood pressure; required for transport of many nutrients into and out of cells	Deficiency is rare except in endurance sports. Deficiency may cause muscle cramping.	Chronically high intakes may lead to hypertension in salt-sensitive persons.	Most foods are naturally low in Na. Processed and prepared foods contain varying levels of added Na.
Iron	Oxygen transport (hemoglobin and myoglobin), respiration and energy metabolism (cytochromes and iron–sulfur proteins), destruction of hydrogen peroxide (hydrogen peroxidase and catalase), and DNA synthesis (ribonucleotide reductase)	Deficiency is widespread. Effects include fatigue, anemia, impaired work capacity, impaired cognitive function, impaired immune response, and poor pregnancy outcomes.	Iron overload leading to increased risk for some cancers and heart disease.	Red meat, cereal products, beans, fortified foods, green leafy vegetables.
Zinc	Cofactor in metalloenzymes, regulation of gene expression	Growth retardation, impaired wound healing, delayed sexual maturation, impaired immune response, and diarrhea.	Inhibition of Cu and Fe absorption, impaired immune response.	Red meat, shellfish, wheat germ, fortified foods.
Iodine	Required for synthesis of thyroid hormones	Goiter, mental retardation, decreased fertility, miscarriage, cretinism, and hypothyroidism.	Rare in iodine replete persons, hyperthyroidism in iodine deficient persons.	Iodized salt, seaweed, seafood, dairy products (if I is added to feed or iodine-containing sanitizers are used).

(Continued)

TABLE 9.2 (*Continued*)
Nutritional and Toxicological Aspects of Minerals

Mineral	Function	Deficiency Effects	Adverse Effects from Excessive Intake	Food Sources
Selenium	Antioxidant (as component in peroxidases)	Myocarditis, osteoarthritis, and increased risk for some cancers.	Hair and nail loss, skin lesions, nausea, increased risk for some cancers.	Cereals grown on high-Se soils, meat from animals supplemented with Se.
Lead	None, not an essential nutrient	None.	Learning and behavioral problems in children, anemia, kidney damage.	Contamination of foods from Pb-soldered cans, exhaust from cars burning leaded gasoline, some ceramic glazes.
Mercury	None, not an essential nutrient	None.	Numbness, vision and hearing loss, kidney damage.	Fish (especially long-lived carnivorous fish).
Cadmium	Unknown	Depressed growth in rats.	Kidney damage, bone disease, cancer.	Grains and vegetables grown on Cd-contaminated soils.

9.3.2 Dietary Reference Intakes for Mineral Nutrients (the United States and Canada)

In 1997, the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (DRIs) of the Food and Nutrition Board of the Institute of Medicine issued a report describing a new approach to the establishment of appropriate dietary nutrient intakes for healthy people in the United States and Canada [119]. These new intake recommendations are termed "Dietary Reference Intakes" and replace the old Recommended Dietary Allowances (RDAs), which were first released in 1941 and have been revised periodically since that time. The last version of the RDAs was published in 1989. DRIs include a subset of values: Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), Adequate Intake (AI), and Tolerable Upper Intake Level (UL). Each of these values is based on specific criteria used in its estimation. Brief descriptions of these are given in the following text. For detailed descriptions, the reader is referred to [69].

1. *Estimated Average Requirement* (*EAR*): EAR is defined as the level of intake of a nutrient that meets the requirements of 50% of the individuals in a particular age and gender group. Presumably, the requirement of the remaining 50% of the individuals is higher than the EAR.
2. *Recommended Dietary Allowance* (*RDA*): RDA is defined as the level of intake of a nutrient sufficient to meet the requirements of nearly all healthy persons in a particular age and gender group. It is set at two standard deviations (SDs) above the EAR: RDA = EAR + 2SD
3. *Adequate Intake* (*AI*): AI is used when the available scientific evidence is insufficient to set an RDA. It is based on estimates of actual average intakes of a nutrient by healthy people, not on results from controlled studies designed to estimate individual requirements for nutrients.
4. *Tolerable Upper Intake Level* (*UL*): UL is the level of intake of a nutrient below which adverse health effects are unlikely to occur. This implies that intakes above the UL may pose a risk of toxicity.

A graphical representation of EAR, RDA, AI, and UL is shown in Figure 9.7.

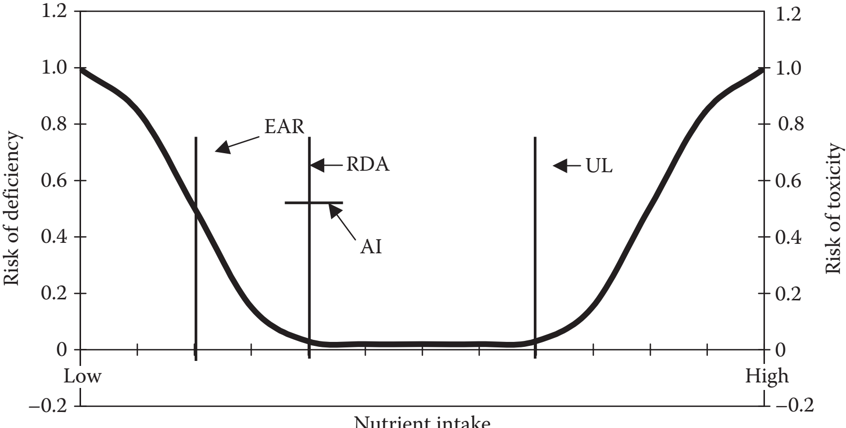


FIGURE 9.7 Risk of deficiency (left vertical axis) or excess (right vertical axis) over a range of intakes of a given nutrient for DRI categories (EAR, RDA, AI, and UL). As intakes increase, risk of deficiency decreases and approaches zero. As intakes increase beyond the safe and adequate range, risk of toxicity rises. (Redrawn from Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, *Dietary Reference Intakes for Calcium, Phosphorous, Vitamin D, and Flouride*, National Academy Press, Washington, DC, 1997.)

DRIs have been set for only 9 of the 25 minerals known to be essential for life: Ca, P, Mg, Fe, Zn, Cu, Cr, Mn, and I. The DRIs for the most important of these are listed in Tables 9.3 and 9.4.

9.3.3 Bioavailability of Minerals

It is well known that the concentration of a nutrient in a food is not necessarily a reliable indicator of the value of that food as a source of the nutrient in question. This led nutritionists to develop the concept of nutrient bioavailability. Bioavailability may be defined as the proportion of an ingested nutrient that is available for utilization in metabolic processes or for deposition in a storage compartment in the body. In the case of mineral nutrients, bioavailability is determined primarily by the efficiency of absorption from the intestinal lumen into the blood. In some cases, however, absorbed nutrients may be in a form that cannot be utilized. For example, iron is bound so tightly in some chelates that even if the iron chelate is absorbed, the iron will not be released to cells for incorporation into iron proteins, rather the intact chelate will be excreted in the urine.

Bioavailabilites of mineral nutrients vary from less than 1% for some forms of iron to greater than 90% for sodium and potassium. The reasons for this wide range are varied and complex since many factors interact to determine the ultimate bioavailability of a nutrient (Table 9.5). One of the most important factors is solubility of the mineral in the contents of the small intestine since insoluble compounds cannot diffuse to the brush border membranes of enterocytes and consequently cannot be absorbed. Therefore, many of the enhancing and inhibiting factors appear to operate through effects on mineral solubility.

9.3.3.1 Bioavailability Enhancers

Organic acids: Several organic acids enhance mineral bioavailability. The magnitude of the effect depends on the composition of the meal, the specific mineral nutrient, and the relative concentrations of the organic acid and the mineral. Organic acids that have received the most attention are ascorbic, citric, and lactic acids. Presumably, these and other organic acids improve bioavailability by forming soluble chelates with the mineral. These chelates protect the mineral from precipitation and/or binding to other ligands that may inhibit absorption.

TABLE 9.3
Dietary Reference Intakes of Nutritionally Essential Minerals (Ca, P, and Mg)[a]

	Calcium (mg/day)	Phosphorus (mg/day)	Magnesium (mg/day)
Life Stage	**RDA/AI/UL**	**RDA/AI/UL**	**RDA/AI/UL**
Infants			
0–6 months	210/ND[b]	**100**/ND	30/ND
7–12 months	270/ND	**275**/ND	75/ND
Children			
1–3 years	500/2500	**460**/3000	**80**/65
4–8 years	800/2500	**500**/3000	**130**/110
Males			
9–13 years	1300/2500	**1250**/4000	**240**/350
14–18 years	1300/2500	**1250**/4000	**410**/350
19–30 years	1000/2500	**700**/4000	**400**/350
31–50 years	1000/2500	**700**/4000	**420**/350
50–70 years	1200/2500	**700**/4000	**400**/350
>70 years	1200/2500	**700**/3000	**400**/350
Females			
9–13 years	1300/2500	**1250**/4000	**240**/350
14–18 years	1300/2500	**1250**/4000	**360**/350
19–30 years	1000/2500	**700**/4000	**310**/350
31–50 years	1000/2500	**700**/4000	**320**/350
50–70 years	1200/2500	**700**/4000	**320**/350
>70 years	1200/2500	**700**/3000	**320**/350
Pregnancy			
≤18 years	1300/2500	**1250**/3500	**400**/350
19–30 years	1000/2500	**700**/3500	**350**/350
31–50 years	1000/2500	**700**/3500	**350**/350
Lactation			
≤18 years	1300/2500	**1250**/4000	**360**/350
19–30 years	1000/2500	**700**/4000	**310**/350
31–50 years	1000/2500	**700**/4000	**320**/350

Sources: Adapted from Food and Nutrition Board (FNB), Institute of Medicine, *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*, National Academy Press, Washington, DC, 2002; Food and Nutrition Board, Institute of Medicine, Dietary reference intake tables: Elements table, 2003, http://www.iom.edu/file.asp?id=7294.

[a] Recommended Dietary Allowances (**RDA**) are printed in bold type and Adequate Intakes (AI) in ordinary type. The first values listed under each element are either **RDA** or AI. For example, only AIs are listed for calcium and only RDAs are listed for phosphorous while for magnesium, some are AIs and some **RDAs**. The values listed following the / are the Upper Limit (UL). In most cases, ULs are for intakes from all sources (food, water, and supplements). In the case of magnesium, however, the ULs are for intakes from supplements and do not include intakes from food and water. See text for an explanation of **RDA**, AI, and UL.

[b] Not determined by the Food and Nutrition Board due to lack of sufficient data for making an estimate.

TABLE 9.4
Dietary Reference Intakes of Nutritionally Essential Trace Minerals (Fe, Zn, Se, I, and F)[a]

	Iron (mg/day)	Zinc (mg/day)	Selenium (μg/day)	Iodine (μg/day)	Fluoride (mg/day)
Life Stage	**RDA or AI/UL**	**RDA or AI/UL**	**RDA or AI/UL**	**RDA or AI/UL**	**RDA or AI/UL**
Infants					
0–6 months	0.27/40	2/4	15/45	110/ND[b]	0.01/0.7
7–12 months	**11**/40	**3**/5	20/60	130/ND	0.5/0.9
Children					
1–3 years	**7**/40	**3**/7	**20**/90	**90**/200	0.7/1.3
4–8 years	**10**/40	**5**/12	**30**/150	**90**/300	1/2.2
Males					
9–13 years	**8**/40	**8**/23	**40**/280	**120**/600	2/10
14–18 years	**11**/45	**11**/34	**55**/400	**150**/900	3/10
19–30 years	**8**/45	**11**/40	**55**/400	**150**/1100	4/10
31–50 years	**8**/45	**11**/40	**55**/400	**150**/1100	4/10
50–70 years	**8**/45	**11**/40	**55**/400	**150**/1100	4/10
>70 years	**8**/45	**11**/40	**55**/400	**150**/1100	4/10
Females					
9–13 years	**8**/40	**8**/23	**40**/280	**120**/600	2/10
14–18 years	**15**/45	**9**/34	**55**/400	**150**/900	3/10
19–30 years	**18**/45	**8**/40	**55**/400	**150**/1100	3/10
31–50 years	**18**/45	**8**/40	**55**/400	**150**/1100	3/10
50–70 years	**8**/45	**8**/40	**55**/400	**150**/1100	3/10
>70 years	**8**/45	**8**/40	**55**/400	**150**/1100	3/10
Pregnancy					
≤18 years	**27**/45	**12**/34	**60**/400	**220**/900	3/10
19–30 years	**27**/45	**11**/40	**60**/400	**220**/1100	3/10
31–50 years	**27**/45	**11**/40	**60**/400	**220**/1100	3/10
Lactation					
≤18 years	**10**/45	**13**/34	**70**/400	**290**/900	3/10
19–30 years	**9**/45	**12**/40	**70**/400	**290**/1100	3/10
31–50 years	**9**/45	**12**/40	**70**/400	**290**/1100	3/10

Sources: Adapted from Food and Nutrition Board (FNB); Institute of Medicine, *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*, National Academy Press, Washington, DC, 2002; Food and Nutrition Board; Institute of Medicine, Dietary reference intake tables: Elements table, 2003, http://www.iom.edu/file.asp?id=7294.

[a] Recommended Dietary Allowances (**RDA**) are printed in bold type and Adequate Intakes (AI) in ordinary type. The first values listed under each element are either **RDA** or AI. For example, **RDAs** are listed for iron but only AIs are listed for fluoride. The values listed following the / are the Upper Limit (UL). See text for an explanation of **RDA**, AI, and UL.

[b] Not determined by the Food and Nutrition Board due to lack of sufficient data for making an estimate.

TABLE 9.5
Factors That May Influence Mineral Bioavailability from Foods

1. Chemical form of the mineral in food
   a. Highly insoluble forms are poorly absorbed.
   b. Soluble chelated forms may be poorly absorbed if chelate has high stability.
   c. Heme iron is absorbed more efficiently than nonheme iron in most diets.
2. Food ligands
   a. Ligands that form soluble chelates with metals may enhance absorption from some foods (e.g., EDTA enhances Fe absorption from some diets).
   b. High-molecular-weight ligands that are poorly digestible may reduce absorption (e.g., dietary fiber, some proteins).
   c. Ligands that form insoluble chelates with minerals may reduce absorption (e.g., oxalate inhibits Ca absorption; phytic acid inhibits Ca, Fe, and Zn absorption).
3. Redox activity of food components
   a. Reductants (e.g., ascorbic acid) enhance absorption of iron but have little effect on other minerals.
   b. Oxidants inhibit the absorption of iron by converting it to the less bioavailable ferric form.
4. Mineral–mineral interactions
   a. High concentrations of one mineral in the diet may inhibit the absorption of another (e.g., Ca inhibits Fe absorption, Fe inhibits Zn absorption, Pb inhibits Fe absorption).
5. Physiological state of individual
   a. Homeostatic regulation of minerals in the body may operate at the site of absorption, resulting in upregulated absorption in deficiency and downregulated absorption in adequacy or overload. This is the case for Fe, Zn, and Ca.
   b. Malabsorption disorders (e.g., Crohn's disease, celiac disease) may reduce absorption of minerals and other nutrients.
   c. Achlorhydria (reduced acid secretion in the stomach) may impair Fe and Ca absorption.
   d. Age may affect mineral absorption: absorption efficiencies often decline with age.
   e. *Pregnancy*: Iron absorption increases during pregnancy.

Ascorbic acid is a particularly potent enhancer of iron absorption because, in addition to its chelating ability, it is a strong reducing agent and reduces Fe^{3+} to the more soluble and bioavailable Fe^{2+}. The following reaction shows how ascorbic acid may reduce iron [120]:

$$\text{Ascobic acid} + 2Fe^{3+} \longrightarrow \text{Dehydroascorbic acid} + 2H^{+} + 2Fe^{2+}$$

Ascorbic acid has a minimal effect on bioavailabilities of other minerals, presumably because they cannot be easily reduced.

Meat factor: Meat, poultry, and fish consistently enhance the absorption of nonheme and heme iron consumed in the same meal [141]. Numerous attempts to identify and isolate the so-called meat factor have proven futile. Meat has a reducing effect on iron [66] so a possible mechanism is the conversion of Fe^{3+} to Fe^{2+} during digestion. In addition, products of meat digestion, including amino acids and polypeptides, may form chelates with iron that are more soluble in the contents of the small intestine.

9.3.3.2 Bioavailability Antagonists

9.3.3.2.1 Phytic Acid

Phytic acid and various phytates are among the most important dietary factors limiting mineral bioavailability [60]. Phytic acid and its mineral complexes (phytates) are the primary storage

FIGURE 9.8 Chemical structure of phytic acid: *myo*-inositol-1,2,3,4,5,6-hexakisphosphate.

FIGURE 9.9 Haworth projection showing possible structure of a phytate-containing chelated magnesium, zinc, calcium, and iron. Ca, Mg, and Zn are divalent cations and Fe is either di- or trivalent. Phytases catalyze the hydrolysis of the phosphate groups yielding a mixture of free inositol, inositol phosphates, inorganic phosphate, and metal cations, some of which would remain bound to the partially hydrolyzed phytic acid. (Redrawn from Lei, X.G. and Stahl, C.H., *Appl. Microbiol. Biotechnol*., 57, 474, 2001.)

forms of phosphorous in plant seeds. Phytic acid, myo-inositol-1,2,3,4,5,6-hexakisphosphate, contains six phosphate groups esterified to inositol (Figure 9.8). These phosphate groups are readily ionized at physiological pH, and thus, phytic acid is a potent chelator of cations, especially di- and trivalent minerals such as Ca^{+2}, Fe^{+2}, Fe^{+3}, Zn^{+2}, and Mg^{+2} (Figure 9.9). The minerals bound in these chelates may have low bioavailability; therefore, phytate is widely perceived as an antinutrient.

In addition to its well-established phosphorous storage function in plant cells, phytic acid and its derivatives serve a wide variety of roles in metabolism, including signal transduction, and possibly ATP, RNA export, DNA repair, and DNA recombination [102]. Phytic acid is readily hydrolyzed by enzymes known as phytases. Partial hydrolysis yields a mixture of inositol phosphates depending on the number of phosphate groups released (Figure 9.9). Phytic acid and its various hydrolysis products are commonly referred to as IP6, IP5, IP4, etc., to indicate the number of phosphate groups esterified to the inositol moiety. The inhibitory effect of phytic acid on mineral absorption is reduced by hydrolysis, but recent evidence suggests that IP5, IP4, and IP3 as well as IP6 may inhibit iron absorption [111].

Concentrations of phytates in foods vary from 1% to 3% (wet basis) in cereals and legumes to a fraction of 1% in roots, tubers, and vegetables [111]. Since most plants contain endogenous phytases that may be activated during food processing, prepared foods contain a mixture of inositol

TABLE 9.6
Content of Inositol Hexakisphosphate (IP6) and Three of Its Hydrolysis Products (IP3, IP4, and IP5) in Selected Foods

Food	IP3	IP4	IP5	IP6
Bread, whole meal	0.3	0.2	0.5	3.2
Textured soy flour	—	0.9	4.4	21.8
Corn grits, Quaker	Tr	0.03	0.3	2.0
Corn flakes, Kellogg's	Tr	0.06	0.09	0.07
Cheerios, General Mills	0.06	2.2	4.6	5.1
Oat bran, Quaker	0.07	1.0	5.6	21.2
Oatmeal, Quaker	0.08	0.7	3.0	10.3
Rice Krispies, Kellogg's	0.05	0.4	0.9	1.2
Shredded wheat, Nabisco	0.1	0.7	3.2	9.7
Wheaties, General Mills	0.6	1.8	3.7	5.1
All-bran, Kellogg's	0.8	3.9	11.5	22.6
Garbanzo beans	0.1	0.56	2.04	5.18
Red kidney beans	0.19	1.02	2.81	9.12

Source: Adapted from Harland. B. and Narula, G., *Nutr. Res.*, 19(6), 947, 1999.
Values are expressed as μmole per gram of food.

hexaphosphate and its various hydrolysis products. Table 9.6 lists the concentrations of these phosphates in selected foods [51]. It is apparent from comparisons of levels in whole cereal brans with refined cereals that phytates are concentrated in the bran layers of the kernel and are quite low in the endosperm. In legume seeds, on the other hand, phytate is more evenly distributed and phytate levels are high in most fractions of these seeds.

Due to the rather consistent evidence supporting the hypothesis that phytic acid reduces the bioavailability of several essential minerals, it is reasonable to infer that reducing phytate concentrations in foods will improve mineral bioavailabilities. This has led to efforts by plant breeders to select for low-phytate varieties of cereal and legume crops as a strategy for reducing the prevalence of trace mineral malnutrition [101]. This approach, while promising, has not yet been sufficiently tested to merit its adoption as a nutritional intervention in humans. Another strategy for reducing phytic acid in foods is to add phytases during food preparation or processing or just prior to consumption. Adding phytase to a maize porridge prior to consumption increased zinc absorption in human subjects by more than 80% [11]. Alternatively, soaking maize flour in water overnight to activate endogenous phytases as a strategy for reducing phytate levels has been tested in a study in Malawi [74]. A small improvement in iron status was observed in children consuming a gruel made from the flour. Unfortunately, the effectiveness of this approach has been inconsistent and disappointing [71].

While reducing phytic acid intakes may benefit mineral nutrition status in some populations, doing so could prove to be unwise because there is compelling evidence from animal studies that phytic acid is protective against some forms of cancer [46,125,129]. The mechanisms involved are poorly understood but may entail antioxidant activity resulting from chelation of iron and copper. Phytic acid is also associated with reduced risk for kidney stone formation, presumably by its ability to inhibit the crystallization of calcium salts [129].

9.3.3.2.2 Polyphenolic Compounds

Foods rich in polyphenolic compounds consistently reduce iron bioavailability from meals [140]. Tea is an especially potent inhibitor, presumably because of its high tannin content. Other polyphenol-rich foods that inhibit iron absorption include coffee, nonwhite beans, raisins, and sorghum [143].

9.3.4 Nutritional Aspects of Essential Minerals: Overview

The process of mineral nutrient digestion and absorption may be described as follows [85]. To start, the food is masticated in the mouth where salivary amylase begins the process of starch digestion. At this stage, only limited changes in mineral species occur. Next, the food is swallowed and enters the stomach where the pH is gradually lowered to about 2 by gastric acid. At this stage, dramatic changes occur in mineral species. Stabilities of complexes are changed by the altered pH and by protein denaturation and hydrolysis. Minerals may be released into solution and may reform complexes with different ligands. In addition, transition metals such as iron may undergo a valance change when the pH is reduced. The redox behavior of iron is strongly pH dependent. At neutral pH, even in the presence of excess reducing agents like ascorbic acid, ferric iron will remain in the 3+ oxidation state. However, when the pH is lowered, ascorbic acid rapidly reduces Fe^{3+} to Fe^{2+}. Since Fe^{2+} has lower affinity than Fe^{3+} for most ligands, this reduction will promote the release of iron from complexes in food.

In the next stage of digestion, the partially digested food in the stomach is emptied into the proximal small intestine where pancreatic secretions containing sodium bicarbonate and digestive enzymes raise the pH and continue the process of protein, lipid, and starch digestion. As digestion proceeds, more new ligands are formed and existing ligands are altered in ways that undoubtedly affect their affinities for metal ions. Thus, a further reshuffling of mineral species occurs in the lumen of the small intestine resulting in a complex mixture of soluble and insoluble and high- and low-molecular-weight species. Soluble species, including unbound mineral ions, may diffuse to the brush border surface of the intestinal epithelial cells where they may be taken by the enterocytes or pass between cells (the paracellular route). Uptake can be facilitated by a membrane carrier or ion channel, which may be an active, energy-requiring process, may be saturable, and may be regulated by physiological processes.

Clearly, the process of mineral absorption and the factors that affect it are extremely complex. Moreover, changes in the speciation of minerals during digestion, although known to occur, are poorly understood. Nevertheless, results from hundreds of studies allow us to identify factors that may influence mineral bioavailability. Some of these are summarized in Table 9.5.

9.3.5 Nutritional Aspects of Essential Minerals: Individual Minerals

For various reasons, deficiencies are common for some mineral elements and rare or nonexistent for others. Moreover, there are large variations in prevalences of specific deficiencies across geographical and socioeconomic divisions. Human dietary deficiencies have been reported for calcium, cobalt (as vitamin B_{12}), chromium, iodine, iron, selenium, and zinc [53]. Calcium, chromium, iron, and zinc occur in bound forms in foods, and bioavailabilities may be low depending on the composition of the food or meal. Thus, deficiencies of these minerals result from a combination of poor bioavailability and low intakes.

Iodine is present in foods and water predominantly as the ionic, unbound form and has high bioavailability. Iodine deficiency is caused primarily by low intakes. Selenium is present in foods principally as selenomethionine, but it is efficiently utilized so deficiency is caused by low intakes. Vitamin B_{12} (cobalt) deficiency is a problem only with persons on strict vegetarian diets that are low in this vitamin or in people suffering from certain malabsorption syndromes. These observations further illustrate the complexities involved in mineral bioavailability. Some bound forms of minerals have low bioavailability, while other bound forms have high bioavailability. Unbound forms generally have high bioavailability. Current thinking on bioavailability and mineral deficiencies is summarized in Figure 9.10 [53].

In the United States, deficiencies of calcium and iron have received the most attention in recent years. In developing countries, iron, zinc, and iodine have been targeted because of high prevalences of deficiencies among populations in these countries.

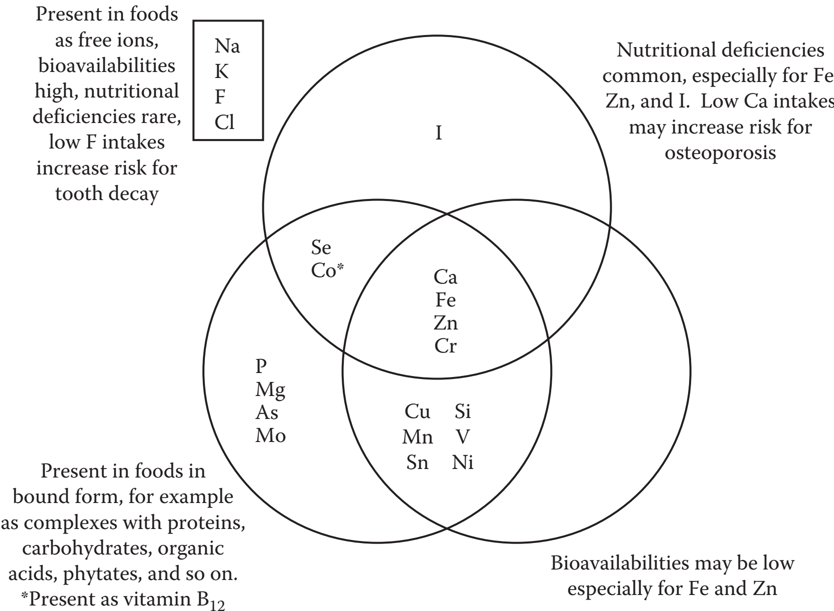


FIGURE 9.10 Essential mineral nutrients grouped according to speciation in foods (metal ions free in solution or bound in complexes or chelates), bioavailabilities, and occurrence of deficiencies in human populations. (Adapted from Hazell, T., *World Rev. Nutr. Diet.*, 46, 1, 1985.)

9.3.5.1 Calcium

Adult male and female bodies contain approximately 1200 and 1000 g of calcium, respectively, making it the most abundant mineral in the body. More than 99% of total body calcium is present in bones [131]. Besides its structural role, calcium plays major regulatory roles in numerous biochemical and physiological processes in both plants and animals. For example, calcium is involved in photosynthesis, oxidative phosphorylation, blood clotting, muscle contraction, cell division, transmission of nerve impulses, enzyme activity, cell membrane function, intercellular adhesion, and hormone secretion.

Calcium is a divalent cation with a radius of 99 picometers. Its multiple roles in living cells are related to its ability to form complexes with proteins, carbohydrates, and lipids. Calcium binding is selective. Its ability to bind to neutral oxygens, including those of alcohols and carbonyl groups, and to bind to two centers simultaneously allows it to function as a cross-linker of proteins and polysaccharides [29].

AI levels for calcium are listed in Table 9.3. They range from 210 mg/day for infants to 1300 mg/day for adolescents and pregnant and lactating women. Calcium intakes for most population groups in the United States are well below the AIs, a cause for concern. Low intakes of calcium are a factor in several chronic diseases including osteoporosis, hypertension, and some forms of cancer. Osteoporosis is characterized by very low bone-mineral density and an increased risk for bone fractures. More than 40 million Americans have osteoporosis or are at high risk for developing it [91]. Osteoporosis is a chronic disease characterized by very low bone-mineral density. People with osteoporosis are at markedly increased risk for bone fractures, especially fractures of the hip, wrist, and vertebrae. While many factors are associated with the disease, low intakes of calcium and vitamin D appear to be among the most important. This putative relationship between calcium intake and bone health has led many health professionals to recommend daily calcium supplements. However, recent meta-analyses have not supported the hypothesis that calcium supplements reduce the risk for bone fractures [105]. Moreover, there is some evidence that taking calcium supplements can increase risk for cardiovascular events, kidney stones, and gastrointestinal problems [105]. Fortunately, there is no evidence that high

calcium intakes from food sources are associated with these adverse health outcomes. Therefore, it seems prudent to get one's calcium from foods rather than from calcium supplements.

9.3.5.1.1 Calcium Bioavailability

The concentration of calcium in the food and the presence of inhibitors or enhancers of calcium absorption determine the absorption of calcium from foods [132]. Calcium absorption efficiency (expressed as a percentage of ingested calcium) is inversely and logarithmically related to the concentration of ingested calcium over a wide range of intakes [54]. The main dietary inhibitors of calcium absorption are oxalate and phytate with oxalate being the more potent. Calcium ions form highly insoluble chelates with oxalate (Figure 9.11). Fiber does not appear to have a major impact on calcium absorption [132].

The calcium content of several dietary sources, the absorption adjusted for calcium load, and the number of servings equivalent to the absorbable calcium in one serving of milk are listed in Table 9.7. Only fortified fruit juices supply more absorbable calcium per serving than milk. These data show that it is difficult to achieve recommended intakes of calcium without consuming milk or other calcium-rich dairy products.

It is apparent from Table 9.7 that both calcium content of foods and absorbability vary widely. The percent absorption of calcium from milk is lower than that for some other foods not because it

FIGURE 9.11 Formation of calcium oxalate from calcium cation and oxalate anion. The solubility of calcium oxalate is only 0.04 mmol/L.

TABLE 9.7
Calcium Content and Bioavailability in Selected Foods

Food	Serving Size (g)	Calcium Content (mg)	Fractional Absorption[a] (%)	Estimated Absorbable Ca/Serving (mg)	Serving to Equal 240 mL Milk (*n*)
Milk	240	300	32.1	96.3	1.0
Almonds	28	80	21.2	17.0	5.7
Pinto beans	86	44.7	17.0	7.6	12.7
Broccoli	71	35	52.6	18.4	5.2
Cabbage, green	75	25	64.9	16.2	5.9
Cauliflower	62	17	68.6	11.7	8.2
Citrus punch, with CCM[b]	240	300	50.0	150	0.64
Kale	65	47	58.8	27.6	3.5
Soy milk	120	5	31.0	1.6	6.4
Spinach	90	122	5.1	6.2	15.5
Tofu, Ca set	126	258	31.0	80.0	1.2
Turnup greens	72	99	51.6	31.1	1.9
Water cress	17	20	67.0	13.4	7.2

Source: Weaver, C.M., and Plawecki, K.L., *Am. J. Clin. Nutr.*, 59, 1238S, 1994.

[a] Percent absorption adjusted for calcium load.

[b] Calcium–citrate–maleate.

is bound in an unavailable form but because it is present at a high concentration. The poor bioavailability of calcium from spinach and pinto beans is probably due to high concentrations of oxalate and phytate, respectively.

9.3.5.2 Phosphorus

Phosphorous is ubiquitous in all living systems due to the vital role it plays in the structure of cell membranes and virtually all metabolic processes. It exists in soft tissues as inorganic phosphate, mostly in the form of HPO_4^{2-}, and as a constituent of numerous organic molecules. The adult human body contains up to 850 g of phosphorous of which 85% is in the skeleton in the form of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. The calcium to phosphate ratio in bone is maintained at a nearly constant mass ratio of approximately 2:1 [4].

Organic phosphates found in living systems include phospholipids, which make up the lipid bilayer in all cell membranes, DNA and RNA, ATP and creatine phosphate, cAMP (an intracellular second messenger), and many others. Thus, phosphorous is required for cell reproduction, cell integrity, transport of nutrients across membranes, energy metabolism, and regulation of metabolic processes.

RDAs for phosphorous range from 100 mg/day in infants to 1250 mg/day in adolescents and pregnant and lactating women (Table 9.3). The phosphorous RDA is very similar to the Ca AI but, unlike the situation for Ca, P deficiency is rare except in persons with certain metabolic diseases. This is because phosphorous is present in significant concentrations on so many foods.

While phosphorous is present in virtually all foods, high-protein foods such as dairy products, meat, poultry, and fish are especially rich sources. Whole grain products and legumes are also high in phosphorous but much of it is present as phytate, the primary storage form of phosphorous in seeds. Unlike inorganic phosphate and most organic phosphates, phytate phosphorous has low bioavailability and may inhibit the absorption of several trace minerals (see Section 9.3.3.2). Phosphates from food additives contribute an increasing proportion of phosphorous intakes. Phosphates are widely used in many processed foods including carbonated beverages, processed cheeses, cured meats, baked products, and many others [36].

9.3.5.3 Sodium, Potassium, and Chloride

Sodium and potassium are classified as alkali metals (group IA of the periodic table). They readily give up one valence electron (ns^1) to form monovalent cations. They exist naturally only as salts. Sodium is the sixth most abundant element in the earth's crust. There are vast underground deposits of sodium chloride. Potassium exists naturally as KCl (sylvite) and $KCl \cdot MgCl_2 \cdot 6H_2O$ (carnallite). The main industrial use of potassium is in fertilizer.

Sodium, potassium, and chloride are essential nutrients but deficiencies are rare because intakes are almost always greater than requirements. An important function of sodium and chloride in the body is to regulate extracellular fluid volume, a key factor affecting blood pressure. Na^+ is the predominant cation in the extracellular fluid and 95% of total body sodium is present in this compartment. Cl^- is the main anion in the extracellular fluid. The functions of Na^+ and Cl^- are closely intertwined, and it is sometimes difficult to separate their roles in metabolism [100]. Potassium, on the other hand, is found primarily in the intracellular fluid. Its functions in the body include maintaining the polarization of membranes, which in turn affects nerve transmission, muscle contraction, and vascular tone [61]. RDAs have not been established for Na, K, or Cl because there are insufficient data available to do so. However, the Institute of Medicine has set AI levels. For adult males and females, AIs for Na, Cl, and K are 1.5, 2.3, and 4.7 g/day, respectively [61]. ULs have been established for sodium and chloride, based on evidence that high sodium intakes increase blood pressure. ULs for sodium and chloride for adult males and females are 2.3 and 3.6 g/day, respectively [61]. A UL for potassium has not been established since there is no evidence of an adverse health effect from consuming too much potassium from foods [61]. For most people, sodium intakes are too high. The third National Health and Nutrition Examination Survey, which was conducted from 1988 to 1994, reported that 95% of men and 75% of

women had intakes of sodium chloride that exceeded the UL. Powles et al. [98] reported that mean global sodium intakes are 3.95 g/day per person with average intakes in North America ranging from 3.4 to 3.8 g/day. The 2010 Dietary Guidelines for Americans recommends that Americans "Reduce daily sodium intake to less than 2,300 milligrams (mg) and further reduce intake to 1,500 mg among persons who are 51 and older and those of any age who are African American or have hypertension, diabetes, or chronic kidney disease. The 1,500 mg recommendation applies to about half of the U.S. population, including children, and the majority of adults" [124]. Clearly, we are a very long way from meeting these guidelines.

9.3.5.3.1 Dietary Sources of Sodium

While sodium is present in foods in many different chemical forms, it is estimated that about 90% of sodium in the U.S. diet is in the form of sodium chloride and that most of this is added during food processing [61]. Table 9.8 provides a summary of the sources of sodium in the American diet. This places considerable pressure on the food industry to reduce the levels of added sodium in its products [16]. Many companies have committed to gradually lowering sodium levels in foods.

There is substantial evidence linking high sodium intakes to elevated blood pressure [62]. This, coupled with evidence showing an association between elevated blood pressure and cardiovascular disease, is the basis for recommendations, like those in the Dietary Guidelines for Americans, to decrease sodium intakes in populations. However, there is also evidence that low sodium intakes may increase risk for death in patients with congestive heart failure [62]. This, along with conflicting evidence on the effectiveness of reducing sodium intakes in populations for preventing chronic diseases, has generated considerable controversy in the literature about the wisdom of public health interventions designed to lower risk of cardiovascular and other diseases by reducing sodium intakes [62]. Part of the controversy is due to the lack of compelling evidence that lowering sodium intakes will actually translate into reduced mortality from cardiovascular and other chronic diseases. A recent meta-analysis may be an indication that more compelling evidence is accumulating. This paper by Mozaffarian et al. [90] concluded that 1.65 million deaths from cardiovascular causes globally can be attributed to sodium intakes above 2.0 g/day.

9.3.5.4 Iron

Iron is the fourth most abundant element in the earth's crust and is an essential nutrient for nearly all living species. In biological systems, it is present almost exclusively as chelates with porphyrin rings or proteins. Adult male and female bodies contain approximately 4 and 2.5 g of iron, respectively. About two-thirds of this iron is functional, meaning that it plays an active role in metabolism. The remaining one-third, in iron replete individuals, is present in iron stores, located primarily in the liver, spleen, and bone marrow. Functional iron plays many key roles in biological

TABLE 9.8
Sources of Salt (NaCl) in the American Diet

Source of Salt	% of Total Salt
Added during food processing	77
Naturally occurring in foods	12
Added at the table	6
Added in the home during cooking	5
Tap water	<1

Source: Adapted from Institute of Medicine, *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*, Otten, J.J., Hellwig, J.P., and Meyers, L.D., eds., The National Academies Press, Washington, DC, 2006.

FIGURE 9.12 Heme, an iron chelate found in many proteins including hemoglobin, myoglobin, cytochromes, and peroxidases. The oxidation state of the iron can be either II or III.

systems, including oxygen transport (hemoglobin and myoglobin), respiration and energy metabolism (cytochromes and iron–sulfur proteins), destruction of hydrogen peroxide (hydrogen peroxidase and catalase), and DNA synthesis (ribonucleotide reductase). Many of the aforementioned proteins contain heme, a complex of iron with protoporphyrin IX (Figure 9.12). Iron's involvement in many of these metabolic reactions depends on its ability to readily accept or donate an electron, that is, to easily redox cycle between the Fe^{2+} and Fe^{3+} forms.

Free iron can be toxic to living cells. Presumably, this toxicity results from the generation of active species of oxygen, which can promote lipid oxidation or attack proteins or DNA molecules (see below, this section and Section 9.5.4). To avoid the toxic consequences of free iron, virtually all living cells have mechanisms for storing extra iron intracellularly in a nontoxic form. The iron is sequestered in the interior of a hollow protein shell called apoferritin. This protein shell is composed of 24 polypeptide subunits arranged as a sphere. Iron is deposited in the cavity of the shell as polymeric ferric oxyhydroxide. Up to 4500 atoms of iron can be stored in one ferritin shell [22]. Ferritin iron is essentially a cellular reserve that can be mobilized when iron is needed for the synthesis of hemoglobin, myoglobin, or other iron proteins.

In spite of iron's abundance in the environment, iron deficiency in humans, some farm animals, and crops grown on some soils is widespread. For example, the World Health Organization estimates that iron deficiency is the most prevalent nutritional disorder in the world [139]. Iron deficiency is a major cause of anemia, which is characterized by low red blood cell counts and low blood hemoglobin concentrations. Approximately one-third of the world's population suffers from anemia. Its health consequences include impaired mental and physical development in children, fatigue, decreased work productivity, and increased child and maternal mortality [139]. Iron deficiency and iron deficiency anemia are particularly widespread in developing countries in South Asia and Africa. Children and women of childbearing age are most affected by iron deficiency.

The paradox of high prevalences of nutritional deficiency of a nutrient present in such abundance in the environment may be explained by the behavior of iron in aqueous solutions. Iron is a transition element, which means that it has unfilled d orbitals. Its oxidation state in most natural forms is either 2+ (ferrous) or 3+ (ferric). Ferrous iron has six d electrons while ferric iron has five. In aqueous solutions under reducing conditions, the ferrous form predominates. Ferrous iron is quite soluble in water at physiological pHs. In the presence of molecular oxygen, however, aqueous Fe^{2+} may react with molecular oxygen to form Fe^{3+} and superoxide anion:

$$Fe_{aq}^{2+} + {}^{\bullet}O_2 \rightarrow Fe_{aq}^{3+} + {}^{\bullet}OL_2^{-}$$

When water molecules bind to iron, their acidity is increased and they may lose protons to form hydroxides. As more and more bound water molecules give up protons, increasingly insoluble ferric hydroxide species form [23]:

$$Fe(H_2O)_6^{+3} + H_2O \rightarrow Fe(H_2O)_5(OH^-)^{+2} + H_3O^+ \rightarrow\rightarrow\rightarrow Fe(OH)_3$$

Because this reaction occurs readily except at very low pH, insoluble ferric hydroxides predominate in aqueous systems, and this explains, in part, the poor bioavailability of dietary nonheme iron.

Iron bioavailability is determined almost totally by the efficiency of iron absorption in the intestine. Total iron intake, composition of the diet, physiological condition (e.g., pregnancy, obesity, infection), and iron status of the individual consuming the diet all play a role in determining the amount of iron absorbed.

Diets in industrialized countries like the United States consistently provide about 6 mg iron per 1000 kcal (4187 kJ) [8]. Iron species in foods may be broadly grouped as either heme or nonheme. Heme iron is firmly bound in the center of a porphyrin ring (Figure 9.12) and does not dissociate from this ligand until after it is taken up by intestinal epithelial cells. It occurs primarily as hemoglobin or myoglobin and thus is found almost exclusively in meat, poultry, and fish. Virtually, all of the iron in plant foods and approximately 40%–60% of the iron in animal tissues is nonheme iron. It is bound primarily to proteins but may also be complexed with citrate, phytate, oxalate, polyphenolics, or other ligands.

The bioavailability of heme iron is relatively unaffected by composition of the diet and is significantly greater than that of nonheme iron. The bioavailability of nonheme iron varies markedly depending on composition of the diet. It is widely assumed that nonheme iron from all sources in a meal (foods as well as fortification iron) enters a common pool during digestion and that absorption of iron from this pool is determined by the totality of ligands present in the digesta as it moves along the gastrointestinal tract.

Several enhancers and inhibitors of nonheme iron absorption have been identified. Enhancers include meat, poultry, fish, ascorbic acid, inulin, and EDTA (in diets where bioavailabilities are low). Inhibitors include polyphenolics (tannins in tea, legumes, and sorghum), phytates (present in legumes and whole grain cereals), some plant proteins (especially legume proteins), calcium (presumably due to competition for transport proteins in the brush border membranes of the intestinal epithelial cells), and phosphates.

The overall bioavailability of iron in a diet is determined by complex interactions of the enhancers and inhibitors present. Iron absorption from diets composed primarily of roots, tubers, legumes, and cereals, with limited meat and ascorbic acid, may be only about 5% even in people with poor iron status. Such a diet would provide only about 0.7 mg of absorbable iron per day, a quantity too small to meet the needs of many individuals. Iron absorption from diets based on roots, cereals, and legumes that contain some meat, poultry, or fish and some foods high in ascorbic acid may be about 10%. These diets provide about 1.4 mg of absorbable iron per day, an amount that is adequate for most men and postmenopausal women but inadequate for up to 50% of women of childbearing age. Diets composed of generous quantities of meat, poultry, fish and foods high in ascorbic acid provide over 2 mg of absorbable iron per day, an amount sufficient to meet the needs of nearly all healthy persons [8].

9.3.5.5 Zinc

Zinc is present in biological systems as a divalent cation, Zn^{2+}. It does not change its valence under most conditions and therefore does not participate directly in redox reactions like its sister transition elements iron and copper. It is a strong Lewis acid and therefore binds to electron donating ligands. Ligands containing sulfhydryl groups (–SH) and amine groups bind Zn^{2+} quite strongly. Therefore, most zinc in biological systems is bound to proteins [27].

Zinc is involved in a wide range of metabolic functions. More than 50 zinc metalloenzymes have been identified. These include RNA polymerases, alkaline phosphatase, and carbonic anhydrase [27]. Zinc plays structural as well as catalytic roles in metalloenzymes. It functions as an antioxidant, presumably as a cofactor of the metalloenzyme Cu/Zn superoxide dismutase. It is also a key player in the regulation of gene expression. RDAs for zinc range from 2 mg/day in infants to 13 mg/day in teenage lactating women (Table 9.4).

In spite of its role in many metabolic processes, a reliable and sensitive indicator for zinc status has not been developed. Plasma zinc concentration is widely used, but it is not very sensitive to changes in zinc status. Nevertheless, plasma zinc concentrations in the range of 12–18 μmol/L are considered normal.

Zinc deficiency in humans and animals causes an impaired immune response, delayed wound healing, and poor appetite. Prasad [99] first described clinical zinc deficiency in 1961 in boys who presented with dwarfism and hypogonadism (delayed sexual maturation). Presumably, these cases were caused by consumption of breads high in phytate [33]. Capacity for storing zinc in the body is limited, and consequently, zinc deficiency can develop rapidly when intakes are low [27].

The content and bioavailability of zinc in foods varies widely. In the United States, meat and milk products are the most important sources [73,75]. Homeostatic regulation of total body zinc occurs primarily in the intestine. When intakes are low, the rate of true absorption increases and endogenous excretion of zinc via the intestine decreases [27]. Fecal excretion of endogenous zinc results from secretions in the pancreatic juice and directly through enterocytes.

Studies on the effects of phytic acid on zinc bioavailability consistently show that phytic acid impairs zinc absorption. Therefore, diets rich in whole grain cereal products and legumes would be expected to increase the risk for zinc deficiency. Products made from refined flour are lower in phytic acid but are also lower in zinc since it is concentrated in the bran and germ fractions of the kernel. Sandström et al. [112] reported that total zinc absorption from whole wheat bread was 50% greater than from white bread even though the percentage absorptions were 17% and 38%, respectively. Zinc deficiency appears to be much more prevalent in developing countries compared to developed countries. In Mexico, 25% of children under the age of 11 had serum zinc levels below 10.0 μmol/L (0.65 mg/L) [110]. A possible explanation for this discrepancy is the lower consumption of meat and dairy products in developing countries. However, in developed countries such as the United States, zinc status in vegetarians does not appear to be significantly lower than in nonvegetarians although some studies have shown lower plasma zinc levels within the normal range [59]. The lack of a sensitive test for marginal zinc deficiency may be one explanation for these results.

9.3.5.6 Iodine

Iodine is an essential nutrient required for the synthesis of the thyroid hormones. These hormones, thyroxine (3,4,3′,5′-tetraiodothyronine, designated as T_4) and 3,5,3′-triiodothyronine (T_3), have multiple functions in the body [118]. They influence neuronal cell growth, physical and mental development in children, and basal metabolic rate. RDAs for iodine range from 90 μg/day in children to 290 μg/day in lactating women (Table 9.4).

Inadequate intakes of iodine cause a variety of diseases known as iodine deficiency disorders (IDD) [30,31]. Goiter is the most widely known IDD, but many other disorders may result from iodine deficiency including decreased fertility, increased rates of perinatal mortality, growth retardation in children, and impaired mental development [31]. Iodine deficiency is the leading cause of mental retardation in the world. Cretinism, its most severe form, occurs in infants whose mothers were severely iodine deficient during their pregnancy. By some estimates, two billion people worldwide have insufficient iodine intakes [58]. The highest prevalences of low intakes are in countries in sub–Saharan Africa and South Asia but intakes in Australia and some European countries are also low [144]. Iodine intakes in the United States appear to be adequate for most people [14]. Dairy products are the single largest contributor to iodine intakes in the United States

but iodized salt is also an important source [95]. The sources of iodine in dairy products include iodine-containing sanitizers and feed supplements. In areas where soil iodine is high, forage crops contain significant concentrations of iodine, and this iodine will be passed into the milk of cows eating the crops.

Iodine deficiency occurs mainly in regions where soil iodine is low due to leaching caused by melting glaciers (e.g., in mountainous regions of Bolivia), heavy rainfall, and flooding [31]. The problem of iodine deficiency may be exacerbated by the ingestion of goitrogens. Goitrogens are substances that promote goiter development. One of these is linamarin, a thioglycoside present in cassava. If it is not removed or degraded by soaking or proper cooking before the cassava is eaten, linamarin is hydrolyzed to cyanide in the intestine, absorbed, and converted to thiocyanate. Thiocyanate interferes with iodide uptake by the thyroid gland. Goitrogens are a factor in goiter development only when iodine intakes are low, as they do not cause goiter in people with AIs of iodine [118].

9.3.5.7 Selenium

Selenium is an essential constituent of at least 25 proteins in the body [80]. These include glutathione (GSH) peroxidase, plasma selenoprotein P, muscle selenoprotein W, and selenoproteins found in the prostate and placenta. GSH peroxidase catalyzes the reduction of hydroperoxides, thereby serving an important antioxidant role. This function explains early observations that selenium could spare vitamin E in humans and animals, that is, the vitamin E requirement increases in selenium deficiency and decreases in selenium adequacy. RDAs for selenium range from 14 μg/day for infants to 70 μg/day for lactating women (Table 9.4).

Se appears in the same group in the periodic table (IVa) as oxygen and sulfur and therefore has similar chemical properties. It is present in animal tissues primarily as selenocysteine, an amino acid with a carbon skeleton identical to serine and cysteine (Figure 9.13).

Proteins that contain Se in stoichiometric proportions are called selenoproteins. Selenocysteine is the active form of Se in proteins of animals. Selenomethionine is also present, but it appears to be a storage form that occurs nonspecifically in both plants and animals as part of the methionine pool [13]. Se is not known to be an essential nutrient in plants, but selenomethionine is present in plant tissues at widely varying concentrations depending on the levels of bioavailable selenium in the soil where the plant was grown.

Selenium deficiency causes serious health problems in both animals and humans. Its prevalence varies widely across regions of the world. High prevalences occur in areas when soil Se levels are low and populations rely heavily on locally produced foods. Keshan disease and Kaschin–Beck

(a) $H_2N-CH(CH_2OH)-C(=O)-OH$
(b) $H_2N-CH(CH_2SH)-C(=O)-OH$
(c) $H_2N-CH(CH_2SeH)-C(=O)-OH$
(d) $H_2N-CH(CH_2-CH_2-Se-CH_3)-C(=O)-OH$

FIGURE 9.13 Chemical structures of (a) serine, (b) cysteine, (c) selenocysteine, and (d) selenomethionine. (Redrawn after from Burk, R.F. and Levander, O.A., Selenium, in: Shills, M.E. Olson, J.A., Shike, M., and Ross, A.C., eds., *Modern Nutrition in Health and Disease*, 9th edn., Lippincott Williams & Wilkins, Philadelphia, PA, 1999, pp. 265–276.)

disease occur in rural areas of China and eastern Siberia where soil Se levels are extremely low [26]. Keshan disease is a myocarditis (an inflammation of the middle muscular layer of the heart wall) that manifests as cardiac insufficiency, enlarged heart, heart arrhythmias, and other heart-related problems. Supplementation with sodium selenite (Na_2SeO_3) tablets have produced a dramatic reduction in the prevalence of this disease in low-Se areas of China in recent years, but it is now recognized that the disease is multifactorial and possibly involves a viral infection that is more virulent in patients with Se deficiency [26]. Kaschin–Beck disease is a form of osteoarthritis that presents as joint deformities and, in severe cases, dwarfism. It has been clearly associated with Se deficiency, but as in the case of Keshan disease, other factors appear to be involved in its causation [13]. These include mycotoxins in grain and unknown organic contaminants in drinking water.

In addition to its role as a nutrient essential for preventing the aforementioned deficiency disorders, there is evidence to suggest that Se intakes above those necessary to prevent deficiencies may prevent cancer. Many observational studies have reported an inverse association between dietary intakes of selenium and cancer rates. A recent meta-analysis of 55 prospective observational studies found that higher selenium intakes were associated with a lower risk of cancer incidence and cancer mortality [128]. However, in the same study, a meta-analysis of randomized controlled trials revealed no clear evidence that selenium supplementation reduced the risk of any cancer. The authors concluded that "To date, no convincing evidence suggests that selenium supplements can prevent cancer" [128]. These conflicting results may be due to a possible selenium–gene interaction whereby the effect of selenium status on cancer risk may be different in different individuals or populations [80]. It is also possible that the baseline selenium status of the populations studied may influence the protective effect, or lack thereof, of selenium on cancer risk. If this is the case, selenium may turn out to be protective in people with low or moderate selenium status but not in people who are well nourished with respect to selenium. In fact, there is growing evidence that taking selenium supplements may increase for nonmelanoma skin cancer and type 2 diabetes in people with adequate selenium status [103].

The primary sources of Se in human diets are cereal products, meats, and seafood [3]. The concentration of Se in these foods is highly variable across regions of the world because of large variations in levels of bioavailable Se in soils. A dramatic example of this is seen in wheat grains. Wheat kernels grown in the Dakotas in the United States may contain more than 2 mg Se/kg, while concentrations in wheat grown in New Zealand may be as low as 0.005 mg/kg. The Se content of animal products is also variable because levels in animal feeds that in turn are influenced by soil levels affect it. In recent decades, the practice of adding Se to animal feed supplements to prevent Se deficiency is becoming increasingly common. This practice has reduced the geographic variation in Se levels in animal products [26]. Table 9.9 lists the Se contents in selected foods available in several countries. Given the differences in Se in foods in

TABLE 9.9
Se Content of Selected Food Categories in μg/g

		Finland[a]		China by Se Regions[b]		
Food	**USA**	**Pre-1984**	**Post-1984**	**Low Se**	**Moderate Se**	**High Se**
Cereal products	0.06–0.66	0.005–0.12	0.01–0.27	0.005–0.02	0.017–0.11	1.06–6.9
Red meats	0.08–0.50	0.05–0.10	0.27–0.91	0.01–0.03	0.05–0.25	—
Dairy products	0.01–0.26	0.01–0.09	0.01–0.25	0.002–0.01	0.01–0.03	—
Fish	0.13–1.48	0.18–0.98	—	0.03–0.20	0.10–0.60	—

Source: Adapted from Combs, G.F., *Br. J. Nutr.*, 85, 517, 2001.

[a] Use of Se fertilizers as a means to increase Se levels in foods began in Finland in 1984.

[b] China has regions of low-, moderate-, and high-Se soils.

TABLE 9.10
Dietary Selenium Intakes from Selected Countries around the World

Country or Region	Se Intake (μg/day, Ranges)
China (low-Se area)	3–11
China (high-Se area)	3200–6690
Finland (1974)	25–60
Finland (1992)	90 (mean)
New Zealand	6–70
UK (1978)	60 (mean)
UK (1995)	29–39
USA	62–216

Source: Adapted from Reilly, C., *Trends Food Sci. Technol.*, 9, 114, 1998.

different countries, it is no surprise that dietary Se intakes also vary across regions of the world. Table 9.10 summarizes Se intakes in several countries.

It is interesting to note that Se intakes in the United Kingdom apparently declined between 1978 and 1995. This decline has been attributed to a shift from bread wheat flour grown in the United States to wheat grown in the United Kingdom [106]. Most bread wheat grown in the United States comes from areas where soil Se levels are high.

9.3.6 Toxicology of Food-Borne Heavy Metals

All metals, including those that are essential nutrients, are toxic when intakes exceed safe levels, but mercury, lead, and cadmium are most commonly considered to pose significant risks in foods.

Heavy metals find their way into foods through a variety of routes. They may be taken up from soils through the roots of plants or be deposited on the surfaces of plant leaves from airborne particulates or aerosols. Animals feeding on contaminated plants, water, or other animals may accumulate the metals in their tissues. Contaminated water may be used for irrigation, food processing, or home preparation of foods. Food processing machinery and food packaging materials may contain heavy metals that leach into foods. Contamination with heavy metals may be from natural as well as man-made sources. Rain may leach heavy metals from rocks and deposit them in a bioavailable form in soils used for food production. Volcanic eruptions often contain high levels of mercury. Man-made sources include fertilizers, fungicides, sewage sludge, solders used to seal cans, clays used in the manufacture of ceramics, pigments used in paints, exhaust from automobiles burning leaded gasoline, emissions from electricity-generating plants, and effluent from manufacturing plants such as paper mills. Fortunately, there has been substantial progress over the past three or four decades in reducing or eliminating contamination from many of these sources. For example, unleaded gasoline has largely replaced leaded gasoline in many countries, manufacturers have applied technologies for removing toxicants from air and water effluents, and fungicides and pesticides containing mercury and arsenic have been replaced by less toxic alternatives. Nevertheless, heavy metal contamination of foods is an ongoing concern that requires constant vigilance and monitoring.

Food processing operations may remove heavy metal contaminants from foods as well as add them. For example, the concentration of cadmium in pasta made from durum wheat was 63% of that in the intact kernel. In contrast, lead levels in the same pasta were 120% that of the intact kernel [28]. Cooking the pasta in water reduced concentrations of cadmium and lead to 33% and 52% of the levels in the intact kernels. It should be noted that levels of both cadmium and lead in the wheat samples were well below the 0.2 μg/g (fresh weight) maximum set by the European Commission in 2001.

9.3.6.1 Lead

Lead (Pb) is a malleable, corrosion-resistant metal and is therefore an attractive material for many uses including water pipes, which were used in many municipal water systems beginning in the 1800s. Some of these pipes remain in use today. Lead has also been used as a gasoline additive, in solder used in plumbing and food cans, and in paint. Fortunately, many of these uses of lead have been banned by governments or voluntarily eliminated by industries, but lead persists as a widespread environmental contaminant.

Lead is a neurotoxin that can cause potentially serious and irreversible damage to health. Children and pregnant women are especially vulnerable to its effects. Signs and symptoms of lead poisoning in children include learning and behavioral problems, anemia, kidney damage, and, when exposure is high, seizures, coma, and even death [39]. In adults with occupational exposure to lead, suppressed immunity, peripheral neuropathy, renal failure, gout, and hypertension have been reported [12]. The U.S. Centers for Disease Control has declared a blood lead level (BLL) of 10 μg/dL as a *level of concern* in children [20]. However, no safe BLL has been identified and BLLs below 10 μg/L have been associated with adverse effects in children including IQ deficits, attention-related behaviors, and poor academic achievement [1]. This has led to calls for the elimination of the *level of concern* designation and a refocusing to primary prevention rather that a response to situations where BLLs exceed 10 μg/L or any other designated *level of concern*. Primary prevention would entail reducing sources of lead exposure even without evidence of elevated BLLs [1].

Fortunately, exposure to lead has dropped dramatically over the past three decades as a result of the U.S. government regulations aimed at reducing lead in the environment. The use of lead in paints was banned in 1978, the addition of lead to gasoline was completely eliminated in 1995 following a 25-year phaseout program, the use of led in plumbing was banned in 1986, and lead solder in food cans was banned in 1995 [12]. The impact of these measures has been remarkable. For example, an FDA Total Diet Study showed that daily intakes of lead from food sources by 2–5-year-olds decreased from 30 μg/day in 1982–1984 to 1.3 μg in 1994–1996. In adults, the decrease over the same period was from 38 to 2.5 μg/day.

The aforementioned measures have also yielded impressive reductions in blood levels in the U.S. population. Geometric mean BLLs in children aged 1–5 years in the United States decreased from 15 μg/dL in 1976–1980 to less than 2 μg/dL in 2007–2008 [12]. However, scientists from the U.S. Centers for Disease and Control reported that analyses of data from the 1999 to 2001 National Health and Nutrition Examination Survey (NHANES) showed that an estimated 434,000 children in the United States still have BLLs higher than 10 μg/dL [82]. Therefore, continued vigilance is required, especially because the adverse effects of lead exposure in children are irreversible.

9.3.6.2 Mercury

Mercury (Hg) is one of the most toxic elements. It has no known physiological function. Mercury occurs naturally in the earth's crust and may find its way into soils, water, and/or the atmosphere through erosion, volcanic eruptions, waste streams from industrial processes, agricultural fungicides, burning of fossil fuels and solid waste, and other anthropogenic activities [19]. Mercury in its various forms has been used to manufacture dental amalgams, agricultural fungicides, antibacterial drugs, thermometers, blood pressure manometers, electrical switches, and many other products. It is only relatively recently that its toxicity has become widely recognized.

9.3.6.2.1 Occurrence and Toxicity

Mercury exists in three oxidation states (0, 1+, and 2+) and three chemical forms: elemental mercury, a liquid commonly known as quicksilver; inorganic salts of mercury; and organic mercury including phenyl and alkyl mercury compounds, for example, methyl mercury, $CH_3–Hg^+$, and diethyl mercury, $(CH_3CH_2)_2Hg$ [2]. Elemental mercury is a liquid at room temperature. It has a relatively

high vapor pressure at 20°C (1.3×10^{-3} mmHg) and therefore can evaporate and be inhaled into the lungs. CH_3–Hg^+ is positively charged and therefore must be combined with a counter ion; chloride appears to be one of the most common, yielding CH_3–Hg–Cl. The Hg–Cl bond in methyl mercury chloride is highly covalent in nature, making the compound lipophilic and therefore able to cross cell membranes. This explains its ability to bioaccumulate [77]. Methyl mercury compounds are formed by biomethylation of inorganic mercury that accumulates in the sediment of lakes, streams, and oceans [121]. These compounds then enter the aquatic food chain and accumulate in fish and marine mammals. Concentrations are highest in long-lived predatory fish like swordfish, shark, pike, and bass [24].

The toxicity of mercury and its compounds varies with the chemical form and usually involves neurological and/or renal pathologies. Elemental mercury is poorly absorbed in the gastrointestinal tract and readily excreted in the feces so toxic effects from oral ingestion are rare except in cases of chronic or high-level exposure [32]. However, inhalation of mercury vapors can be toxic [24] and the use of elemental mercury is being phased out in many applications including laboratory thermometers and manometers used to measure blood pressure in doctors' offices. Mercury salts and organic mercury compounds, on the other hand, are highly toxic at low exposure levels. Organomercury compounds are the most toxic. Methyl mercury compounds were first synthesized in London in the 1860s, and two lab technicians working on the project died from mercury poisoning [25]. A Dartmouth College chemistry professor died in 1997, 298 days after she accidentally spilled a small amount of dimethyl mercury on her gloved hand [93]. Clinical signs and symptoms involving the kidney may include glomerulonephritis and proteinuria [2]. Neurological effects can include paresthesia (numbness or tingling), ataxia (loss of coordination of voluntary muscles), neurasthenia (emotional and psychological problems), vision and hearing loss, coma, and even death [2].

Mercury compounds have a strong affinity for thiol (–SH) groups and this property appears to be related to its toxicity. In mitochondria, mercury binds to GSH causing a depletion of free GSH. GSH is an important antioxidant within cells and its depletion leads to the accumulation of free radicals and oxidative stress [19].

Several tragic episodes of mercury poisoning resulting from food contamination have been documented. An outbreak in Minamata, Japan, was caused by consumption of fish caught in Minamata Bay [108]. The bay was heavily contaminated with mercury from industrial wastewater [32]. In another case in the winter of 1971–1972, an outbreak in Iraq affecting hundreds of people was caused by the mistaken use of wheat seeds treated with a fungicide containing methyl mercury for bread baking. The seeds were meant for planting but somehow got diverted to a flourmill. There were more than 6000 cases of poisoning and 500 people died. The Environmental Protection Agency in the United States has since banned the use of alkyl mercury compounds in agriculture [2].

Now that mercury compounds have been banned from use as a fungicide, fish and marine mammals are the primary source of exposure to methyl mercury [24]. Levels of mercury in fish can vary widely as indicated in Table 9.11. While commercially caught marine fish appear to pose the greatest risk, freshwater fish may also be contaminated with mercury.

The known contamination of fish and other seafood with mercury poses a bit of a dilemma for institutions that make dietary recommendations. Seafood is our primary source of the long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Consumption of EPA and DHA is associated with reduced risk for cardiovascular disease. There is also moderate evidence that intakes of omega-3 fatty acids by pregnant and lactating women are associated with improved visual and cognitive development in their infants. Therefore, the 2010 Dietary Guidelines for Americans recommend that adults consume 8 oz of seafood per week and pregnant and lactating women consume at least 8–12 oz of seafood per week [124]. The dietary guidelines state that "Moderate, consistent evidence shows that the health benefits from consuming a variety of seafood in amounts recommended outweigh the health risks associated with methyl mercury, a heavy metal found in seafood in varying levels."

TABLE 9.11
Levels of Mercury in Some Species of Seafood

Species	Mean (ppm)	Range (ppm)
Tilefish (Gulf of Mexico)	1.45	0.65–3.73
Swordfish	1.00	N.D.–3.22
King mackerel	0.73	0.23–1.67
Shark	0.98	N.D.–4.54
Tuna (fresh or frozen, Albacore)	0.35	N.D.–0.85
Lobster (northern, American)	0.11	N.D.–0.23
Tuna (canned, Albacore)	0.35	N.D.–0.85
Salmon (fresh or frozen)	N.D.	N.D.–0.19
Shrimps	0.01	N.D.–0.05

Source: Adapted from Food and Drug Administration, Mercury levels in commercial fish and shellfish (1990–2010), 2006, http://www.fda.gov/food/foodborneillnesscontaminants/metals/ucm115644.htm, accessed on August 26, 2014.

N.D., not detectable.

The FDA does recommend that pregnant women choose fish that tend to contain lower levels of mercury [146]. These include salmon, shrimp, pollock, tuna, tilapia, catfish, and cod. The FDA recommends avoiding fish that often contain higher levels of mercury including tilefish from the Gulf of Mexico, shark, swordfish, and king mackerel.

9.3.6.3 Cadmium

Chronic cadmium toxicity is associated with kidney dysfunction, bone disease, and some forms of cancer [64]. Sources of cadmium exposure include food, tobacco smoke, emissions from burning fossil fuels, and some industrial processes. The FAO/WHO Joint Expert Committee on Food Additives (JECFA) has published a provisional tolerable weekly intake (PTWI) level of 7 μg/kg body weight per week (1 μg/kg body weight/day) for cadmium. JECFA defines PTWI as the level of intake that can be safely ingested weekly over a lifetime without significant risk for adverse health effects [113,115]. Recently, some authors have suggested that risks for kidney dysfunction increase at intakes below current PTWI levels [64,113,114].

Cadmium occurs naturally in soils, water, and sediments in lakes, streams, and oceans [88]. A comparison of the cadmium content of agricultural and nonagricultural soils in Australia revealed that the levels in the agricultural soils were significantly higher than in nonagricultural soils [115]. The likely explanation is the use of cadmium-contaminated phosphate fertilizers, but the application of sewage sludge to soils may also be a factor. This is a concern since cadmium in soils is known to be more bioavailable to plants than lead or mercury, and food crops grown on cadmium-contaminated soils are the primary source of cadmium exposure in the general population [115].

In the United States, the primary source of cadmium exposure among nonsmokers is the diet. Table 9.12 provides estimates of the cadmium content of various foods, consumption of those foods, and daily cadmium intakes. Leafy vegetables, grains, legumes, and kidney meats tend to contain higher levels than other foods [147]. Some plants and animals are bioaccumulators of cadmium. For example, sunflower seeds typically contain higher levels of cadmium than other crops grown on the same soil. Crustaceans and mollusks are also accumulators. Fortunately, consumption of these foods is generally low. The estimated typical daily intake of cadmium is 30 μg/day, which is below the 70 μg/day the FAO/WHO has set as a safe level of intake.

TABLE 9.12
Content of Cadmium in Food Categories and Estimates of Intakes in Human Populations

Food	Cd Content of the Food (mg/kg)		Typical Intake of the Food (g/day)	Exposure (µg/day)	
	Maximum	Typical		Extreme	Typical
Vegetables, including potatoes	0.1	0.05	250	25	12.5
Cereals and legumes	0.2	0.05	200	40	10
Fruit	0.05	0.01	150	7.5	1.5
Oilseeds and cocoa beans	1.0	0.5	1	1	0.5
Meat and poultry	0.1	0.02	150	15	3.0
Liver (cattle, sheep, poultry, pig)	0.5	1.0	5	2.5	0.5
Kidney (cattle, sheep, poultry, pig)	2.0	0.5	1	2	0.5
Fish	0.05	0.02	30	1.5	0.6
Crustaceans, molluscs	2	0.25	3	6	0.75
Total				93.5	30

Source: Adapted from Satarug, S. et al., *Br. J. Nutr.*, 84(6), 791, 2000.

9.4 MINERAL COMPOSITION OF FOODS

9.4.1 Ash: Definition and Significance in Food Analysis

Ash is included in nutrient databases as one of the proximate components of foods. It is determined by weighing the residue left following complete combustion of the organic matter in the food and provides an estimate of the total mineral content of foods [50]. Methods for determination of ash in specific foods and food groups are described in official publications [5]. Minerals in the ash are in the form of metal oxides, sulfates, phosphates, nitrates, chlorides, and other halides. Thus, ash content overestimates total mineral content by a considerable extent since oxygen is present in many of the anions. It does, however, provide a crude idea of mineral content and it is required for calculation of total carbohydrate in the proximate analysis scheme.

9.4.2 Individual Minerals

Individual minerals in foods are determined by ashing the food, dissolving the ash (usually in acid), and measuring mineral concentrations in the resulting solution [18,50,86]. Both chemical and instrumental methods are used to measure mineral concentrations, but instrumental methods are generally more rapid, precise, and accurate. Atomic absorption spectroscopy has been available since the 1960s and is still widely used. It is a reliable technique but can measure only one mineral at a time. Inductively coupled plasma spectrometers have gained popularity in recent years primarily because they are capable of quantifying several mineral elements simultaneously from a single sample [86].

Nutrient composition data are available online at the USDA Nutrient Database for Standard Reference [148]. This searchable database provides composition data for over 8000 foods, including many brand-name products. Values for water, protein, fat, carbohydrate, vitamins, and minerals are given. Ca, Fe, Mg, P, K, Na, and Zn are listed for most foods. Values are means of multiple, presumably representative, samples, so values for a given food sample may deviate considerably from the mean.

9.4.3 Factors Affecting the Mineral Composition of Foods

Many factors interact to affect the mineral composition of foods so compositions can vary greatly.

9.4.3.1 Factors Affecting the Mineral Composition of Plant Foods

In order for plants to grow, they must take up water and essential mineral nutrients from the soil. Once taken up by plant roots, nutrients are transported to other parts of the plant. The ultimate composition of the edible parts of plants is influenced and controlled by genetics of the plant, fertility of the soil, and the environment in which it grows (Figure 9.14). Recent evidence suggests that the variation in the trace mineral content of cereal grains and beans is quite large (Table 9.13).

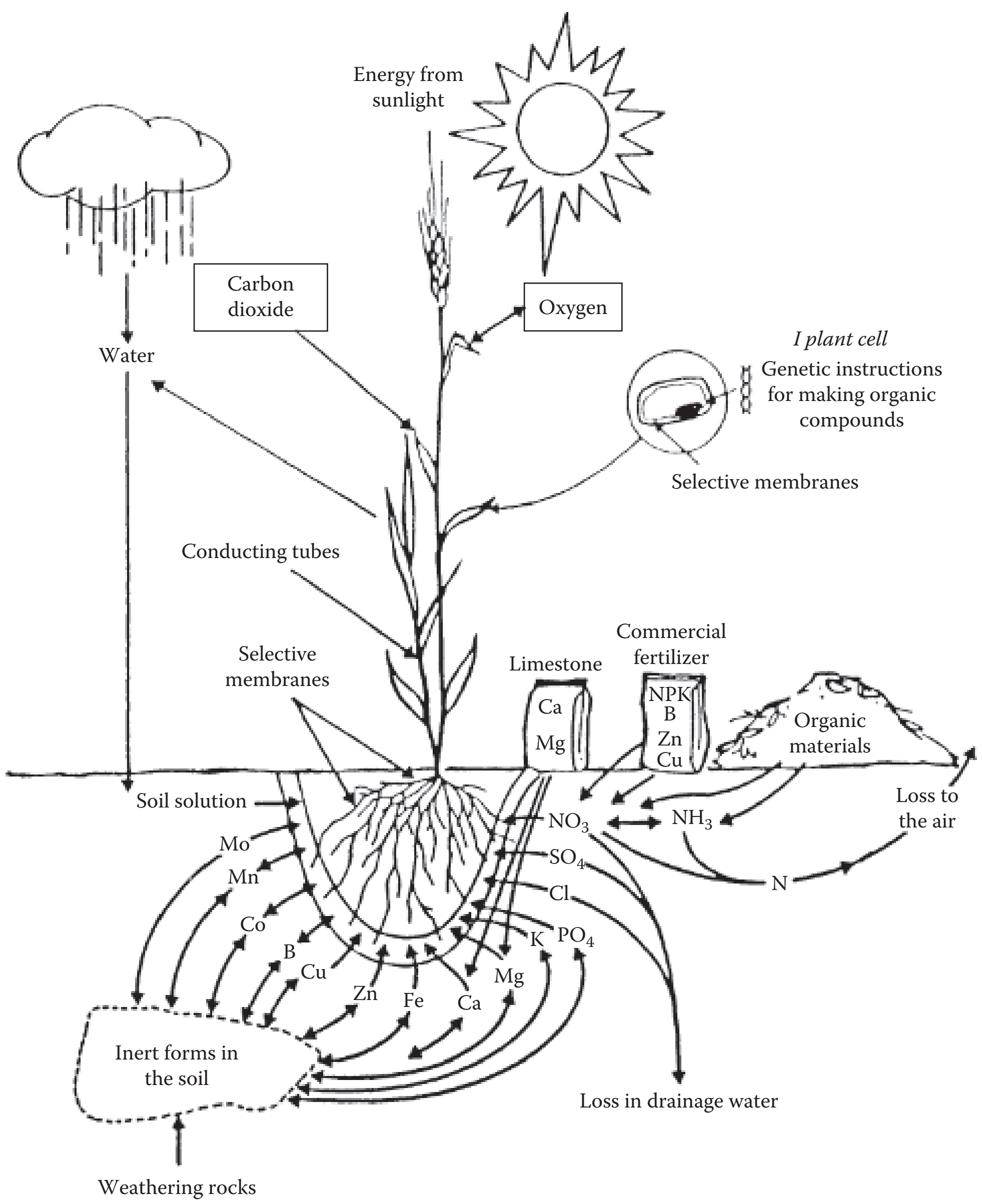


FIGURE 9.14 Plants obtain nutrients from the soil solution surrounding the roots. Sources of these nutrients include fertilizer, decaying organic matter, and weathering rocks. The minerals are taken up into the roots by a selective process and transported upward to all parts of the plant. The whole process is regulated according to instructions encoded in the plant's genome. (From Allaway, W.H., The effects of soils and fertilizers on human and animal nutrition, Agriculture Information Bulletin No. 378, U.S. Department of Agriculture, Washington, DC, 1975.)

TABLE 9.13
Variation in the Content of Iron and Zinc (Dry Weight Basis) in Selected Genotypes of Rice, Wheat, and Common Beans

	Fe (μg/g)		Zn (μg/g)	
Crop	**Mean**	**Range**	**Mean**	**Range**
Brown rice	13	9–23	24	13–42
Wheat	37	29–57	35	25–53
Beans	55	34–89	35	21–54

Source: Data are from Welch, R.M. and House, W.A., Factors affecting the bioavailability of mineral nutrients in plant foods, in: Welch, R.M., ed., *Crops as Sources of Nutrients for Humans*, Soil Science Society of America, Madison, WI, 1984, pp. 37–54.

9.4.3.2 Adequacy of Plant Foods for Supplying the Mineral Needs of Humans

Plant-based foods are the primary source of nutrients for much of the world's population. Therefore, it is important to understand whether plants can meet human nutrient requirements and how nutrient levels can be manipulated to enhance nutritional quality. This raises a number of questions. Do plants and humans require the same mineral nutrients? Are the concentrations of mineral nutrients in plants sufficient to meet human requirements? Can mineral concentrations in plants be altered by agricultural or genetic means to enhance the nutritional quality of plants? Are plants grown on depleted soils nutritionally inferior to plants grown on more fertile soils?

The list of essential minerals for plants is similar but not identical to the list for humans. F, Se, and I are essential for humans but not for most plants. Thus, we might expect to see human deficiencies of these elements in populations that depend on plants grown locally where soil concentrations of these elements are low. In fact, serious human deficiencies of selenium and iodine do occur in several areas of the world [26,31].

For nutrients required by both plants and animals, we might expect human deficiencies to be less of a problem because the elements will necessarily be present in plant foods. Unfortunately, concentrations of minerals in plants are sometimes too low to meet human needs, or the minerals may be present in forms that cannot be efficiently utilized by humans (see Sections 9.3.5.1 and 9.3.5.4). These situations apply, respectively, to calcium and iron. The calcium content of some plants is extremely low. Rice, for example, contains only about 10 mg calcium per 100 kcal. Thus, persons consuming rice-based diets must depend on other foods to meet calcium requirements. Iron is more uniformly distributed in plant foods than calcium, but its bioavailability can be extremely poor so diets based on cereals and legumes are often inadequate in iron [69]. For example, Joy et al. [65] used data from food balance sheets and food composition tables to estimate the per capita supply of several minerals in 46 countries in Africa. According to their analysis, 54% of the population is at risk for calcium deficiency, 40% for zinc deficiency, and 28% for selenium deficiency. In many African countries, animal-source foods make up a relatively small proportion of the diet. This suggests that it is difficult to meet mineral nutrient requirements from plant foods.

While it is possible in some cases to enhance the nutritional quality of crops through agronomic practices and plant breeding, the movement of mineral nutrients from the soil to the plant and from the plant to the animal or human is an extremely complicated process. Soils differ considerably in their mineral composition. Moreover, the concentration of an element in the soil may not be a good indicator of the amount that can be taken up by plant roots since the chemical form of the element and soil pH have marked effects on mineral bioavailability to plants. For example, increasing soil pH by adding lime will lower availability of iron, zinc, manganese, and nickel to plants and will increase availability of molybdenum and selenium [134]. Also, plants generally

possess physiological mechanisms for regulating amounts of nutrients taken up from the soil. Therefore, we might expect that attempts to alter the mineral composition of food crops would meet with mixed results. For example, application of fertilizer does not significantly increase iron, manganese, or calcium content of food crops [134]. On the other hand, fertilization with zinc at levels in excess of the zinc requirement of the plant has been shown to increase the level of zinc in pea seeds [135]. Moreover, there is growing evidence that genetic factors play a major role in determining mineral content of plants and that the variation among genotypes can be quite large [9,133]. This suggests that it should be possible to enhance the trace mineral content of these important food staples through conventional plant breeding practices, a strategy that has been termed biofortification.

Biofortification is a strategy whereby plant breeding and agronomic practices are used to increase the concentrations and/or bioavailabilities of key nutrients in food crops [133]. A major international effort led by the HarvestPlus program is working with plant breeders, seed companies, farmers, nutritionists, and others to prevent micronutrient malnutrition in resource-poor regions by providing local farmers with biofortified seeds [52]. The idea is that crops grown from these seeds will deliver increased amounts of nutrients to people who consume them. HarvestPlus is focused on biofortifying staple food crops with iron, zinc, and provitamin A, which are the three micronutrients that are deficient in the diets of millions of people around the world. Their target crops include beans, cassava, maize, pearl millet, rice, sweet potato, and wheat.

9.4.3.3 Factors Affecting the Mineral Composition of Animal Foods

Mineral concentrations in animal foods vary less than mineral concentrations in plant foods. In general, changes in dietary intake of the animal have only a small effect on mineral concentrations in meat, milk, and eggs. This is because homeostatic mechanisms operating in the animal regulate tissue concentrations of essential nutrients. An exception to this is the significantly lower iron content of veal compared to beef that are range-fed or fed cereal and legume-based rations. Both are bovines, but veal calves are usually fed milk-based diets that are low in iron and consequently are often iron deficient at slaughter. This can affect the iron content of the meat. For example, the iron content of braised top round of veal, separable lean only, is 1.32 mg/100 g compared to 3.32 mg/100 g for the same cut of braised beef [123].

9.4.3.4 Adequacy of Animal Foods for Supplying Mineral Needs of Humans

The composition of animal tissues is similar to that of humans; thus, we might expect animal foods to be good sources of nutrients. Meat, poultry, and fish are good sources of iron, zinc, phosphate, and cobalt (as vitamin B_{12}). However, these products are not good sources of calcium unless bones are consumed, which is usually not the case. Also, the iodine content of animal foods, except marine fish, may be low. Dairy products are excellent sources of calcium. Thus, consumption of a variety of animal foods along with a variety of plant foods is the best way to ensure AIs of all essential minerals.

9.4.4 Fortification and Enrichment of Foods with Minerals

Fortification of the U.S. food supply began in 1924 with the addition of iodine to salt to prevent goiter, a prevalent public health problem in the United States at the time [17]. In the early 1940s, food fortification was expanded further when it became apparent that many young adults were failing Army physical exams due to poor nutritional status. In 1943, the government issued an order making it mandatory to enrich of flour with iron (along with riboflavin, thiamin, and niacin). Many other fortification initiatives have dealt with nutrients other than minerals, including vitamin D in 1933 and folic acid in 1998 [6].

Since the introduction of fortification back in the 1920s, there has been a dramatic reduction in the prevalences of many nutrient deficiency diseases in the United States, including iron, iodine,

TABLE 9.14
FDA Standards for the Enrichment of Cereal Products with Iron and Calcium. Products Labeled as Enriched Must Conform to These Standards

Food	Iron (mg/lb) (Shall Contain)	Calcium (mg/lb) (May Contain)
Enriched flour[a]	20	960
Enriched bread, rolls, and buns[a]	12.5	600
Enriched macaroni and noodle products[b]	Not less than 13	Not less than 500
	Not more than 16.5	Not more than 625
Enriched rice[b]	Not less than 13	Not less than 500
	Not more than 26	Not more than 1000
Enriched corn meals[b]	Not less than 13	Not less than 500
	Not more than 26	Not more than 750

Source: Adapted from Food and Drug Administration, Code of federal regulations, U.S. Government Printing Office, Washington, DC, Chapter I, Parts 136, 137, 139, 2003, http://www.gpoaccess.gov/cfr/index.html.

[a] May be from any safe and suitable substance.

[b] Must be from iron and/or calcium sources that are harmless and assimilable.

niacin, and vitamin D deficiencies. While general improvements in diets were major factors in this improvement in nutritional status, fortification undoubtedly deserves much of the credit for the low prevalences of nutrient deficiency diseases in the United States today. Rates of anemia in children in the United States have gradually declined since 1970 and they continue to fall [142]. This decline coincides with an increase in the quality and quantity of iron-fortified infant formulas and cereals, suggesting that fortification does make a difference. Another example of a successful fortification program is Chile where prevalence of iron deficiency among children has dramatically decreased following a national program to fortify milk products with iron [142].

In the United States today, most foods containing refined cereal grains (e.g., white flour, white rice, corn meal) are enriched with iron, niacin, riboflavin, thiamin, and folic acid. Current FDA standards for enriched flour, bread, rice, corn, and macaroni products are listed in Table 9.14. Most salt destined for domestic use is iodized. In addition, calcium, zinc, and other trace minerals are sometimes added to breakfast cereals and other products. Infant formulas contain the largest number of added minerals since they must be nutritionally complete.

9.4.4.1 Iron

In 4000 B.C., a Persian physician named Melampus made the first recorded recommendation for iron fortification [107]. He recommended that sailors consume sweet wine laced with iron filings to strengthen their resistance to spears and arrows and to enhance sexual potency. Widespread iron fortification began in the United States in 1943 when War Food Order No. 1 made enrichment of white flour sold in interstate commerce mandatory. Federal regulations no longer require flour enrichment but many state regulations do.

Addition of iron to foods is a difficult balancing act because some forms of iron catalyze oxidation of unsaturated fatty acids and vitamins A, C, and E [83]. These oxidation reactions and other interactions of the added iron with food components may produce undesirable changes in color, odor, and/or taste. In many cases, forms that are highly bioavailable are also the most active catalytically, and forms that are relatively chemically inert tend to have poor bioavailability. In general, the more water soluble the iron compound, the higher its bioavailability and the greater the tendency to adversely affect sensory properties of foods. Some commonly used iron fortificants and their properties are listed in Table 9.15.

TABLE 9.15
Characteristics of Selected Iron Fortificants Used to Fortify Food Products

Chemical Name	Formula/Formula Weight	Iron Content (g/kg Fortificant)	Solubility	Relative Bioavailability[a]
Ferrous sulfate	$FeSO_4 \cdot 7H_2O$ F.W. = 278	200	Soluble in H_2O and dilute HCl	100
Ferrous gluconate	$FeC_{12}H_{22}O_{14} \cdot H_2O$ F.W. = 482	116	Soluble in H_2O and dilute HCl	89
Ferrous fumarate	$FeC_4H_2O_4$ F.W. = 170	330	Soluble in H_2O and dilute HCl	27–200
Ferric pyrophosphate	$Fe_4(P_2O_7)_3 \cdot xH_2O$ F.W. = 745	240	Insoluble in H_2O, soluble in dilute HCl	21–74
Ferric pyrophosphate nanoparticles	$FePO_4 \cdot 2H_2O$ F.W. = 187	300	Soluble in H_2O and dilute HCl	96
Ferrous ammonium phosphate	$FeNH_4PO_4$	240–300	Insoluble in H_2O, soluble in dilute HCl	70
Micronized ferric pyrophosphate	$Fe_4(P_2O_7)_3 \cdot xH_2O$ F.W. = 745	240	Water dispersible	100
Ferrous bisglycinate	$FeC_4H_8O_4 \cdot H_2O$ F.W. = 240	230	Soluble in H_2O and dilute HCl	90–350
Ferric sodium ethylenediaminetetraacetate	$FeNaC_{10}H_{12}N_2O_8 \cdot 3H_2O$ F.W. = 421	130	Soluble in H_2O and dilute HCl	30–390
Electrolytic iron powder	Fe F.W. = 56	970	Insoluble in H_2O, soluble in dilute HCl	75
Hydrogen-reduced iron powder	Fe F.W. = 56	97	Insoluble in H_2O, soluble in dilute HCl	13–148
Carbonyl iron powder	Fe F.W. = 56	99	Insoluble in H_2O, soluble in dilute HCl	5–20

Sources: Adapted from Miller, D.D., Iron fortification of the food supply: A balancing act between bioavailability and iron-catalyzed oxidation reactions, in: Lyons, T.P. and Jacques, K.A., eds., *Nutritional Biotechnology in the Feed and Food Industries*, Nottingham University Press, Nottingham, England, 2002. Additional data from Bothwell, T.H. and MacPhail, A.P., *Int. J. Vitam. Nutr. Res.*, 74(6), 421, 2004; Fidler, M.C. et al., *Br. J. Nutr.*, 91, 107, 2004; *Food Chemicals Codex*, 9th edn., National Academy Press, Washington, DC, 2014; Hertrampf, E. and Olivares, M. *Int. J. Vitam. Nutr. Res.*, 74(6), 435, 2004; Walczyk, T. et al., *Eur. J. Nutr.*, 52(4), 1361, 2013; Zimmermann, M.B. and Hilty, F.M., *Nanoscale*, 3(6), 2390, 2011.

[a] Relative bioavailability is bioavailability compared to ferrous sulfate that is set at 100.

Ferrous sulfate is one of the cheapest iron sources for food fortification. It is routinely used as the reference standard in iron bioavailability studies because of its relatively high bioavailability in many foods (Table 9.15). Results of several studies have indicated that off-odors and off-flavors occur in bakery products made from flour that was heavily fortified with ferrous sulfate and stored for extended periods of time. Barrett and Ranum [7] made the following recommendations for minimizing oxidation problems in bakery products that have been fortified with ferrous sulfate:

1. Ferrous sulfate is the preferred iron source as an additive in bakery products.
2. Ferrous sulfate may be used to fortify wheat flour provided that iron levels are kept below 40 ppm and the flour is stored at moderate temperatures and humidities for periods not to exceed 3 months.

3. Ferrous sulfate should not be used to fortify flour that may be stored for extended periods of time (as is the case with all-purpose flour intended for domestic use) or for flour that is to be used in mixes containing added fats, oils, or other easily oxidized ingredients.
4. Concentrated premixes containing ferrous sulfate and wheat flour for later addition to flour should not be used because rancidity may develop in the premix.

When fortification with ferrous sulfate is likely to cause problems in a food, other sources are commonly used. In recent years, elemental iron powders have been the sources of choice for fortification of flour for domestic use, breakfast cereals, and infant cereals. These are all products with long shelf lives.

As the name implies, elemental iron powders consist of elemental iron in a finely divided form. These forms are nearly pure iron with some contamination with other trace minerals and iron oxides. Elemental iron is insoluble in water, and thus, it is likely that it must be oxidized to a higher oxidation state before it can be absorbed from the intestine. Presumably, this oxidation occurs in the stomach when the iron is exposed to stomach acid:

$$Fe^0 + 2H^+ \rightarrow Fe^{2+} + H_2 \uparrow$$

Alternatively, oxygen could serve as the electron acceptor in the oxidation reaction:

$$Fe^0 + O_2 + 4H^+ \rightarrow Fe^{2+} + 2H_2O$$

Reaction with oxygen could occur during food processing operations such as bread baking.

Three different types of elemental iron powders are available [94]:

1. *Reduced iron*: This form is produced by reducing iron oxide with hydrogen or carbon monoxide gas and then milling to a fine powder. It is the least pure of the three types and purity depends largely on the purity of the iron oxide used [94].
2. *Electrolytic iron*: This form is produced by the electrolytic deposition of iron onto a cathode made of flexible sheets of stainless steel. The deposited iron is removed by flexing the sheets and it is then milled to a fine powder. The purity of electrolytic iron is greater than that of reduced iron. The main impurity is the iron oxide that forms on the surface during grinding and storage [94].
3. *Carbonyl iron*: This form is produced by heating scrap or reduced iron in the presence of CO under high pressure to form iron pentacarbonyl, $Fe(CO)_5$. The pentacarbonyl is then decomposed by heating to yield a very fine powder of high purity [94].

Elemental iron powders are relatively stable and don't appear to cause serious problems with oxidation in foods. However, the bioavailability of the powders is variable, probably due to differences in particle size. Iron powders are dark gray in color and may cause a slight darkening of white flour but this is not considered to be a problem [7].

Recently, there has been renewed interest in using chelated forms of iron as fortificants with sodium iron ethylenediaminetetraacetate [NaFe(III)EDTA] showing considerable promise. Studies with rats revealed that iron from NaFe(III)EDTA is absorbed as well or better than iron from $FeSO_4$ [35]. Numerous human trials showed that iron bioavailability from NaFe(III)EDTA in diets containing significant amounts of iron absorption inhibitors is higher than iron bioavailability from the same diets fortified with $FeSO_4$ [10,63]. Ethylenediaminetetraacetic acid (EDTA) binds ferric and ferrous iron with higher affinity than other ligands such as citric acid and polyphenolic compounds [55,117]. This high affinity produces a stable chelate that may not dissociate during gastrointestinal digestion, thereby preventing the iron from binding to iron absorption inhibitors. In the absence of iron absorption inhibitors, bioavailability from NaFeEDTA may

be lower than from ferrous sulfate, this explains the wide variation in relative bioavailabilities from NaFeEDTA shown in Table 9.15. In a recent prospective, double-blind, controlled study in Vietnam, Van Thuy et al. [127] showed that the prevalence of iron deficiency in women receiving fish sauce fortified with NaFeEDTA for 6 months was about 50% lower than in women in a control group who received unfortified fish sauce. A similar efficacy trial in China demonstrated that NaFeEDTA-fortified soy sauce significantly reduced prevalences of iron deficiency anemia in men, women, and children [21].

Iron amino acid chelates are also promising as food fortificants [57]. The most studied of these is ferrous bisglycinate, which is ferrous iron chelated with the amino acid glycine in a 1–2 molar ratio. Ferrous bisglycinate is less affected by iron absorption inhibitors than ferrous sulfate. It appears to especially effective in meals containing whole grain cereals. A major drawback to amino acid chelates is their high cost relative to ferrous sulfate or elemental iron powders [57].

As indicated earlier, reducing the particle size of elemental iron powders improves their bioavailability. Now there is evidence that reducing particle size of iron compounds may also improve bioavailability. Zimmermann and Hilty [145] prepared *nanostructured* iron oxides and phosphates using a process called flame spray pyrolysis. They prepared ferric orthophosphate and other iron compounds with particles sizes in the 10 nm range. Commercially available ferric orthophosphate has particle sizes in the micron range and low solubility in water. Therefore, it has minimal adverse impact on the color and odor of foods. Unfortunately, it also has low bioavailability. Nano ferric orthophosphate had iron bioavailabilities similar to ferrous sulfate when compared in a rat model. These nanocomplexes have yet to be tested in humans, but they show promise as fortificants with good bioavailability and minimal reactivity in foods [84].

9.4.4.2 Zinc

Given the apparently widespread occurrence of marginal zinc deficiency, many nutritionists advocate zinc fortification of foods as a strategy for addressing the problem. In the United States, five zinc compounds are listed as GRAS: zinc sulfate, zinc chloride, zinc gluconate, zinc oxide, and zinc stearate [110]. Of these, zinc oxide is the most commonly used for food fortification. It is more stable in foods due, in part, to its lower solubility. However, its bioavailability appears to be equal to that of zinc sulfate, which is more soluble. Fractional absorption rates of zinc from zinc oxide and zinc sulfate added to corn tortillas were 36.8% and 37.2%, respectively [110]. Moreover, zinc added as zinc sulfate to an iron-fortified wheat flour dumpling decreased iron absorption in 4–8-year-old children, but the same amount of zinc added as zinc oxide had no effect on iron absorption [56]. Rosado [110] recommends a fortification level of 20–50 mg Zn/kg of corn flour in Mexico.

9.4.4.3 Iodine

As mentioned earlier, a program for iodization of salt was adopted in the United States in 1924. In spite of the relatively simple process for adding iodine to salt and the widely recognized success of the program in the United States and other developed countries, as recently as 25 years ago, salt iodization was not common in many developing countries and iodine deficiency continues to be a problem today. Fortunately, the World Health Organization adopted an intervention strategy called Universal Salt Iodization (USI) in 1993 to tackle the problem. USI interventions strive for iodization of all salt for humans and livestock, including salt used in food processing [30]. The number of countries with a salt iodization policy increased from 43 in 1993 to 93 in 2003 and rates of goiter and mental retardation have fallen significantly as a result [138]. Unfortunately, however, IDD is still a significant problem in many areas of the world for a variety of reasons including the abundance of noniodized salt, which is cheaper and often locally produced.

Either sodium iodide, NaI, or sodium iodate, $NaIO_3$, may be used to fortify salt. Sodium iodate is often preferred because it is more stable during prolonged storage than sodium iodide, especially under conditions of high humidity and temperature [30].

9.4.5 Effects of Processing

Mineral elements, unlike vitamins and amino acids, cannot be destroyed by exposure to heat, light, oxidizing agents, extremes in pH, or other factors that affect organic nutrients. In essence, minerals are indestructible. Minerals can, however, be removed from foods by leaching or physical separation. Also, the bioavailabilities of minerals may be altered by the factors mentioned earlier (see Section 9.3.3).

The most important factor causing mineral loss in foods is milling of cereals. Mineral elements in grain kernels tend to be concentrated in the bran layers and the germ. Removal of bran and germ leaves pure endosperm, which is mineral poor. Mineral concentrations in whole wheat, white flour, wheat bran, and wheat germ are shown in Table 9.16. Similar losses occur during milling of rice and other cereals. These are substantial losses. During fortification of milled products in the United States, iron is the only mineral commonly added.

Retention of calcium in cheese can be dramatically affected by manufacturing conditions. In cheeses where the pH is low, substantial losses of calcium occur when the whey is drained. Calcium and phosphate contents of various cheeses are shown in Table 9.17. Compositions are expressed both as mg/100 g cheese and as a Ca–protein ratio. The latter expression gives a better comparison of Ca losses because the water content of cheeses varies from one variety

TABLE 9.16
Minerals in Whole Wheat and Milled Products

Mineral	Whole Wheat	White Flour	Wheat Germ	Millfeeds (Bran)	Loss from Wheat to Flour (%)
Iron	43	10.5	67	47–78	76
Zinc	35	8	101	54–130	78
Manganese	46	6.5	137	64–119	86
Copper	5	2	7	7–17	68
Selenium	0.6	0.5	1.1	0.5–0.8	16

Source: Rotruck, J.T., in *Handbook of Nutritive Value Processed Food*, 3rd edn., M. Rechcigl, Jr., ed., CRC Press, Boca Raton, FL, Vol. I, 1982, pp. 521–528.
Note: Values are mg mineral/kg product.

TABLE 9.17
Ca and P in Selected Cheeses

Cheese Variety	Protein (%)	Ca (mg/100 g)	Ca:Protein (mg:g)	PO_4 (mg/100 g)	PO_4:Protein (mg:g)
Cottage	15.2	80	5.4	90	16.7
Cheddar	25.4	800	31.5	860	27.3
Emmental	27.9	920	33.1	980	29.6

Sources: Guinee, T.P. et al., in: *Cheese*: *Chemistry*, *Physics and Microbiology*, Vol. 2, 2nd edn., P. F. Fox, ed., Chapman & Hall, London, 1993, pp. 369–371; Lucey, J.A. and Fox, P.F., *J. Dairy Sci*., 76(6), 1714–1724, 1993.

to another. Cottage cheese has the smallest calcium concentration because the pH at time of whey removal is typically less than 5 [49]. In cheddar and emmental cheeses, the whey is normally drained at pH 6.1 and 6.5, respectively. Colloidal calcium phosphate, the major fraction of Ca in milk, becomes increasingly soluble as the pH declines. Soluble Ca partitions to the whey fraction during cheese making and is lost when the whey is drained. This explains the lower Ca content in cottage cheese [72].

Since many minerals have significant solubility in water, it is reasonable to expect that cooking in water would result in some losses of minerals. Unfortunately, few controlled studies have been done. In general, boiling in water causes greater loss of minerals from vegetables than steaming [68]. Mineral losses during cooking of pasta are minimal for iron but more than 50% for potassium [68]. This is predictable because potassium is present in foods as the free ion, while iron is bound to proteins and other high- and low-molecular-weight ligands in the food.

9.5 CHEMICAL AND FUNCTIONAL PROPERTIES OF MINERALS IN FOODS

Even though minerals are present in foods at relatively low concentrations, they often have profound effects on physical and chemical properties of foods because of interactions with other food components. Details of mineral–food interactions for the broad array of minerals found in foods are given mainly in other chapters, and these interactions and their roles are summarized in Table 9.18. A more detailed treatment of selected minerals follows.

9.5.1 Calcium

The functional role of calcium in milk and milk products has been studied extensively and serves as an example of mineral interactions in a food system (see Chapter 14). Milk contains a complex mixture of minerals including calcium, magnesium, sodium, potassium, chloride, sulfate, and phosphate. Calcium in milk is distributed between the milk serum and the casein micelles. The calcium in serum is in solution and comprises about 30% of the total milk calcium. The remainder of the calcium is associated with casein micelles and is present primarily as colloidal calcium phosphate. It is likely that association of submicelles involves calcium bridges between phosphate groups esterified to serine residues in casein and inorganic phosphate ions.

Calcium and phosphate play an important functional role in the manufacture of cheese. Addition of calcium prior to renneting shortens coagulation time [72]. Curds with lower Ca content tend to be crumbly while cheeses higher in Ca are more elastic.

Calcium salts are widely used in the fruit and vegetable industry to enhance texture. Calcium ions, which are divalent, can slow the decline in firmness in fresh-cut fruits and vegetables by forming cross-links between galacturonic acid residues in the cell wall pectins [109]. Treatments normally involve dipping the product in a solution containing dissolved calcium salts such as calcium chloride or calcium lactate. Calcium lactate is preferred because calcium chloride may cause bitterness [76].

9.5.2 Phosphates

Phosphates occur in foods in many different forms, both as naturally occurring compounds intrinsic to plant and animal tissues and as components in food additives. A voluminous literature exists on the use of phosphates in foods. See Ellinger [37] and Molins [87] for in-depth treatments of this topic. Several phosphates are approved food additives. These include phosphoric acid, the orthophosphates, pyrophosphates, tripolyphosphates, and higher polyphosphates. Structures are shown in Figure 9.15.

TABLE 9.18
Functional Roles of Minerals and Mineral Salts/Complexes in Foods

Mineral	Food Sources	Function
Aluminum	Low and variable in foods; food additives (leavening acids, coloring agents) are a major source. Endogenous Al in plant food and contamination from Al cooking vessels also contribute.	*Leavening acid*: As sodium aluminum sulfate ($Na_2SO_4 \cdot Al_2(SO_4)_3$). *Colorant*: Al lakes of food dyes. *Emulsifying agent*: $Na_3Al(PO_4)_2$ in processed cheese.
Bromine	Brominated flour.	*Dough improver*: $KBrO_3$ improves baking quality of wheat flour. It has largely been replaced by ascorbic acid in the United States.
Calcium	Dairy products, green leafy vegetables, tofu, fish bones, Ca-fortified foods.	*Texture modifier*: Forms gels with negatively charged macromolecules such as alginates, low methoxy pectins, soy proteins, and caseins. Increases viscosity of alginate solutions. Firms canned vegetables when added to canning brine.
Copper	Organ meats, seafood, nuts, seeds.	*Catalyst*: Lipid peroxidation, ascorbic acid oxidation, nonenzymatic oxidative browning. *Color modifier*: May cause black discoloration in canned, cured meats. *Enzyme cofactor*: Polyphenol oxidase *Texture stabilizer*: Stabilizes egg white foams.
Iodine	Iodized salt, seafood, plants and animals grown in areas where soil iodine is not depleted.	*Dough improver*: KIO_3 improves baking quality of wheat flour.
Iron	Cereals, legumes, meat, contamination from iron utensils and soil, enriched or fortified foods.	*Catalyst*: Fe^{2+} and Fe^{3+} catalyze lipid peroxidation in foods. *Color modifier*: Color of fresh meat depends on the valence of Fe in myoglobin and hemoglobin: Fe^{2+} is red. Fe^{3+} is brown. Forms green, blue, or black complexes with polyphenolic compounds. Reacts with S^{2-} to form black FeS in canned foods. *Enzyme cofactor*: Lipoxygenase, cytochromes, ribonucleotide reductase, etc.
Magnesium	Whole grains, nuts, legumes, green leafy vegetables.	*Color modifier*: Removal of Mg from chlorophyll changes color from green to olive brown.
Manganese	Whole grains, fruits, vegetables.	*Enzyme cofactor*: Pyruvate carboxylase, superoxide dismutase.
Nickel	Plant foods.	*Catalyst*: Hydrogenation of vegetable oils and reducing sugars—finely divided, elemental Ni is the most widely used catalyst for this process.
Phosphates	Ubiquitous, animal products tend to be good sources; widely used food additive.	*Acidulent*: H_3PO_4 in soft drinks. *Leavening acid*: $Ca(HPO_4)_2$ is a fast-acting leavening acid. *Moisture retention in meats*: Sodium tripolyphosphate improves moisture retention in cured meats. *Emulsification aid*: Phosphates are used to aid emulsification in comminuted meats and in process cheeses.
Potassium	Fruits, vegetables, meats.	*Salt substitute*: KCl may be used as a salt substitute; may cause bitter flavor. *Leavening acid*: Potassium acid tartrate.
Selenium	Seafood, organ meats, cereals (levels vary depending on soil levels).	*Enzyme cofactor*: Glutathione peroxidase.

(*Continued*)

TABLE 9.18 (*Continued*)
Functional Roles of Minerals and Mineral Salts/Complexes in Foods

Mineral	Food Sources	Function
Sodium	NaCl, MSG, other food additives, milk; low in most raw foods.	*Flavor modifier*: NaCl elicits the classic salty taste in foods and enhances other flavors. *Preservative*: NaCl may be used to lower water activity in foods to prevent or control microbial growth. *Leavening agents*: Many leaving agents are sodium salts, e.g., sodium bicarbonate, sodium aluminum sulfate, and sodium acid pyrophosphate.
Sulfur	Widely distributed as component of sulfur-containing amino acids, food additives (sulfites, SO_2).	*Browning inhibitor*: Sulfur dioxide and sulfites inhibit both enzymatic and nonenzymatic browning; widely used in dried fruits. *Antimicrobial*: Prevents and controls microbial growth; widely used in wine making.
Zinc	Meats, cereals, fortified foods.	ZnO is used in the lining of cans for proteinaceous foods to lessen formation of black FeS during heating.

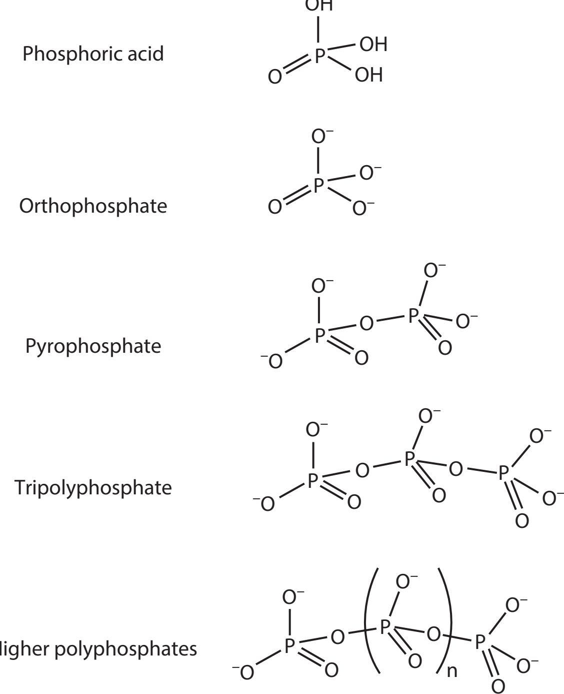


FIGURE 9.15 Structures of phosphoric acid and phosphate ions important in foods.

Phosphate food additives serve many functions including acidification (soft drinks), buffering (various beverages), anticaking, leavening, stabilizing, emulsifying, water binding, and protection against oxidation. The chemistry responsible for the wide array of functional properties of phosphates is not fully understood but undoubtedly is related to the acidity of protons associated with phosphates and the charge on phosphate ions. At pHs common in foods, phosphates carry negative charges and polyphosphates behave as polyelectrolytes. These negative charges give phosphates strong Lewis base character and thus strong tendencies to bind metal cations. An ability to bind metal ions may underlie several of the

functional properties noted earlier. It should be mentioned, however, that there is considerable controversy about mechanisms of phosphate functionality, particularly as it relates to enhanced water-holding capacity in meats and fish.

9.5.3 Sodium Chloride

Sodium chloride (salt) is a widely used food additive. Its beneficial functions in food include enhanced flavor, control of microbial growth, improved water-holding capacity in meats, and enhanced color. Salt not only adds flavor as a single ingredient but also enhances other flavors in foods and reduces bitterness. Many foods with added salt, for example, breads and other cereal products, do not taste salty, and therefore, it is difficult for consumers to judge the salt content of foods based on taste. Sources of sodium in the U.S. food supply are listed in Table 9.19.

Salt is an essential ingredient in most cheeses. It adds to the flavor, helps to control the growth of undesirable bacteria by lowering water activity, controls the rate of lactic acid fermentation, and modifies the texture [104].

In processed meats such as sausages, salt functions as a preservative by lowering water activity. It also promotes the solubilization of muscle proteins (a salting-in phenomenon) that then function as emulsifying agents [47].

In bakery products, salt enhances flavor without imparting a salty taste, controls the rate of fermentation in yeast-leavened products, and functions as a dough improver through its interactions with gluten proteins [104].

In spite of the controversy over sodium reduction mentioned earlier, there is widespread consensus that reducing sodium in foods will lower blood pressure and death rates from cardiovascular and possibly other chronic diseases. Member states of the World Health Organization have set a voluntary target of a 30% reduction salt intakes in the global population by 2025 (http://www.who.int/dietphysicalactivity/reducingsalt/en/, accessed 8/20/2014). Individual food companies have been working to reduce sodium in their products [34]. Several strategies are being pursued including gradually reducing the amount of salt added to foods, adding other flavors that can enhance the salty taste, and using salt substitutes. Reducing salt intakes gradually over time has been shown to increase sensitivity to the salty taste so that consumers do not perceive a decrease in taste quality. This is a promising strategy but it will take time and will require industry-wide cooperation [34]. Other flavor compounds are known to enhance saltiness in foods. These include sour compounds such as organic acids, glutamate and other amino acids, nucleotides, and yeast extracts. Another strategy is to use salt substitutes. Potassium chloride is the most widely used substitute. It has the

TABLE 9.19
Contributions of Food Groups to Sodium Intakes in the U.S. Population

Food Category	Contribution to Na Intake (% of Total Intake)
Milk and milk products	6.5
Grain products	22.0
Fruits and vegetables	6.6
Meat, fish, poultry, and eggs	26.1
Mixed dishes (casseroles, soups, etc.)	22.1
Fats, oils, and sauces	8.2
Desserts and sweets	4.8
Others	3.8

Source: Adapted from Engstrom. A. et al., *Am. J. Clin. Nutr.*, 65(Suppl), 704S, 1997.

added advantage that increasing potassium intakes lowers blood pressure. The disadvantage is that it may produce a bitter flavor in the food.

9.5.4 Iron

It is well established that iron can promote lipid peroxidation in foods. Iron appears to catalyze both the initiation and propagation stages of lipid peroxidation. The chemistry is exceedingly complex but several probable mechanisms have been suggested. In the presence of reducing agents such as thiol groups and ascorbic acid, ferric iron promotes the formation of the superoxide anion [137]:

$$Fe^{3+} + RSH \rightarrow Fe^{2+} + RS^{\bullet} + H^{+}$$

$$RSH + RS^{\bullet} + O_2 \rightarrow RSSR + H^{+} + {}^{\bullet}O_2^{-}$$

The superoxide anion may then react with protons to form hydrogen peroxide or reduce ferric iron to the ferrous form:

$$2H^{+} + 2{}^{\bullet}O_2^{-} \rightarrow H_2O_2 + O_2$$

$$Fe^{3+} + {}^{\bullet}O_2^{-} \rightarrow Fe^{2+} + O_2$$

Ferrous ion promotes decomposition of hydrogen peroxide to hydroxyl radicals by the Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + {}^{\bullet}OH$$

The hydroxyl radical is highly reactive and may rapidly generate lipid free radicals by abstracting hydrogen atoms from unsaturated fatty acids. This initiates the lipid peroxidation chain reaction.

Iron can also catalyze lipid peroxidation by accelerating decomposition of lipid hydroperoxides present in foods:

$$Fe^{2+} + LOOH \rightarrow Fe^{3+} + LO^{\bullet} + OH^{-}$$

or

$$Fe^{3+} + LOOH \rightarrow Fe^{2+} + LOO^{\bullet} + H^{+}$$

The rate of the first reaction is greater than the second by an order of magnitude. This explains why ascorbic acid may function as a prooxidant in some food systems since it can reduce ferric iron to the ferrous form.

9.5.5 Nickel

While nickel deficiency has never been documented in humans, there is substantial evidence of its essentiality in several animal species [92]. There is no RDA or AI for nickel. Food sources include chocolates, nuts, beans, and grains [92]. The primary significance of nickel from a food processing perspective is its use as a catalyst for the hydrogenation of edible oils [89] (see Chapter 4) and to produce sugar alcohols (Chapter 3).

9.5.6 Copper

Copper, like iron, is a transition element and exists in foods in two oxidation states, Cu^{+} and Cu^{2+}. It is a cofactor in many enzymes including phenolase and is at the active center of hemocyanin, an

oxygen-carrying protein in some arthropods. Both Cu^{+} and Cu^{2+} bind tightly to organic molecules and thus exist primarily as complexes and chelates in foods. On the negative side, copper is a potent catalyst of lipid oxidation in foods.

An intriguing functional role of copper has been exploited in Western cuisine for at least 300 years [78]. Many recipes for meringues specify copper bowls as the preferred vessel for whipping egg whites. A common problem with egg white foams is collapse resulting from overwhipping. Presumably, foam stability is reduced when the proteins at the air–liquid interface are excessively denatured by whipping. Egg white contains conalbumin, a protein analogous to the plasma iron-binding protein transferrin. Conalbumin binds Cu^{2+} as well as Fe^{3+} and the presence of bound copper or iron stabilizes conalbumin against excessive denaturation [96].

9.6 GLOSSARY OF TERMS

Minerals are present in foods at low but variable concentrations and in multiple chemical forms. These species undergo complex changes during processing, storage, and digestion of foods. With the exception of group IA and VIIA elements, minerals exist in foods as complexes, chelates, or oxyanions. While understanding of the chemical forms and properties of many of these mineral species remains limited, their behavior in foods often can be predicted by applying principles of inorganic, organic, physical, and biological chemistry.

The primary role of minerals in foods is to provide a reliable source of essential nutrients in a balanced and bioavailable form. In cases where concentrations and/or bioavailabilites in the food supply are low, fortification has been used to help assure AIs by all segments of the population. Fortification with iron and iodine has dramatically reduced deficiency diseases associated with these nutrients in the United States and other industrialized countries. Unfortunately, it has not been possible to fortify appropriate staple foods in many developing countries leaving hundreds of millions of people in these countries to suffer the tragic consequences of iron, iodine, zinc, and other deficiencies.

Minerals also play key functional roles in foods. For example, minerals may dramatically alter the color, texture, flavor, and stability of foods. Thus, minerals may be added or removed from foods to achieve a particular functional effect. When manipulation of concentrations of minerals in foods is not practical, chelating agents such as EDTA (when allowed) can be used to alter their behavior.

- *Minerals*: Elements other than C, H, O, and N that remain after the organic matter in a food is removed by combustion or oxidizing acids.
- *Essential mineral element*: A mineral required for a critical physiological function in the body. Inadequate intake of an essential mineral will lead to an impairment of one or more physiological functions.
- *Mineral specie*: The chemical form of mineral elements. Minerals are present in foods in many different chemical forms including free ions, complexes, chelates, and compounds.
- *Lewis acid*: An electron pair acceptor.
- *Lewis base*: An electron pair donor.
- *Ligands*: Chemical species capable of donating electron pairs to a metal ion to form complexes and chelates. Principal electron donating atoms in ligands include oxygen, nitrogen, and sulfur.
- *Chelate*: A metal complex in which the ligand forms two or more bonds with the metal ion forming a ring structure. Chelates are more stable than similar complexes that are not chelates.
- *Homeostasis*: Processes whereby an organism maintains tissue levels of nutrients within narrow and constant ranges even when intakes of the nutrients may be low or high.

- *Dietary Reference Intakes* (*DRIs*): Estimates of nutrient requirements of healthy people. DRIs encompass four subsets of values that include the *EAR*, the *RDA*, the *AI*, and the *UL*.
- *Estimated Average Requirement* (*EAR*): EAR is defined as the level of intake of a nutrient that meets the requirements of 50% of the individuals in a particular age and gender group. *Presumably*, the requirement of the remaining 50% of the individuals is higher than the EAR.
- *Recommended Dietary Allowance* (*RDA*): RDA is defined as the level of intake of a nutrient sufficient to meet the requirements of nearly all healthy persons in a particular age and gender group. It is set at two SDs above the EAR: RDA = EAR + 2SD.
- *Adequate Intake* (*AI*): AI is used when the available scientific evidence is insufficient to set an RDA. It is based on estimates of actual average intakes of a nutrient by healthy people, not on results from controlled studies designed to estimate individual requirements for nutrients.
- *Tolerable Upper Intake Level* (*UL*): UL is the level of intake of a nutrient below which adverse health effects are unlikely to occur. This implies that intakes above the UL may pose a risk of toxicity.
- *Nutritional aspects of minerals*: Twenty-five minerals are considered essential nutrients for humans. See Table 9.2 for a summary of nutritional aspects of mineral nutrients.
- *Bioavailability*: The proportion of an ingested nutrient that is available for utilization in a metabolic process or for deposition in a storage compartment in the body. The bioavailability of a nutrient in a food is influenced by many factors including the chemical form of the nutrient, ligands that may be present in the food or formed during digestion, the redox activity of the food matrix, the concentrations of other nutrients in the food or meal, and the physiological state of the consumer.
- *Phytic acid*: *Myo*-inositol-1,2,3,4,5,6-hexakisphosphate. The primary storage form of phosphorous in plant seeds. Phytic acid and its salts are potent inhibitors of absorption for some minerals including iron, zinc, and calcium.
- *Mineral composition of plant-based foods*: The mineral composition of plant foods are influenced by many factors including the genetics of the plant, the quality and fertility of the soil on which the plant is grown, the amount of rainfall and sunshine during the growing season, and the maturity of the plant at harvest.
- *Mineral composition of animal-based foods*: Mineral composition of animal foods is less variable than is the case with plant foods. Animals fed diets that are low in iodine and selenium have reduced levels of these minerals in their tissues, milk, and/or eggs. Veal calves fed low-iron diets have low levels of iron in their muscles.
- *Fortification*: The addition of one or more nutrients to a food for the purpose of preventing nutrient deficiencies in populations.
- *Biofortification*: The application of conventional plant breeding or genetic engineering technologies to enhance the concentrations and/or bioavailabilities of micronutrients in staple food crops. The improved seeds are then distributed to farmers in resource-poor areas who plant them and subsequently harvest nutritionally enhanced crops. Distribution of these crops to local consumers results in increased intakes of micronutrients that may be deficient in their traditional diets. Biofortification as a strategy for preventing micronutrient malnutrition is especially advantageous in rural regions where processed, commercially fortified foods are not available or affordable.
- *Impact of fortification on food quality*: In most cases, the addition of mineral nutrients to foods has a minimal effect on quality. The addition of iron is an exception. Iron, a redox-active mineral, may catalyze lipid oxidation in the food leading to the formation of off-odors and off-flavors. Some forms of iron may also catalyze the destruction of food antioxidants such as vitamins A, C, and E. Elemental iron powders, iron chelates such as ferric sodium EDTA, and ferric pyrophosphate nanoparticles are less active as oxidation catalysts than ferrous sulfate and other iron salts.

- *Mineral toxicants*: All minerals, including essential mineral nutrients, can be toxic when present at excessive levels in the diet. However, most mineral essential nutrients are rarely present at toxic levels in foods. The mineral elements of most concern from a toxicity perspective are not essential nutrients. They include lead, mercury, arsenic, and cadmium. These highly toxic minerals may find their way into foods through uptake from the soil by plant roots, contamination of water and air by natural and industrial processes, ingestion of contaminated feed by animals, leaching from food packages, and other mechanisms. Lead levels in foods have been substantially reduced in recent years due to the banning of leaded gasoline and lead-containing solder used in the manufacture of food cans; replacement of lead water pipes with iron, copper, or synthetic polymer pipes; the banning of mercury-containing agricultural fungicides; and other strategies. Methyl mercury, a highly toxic mercury compound, remains a concern due to its accumulation in some fish, especially long-lived predatory fish such as swordfish, tilefish, shark, and king mackerel.
- *Effects of food processing on the mineral content of foods*: Minerals are essentially indestructible but certain processing operations may either decrease or increase the concentration of minerals in foods. Milling of cereal grains to remove the bran and germ decreases the concentration of several minerals in whole grains. Cheese manufacturing leads to significant losses of calcium and potassium when whey is drained away. The addition of salt (NaCl) and other sodium-containing food additives increases the sodium content of foods. It is estimated that 77% of the total salt in the American diet is from salt added during food processing. Phosphates are added to many foods as functional ingredients. Some minerals may be added to foods to fortify them with nutrients that are deficient in the diet. Iron and iodine are the most commonly added mineral nutrients but calcium and zinc are also added to some foods.
- *Functional roles of minerals in foods*: Minerals play a variety of important functional roles in foods ranging from acidification to color modification to water activity control to flavor enhancement. See Table 9.18 for a summary of the functional roles of minerals in foods.

REFERENCES

1. Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) (2012). Low level lead exposure harms children: A renewed call for primary prevention. http://www.cdc.gov/nceh/lead/acclpp/final_document_030712.pdf (accessed August 26, 2014).
2. Ahmed, F.E. (1999). Trace metal contaminants in food, in: *Environmental Contaminants in Food*, C.F. Moffat and Whittle, K.J., eds., Sheffield Academic Press, Sheffield, U.K., Chapter 6, pp. 146–214.
3. Allaway, W.H. (1975). The effects of soils and fertilizers on human and animal nutrition. Agriculture Information Bulletin No. 378, U.S. Department of Agriculture, Washington, DC.
4. Anderson, J.J.B., M.L. Sell, S.C. Garner, and M.S. Calvo (2001). Phosphorus, in: *Present Knowledge in Nutrition*, 8th edn., B.A. Bowman and R.M. Russell, eds., ILSI Press, Washington, DC, Chapter 27, pp. 281–291.
5. AOAC International (2012). *Official Methods of Analysis*, 19th edn., AOAC International, Washington, DC.
6. Bailey, L.B., S. Moyers, and J.F. Gregory (2001). Folate, in: *Present Knowledge in Nutrition*, 8th edn., B.A. Bowman and R.M. Russell, eds., ILSI Press, Washington, DC, Chapter 21, pp. 214–229.
7. Barrett, F. and P. Ranum (1985). Wheat and blended cereal foods, in: *Iron Fortification of Foods*, F.M. Clydesdale and K.L. Wiemer, eds., Academic Press, Orlando, FL, pp. 75–109.
8. Baynes, R.D. and T.H. Bothwell (1990). Iron deficiency. *Annu Rev Nutr* 10: 133–148.
9. Blair, M.W., P. Izquierdo, C. Astudillo, and M.A. Grusak (2013). A legume biofortification quandary: Variability and genetic control of seed coat micronutrient accumulation in common beans. *Front Plant Sci* 4: 1–14.
10. Bothwell, T.H. and A.P. MacPhail (2004). The potential role of NaFeEDTA as an iron fortificant. *Int J Vitam Nutr Res* 74(6): 421–434.
11. Brnic, M., R. Wegmuller, C. Zeder, G. Senti, and R.F. Hurrell (2014). Influence of phytase, EDTA, and polyphenols on zinc absorption in adults from porridges fortified with zinc sulfate or zinc oxide. *J Nutr* 144: 1467–1473.

12. Brown, M.J. and S. Margolis (2012). Lead in drinking water and human blood levels in the United States. *MMWR Morb Mortal Wkly Rep* 61(Suppl.): 1–9.
13. Burk, R.F. and O.A. Levander (1999). Selenium, in: *Modern Nutrition in Health and Disease*, 9th edn., M.E. Shills, J.A. Olson, M. Shike, and A.C. Ross, eds., Lippincott Williams & Wilkins, Philadelphia, PA, pp. 265–276.
14. Caldwell, K.L., A. Makhmudov, E. Ely, R.L. Jones, and R.Y. Wang (2011). Iodine status of the U.S. population, National Health and Nutrition Examination Survey, 2005–2006 and 2007–2008. *Thyroid* 21(4): 419–427.
15. Canfield, R.L., C.R. Henderson Jr., D.A. Cory-Slechta, C. Cox, T.A. Jusko, and B.P. Lanphear (2003). Intellectual impairment in children with blood lead concentrations below 10 μg per deciliter. *N Engl J Med* 348(16): 1517–1526.
16. Cappuccio, F.P., S. Capewell, F.J. He, and G.A. MacGregor (2014). Salt: The dying echoes of the food industry. *Am J Hypertens* 27(2): 279–281.
17. Carpenter, K.J. (1995). Episodes in the history of food fortification. *Cereal Foods World* 42(2): 54–57.
18. Ward, C.R.E. and C.E. Carpenter (2010). Traditional methods for mineral analysis, in: *Food Analysis*, 4th edn., S.S. Nielsen, ed., Springer, New York, Dordrecht, Heidleberg, London, pp. 201–215.
19. Carocci, A., N. Rovito, M.S. Sinicropi, and G. Genchi (2014). Mercury toxicity and neurodegenerative effects. *Rev Environ Contam Toxicol* 229: 1–18.
20. Centers for Disease Control and Prevention (2005). Preventing lead poisoning in young children. CDC, Atlanta, GA. http://www.cdc.gov/nceh/lead/publications/PrevLeadPoisoning.pdf (accessed August 26, 2014).
21. Chen, J., X. Zhao, X. Zhang, S. Yin, J. Piao, J. Huo, B. Yu, N. Qu, Q. Lu, S. Wang, and C. Chen (2005). Studies on the effectiveness of NaFeEDTA-fortified soy sauce for controlling iron deficiency: A population-based intervention trial. *Food Nutr Bull* 26(2): 177–186.
22. Chiancone, E., P. Ceci, A. Ilari, F. Ribacchi, and S. Steranini (2004). Iron and proteins for iron storage and detoxification. *Biometals* 17(3): 197–202.
23. Chrichton, R.R. (1991). *Inorganic Biochemistry of Iron Metabolism*, Ellis Horwood Series in Inorganic Chemistry, J. Burgess, series ed., Ellis Horwood, New York.
24. Clarkson, T.W., L. Magos, and G.J. Meyers (2003). The toxicology of mercury. *N Eng J Med* 349(18): 1731–1737.
25. Clarkson, T.W. and J.J. Strain (2003). Nutritional factors may modify the toxic action of methyl mercury in fish-eating populations. *J Nutr* 133(5 Suppl 1): 1539S–1543S.
26. Combs, G.F. (2001). Selenium in global food systems. *Br J Nutr* 85: 517–547.
27. Cousins, R.J. (1996). Zinc, in: *Present Knowledge in Nutrition*, 7th edn., E.E. Ziegler and L.J. Filer Jr., eds., ILSI Press, Washington, DC, Chapter 29, pp. 293–306.
28. Cubadda, F., A. Raggi, F. Zanasi, and M. Carcea (2003). From durum wheat to pasta: Effect of technological processing on the levels of arsenic, cadmium, lead and nickel—A pilot study. *Food Addit Contam* 20(4): 353–360.
29. da Silva, J.J.F.R. and R.J.P. Williams (1991). *The Biological Chemistry of the Elements: The Inorganic Chemistry of Life*, Clarendon Press, Oxford, U.K.
30. Delange, F., H. Burgi, Z.P. Chen, and J.T. Dunn (2002). World status of monitoring of iodine deficiency disorders control programs. *Thyroid* 12(10): 915–924.
31. Delange, F., B. de Benoist, E. Pretell, and J.T. Dunn (2001). Iodine deficiency in the world: where do we stand at the turn of the century? *Thyroid* 11(5): 437–447.
32. Deshpande, S.S. (2002). Toxic metals, radionuclides, and food packaging contaminants, in: *Handbook of Food Toxicology*, S.S. Deshpande, ed., Marcel Dekker, Inc., New York, Chapter 16, pp. 783–812.
33. Dibley, M.J. (2001). Zinc, in: *Present Knowledge in Nutrition*, 8th edn., B.A. Bowman and R.M. Russell, eds., ILSI Press, Washington, DC, Chapter 31, pp. 329–343.
34. Dotsch, M., J. Busch, M. Batenburg, G. Liem, E. Tareilus, R. Mueller, and G. Meijer (2009). Strategies to reduce sodium consumption: A food industry perspective. *Crit Rev Food Sci Nutr* 49(10): 841–851.
35. Dutra-de-oliveira, J.E., M.L.S. Freitas, J.F. Ferreira, A.L. Goncalves, and J.S. Marchini (1995). Iron from complex salts and its bioavailability to rats. *Int J Vitam Nutr Res* 65: 272–275.
36. Dziezak, J.D. (1990). Phosphates improve many foods. *Food Technol* 44(4): 80–92.
37. Ellinger, R.H. (1972). Phosphates in food processing, in: *Handbook of Food Additives*, 2nd edn., T.E. Furia, ed., CRC Press, Cleveland, OH, pp. 617–780.
38. Engstrom, A., R.C. Tobelmann, and A.M. Albertson (1997). Sodium intake trends and food choices. *Am J Clin Nutr* 65(Suppl.): 704S–707S.

39. Farley, D. (1998). Dangers of lead still linger. *FDA Consum* 32(1): 16–21.
40. Fidler, M.C., T. Walczyk, L. Davidsson, C. Zeder, N. Sakaguchi, L.R. Juneja, and R.F. Hurrell (2004). A micronized, dispersible ferric pyrophosphate with high relative bioavailability in man. *Br J Nutr* 91: 107–120.
41. Food and Drug Administration (2003). Code of federal regulations. U.S. Government Printing Office, Washington, DC, Chapter I, Parts 136, 137, 139. http://www.gpoaccess.gov/cfr/index.html (accessed August 26, 2014).
42. Food and Drug Administration (2006). Mercury levels in commercial fish and shellfish (1990–2010). http://www.fda.gov/food/foodborneillnesscontaminants/metals/ucm115644.htm (accessed August 26, 2014).
43. Food and Nutrition Board (FNB); Institute of Medicine (2002). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*, National Academy Press, Washington, DC.
44. Food and Nutrition Board; Institute of Medicine (2003). Dietary reference intake tables: Elements table. http://www.iom.edu/file.asp?id=7294 (accessed August 26, 2014).
45. Committee on Food Chemicals Codex. (2014). Food chemicals codex, 9th edn. National Academy Press, Washington, DC.
46. Fox, C.H. and M. Eberl (2002). Phytic acid (IP6), novel broad spectrum anti-neoplastic agent: A systematic review. *Complement Ther Med* 10(4): 229–234.
47. Gelabert, J., P. Gou, L. Guerrero, and J. Arnau (2003). Effect of sodium chloride replacement on some characteristics of fermented sausages. *Meat Sci* 65: 833–839.
48. Graham, R.D., R.M. Welch, and H.E. Bouis (2001). Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: Principles, perspectives, and knowledge gaps. *Adv Agron* 70: 77–142.
49. Guinee, T.P., P.D. Pudja, and N.Y. Farkye (1993). Fresh acid-cured cheese varieties, in: *Cheese: Chemistry, Physics and Microbiology*, Vol. 2, 2nd edn., P.F. Fox, ed., Chapman & Hall, London, U.K., pp. 369–371.
50. Marshall, M.R. (2010). Ash analysis, in: *Food Analysis*, 4th edn., S.S. Nielsen, ed., Springer, New York, Dordrecht, Heidelberg, London, pp. 105–115.
51. Harland, B. and G. Narula (1999). Phytate and its hydrolysis products. *Nutr Res* 19(6): 947–961.
52. HarvestPlus (2014). It all starts with a seed. http://www.harvestplus.org/ (accessed September 5, 2014).
53. Hazell, T. (1985). Minerals in foods: Dietary sources, chemical forms, interactions, bioavailability. *World Rev Nutr Diet* 46: 1–123.
54. Heaney, R.P., C.M. Weaver, and M.L. Fitzsimmons (1990). Influence of calcium load on absorption fraction. *J Bone Miner Res* 5: 1135–1138.
55. Hegenauer, J., P. Saltman, and G. Nace (1979). Iron III phosphoprotein chelates: Stoichiometric equilibrium constant for interaction of iron III and phosphorylserine residues of phosvitin and casein. *Biochemistry* 18: 3865–3879.
56. Herman, S., I.J. Griffin, S. Suwarti, F. Ernawati, D. Permaesih, D. Pambudi, and S.A. Abrams (2002). Cofortification of iron-fortified flour with zinc sulfate, but not zinc oxide, decreases iron absorption in Indonesian children. *Am J Clin Nutr* 76(4): 813–817.
57. Hertrampf, E. and M. Olivares (2004). Iron amino acid chelates. *Int J Vitam Nutr Res* 74(6): 435–443.
58. Horton, S., V. Mannar, and A. Wesley (2008). Best practice paper: Food fortification with iron and iodine. Copenhagen Consensus Center, Copenhagen Business School, Copenhagen, Denmark.
59. Hunt, J.R. (2003). Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. *Am J Clin Nutr* 78: 633S–639S.
60. Hurell, R.F. (2004). Phytic acid degradation as a means of improving iron absorption. *Int J Vitam Nutr Res* 74(6): 445–452.
61. Institute of Medicine (2006). Introduction to the dietary reference intakes, in *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*, J.J. Otten, J.P. Hellwig, and L.D. Meyers, eds., The National Academies Press, Washington, DC, pp. 5–17.
62. Institute of Medicine (2013). *Sodium Intake in Populations: Assessment of Evidence*, The National Academies Press, Washington, DC.
63. International Nutritional Anemia Consultative Group (INACG) (1993). Iron EDTA for food fortification. ILSI Research Foundation, Washington, DC, pp. 27–35.
64. Jarup, L. (2002). Cadmium overload and toxicity. *Nephrol Dial Transplant* 17(Suppl. 2): 35–39.
65. Joy, E.J., E.L Ander, S.D. Young, C.R. Black, M.J. Watts, A.D. Chilimba, B. Chilima et al. (2014). Dietary mineral supplies in Africa. *Physiol Plant* 151(3): 208–229.

66. Kapsokefalou, M. and D.D. Miller (1991). Effects of meat and selected food components on the valence of nonheme iron during in vitro digestion. *J Food Sci* 56(2): 352–355, 358.
67. Kratzer, F.H. and P. Vohra (1986). *Chelates in Nutrition*, CRC Press, Boca Raton, FL.
68. Lachance, P.A. and M.C. Fisher (1988). Effects of food preparation procedures in nutrient retention with emphasis on food service practices, in: *Nutritional Evaluation of Food Processing*, E. Karmas and R.S. Harris, eds., Van Nostrand Reinhold, New York, pp. 505–556.
69. La Frano, M.R., F.F. de Moura, E. Boy, B. Lonnerdal, and B.J. Burri (2014). Bioavailability of iron, zinc, and provitamin A carotenoids in biofortified staple crops. *Nutr Rev* 72(5): 289–307.
70. Lei, X.G. and C.H. Stahl (2001). Biotechnological development of effective phytases for mineral nutrition and environmental protection. *Appl Microbiol Biotechnol* 57: 474–481.
71. Lind, T., B. Lonnerdal, L.A. Persson, H. Stenlund, C. Tennefors, and O. Hernell (2003). Effects of weaning cereals with different phytate contents on hemoglobin, iron stores, and serum zinc: A randomized intervention in infants from 6 to 12 mo of age. *Am J Clin Nutr* 78(1): 168–175.
72. Lucey, J.A. and P.F. Fox (1993). Importance of calcium and phosphate in cheese manufacture: A review. *J Dairy Sci* 76(6): 1714–1724.
73. Ma, J. and N.M. Betts (2000). Zinc and copper intakes and their major food sources for older adults in the 1994–96 continuing survey of food intakes by individuals (CSFII). *J Nutr* 130(11): 2838–2843.
74. Manary, M.J., N.F. Krebs, R.S. Gibson, R.L. Broadhead, and K.M. Hambidge (2002). Community-based dietary phytate reduction and its effect on iron status in Malawian children. *Ann Trop Paediatr* 22(2): 133–136.
75. Mares-Perlman, J.A., A.F. Subar, G. Block, J.L. Greger, and M.H. Luby (1995). Zinc intake and sources in the US adult population: 1976–1980. *J Am Coll Nutr* 14(4): 349–357.
76. Martín-Diana, A.B., D. Rico, J.M. Frías, J.M. Barat, G.T.M. Henehan, and C. Barry-Ryan (2007). Calcium for extending the shelf life of fresh whole and minimally processed fruits and vegetables: A review. *Trends Food Sci Technol* 18: 210–218.
77. McElwee, M.K., L.A. Ho, J.W. Chou, M.V. Smith, and J.H. Freedman (2013). Comparative toxicogenomic responses of mercuric and methyl-mercury. *BMC Genomics* 14: 698.
78. McGee, H.J., S.R. Long, and W.R. Briggs (1984). Why whip egg whites in copper bowls? *Nature* 308: 667–668.
79. Meneton, P., X. Jeunemaitre, H.E. de Wardener, and G.A. MacGregor (2005). Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. *Physiol Rev* 85(2): 679–715.
80. Méplan, C. and J. Hesketh (2014). Selenium and cancer: A story that should not be forgotten—Insights from genomics. *Cancer Treat Res* 159: 145–166.
81. Mertz, W. (1984). The essential elements: Nutritional aspects. *Nutr Today* 19(1): 22–30.
82. Meyer, P.A., T. Pivetz, T.A. Dignan, D.M. Homa, J. Schoonover, and D. Brody (2003). Surveillance for elevated blood lead levels among children—United States, 1997–2001. *MMWR Surveill Summ* 52(SS-10): 1–21.
83. Miller, D.D. (2002). Iron fortification of the food supply: A balancing act between bioavailability and iron-catalyzed oxidation reactions, in: *Nutritional Biotechnology in the Feed and Food Industries*, T.P. Lyons and K.A. Jacques, eds., Nottingham University Press, Nottingham, England.
84. Miller, D.D. (2010). Food nanotechnology: New leverage against iron deficiency. *Nat Nanotechnol* 5(5): 318–319.
85. Miller, D.D. and L.A. Berner (1989). Is solubility in vitro a reliable predictor of iron bioavailability? *Biol Trace Elem Res* 19: 11–24.
86. Miller, D.D. and M.A. Rutzke (2010). Atomic absorption and emission spectroscopy, in: *Food Analysis*, 4th edn., S.S. Nielsen, ed., Springer Science + Business Media, New York, pp. 421–442.
87. Molins, R.A. (1991). *Phosphates in Food*, CRC Press, Boca Raton, FL.
88. Morgan, J.N. (1999). Effects of processing on heavy metal content of foods, in: *Impact of Processing on Food Safety*, L.S. Jackson, M.G. Kinze, and J.N. Morgan, eds., Kluwer Academic/Plenum Publishers, New York, Chapter 13, pp. 195–211.
89. Mounts, T.L. (1987). Alternative catalysts for hydrogenation of edible oils, in: *Hydrogenation: Proceedings of an AOCS Colloquium*, R. Hastert, ed., American Oil Chemists Society, Champaign, IL.
90. Mozaffarian, D., S. Fahimi, G.M. Singh, R. Micha, S. Khatibzadeh, R.E. Engell, S. Lim, G. Danaei, M. Ezzati, and J. Powles (2014).Global sodium consumption and death from cardiovascular causes. *N Engl J Med* 371: 624–634.
91. National Institutes of Health (2014). Osteoporosis basics. http://www.niams.nih.gov/Health_Info/Bone/Osteoporosis/osteoporosis_ff.asp (accessed August 22, 2014).
92. Nielsen, F.H. (2001). Boron, manganese, molybdenum, and other trace elements, in: *Present Knowledge in Nutrition*, 8th edn., B.A. Bowman and R.M. Russel, eds., ILSI Press, Washington, DC, pp. 392–393.

93. Nierenberg, D.W., R.E. Nordgren, M.B. Chang, R.W. Siegler, M.B. Blayney, F. Hochberg, T.Y. Toribara, E. Cernichiari, and T. Clarkson (1998). Delayed cerebellar disease and death after accidental exposure to dimethylmercury. *N Engl J Med* 338(23): 1672–1676.
94. Patrick, J. (1985). Elemental sources, in: *Iron Fortification of Foods*, F.M. Clydesdale and K.L. Wiemer, eds., Academic Press, Orlando, FL, pp. 31–38.
95. Perrine, C.G., K.M. Sullivan, R. Flores, K.L. Caldwell, and L.M. Grummer-Strawn (2013). Intakes of dairy products and dietary supplements are positively associated with iodine status among U.S. children. *J Nutr* 143(7): 1155–1160.
96. Phillips, L.G., Z. Haque, and J.E. Kinsella (1987). A method for measurement of foam formation and stability. *J Food Sci* 52: 1047–1049.
97. Porterfield, W. (1993). *Inorganic Chemistry: A Unified Approach*, 2nd edn., Academic Press, San Diego, CA.
98. Powles, J., S. Fahimi, R. Micha, S. Khatibzadeh, P. Shi, M. Ezzati, R.E. Engell, S.S. Lim, G. Danaei, D. Mozaffarian; on behalf of the Global Burden of Diseases Nutrition and Chronic Diseases Expert Group (NutriCoDE) (2013). Global, regional and national sodium intakes in 1990 and 2010: A systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. *BMJ Open* 3(12): e003733. doi: 10.1136/bmjopen-2013-003733.
99. Prasad, A.S., J.A. Halsted, and M. Nadimi (1961). Syndrome of iron deficiency, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. *Am J Med* 31: 532–546.
100. Preuss, H.G. (2001). Sodium, chloride, and potassium, in: *Present Knowledge in Nutrition*, 8th edn., ILSI Press, Washington, DC, Chapter 29, pp. 302–310.
101. Raboy, V. (2007). The ABCs of low-phytate crops. *Nat Biotechnol* 25: 874–875.
102. Raboy, V. (2003). Myo-inositol-1,2,3,4,5,6-hexakisphosphate. *Phytochemistry* 64(6): 1033–1043.
103. Rayman, M.P. (2012). Selenium and human health. *Lancet* 279(9822): 1256–1268.
104. Reddy, K.A. and E.H. Marth (1991). Reducing the sodium content of foods: A review. *J Food Protect* 54(2): 138–150.
105. Reid, I.R. (2014). Should we prescribe calcium supplements for osteoporosis prevention? *J Bone Metab* 21(1): 21–28.
106. Reilly, C. (1998). Selenium: A new entrant into the functional food arena. *Trends Food Sci Technol* 9: 114–118.
107. Richardson, D.P. (1990). Food fortification. *Proc Nutr Soc* 49: 39–50.
108. Rice, D.C., R. Schoeny, and K. Mahaffey (2003). Methods and rationale for derivation of a reference dose for methylmercury by the U.S. EPA. *Risk Anal* 23(1): 107–115.
109. Rico, D., A.B. Martín-Diana, J.M. Barat, and C. Barry-Ryan (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: A review. *Trends Food Sci Technol* 18: 373–386.
110. Rosado, J.L. (2003). Zinc and copper: Proposed fortification levels and recommended zinc compounds. *J Nutr* 133(9): 2985S–2989S.
111. Sandberg, A.-S. (2002). Bioavailability of minerals in legumes. *Br J Nutr* 88: S281–S285.
112. Sandström, B., Arvidsson, B., Cederblad, A., and Bjorn-Rasmussen, E. (1980). Zinc absorption from composite meals, I: The significance of wheat extraction rate, zinc, calcium, and protein content in meals based on bread. *Am J Clin Nutr* 33: 739–745.
113. Satarug, S., J.R. Baker, S. Urbenjapol, M. Haswell-Elkins, P.E. Reilly, D.J. Williams, and M.R. Moore (2003). A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. *Toxicol Lett* 137(1–2): 65–83.
114. Satarug, S., S.H. Garrett, M.A. Sens, and D.A. Sens (2010). Cadmium, environmental exposure, and health outcomes. *Environ Health Perspect* 118(2): 182–190.
115. Satarug, S., M.R. Haswell-Elkins, and M.R. Moore (2000). Safe levels of cadmium intake to prevent renal toxicity in human subjects. *Br J Nutr* 84(6): 791–802.
116. Shriver, D.F., P.W. Atkins, and C.H. Langford (1994). *Inorganic Chemistry*, 2nd edn., W.H. Freeman, New York.
117. South, P.K. and D.D. Miller (1998). Iron binding by tannic acid: Effects of selected ligands. *Food Chem* 63: 167–172.
118. Stanbury, J.B. (1996). Iodine deficiency and iodine deficiency disorders, in: *Present Knowledge in Nutrition*, 7th edn., E.E. Ziegler and L.J. Filer, eds., ILSI Press, Washington, DC, pp. 378–383.
119. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes; Food and Nutrition Board; Institute of Medicine (1997). *Dietary Reference Intakes for Calcium, Phosphorous, Vitamin D, and Flouride*, National Academy Press, Washington, DC.

120. Suh, J., B.Z. Zhu, and B. Frei (2003). Ascorbate does not act as a pro-oxidant towards lipids and proteins in human plasma exposed to redox-active transition metal ions and hydrogen peroxide. *Free Radic Biol Med* 34(10): 1306–1314.
121. Tchounwou, P.B., W.K. Ayensu, N. Ninashvili, and D. Sutton (2003). Environmental exposure to mercury and its toxicopathologic implications for public health. *Environ Toxicol* 18: 149–175.
122. Underwood, E.J. and W. Mertz (1987). Introduction, in: *Trace Elements in Human and Animal Nutrition*, 5th edn., W. Mertz, ed., Academic Press, San Diego, CA, pp. 1–19.
123. USDA Agricultural Research Service. USDA Food Composition Databases. 2016. https://ndb.nal.usda.gov/ndb/ (accessed October 6, 2016).
124. U.S. Department of Agriculture and U.S. Department of Health and Human Services (2010). Dietary guidelines for Americans, 2010, 7th edn., U.S. Government Printing Office, Washington, DC.
125. Urbano, G., M. Lopez-Jurado, P. Aranda, C. Vidal-Valverde, E. Tenorio, and J. Porres (2000). The role of phytic acid in legumes: Antinutrient or beneficial function? *J Physiol Biochem* 56(3): 283–294.
126. van Dokum, W. (1989). The significance of speciation for predicting mineral bioavailability, in: *Nutrient Availability: Chemical and Biological Aspects*, D. Southgate, I. Johnson, and G.R. Fenwick, eds., Roy Society of Chemistry, Cambridge, England, pp. 89–96.
127. Van Thuy, P., J. Berger, L. Davidsson, N. Cong Khan, N. Thi Lam, J.D. Cook, R.F. Hurrell, and H. Huy Khoi (2003). Regular consumption of NaFeEDTA-fortified fish sauce improves iron status and reduces the prevalence of anemia in anemic Vietnamese women. *Am J Clin Nutr* 78: 284–290.
128. Vinceti, M., G. Dennert, C.M. Crespi, M. Zwahlen, M. Brinkman, M.P.A. Zeegers, M. Horneber, R. D'Amico, and C. Del Giovane (2014). Selenium for preventing cancer. *Cochrane Database Syst Rev* (3): Art. No. CD005195. doi: 10.1002/14651858.CD005195.pub3.
129. Vucenik, I. and A.M. Shamsuddin (2006). Protection against cancer by dietary IP_6 and inositol. *Nutr Cancer* 55(2): 109–125.
130. Walczyk, T., P. Kastenmayer, S. Storcksdieck Genannt Bonsmann, C. Zeder, D. Grathwohl, and R.F. Hurrell (2013). Ferrous ammonium phosphate ($FeNH_4PO_4$) as a new food fortification: Iron bioavailability compared to ferrous sulfate and ferric pyrophosphate form an instant milk drink. *Eur J Nutr* 52(4): 1361–1368.
131. Weaver, C.M. (2001). Calcium, in: *Present Knowledge in Nutrition*, 8th edn., B.A. Bowman and R.M. Russell, eds., ILSI Press, Washington, DC, Chapter 26, pp. 273–280.
132. Weaver, C.M. and K.L. Plawecki (1994). Dietary calcium: Adequacy of a vegetarian diet. *Am J Clin Nutr* 59: 1238S–1241S.
133. Welch, R.M. and R.D. Graham (2002). Breeding crops for enhanced micronutrient content. *Plant Soil* 245(1): 205–214.
134. Welch, R.M. and W.A. House (1984). Factors affecting the bioavailability of mineral nutrients in plant foods, in: *Crops as Sources of Nutrients for Humans*, R.M. Welch, ed., Soil Science Society of America, Madison, WI, pp. 37–54.
135. Welch, R.M., W.A. House, and W.H. Allaway (1974). Availability of zinc from pea seeds to rats. *J Nutr* 104: 733–740.
136. Whittaker, P., J.E. Vanderveen, M.J. DiNovi, P.M. Kuznesof, and V.C. Dunkel (1993). Toxicological profile, current use and regulatory issues of EDTA compound for assessing potential use of sodium iron EDTA for food fortification. *Regulat Toxicol Pharmacol* 18: 419–427.
137. Wong, D.W.S. (1989). *Mechanism and Theory in Food Chemistry*, Van Nostrand Reinhold, New York, pp. 5–7.
138. World Health Organization (2003). Eliminating iodine deficiency disorders. http://www.who.int/nut/idd.htm (accessed August 22, 2014).
139. World Health Organization (2014). Micronutrient deficiencies. http://www.who.int/nutrition/topics/ida/en/ (accessed August 22, 2014).
140. Yeung, C.K., R.P. Glahn, X. Wu, R.H. Liu, and D.D. Miller (2003). In vitro iron bioavailability and antioxidant activity of raisins. *J Food Sci* 68(2): 701–705.
141. Yip, R. (2001). Iron, in: *Present Knowledge in Nutrition*, 8th edn., B.A. Bowman and R.M. Russell, eds., ILSI Press, Washington, DC, Chapter 23, pp. 311–328.
142. Yip, R. and U. Ramakrishnan (2002). Experiences and challenges in developing countries. *J Nutr* 132: 827S–830S.
143. Zijp, I.M., O. Korver, and L.B. Tijburg (2000). Effect of tea and other dietary factors on iron absorption. *Crit Rev Food Sci Nutr* 40(5): 371–398.
144. Zimmermann, M.B. (2009). Iodine deficiency. *Endocr Rev* 30(4): 376–408.

145. Zimmermann, M.B. and F.M. Hilty (2011). Nanocompounds of iron and zinc: Their potential in nutrition. *Nanoscale* 3(6): 2390–2398.
146. FDA (2014). Fish: What pregnant women and parents should know. http://www.fda.gov/food/foodborneillnesscontaminants/metals/ucm393070.htm (accessed August 28, 2014).
147. Agency for Toxic Substances & Disease Registry. Toxic substances portal—cadmium. http://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=47&tid=15 (accessed October 5, 2016).
148. USDA. USDA Food Composition Databases. Nutrient Data Laboratory, Beltsville Human Nutrition Research Center. http://ndb.nal.usda.gov/ (accessed October 5, 2016).

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

10 Colorants

Steven J. Schwartz, Jessica L. Cooperstone, Morgan J. Cichon, Joachim H. von Elbe, and M. Monica Giusti

CONTENTS

10.1 Introduction 682
10.2 Pigments in Animal and Plant Tissues 683
10.2.1 Heme Compounds 683
10.2.1.1 Myoglobin/Hemoglobin 684
10.2.1.2 Cured Meat Pigments 688
10.2.1.3 Stability of Meat Pigments 688
10.2.1.4 Packaging Considerations 689
10.2.2 Chlorophyll 689
10.2.2.1 Structure and Nomenclature 690
10.2.2.2 Physical Characteristics and Analysis 691
10.2.2.3 Alterations of Chlorophyll 692
10.2.2.4 Color Loss during Thermal Processing 699
10.2.2.5 Technology of Color Preservation 699
10.2.3 Carotenoids 702
10.2.3.1 Structures of Carotenoids 702
10.2.3.2 Occurrence and Distribution 705
10.2.3.3 Physical Properties, Extraction, and Analysis 706
10.2.3.4 Chemical Properties 706
10.2.3.5 Stability during Processing 708
10.2.4 Anthocyanins and Other Phenols 708
10.2.4.1 Anthocyanins 708
10.2.4.2 Other Flavonoids 720
10.2.4.3 Quinoids and Xanthones 724
10.2.5 Betalains 725
10.2.5.1 Structure 725
10.2.5.2 Physical Properties 726
10.2.5.3 Chemical Properties 727
10.3 Food Colorants 731
10.3.1 Regulatory Aspects 731
10.3.1.1 United States 731
10.3.1.2 International 734
10.3.2 Properties of Certified Dyes 735
10.3.3 Use of Certified Colors 739
10.3.4 Colors Exempt from Certification 741
10.3.5 Use of Colors Exempt from Certification 743
References 743
Further Reading 752

10.1 INTRODUCTION

Color is perhaps one of the most important attributes dictating liking and willingness to eat for a particular food. "Color can be defined as the interpretation by the brain of a light signal coming from a sample" [71]. A colorant is any chemical, either natural or synthetic, that imparts color. Foods have color because of their ability to reflect or emit different quantities of energy at wavelengths able to stimulate the retina in the eye. The energy range to which the eye is sensitive is referred to as visible light. Visible light, depending on an individual's sensitivity, encompasses wavelengths of approximately 380–770 nm. This range makes up a very small portion of the electromagnetic spectrum (Figure 10.1). In addition to obvious colors (hues), black, white, and intermediate grays are also regarded as colors.

Pigments are substances (often in the cells and tissues of plants and animals) that impart color. Dyes are any substances added to lend color to materials. The term dye is commonly used in the textile industry. In the U.S. food industry, a dye is a food-grade water-soluble colorant certified by the U.S. Food and Drug Administration (FDA). These specific dyes are referred to as "certified colors," and each one is assigned an FD&C number. The FD&C designation means that the dye may be used in foods, drugs, and cosmetics. Added to the approved list of certified colors are the FD&C lakes. Lakes are dyes extended on a substratum and they are oil dispersible. The dye/substratum combination is achieved by adsorption, coprecipitation, or chemical reaction. The complex involves a salt of a water-soluble primary dye and an approved insoluble base stratum. Alumina is the only approved substratum for preparing FD&C lakes. In addition, there are other dyes or lakes approved for use in other countries, where specifications are established by the European Union (EU) or the World Health Organization (WHO). Colorants exempt from certification may also be used. These are natural pigments or substances that have been synthesized but are considered "nature identical" to the natural pigment. A classification of colorants and an example within each class are given in Table 10.1.

Pigments play important roles in plants, with a number of different functions. Some pigments are involved in photosynthesis as part of the plant machinery that absorbs, transfers, and converts light into energy [149]. Pigments are also used as attractants or signals between plants and animals/insects, to filter out light of undesired wavelengths and to quench high-energy intermediates from light harvesting [149].

It is because of our ability to easily perceive both color and appearance that these factors are the first to be evaluated by the consumer when purchasing foods. One can provide consumers nutritious, safe, and cost-effective foods, but if they are not attractive, purchase of them will be limited. The consumer also relates the color of foods to quality. Specific colors are often associated with maturity and freshness; for example, the redness of raw meat is often associated with freshness

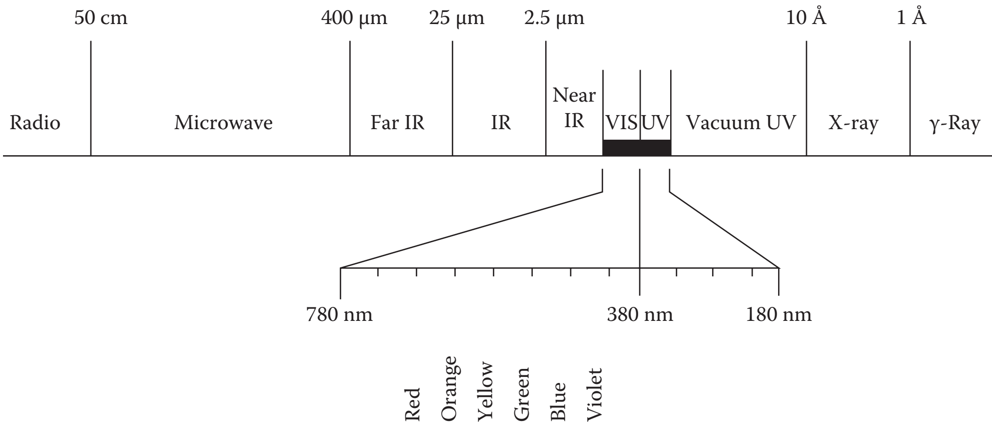


FIGURE 10.1 Electromagnetic spectrum.

TABLE 10.1
Classification of Colorants

Colorant	Example
A. Certified	
1. Dye	FD&C Red No. 40
2. Lake	Lake of FD&C Red No. 40
B. Exempt from certification	
1. Natural pigments	Anthocyanin, juice concentrate, annatto extract
2. Synthetic (nature identical)	β-Carotene

and a green strawberry may be judged as immature. Color also influences flavor perception. The consumer expects beverages to have a certain flavor based on color, for example, red drinks to be berry/cherry/watermelon flavored, yellow to be lemon flavored, and green to be lime flavored [155]. The impact of color on sweetness perception has also been demonstrated, with more pigmented beverages being perceived as sweeter [107].

It should also be noted that many compounds responsible for the brilliant colors of fruits and vegetables might exhibit bioactivity within foods and/or potential health benefits when consumed. It is clear, therefore, that the color of foods can have multiple effects on consumers and is not purely cosmetic.

Many food pigments are, unfortunately, unstable during processing and storage. Complete prevention of undesirable changes is often difficult or impossible. Depending on the pigment, stability is impacted by factors such as the presence or absence of light, oxygen, metals and oxidizing/reducing agents, temperature, water activity, and pH. Because of the relative instability of naturally occurring pigments, colorants are sometimes added to foods [50].

The purpose of this chapter is to provide an understanding of colorant chemistry—an essential prerequisite to managing the color and color stability of foods.

10.2 PIGMENTS IN ANIMAL AND PLANT TISSUES

Naturally occurring pigments in plants and animal tissues are those that are synthesized and accumulated, or excreted from living cells. In addition, pigment transformations occurring in foods during processing may result in the formation or alteration of color. Pigments indigenous to animals and plants have historically the formed part of the normal human diet and have a history of safety. Their chemical structures can be used to classify them as shown in Table 10.2.

10.2.1 Heme Compounds

Heme pigments are responsible for the color of meat. Myoglobin (sometimes referred to myohemoglobin in older publications) is the primary pigment in muscle, while hemoglobin, the pigment of blood, is of secondary importance in meat. Most of the hemoglobin in muscle is removed when animals are slaughtered and bled. Thus, in properly bled tissue myoglobin is responsible for 90% or more of the muscle pigmentation. The myoglobin content varies considerably among muscle tissues and is influenced by factors including species, muscle type, age, sex, rearing conditions, and physical activity. For example, pale-colored veal has lower myoglobin content than red-colored beef. Such is the case with poultry, where light-colored breast muscle is easily distinguished from the dark color of leg and thigh muscles. Listed in Table 10.3 are the major pigments found in fresh, cured, and cooked meat. Other minor pigments present in muscle tissue include the cytochrome enzymes, flavins, and vitamin B_{12}.

TABLE 10.2
Classification of Plant and Animal Pigments Based on Their Chemical Structure

Chemical Group	Pigment	Examples	Coloration	Occurrence (Examples)
Tetrapyrroles	Heme compounds	Oxymyoglobin	Red	Fresh meats
		Myoglobin	Purple/red	
		Metmyoglobin	Brown	Aged meats
	Chlorophylls	Chlorophyll *a*	Blue green	Broccoli, lettuce, spinach
		Chlorophyll *b*	Green	
Isoprenoids/tetraterpenoids	Carotenoids	β-Carotene	Yellow/orange	Carrots, melons, peaches, peppers
		Lycopene	Red/pink/orange	Tomatoes, watermelon, pink grapefruit
O-heterocyclic compounds/quinones	Flavonoids/phenolics	Anthocyanins	Orange/red/blue	Berries, red apple, red cabbage, radish
		Flavonols	White/yellow	Onions, cauliflower
		Tannins	Red brown	Wine, black tea
N-heterocyclic compounds	Betalains	Betacyanins	Purple/red	Red beets, Swiss chard, cactus pear
		Betaxanthins	Yellow/orange	Yellow beets

10.2.1.1 Myoglobin/Hemoglobin

10.2.1.1.1 Structure of Heme Compounds

Myoglobin is a globular protein that binds both iron and oxygen and consists of a single polypeptide chain. Its three-dimensional structure was determined in 1958 and resulted in a Nobel Prize in chemistry for its discovery [112]. Its molecular mass is 16.8 kDa and it is comprised of 153 amino acids. This protein portion of the molecule is a globulin. The chromophore component responsible for light absorption and color is a porphyrin known as heme, which is composed of four pyrrole rings joined together and linked to a central iron atom (Figure 10.2). The oxidation state of this iron atom, the status of the hematin nucleus, and the state of the globin protein are important for determining the color of meat. The heme porphyrin is present within a hydrophobic pocket of the globin protein and bound to a histidine residue (Figure 10.3) [127]. The centrally located iron atom possesses six coordination sites, four of which are occupied by the nitrogen atoms within the tetrapyrrole ring. The fifth coordination site is bound by the histidine residue of globin, leaving the sixth site available to complex with electronegative atoms donated by various ligands, primarily O_2, NO, and CO [26].

Hemoglobin consists of four myoglobins linked together as a tetramer. Hemoglobin, a component of red blood cells, forms reversible complexes with oxygen in the lung. This complex is distributed via the blood to various tissues and cells throughout the animal where oxygen is needed. Myoglobin within tissue acts in a similar fashion, accepting the oxygen carried by hemoglobin and storing it within the tissues for metabolism.

10.2.1.1.2 Chemistry and Color: Oxidation State

Meat color is determined by the chemistry of myoglobin, including its state of oxidation, the type of ligands bounds to heme, and the state of the globin protein. The heme iron within the porphyrin ring may exist in two oxidation states: either as the reduced ferrous (Fe^{2+}) or oxidized ferric (Fe^{3+}) form. This state of *oxidation* for the iron atom within heme should be distinguished from *oxygenation* of myoglobin. *Oxygenation* occurs when molecular oxygen binds to myoglobin and oxymyoglobin is formed. When *oxidation* of myoglobin occurs, the iron atom is converted from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state, forming metmyoglobin.

TABLE 10.3
Major Pigments Found in Fresh, Cured and Cooked Meat

Pigment	Mode of Formation	State of Iron	State of Hematin Nucleus	State of Globin	Color
1. Myoglobin	Reduction of metmyoglobin; deoxygenation of oxymyoglobin	Fe^{2+}	Intact	Native	Purplish-red
2. Oxymyoglobin	Oxygenation of myoglobin	Fe^{2+}	Intact	Native	Bright red
3. Metmyoglobin	Oxidation of myoglobin, oxymyoglobin	Fe^{3+}	Intact	Native	Brown
4. Nitric oxide myoglobin (nitrosomyoglobin)	Combination of myoglobin with nitric oxide	Fe^{2+}	Intact	Native	Bright red (pink)
5. Nitric oxide metmyoglobin (nitrosometmyoglobin)	Combination of metmyoglobin with nitric oxide	Fe^{3+}	Intact	Native	Crimson
6. Metmyoglobin nitrite	Combination of metmyoglobin with excess nitrite	Fe^{3+}	Intact	Native	Reddish-brown
7. Globin myohemochromogen	Effect of heat, denaturing agents on myoglobin, oxymyoglobin; irradiation of globin hemichromogen	Fe^{2+}	Intact (usually bound to denatured protein other than globin)	Denatured (usually detached)	Dull red
8. Globin myohemichromogen	Effect of heat, denaturing agents on myoglobin, oxymoglobin, metmyoglobin, hemochromogen	Fe^{3+}	Intact (usually bound to denatured protein other than globin)	Denatured (usually detached)	Brown (sometimes greyish)
9. Nitric oxide myohemochromogen	Effect of heat, denaturing agents on nitric oxide myoglobin	Fe^{2+}	Intact	Denatured	Bright red (pink)
10. Sulfmyoglobin	Effect of H_2S and oxygen on myoglobin	Fe^{3+}	Intact but one double bond saturated	Native	Green
11. Metsulfmyoglobin	Oxidation of sulfmyoglobin	Fe^{3+}	Intact but one double bond saturated	Native	Red
12. Choleglobin	Effect of hydrogen peroxide on myoglobin or oxymyoglobin; effect of ascorbine or other reducing agent on oxymyoglobin	Fe^{2+} or Fe^{3+}	Intact but one double bond saturated	Native	Green
13. Nitrihemin	Effect of large excess nitrite and heat on 5	Fe^{3+}	Intact but reduced	Absent	Green
14. Verdohaem	Effect of reagents as in 7–9 in excess	Fe^{3+}	Porphyrin ring opened	Absent	Green
15. Bile pigments	Effect of reagents as in 7–9 in large excess	Fe absent	Porphyrin ring destroyed Chain of porphyrins	Absent	Yellow or colorless

Source: From Lawrie, R. A. (1985). *Meat Science*, 4th edn. Pergamon Press, New York.

FIGURE 10.2 Chemical structure of heme from hemoglobin or myoglobin.

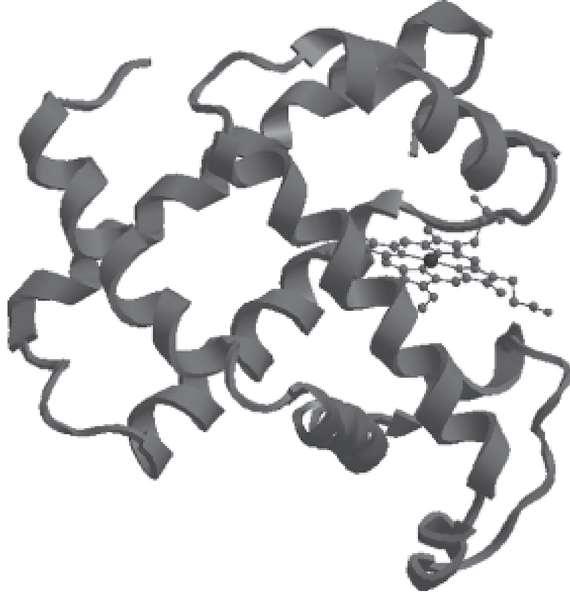


FIGURE 10.3 Tertiary structure of myoglobin showing coordination of the heme molecule with the globin protein. (Adapted from PDB ID 1co8; Liong, E.C. et al., *J. Biol. Chem.*, 276, 9093, 2001.)

Meat tissue that contains primarily myoglobin (also referred to as deoxymyoglobin) is purplish red in color. Binding of molecular oxygen at the sixth ligand yields oxymyoglobin, and the color of the tissue changes to bright red, a desirable color for meat consumers. This color is often present on the surface of the meat as the muscle still contains active cytochrome enzymes, which can utilize oxygen postmortem [126]. Both the purple myoglobin and the red oxymyoglobin can oxidize, changing the state of the iron from ferrous to ferric. If this change in state occurs through autoxidation, these pigments acquire the undesirable brownish-red color of metmyoglobin. In this state, metmyoglobin is not capable of binding oxygen and the sixth coordination position is occupied by water [60]. Metmyoglobin can be reduced back to myoglobin both enzymatically and nonenzymatically. The main pathway seems to be by action of a metmyoglobin reductase that in the presence of NADH can effectively reduce metmyoglobin to the ferrous state [88,148,156]. Shown in Figure 10.4 are the various reactions of the heme pigments. Color reactions in fresh meat are dynamic and determined by conditions in the muscle and the resulting ratios of myoglobin, metmyoglobin, and oxymyoglobin. While interconversion among myoglobin and oxymyoglobin can occur readily (and spontaneously)

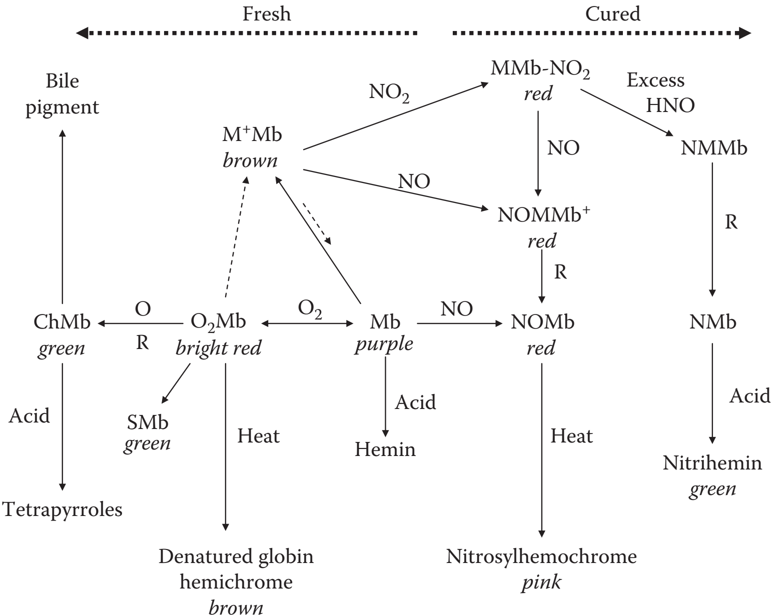


FIGURE 10.4 Myoglobin reactions in fresh and cured meats. ChMb, cholemyoglobin (oxidized porphyrin ring); O_2Mb, oxymyoglobin (Fe^{2+}); MMb, metmyoglobin (Fe^{3+}); Mb, myoglobin (Fe^{2+}); MMb-NO_2, metmyoglobin nitrite; NOMMb, nitrosylmetmyoglobin; NOMb, nitrosylmyoglobin; NMMb, nitrometmyoglobin; NMb, nitromyoglobin, the latter two being reaction products of nitrous acid and the heme portion of the molecule; SMb, sulfomyoglobin; R, reductant; O, strong oxidizing conditions. (From Fox Jr., J.B., *J. Agric. Food Chem.*, 14, 207, 1966.)

depending on oxygen tension, the conversion of metmyoglobin to the other forms would require enzymatic or nonenzymatic reduction of the ferric to the ferrous state.

Shown in Figure 10.5 is the relationship between oxygen partial pressure and the percentage of each type of heme pigment. A high partial pressure of oxygen favors oxygenation, forming bright red oxymyoglobin. Freshly cut meats will *bloom* or rapidly develop a bright red coloration, a result of the rapid conversion of myoglobin to oxymyoglobin, when exposed to oxygen in the environment [70]. Conversely, at low oxygen partial pressures myoglobin and metmyoglobin are favored. In order to enhance oxymyoglobin formation, saturation levels of oxygen in the environment are useful.

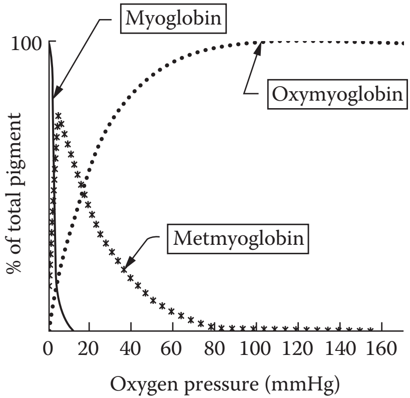


FIGURE 10.5 Influence of oxygen partial pressure on the three chemical states of myoglobin. (From Forrest, J. et al., *Principles of Meat Science*, W.H. Freeman, San Francisco, CA, 1975.)

The rate of metmyoglobin formation, caused by heme oxidation (Fe^{2+} to Fe^{3+}), can be minimized if oxygen is totally excluded. Different muscles have varying oxygen partial pressures causing ratios of pigment forms to vary in a tissue-dependent manner.

The presence of the globin protein can decrease the rate of heme oxidation. In addition, oxidation occurs more rapidly at lower pH values, and the rate of autoxidation of oxymyoglobin occurs more slowly than that of myoglobin. The presence of trace metals, especially copper ions, is known to promote autoxidation.

10.2.1.1.3 Chemistry and Color: Discoloration

Two different reactions can cause green discoloration of myoglobin [126]. Hydrogen peroxide can react with iron in either the ferrous or ferric state, resulting in choleglobin, a green-colored pigment. Also, in the presence of hydrogen sulfide and oxygen, green sulfomyoglobin can form. It is thought that hydrogen peroxide and/or hydrogen sulfide arise from bacterial growth. A third mechanism for green pigmentation (the heating of nitrimyoglobin in a reducing environment) occurs in cured meats and is described in Section 10.2.1.2.

10.2.1.2 Cured Meat Pigments

In the manufacture of most cured meats, nitrates or nitrites are added to inhibit germination of *Clostridium botulinum* spores, with the additional benefit of improving both color and flavor. During the curing process, specific reactions occur that are responsible for the stable pink color of cured meat products. These reactions are outlined in Figure 10.4 and the compounds responsible for the reactions are listed in Table 10.3.

The first reaction occurs between nitric oxide and myoglobin to produce nitric oxide myoglobin, also known as nitrosylmyoglobin. Nitrosylmyoglobin is bright red and unstable. Upon heating, the more stable nitric oxide myohemochromogen (nitrosylhemochrome) forms. This product is responsible for the desirable pink color of cured meats. Heating of this pigment denatures globin, but the pink color persists. If metmyoglobin is present, it has been postulated that reducing agents are required to convert metmyoglobin to myoglobin before the reaction with nitric oxide can take place. Alternatively, nitrite can interact directly with metmyoglobin. In the presence of excess nitrous acid, nitrimyoglobin will form. Upon heating in a reducing environment, nitrimyoglobin is converted to nitrihemin, a green pigment. This series of reactions causes a defect known as "nitrite burn."

In the absence of oxygen, nitric oxide complexes of myoglobin are relatively stable. However, under aerobic conditions, these pigments are sensitive to light. If reductants are added, such as ascorbate or sulfhydryl compounds, the reductive conversion of nitrite to nitric oxide is favored. Thus, under these conditions, formation of nitric oxide myoglobin occurs more readily.

Parma hams (prosciutto di Parma) are a special type of ham manufactured using only pork and salt, without addition of nitrates or nitrites. During dry curing of these products, a new pigment was discovered, zinc protoporphyrin, in which the iron from heme is substituted by zinc. These pigments are responsible for the stable bright red color of Parma hams in the absence of a curing agent [218].

Detailed reviews on the chemistry of cured meat pigments are available elsewhere [69,126,129,161].

10.2.1.3 Stability of Meat Pigments

The main factor determining consumer acceptance of meats is muscle color. Many factors in a complex food system can influence the stability of meat pigments. Generally, when the globin part of the compound is not intact, myoglobin cannot bind to oxygen and the prevalence of iron oxidized in the ferric form is increased. This results in a preference toward metmyoglobin when the globin is, for any reason, denatured [126]. In addition, interactions between various factors are critical and make it difficult to determine absolute cause and effect relationships. Some environmental conditions that have important effects on meat color and pigment stability include exposure to light, temperature, relative humidity, pH, and the presence of specific bacteria. Review papers on this subject are available [60,121].

Specific reactions, such as lipid oxidation, are known to increase the rate of pigment oxidation [59]. Color stability can be improved by the addition of antioxidants such as ascorbic acid, vitamin E, butylated hydroxyanisole (BHA), or propyl gallate [83]. Vitamin E supplementation of beef cattle diets is an effective procedure for enhancing the lipid and color stability of meat products obtained from these animals [61]. These compounds have been shown to delay lipid oxidation and improve retention of color in tissues. Other biochemical factors, such as the rate of oxygen consumption prior to slaughter and activity of metmyoglobin reductase, can influence the color stability of fresh meat [136].

Irradiation of meats can also cause color changes because of the susceptibility of the myoglobin molecule, especially the iron, to alterations in the chemical environment and to energy input. Stable red pigments, brown pigments, and even green discoloration may occur during irradiation. A combination of preslaughter feeding of livestock with antioxidants, optimizing the conditions of the meat prior to irradiation, addition of antioxidants to the meat, use of modified atmosphere packaging (MAP), and controlling temperature may all contribute to optimize color during irradiation [20].

Many consumers use the internal cooked appearance of meat (e.g., ground beef patties) to evaluate thoroughness of cooking and thus inactivation of harmful microorganisms. However, two phenomena prevent using internal cooked color as an indicator: premature browning and persistent pink color. In premature browning, meat appears cooked (brown), even though it has not reached the internal temperature required to kill pathogens. On the other hand, the color of some meats remains pink even after reaching safe internal cooking temperatures, so consumers overcook them. Persistent pink coloration can be influenced by meat pH and concentration of meat pigments [146]. A higher pH (>6.0) protects myoglobin from denaturation [84]. Meat from older animals and those who have been stressed is of higher pH and more susceptible to persistent pink color. Therefore, it is important for consumers to understand that meat color should not be used as an indicator of meat doneness [98,116].

10.2.1.4 Packaging Considerations

An important means of stabilizing meat color is to store under appropriate environmental conditions. The use of MAP can extend the shelf life of meat products. This technique requires the use of packaging films with low gas permeability. After packaging, air is removed from the package and the storage gas is injected, creating conditions that minimize the discoloration caused by heme iron oxidation from ferric to ferrous. By employing oxygen-enriched or -devoid atmospheres, color stability can be enhanced [154]. Muscle tissue stored under conditions devoid of O_2 (100% CO_2) and in the presence of an oxygen scavenger also exhibit good color stability [174,212]. Use of MAP techniques may result in other chemical and biochemical alterations that can influence the acceptability of meat products. Part of the effect of modified atmospheres on pigment stability relates to its influence on microbial growth. Combinations of O_2, CO_2, and N_2 have been used to maintain the quality of fresh red meat through optimization of both microbiological and organoleptic properties. Addition of low levels of CO has resulted in extended shelf life though formation of carboxymyoglobin, which is more stable to oxidation than oxymyoglobin and gives an attractive cherry-red color to meat, although not legally permitted in foods in the United States [135]. Further information on use of modified atmospheres for fresh meat storage can be found in a review article by Seideman and Durland [190] and in Luño et al. [135].

10.2.2 Chlorophyll

Chlorophylls are the major light-harvesting pigments in green plants, algae, and photosynthetic bacteria. They are responsible for the bright-green color of many fresh vegetables and are linked to consumer perception of quality. Loss of green color during vegetable processing and storage can be attributed to chlorophyll degradation.

10.2.2.1 Structure and Nomenclature

Chlorophyll molecules are substituted cyclic tetrapyrroles with a centrally coordinated magnesium atom. They are derived from porphyrin, which is a fully unsaturated macrocyclic structure containing four pyrrole rings linked through methine bridges. The pyrrole rings are letter designated A through D (Figure 10.6). According to the Fischer numbering system, the peripheral pyrrolic carbons are numbered 1 through 8, while the bridging carbons are designated α, β, γ, and δ (Figure 10.6a). Due to the large number of trivial names for substituted porphyrins in the Fischer system, a 1–24 numbering scheme (Figure 10.6b) was developed for porphyrins by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [147]. While the 1–24 numbering scheme simplifies the nomenclature for porphyrins, the Fischer numbering system is still commonly used for chlorophylls.

Phorbin is considered to be the nucleus of all chlorophylls and is formed by the addition of a fifth isocyclic ring (E) to porphyrin (Figure 10.6). Chlorophylls are tetradentate ligands, binding Mg^{2+} through the nitrogen atoms in the porphyrin ring. They are also characterized by the presence of propionic acid at the C-7 position. Several chlorophylls are found in nature and their structures differ in the substituents around the phorbin nucleus. Chlorophylls *a* and *b* are the predominant chlorophylls in foods and are found in green plants in an approximate ratio of 3:1. They differ in the C-3 substituent; chlorophyll *a* contains a methyl group while chlorophyll *b* contains a formyl group (Figure 10.6c). Both chlorophylls have a vinyl group at the C-2 position, an ethyl group at the C-4 position, a carbomethoxy group at the C-10 position of ring E, and a phytol group esterified to propionate at the C-7 position. Phytol is a 20-carbon monounsaturated isoprenoid alcohol responsible for most of the lipophilicity of chlorophyll and binds the chlorophyll molecule to the hydrophobic regions of the thylakoid membrane within the chloroplast. Other naturally occurring chlorophylls include chlorophylls *c* and *d*. Chlorophyll *c* is found in association with chlorophyll *a* in brown algae, dinoflagellates, and diatoms. Chlorophyll *d* is a minor constituent accompanying chlorophyll

FIGURE 10.6 Structures of porphin using the Fischer numbering scheme (a), porphin using the 1–24 numbering scheme (b), and chlorophyll (c).

TABLE 10.4
Nomenclature of Chlorophyll Derivatives

Phyllins	Chlorophyll derivatives containing magnesium
Pheophytins	The magnesium-free derivatives of the chlorophylls
Chlorophylllides	The products containing a C-7 propionic acid resulting from enzymic or chemical hydrolysis of the phytyl ester
Pheophorbides	The magnesium-free derivatives containing a C-7 propionic acid resulting from enzymatic or chemical hydrolysis of the phytyl ester
Methyl or ethyl pheophorbides	The corresponding 7-propionate methyl of ethyl propionate
Pyro compounds	Derivatives in which the C-10 carbomethoxy group has been replaced by hydrogen
Meso compounds	Derivatives in which the C-2 vinyl group has been reduced to an ethyl group
Chlorins e	Derivatives of pheophorbide *a* resulting from cleavage of the isocyclic ring E
Rhodins g	The corresponding derivatives from pheophorbide *b*

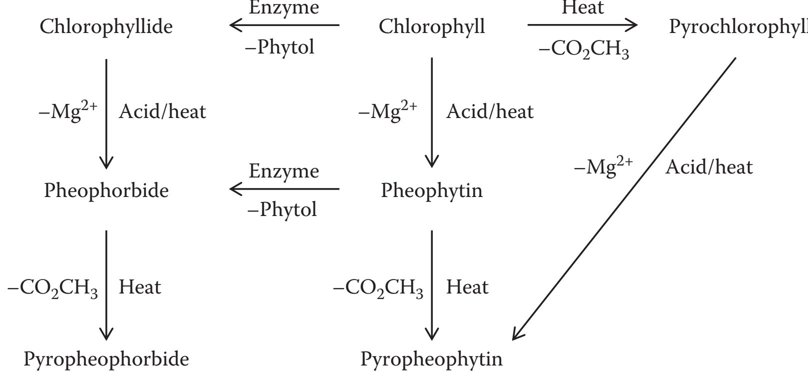


FIGURE 10.7 Relationship between chlorophyll and its derivatives.

a in red algae. Bacteriochlorophylls and chlorobium chlorophylls are chlorophyll-related pigments found in purple photosynthetic bacteria and green sulfur bacteria, respectively. Trivial names are widely used for chlorophylls and their derivatives [104]. Listed in Table 10.4 are the most commonly used names. Figure 10.7 shows a schematic representation of the structural relationships of chlorophyll and some of its derivatives.

10.2.2.2 Physical Characteristics and Analysis

Chlorophylls are located in the lamellae of intercellular organelles of green plants known as chloroplasts. They are associated with carotenoids, lipids, and lipoproteins. Weak linkages (noncovalent bonds) exist between these molecules. As these bonds are easily broken, chlorophylls can be effectively extracted by macerating plant tissue in organic solvents. Due to the varying polarities of chlorophylls and their derivatives, solvent choice for extraction is important. Lipophilic chlorophylls and chlorophyll derivatives with an intact phytol chain are commonly extracted with acetone or ether. Depending on the sample, separation of chlorophylls from coextracted lipids is sometimes necessary prior to analysis [6,170]. Derivatives that lack the phytol group, such as chlorophyllides and pheophorbides, are water soluble and better extracted using more polar solvents. High-performance liquid chromatography (HPLC) is commonly used for separating individual chlorophylls and their derivatives [36,62,179].

Chlorophylls are highly conjugated systems that conform to Hückel's $4n + 2$ rule of aromaticity. Given this, chlorophylls have unique chromophores and can be identified based on their characteristic absorption spectra. Chlorophylls *a* and *b* and their derivatives exhibit sharp absorption bands between

TABLE 10.5
Spectral Properties in Ethyl Ether of Chlorophyll *a* and *b* and Their Derivatives

Compound	Absorption Maxima (nm) "Red" Region	Absorption Maxima (nm) "Blue" Region	Ratio of Absorption ("Blue"/"Red")	Molar Absorptivity ("Red" Region)
Chlorophyll *a*	660.5	428.5	1.30	86,300[a]
Methyl chlorophyllide *a*	660.5	427.5	1.30	83,000[b]
Chlorophyll *b*	642.0	452.5	2.84	56,100[a]
Methyl chlorophyllide *b*	641.5	451.0	2.84	—[b]
Pheophytin *a*	667.0	409.0	2.09	61,000[b]
Methyl pheophorbide *a*	667.0	408.5	2.07	59,000[b]
Pheophytin *b*	655	434	—	37,000[c]
Pyropheophytin *a*	667.0	409.0	2.09	49,000[b]
Zinc pheophytin *a*	653	423	1.38	90,300[d]
Zinc pheophytin *b*	634	446	2.94	60,200[d]
Copper pheophytin *a*	648	421	1.36	67,900[d]
Copper pheophytin *b*	627	438	2.53	49,800[d]

[a] Strain et al. [205].
[b] Pennington et al. [164].
[c] Davidson [47].
[d] Jones et al. [110].

600 and 700 nm (red regions) and between 400 and 500 nm (blue regions) (Table 10.5). The band in the blue region is referred to as the Soret band and is common to all porphyrins, while the band in the red region is particular to chlorophylls [95]. The wavelengths of maximum absorption for chlorophylls *a* and *b* dissolved in ethyl ether are, respectively, 660.5 and 642 nm in the red region and 428.5 and 452.5 nm in the blue region [205]. The bathochromic shift in the Soret band from chlorophyll *a* to *b* can be attributed to the increase in resonance structures with the formyl substituent on chlorophyll *b*. Mass spectroscopic techniques employing atmospheric pressure chemical ionization (APCI) and electrospray ionization in conjunction with chromatographic separation have also been used for structure elucidation of chlorophyll derivatives produced during food processing [99,170,227].

10.2.2.3 Alterations of Chlorophyll

10.2.2.3.1 Enzymatic

Chlorophyllase and pheophytinase are two enzymes known to catalyze the degradation of chlorophyll during plant senescence, fruit ripening, and under some vegetable processing conditions. Chlorophyllase is an esterase that catalyzes the cleavage of phytol from chlorophylls, forming green chlorophyllides (Figure 10.7). The loss of the phytol chain significantly increases the hydrophilicity of the resulting phorbin unit, but as the chromophore is unaltered, the absorption spectrum remains the same. However, chlorophyllides have been shown to be less heat stable and more likely to degrade to magnesium-free derivatives than chlorophyll [36].

Chlorophyllase activity is limited to porphyrins with a carbomethoxy group at C-10 and hydrogens at positions C-7 and C-8 [145]. The enzyme is active in solutions containing alcohols, acetone, or hot water [222]. In the presence of large amounts of alcohols such as methanol or ethanol, the phytol group is removed and the chlorophyllide is esterified to form either methyl or ethyl chlorophyllide. Degradation rates of chlorophylls *a* and *b* and their respective methyl, ethyl, and free chlorophyllides in acidic acetone increase as the length of the C-7 chain is decreased, suggesting that steric hindrance from the C-7 chain affects the rate of hydrogen ion attack and the subsequent loss of magnesium from the porphyrin ring [176]. The optimum temperature for chlorophyllide formation in vegetables ranges

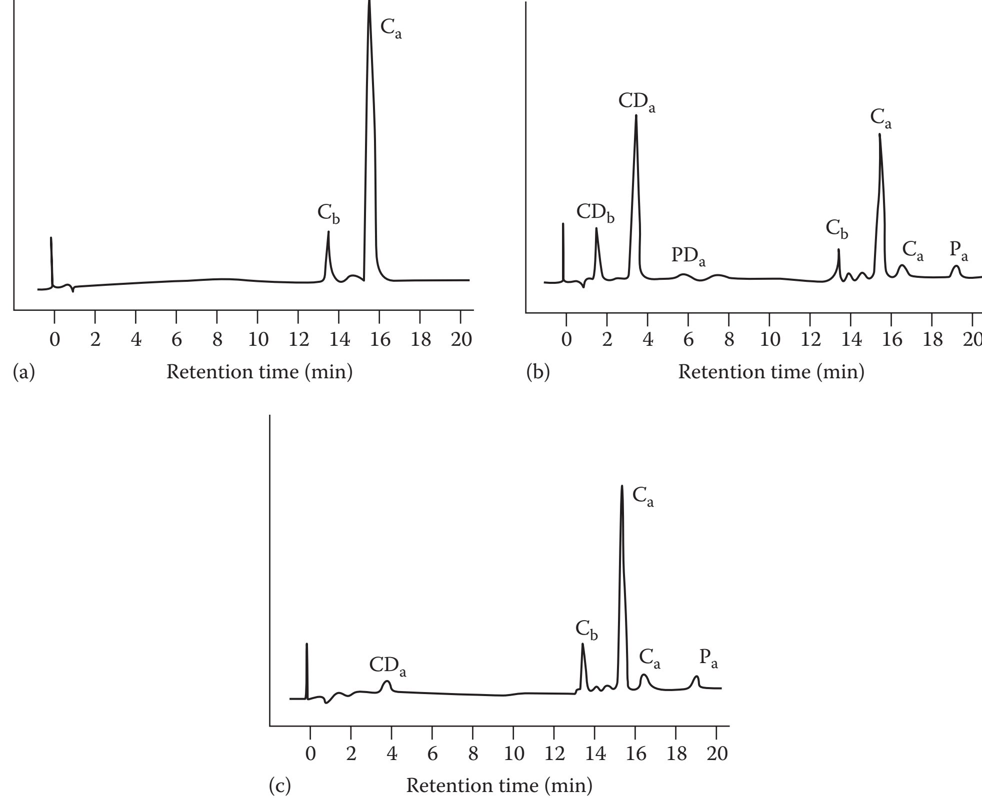


FIGURE 10.8 Reversed phase high-performance liquid chromatography chromatograms of chlorophyll and chlorophyll derivatives in spinach: (a) unblanched, (b) blanched 3 min at 71°C, (c) blanched 3 min at 88°C. C_a, chlorophyll *a* (different retention times correspond to isomeric forms); C_b, chlorophyll *b*; P_a, pheophytin *a*; PD_a, pheophorbide *a* CD_a, chlorophyllide *a*; CD_b, chlorophyllide *b*. (From von Elbe, J.H. and Laborde, L.F., Chemistry of color improvement in thermally processed green vegetables, ACS Symposium Series 405, in: Jen, J.J., ed., *Quality Factors of Fruits and Vegetables*, American Chemical Society, Washington, DC, 1989, pp. 12–28.)

from 60°C to 82.2°C [108,132]. Enzyme activity is essentially lost when plant tissue is heated to 100°C [108,132]. In spinach, chlorophyllase activity fluctuates during growing with maximum activity observed at the time the plant begins flowering. Postharvest storage of fresh spinach at 5°C decreases enzyme activity compared to activity measured during plant growth and at the time of harvest [183].

The conversion of chlorophylls to chlorophyllides in heated spinach leaves is shown in Figure 10.8. The unblanched spinach contains only chlorophylls *a* and *b*. Activity of chlorophyllase in spinach blanched at 71°C is illustrated by the formation of chlorophyllides, while the absence of almost all chlorophyllides in spinach blanched at 88°C results from inactivation of the enzyme.

Pheophytinase is a more recently discovered hydrolase that cleaves phytol from magnesium-free pheophytins to form olive-brown pheophorbides. Pheophytinase is believed to play a critical role in chlorophyll degradation during leaf senescence [178]. Gene expression of pheophytinase under various postharvest treatments of broccoli has been found to better correlate with chlorophyll loss than chlorophyllase expression [25].

10.2.2.3.2 Heat and Acid

Chlorophyll derivatives formed during heating or thermal processing can be classified into two groups based on the presence or absence of the magnesium atom in the tetrapyrrole center. Magnesium-containing derivatives are green in color, while magnesium-free derivatives are

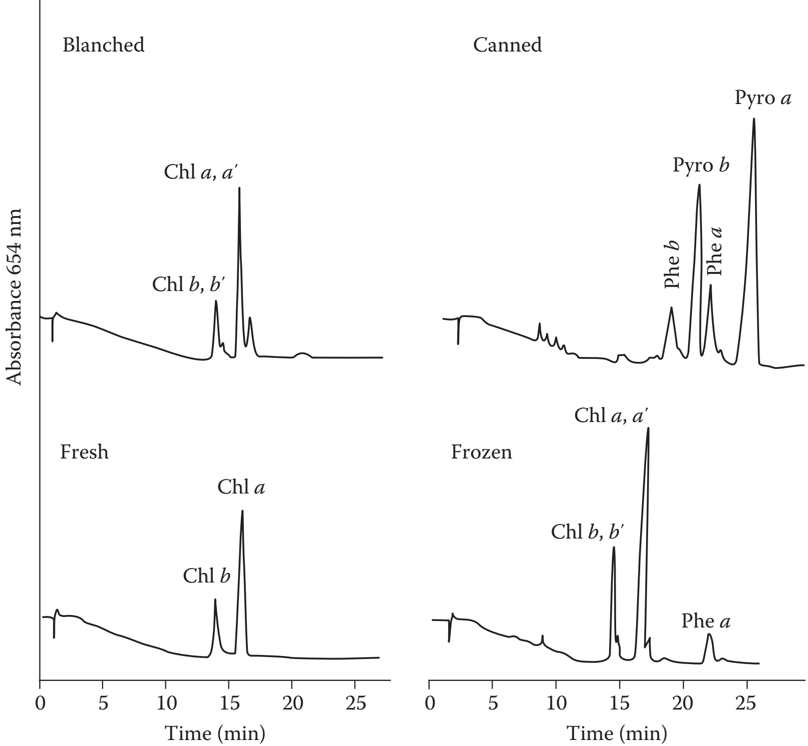


FIGURE 10.9 Reversed phase high-performance liquid chromatography chromatograms of chlorophylls (chl) and their derivatives, pheophytin (pheo) and pyropheophytin (pyro), in fresh, blanched, frozen, and canned spinach. (From Schwartz, S.J. et al., *J. Agric. Food Chem.*, 29, 533, 1981.)

olive-brown in color. The latter are chelators, and when, for example, sufficient zinc or copper atoms are available, they can form green zinc or copper complexes (see Section 10.2.2.3.3).

The first change observed when the chlorophyll molecule is exposed to heat is isomerization. Chlorophyll isomers are formed by inversion of the C-10 carbomethoxy group and are designated *a*′ and *b*′. Isomerization occurs rapidly in heated plant tissue or in organic solvents. Establishment of equilibrium in leaves results in conversion of 5%–10% of chlorophyll *a* and *b* to *a*′ and *b*′, respectively, after heating for 10 min at 100°C [12,183,221]. Chromatograms of chlorophyll extracts from fresh versus blanched spinach in Figure 10.9 show chromatographic separation of isomers formed during heating [183].

The magnesium atom in chlorophyll is easily displaced by two hydrogen atoms, resulting in the formation of olive-brown pheophytin (Figure 10.7). This reaction is irreversible. Compared to their parent compounds, pheophytin *a* and *b* are less polar. As chlorophyll *b* is more heat stable than chlorophyll *a*, formation of the respective pheophytins occurs more rapidly from chlorophyll *a* than from chlorophyll *b* [180]. The greater stability of chlorophyll *b* is attributed to the electron withdrawing formyl group at the C-3 position. Transfer of electrons away from the center of the molecule occurs because of the conjugated structure of chlorophyll. The resulting increase in positive charge on the four pyrrole nitrogens reduces the equilibrium constant for hydrogenation at this position, so pheophytin formation is less favored.

The rate of pheophytin formation during processing can be affected by such factors as the food matrix, pH, and temperature [87,172,180,223]. Chlorophyll degradation in heated vegetable tissue is greatly affected by tissue pH. In a basic media (pH 9.0) chlorophyll is very heat stable, whereas in an acidic media (pH 3.0) it is unstable. A decrease of 1 pH unit can occur during heating of plant tissue through the release of organic acids, which encourages

chlorophyll degradation. In a study by Haisman and Clarke [89], chlorophyll degradation in sugar beet leaves held in a heated buffer was not initiated until the temperature reached 60°C. Conversion of chlorophyll to pheophytin after holding for 60 min at 60°C or 90°C was 32% and 97%, respectively. It was proposed that pheophytin formation in plant cells is initiated by heat-induced chloroplast disruption, which, in turn, increases permeability of hydrogen ions across cell membranes. The critical temperature for initiation of pheophytin formation coincided with gross changes in membrane organization as observed using electron microscopy. Therefore, pheophytin formation in intact plant tissue postharvest is mediated by the availability of hydrogen ions to displace magnesium from chlorophyll.

The addition of chloride salts of sodium, magnesium, or calcium (1.0 M) decreases the rate of pheophytin formation in tobacco leaves heated at 90°C by approximately 47%, 70%, and 77%, respectively. The decrease in chlorophyll degradation was attributed to the electrostatic shielding effect of the salts [89]. It has been proposed that the addition of cations neutralizes the negative surface charge of the fatty acids and proteins in the chloroplast membrane, thereby reducing the attraction of hydrogen ions to the membrane surface [153].

The permeability of hydrogen across the chloroplast membrane can also be affected by the addition of detergents that adsorb on the surface of the membrane. Cationic detergents repel hydrogen ions, limiting their diffusion across the membrane and decreasing chlorophyll degradation. Anionic detergents attract hydrogen ions, increasing the rate of hydrogen diffusion across the membrane, which, in turn, increases the degradation of chlorophyll. In the case of neutral detergents, the negative surface charge on the membrane is diluted, and therefore, the attraction of hydrogen ions and consequent degradation of chlorophyll is decreased [40,89].

Extensive heat treatment can also result in the loss of the C-10 carbomethoxy group from pheophytin, resulting in the formation of olive-colored pyropheophytin. This modification does not alter the chromophore of the molecule, so both the absorption spectrum and color of pyropheophytin are identical to those for pheophytin in both the red and blue regions (Table 10.5). Pyropheophytins *a* and *b* are more nonpolar than their respective pheophytins (Figures 10.8 and 10.9).

The data in Table 10.6 show that for the first 15 min of heating, chlorophyll decreases rapidly while pheophytin increases rapidly [180]. With further heating, pheophytin decreases and

TABLE 10.6
Concentration (mg/g Dry Weight)[a] of Chlorophylls, Pheophytins and Pyropheophytins *a* and *b* in Fresh, Blanched, and Heated Spinach Processed at 121°C for Various Times

	Chlorophyll		Pheophytin		Pyropheophytin		
	a	***b***	***a***	***b***	***a***	***b***	**pH[b]**
Fresh	6.98	2.49					
Blanched	6.78	2.47				7.06	
Processed (min)[c]							
2	5.72	2.46	1.36	0.13			6.90
4	4.59	2.21	2.20	0.29	0.12		6.77
7	2.81	1.75	3.12	0.57	0.35		6.60
15	0.59	0.89	3.32	0.78	1.09	0.27	6.32
30		0.24	2.45	0.66	1.74	0.57	6.00
60			1.01	0.32	3.62	1.24	5.65

Source: Schwartz, S.J. and von Elbe, J., *J. Food Sci*., 48, 1303, 1983.

[a] Estimated error ±2%; each value represents a mean of 3 determinations.

[b] The pH was measured after processing and before pigment extraction.

[c] Times listed were measured after the internal product temperature reached 121°C.

TABLE 10.7
Pheophytins and Pyropheophytins *a* and *b* in Commercially Canned Vegetables

	Pheophytin[a] (μg/g dry weight)		Pyropheophytin[a] (μg/g dry weight)	
Product	***a***	***b***	***a***	***b***
Spinach	830	200	4000	1400
Beans	340	120	260	95
Asparagus	180	51	110	30
Peas	34	13	33	12

Source: Schwartz, S.J. and von Elbe, J., *J. Food Sci.*, 48, 1303, 1983.
[a] Estimated error ±2%.

pyropheophytin rapidly increases. Although a small amount of pyropheophytin is evident after 4 min of heating, accumulation does not become appreciable until after 15 min. Depending on the temperature, the first-order rate constant for conversion of pheophytin *b* to pyropheophytin *b* is 31%–57% greater than that for conversion of pheophytin *a* to pyropheophytin *a* [180]. Activation energies for the removal of the C-10 carbomethoxy group from pheophytins *a* and *b* are lower than the activation energies for the formation of pheophytins *a* and *b*, indicating a slightly lower temperature dependency for the formation of pyropheophytins over pheophytins.

Listed in Table 10.7 are the concentrations of pheophytins *a* and *b* and pyropheophytins *a* and *b* in commercially canned vegetable products [180]. These data indicate that pyropheophytins *a* and *b* are the major chlorophyll derivatives responsible for the olive-green color in many canned vegetables. It is also important to note that the amount of pyropheophytin formed is reflective of the severity of the heat treatment. Comparing commercial sterility of spinach, green beans, cut asparagus, and green peas processed at 121°C, the percentages of pyropheophytins relative to total pheo compounds for these products correspond fairly well to the heating times (Table 10.6).

While not as common, it is possible for chlorophylls to lose the C-10 carbomethoxy group prior to displacement of the magnesium from the porphyrin ring, forming green pyrochlorophylls (Figure 10.10). Pyrochlorophylls have the same absorption spectra as their parent chlorophylls and therefore are difficult to differentiate by UV–Vis spectroscopy alone. Pyrochlorophylls *a* and *b* were identified in roasted pistachios using mass spectrometry with atmospheric pressure chemical ionization [170]. After roasting for 60 min at 138°C, pyrochlorophylls and pyropheophytins were the predominant chlorophyll degradation products detected in pistachios. Pyrochlorophylls have also been reported in spinach leaves after microwave heat treatment [210]. It has been hypothesized that high temperatures and low moisture encourage the formation of pyrochlorophylls in foods [170].

As previously discussed, olive-green pheophorbides can form from the enzymatic cleavage of the phytol chain from pheophytin. Pheophorbides can also form from the chemical displacement of magnesium from green chlorophyllides under thermal processing conditions. Pheophorbide *a* and *b* are more water soluble than their respective pheophytins but maintain the same spectral characteristics (Table 10.5).

10.2.2.3.3 Metallocomplex Formation

The two hydrogen atoms within the tetrapyrrole nucleus of magnesium-free chlorophyll derivatives are easily displaced by zinc or copper ions to form green metallocomplexes. Formation of metallocomplexes from pheophytins *a* and *b* causes the red absorption maximum to shift to a shorter wavelength and the blue absorption maximum to a longer wavelength (Table 10.5) [110]. Spectral characteristics of the phytol-free metallocomplexes are identical to their parent compounds.

FIGURE 10.10 Formation of pheophytin, pyropheophytin, and pyrochlorophyll from chlorophyll.

The zinc and copper complexes are more stable in acid than in alkaline solutions. Magnesium, as mentioned, is easily displaced by the addition of acid at room temperature, while zinc pheophytin *a* is stable in solution at pH 2. Removal of copper is achieved only at pH values sufficiently low to begin degradation of the porphyrin ring.

Incorporation of metal ions into the neutral porphyrin is a bimolecular reaction. It is believed to be an S_N2 reaction involving the attachment of the metal ion to a pyrrole nitrogen and the simultaneous displacement of two hydrogen atoms [58]. Formation of metallocomplexes is affected by substituent groups because of steric hindrance and the highly conjugated structure of the tetrapyrrole nucleus [55,199].

Metallocomplexes of chlorophyll derivatives are known to form in plant tissue, with the *a* complexes forming faster than the *b* complexes. The slower formation of the *b* complexes has been attributed to the electron withdrawing C-3 formyl group. Migration of electrons away from the conjugated porphyrin ring system causes pyrrole nitrogen atoms to become more positively charged and therefore less reactive with metal cations.

Steric hindrance from the phytol chain also decreases the rate of complex formation. Pheophorbide *a* in ethanol reacts four times faster with copper ions than does pheophytin *a* [109]. Similarly, in acetone/water (80/20), formation of zinc pyropheophorbide *a* occurs most rapidly, followed by pheophorbide *a*, methyl pheophorbide *a*, ethyl pheophorbide *a*, pyropheophytin *a*, and pheophytin *a*. Not only do reaction rates decrease as the length of the C-7 chain increases, but they are also slowed by the presence of the carbomethoxy group at the C-10 carbon. This demonstrates the importance of the porphyrin substituents in metallocomplex formation, which can be attributed to the effects on steric hindrance and charge distribution [164,214].

Comparative studies on the formation of metallocomplexes in vegetable purées indicate that copper is chelated more rapidly than zinc. Copper complexes are detectable in pea purée when the concentration of Cu^{2+} is as low as 1–2 ppm. In contrast, zinc complex formation under similar conditions does not occur in purée containing less than 25 ppm Zn^{2+}. When both Zn^{2+} and Cu^{2+} are present, formation of copper complexes dominates [177]. Copper complexes of chlorophyll derivatives have been identified in bright-green table olives by HPLC-MS [6]. These complexes are responsible for blue-green color defects on the surfaces of olives, an effect known as "green staining."

The pH is also a factor in the rate of complex formation. Increasing the pH of spinach purée from 4.0 to 8.5 results in an 11-fold increase in the amount of zinc pyropheophytin *a* formed during heating for 60 min at 121°C. A decrease in the rate of complex formation occurs when the pH is raised to 10, presumably because of precipitation of Zn^{2+} [123].

These metallocomplexes are of interest because of the green color they impart. Copper complexes, due to their stability under most food processing conditions, are used as colorants in the EU. However, the addition of copper during food processing is not approved in the United States. A process that improves the green color of canned vegetables based on the formation of zinc metallocomplexes was introduced into the United States in 1990 and is discussed in a later section (10.2.2.5.4).

10.2.2.3.4 Allomerization

Chlorophylls can oxidize when dissolved in alcohol or other solvents and exposed to air, a process referred to as allomerization. This process is associated with the uptake of oxygen equimolar to the chlorophylls present and oxidation of ring E (Figure 10.6) at the C-10 position [188]. The primary products of allomerization have been identified as 10-hydroxychlorophylls, 10-methoxychlorophylls, and 10-methoxylactones (Figure 10.11) [122,175].

10.2.2.3.5 Photodegradation

Chlorophylls are protected from destruction by light during photosynthesis in healthy plant cells by surrounding carotenoids and other lipids. Chlorophylls can act as sensitizers and generate singlet oxygen while carotenoids are known to quench reactive oxygen species and protect the plant from photodegradation. Once this protection is lost during plant senescence, pigment extraction from the tissue, or cell damage caused during processing, chlorophylls are susceptible to photodegradation [130,131]. When these conditions prevail and light and oxygen are present, chlorophylls are irreversibly bleached.

Many researchers have tried to identify colorless photodegradation products of chlorophylls. Methyl ethyl maleimide has been identified by Jen and Mackinney [106]. In a study by Llewellyn et al. [130,131], glycerol was found to be the major breakdown product, with lactic, citric, succinic, and malonic acids and alanine occurring in lesser amounts. The reacted pigments were completely bleached.

FIGURE 10.11 Structures of 10-hydroxychlorophyll *a* (R=OH) and 10-methoxychlorophyll *a* (R=OCH_3) (a) and 10-methoxylactone of chlorophyll *a* (b).

It is believed that photodegradation of chlorophylls results in the opening of the tetrapyrrole ring and fragmentation into smaller molecular weight compounds. It has been suggested that photodegradation begins with ring opening at one of the methine bridges to form oxidized linear tetrapyrroles [206]. Singlet oxygen and hydroxyl radicals are known to be produced during exposure of chlorophylls or similar porphyrins to light in the presence of oxygen [65]. Once formed, singlet oxygen or hydroxyl radicals will react further with tetrapyrroles to form peroxides and additional free radicals, eventually leading to destruction of the porphyrins and total loss of color.

10.2.2.4 Color Loss during Thermal Processing

Loss of green color in thermally processed vegetables results from degradation of chlorophylls and the subsequent formation of pheophytins and pyropheophytins. Commercial heat sterilization can reduce chlorophyll content by as much as 80%–100% [181,183]. Evidence that a small amount of pheophytin is formed during blanching before commercial sterilization is provided in Figure 10.9. The greater amount of pheophytin detected in frozen spinach as compared to spinach blanched for canning can most likely be attributed to the greater severity of the blanch treatment that is generally applied to vegetables intended for freezing. One of the major reasons for blanching of spinach prior to canning is to wilt the tissue and facilitate packaging, whereas blanching prior to freezing must be sufficient not only to wilt the tissue, but also to inactivate enzymes. The pigment composition for the canned sample indicates that the total conversion of chlorophylls to pheophytins and pyropheophytins has occurred (Table 10.6).

Degradation of chlorophylls within processed plant tissues is initiated by heat-induced decompartmentalization of cellular acids as well as the synthesis of new acids [89]. In vegetables several acids have been identified, including oxalic, malic, citric, acetic, succinic, and pyrrolidone carboxylic acid (PCA). Thermal degradation of glutamine to form PCA is believed to be the major cause of the increase in acidity of vegetables during heating [41]. The pH decrease occurring during thermal processing of spinach purée is shown in Table 10.6. Other weak acids released during thermal processing include: fatty acids formed by lipid hydrolysis, dissolved hydrogen sulfide liberated from proteins or amino acids, and dissolved carbon dioxide from browning reactions.

10.2.2.5 Technology of Color Preservation

Efforts to preserve green color in canned vegetables have concentrated on retaining chlorophyll or creating a more acceptable green color through the formation of metallocomplexes.

10.2.2.5.1 Acid Neutralization to Retain Chlorophyll

The addition of alkalizing agents to canned green vegetables can result in improved retention of chlorophylls during processing by preventing acid-induced degradation. Techniques have included the addition of calcium oxide and sodium dihydrogen phosphate in blanch water to maintain or to raise the pH to 7.0. Magnesium carbonate or sodium carbonate in combination with sodium phosphate has been tested for this purpose. However, all of these treatments result in softening of the tissue and an *alkaline* flavor.

In 1940, James Blair recognized the toughening effect of calcium and magnesium when added to vegetables. This observation led to the use of calcium or magnesium hydroxide for the purpose of raising pH and maintaining texture, part of a treatment known as the "Blair process" [17]. Commercial application of this process has not been successful because of the inability of the alkalizing agents to effectively neutralize interior tissue acids over a long period of time, resulting in substantial color loss after less than 2 months of storage.

Another technique to retain chlorophyll involves coating the can interior with ethyl cellulose and 5% magnesium hydroxide. It was claimed that slow leaching of magnesium oxide from the lining would maintain the pH at or near 8.0 for a longer time and would therefore help stabilize the green color [137,138]. These efforts were only partially successful because increasing the pH of canned vegetables can also cause hydrolysis of amides, such as glutamine or asparagine, resulting

in formation of undesirable ammonia-like odors. In addition, fatty acids formed by lipid hydrolysis during high-pH blanching may oxidize to form rancid off-flavors. In peas, an elevated pH (8.0 or above) can cause formation of struvite, glass-like crystals of magnesium ammonium phosphate. Struvite is believed to result from the reaction of magnesium with ammonium generated from the protein in peas during heating [77].

10.2.2.5.2 Novel Processing Techniques

The effects of novel food processing techniques on the degradation of chlorophylls have been investigated. High-temperature short-time (HTST) processing has been shown to be effective in preserving color in spinach puree because the relative proportion of *C. botulinum* spores inactivated to amount of chlorophyll degraded increases with increasing process temperature [82,168]. Other studies on vegetables have combined HTST processing with pH adjustment. Samples treated in this manner were initially greener and contained more chlorophyll than control samples (typical processing and pH). However, the improvement in color following HTST treatment was generally lost during storage [28,87]. High-pressure processing has also been shown to retain vitamins, flavor, and color better than conventional food processing techniques. Chlorophylls *a* and *b* were found to be relatively stable under high pressure (800 MPa) in broccoli juice at temperatures below 50°C [133].

10.2.2.5.3 Commercial Application of Metallocomplexes

Current efforts to improve the color of green processed vegetables and to prepare chlorophylls that might be used as food colorants have involved the use of either zinc or copper complexes of chlorophyll derivatives. Copper complexes of pheophytin and pheophorbide are available commercially under the names copper chlorophyll and copper chlorophyllin, respectively. These chlorophyll derivatives have a limited approved use in foods in the United States. Their use in canned foods, soups, candy, and dairy products is permitted in most European countries under regulatory control of the EU. The Food and Agriculture Organization (FAO) of the United Nations has certified their use as safe in foods, provided no more than 200 ppm of free ionizable copper is present in the additive.

Commercial production of the copper chlorophylls was described by Humphrey [97]. Chlorophyll is extracted from dried grass or alfalfa with acetone or chlorinated hydrocarbons. Sufficient water is added, depending on the moisture content of the plant material, to aid penetration of the solvent while avoiding activation of chlorophyllase. Some pheophytin forms spontaneously during extraction. Copper acetate is added to form oil-soluble copper chlorophyll. Alternatively, pheophytin can be acid hydrolyzed before copper ion is added, resulting in formation of water-soluble copper chlorophyllin. The copper complexes have greater stability than comparable magnesium complexes.

10.2.2.5.4 Regreening of Thermal-Processed Vegetables

It has been observed that when vegetable purées are commercially sterilized, small bright-green areas occasionally appear, and it was found that pigments in the bright-green areas contained zinc and copper. The formation of these bright-green areas in vegetables was termed "regreening." Regreening of commercially processed vegetables has been observed when zinc and/or copper ions are present in process solutions. When okra is processed in a brine solution containing zinc chloride, it retains its bright-green color, which is attributed to the formation of zinc complexes of chlorophyll derivatives [63,207,209].

A patent was issued to Continental Can Company (now Crown Holdings, Inc.) for the commercial canning of vegetables with metal salts in the blanch or brine solution. The process involved blanching vegetables in water containing sufficient amounts of Zn^{2+} or Cu^{2+} salts to raise the tissue concentration of the metal ions to between 100 and 200 ppm. Green vegetables processed in modified blanch water were claimed to be greener than conventionally processed vegetables. Other bi-or trivalent metal ions were either less effective or ineffective as compared to the use of copper or zinc salts [189]. This approach is known as the Veri-Green process. Pigments present in canned green beans processed by the Veri-Green process were identified as zinc pheophytin and zinc pyropheophytin [55].

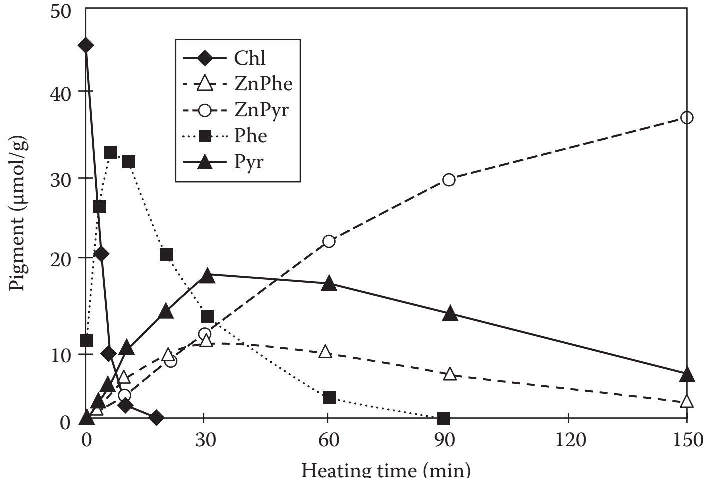


FIGURE 10.12 Transformation of pigments in pea purée containing 300 ppm of Zn^{2+} after heating at 121°C for up to 150 min. Chl, chlorophyll; ZnPhe, zinc pheophytin; ZnPyr, zinc pyropheophytin; Phe, pheophytin; Pyr, pyropheophytin. (From von Elbe, J.H. and Laborde, L.F., Chemistry of color improvement in thermally processed green vegetables, ACS Symposium Series 405, in: Jen, J.J., ed., *Quality Factors of Fruits and Vegetables*, American Chemical Society, Washington, DC, 1989, pp. 12–28.)

Presently, commercial production of zinc-processed green beans exists, but application of this process to other vegetables has had mixed results. Shown in Figure 10.12 is the sequence of pigment changes occurring when pea purée is heated in the presence of 300 ppm Zn^{2+}. Chlorophyll *a* decreases to trace levels after only 20 min of heating. Accompanying this rapid decrease in chlorophyll is the formation of zinc complexes of pheophytin *a* and pyropheophytin *a*. Further heating increases the zinc pyropheophytin concentration at the expense of a decrease in zinc pheophytin (Figure 10.12). Zinc pyropheophytin may form through decarboxymethylation of zinc pheophytin or by reaction of pyropheophytin with Zn^{2+} (Figure 10.13). These results suggest that the green color in vegetables processed in the presence of zinc is largely due to the presence of zinc pyropheophytin.

Formation of zinc complexes occurs most rapidly between pH 4.0 and 6.0, and the rate decreases markedly at pH 8.0. The reason for the decrease is that Mg^{2+} within chlorophyll is retained at the high pH, thereby limiting the amount of chlorophyll derivatives available for metallocomplex

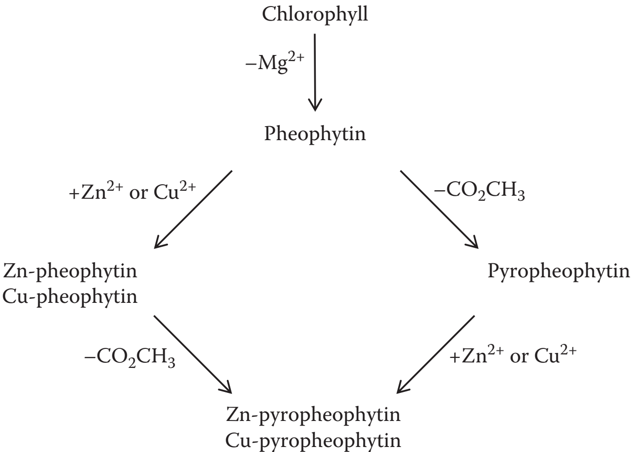


FIGURE 10.13 Chemical reactions occurring in heated green vegetables containing zinc or copper.

formation [124,125]. It has further been shown that zinc complex formation can be influenced by the presence of surface-active anionic compounds. Such compounds adsorb onto the chloroplast membranes, increasing the negative surface charge, thereby increasing complex formation [124,125].

Currently, the best process for attaining a desirable green color in canned vegetables involves adding zinc to the blanch solution, increasing membrane permeability by heating the tissue prior to blanching at or slightly above 60°C, adjusting to a pH that favors formation of metallocomplexes, and using anions to alter surface charge at the chloroplast membrane.

10.2.3 Carotenoids

Carotenoids are nature's most widespread pigments. They provide the characteristic yellow, orange, and red colors of many fruits, vegetables, and plant life; however, when bound to proteins, they can elicit green, blue, and purple colors as well [22]. A large majority of these pigments are biosynthesized by the ocean algae population. Carotenoids were first discovered in the early nineteenth century and found to be both heat sensitive and lipophilic [57]. Over 700 carotenoids have been identified in nature, while only 60 or so exist in foods consumed by humans [235]. Carotenoids exist in all photosynthetic organisms and can be additionally produced by some bacteria, yeasts, and fungi [120]. In higher plants, carotenoids in chloroplasts are often masked by the more dominant chlorophyll pigments. In the autumn season when chloroplasts decompose during plant senescence, the yellow-orange color of carotenoids becomes evident [15].

Carotenoids play important functions in photosynthesis and photoprotection in plant tissues [82]. In all chlorophyll-containing tissues, carotenoids function as secondary pigments in harvesting energy from light via photosynthesis. The photoprotective role of carotenoids stems from their ability to quench reactive oxygen species (particularly singlet oxygen) formed by exposure to light and air. In addition, specific carotenoids present in roots and leaves serve as precursors to abscisic acid, a plant hormone that functions as a chemical messenger and crucial growth regulator [48,158]. Carotenoids also signal immunocompetence in birds, influencing mate selection [197] and affecting attraction of pollinators [113].

The most prominent role of carotenoid pigments in the diet of humans and other animals is their ability to serve as precursors of vitamin A. Although the carotenoid β-carotene possesses the greatest provitamin A activity because of its ability to form two molecules of retinol, other commonly consumed carotenoids, such as α-carotene and β-cryptoxanthin, also possess provitamin A activity. Provitamin A carotenoids present in fruits and vegetables are estimated to provide 30%–100% of the vitamin A requirement in human populations [15,37]. A prerequisite to vitamin A activity is the existence of an unsubstituted β-ionone ring in the carotenoid. Thus, only a few carotenoids possess vitamin activity. This topic is covered thoroughly in Chapter 8.

In 1981, Peto et al. [165] drew attention to these pigments because of the epidemiological findings that consumption of fruits and vegetables high in carotenoids was associated with a decreased incidence of specific cancers in humans. More recently, interest has focused on the presence of carotenoids in the diet and on their physiological significance. These findings have stimulated a substantial increase in carotenoid research. An overview on the impact of carotenoids on health and disease can be found elsewhere [43a,120,236].

10.2.3.1 Structures of Carotenoids

Carotenoids are comprised of two classes: the hydrocarbon carotenes and the oxygenated xanthophylls (Figure 10.14). Xanthophylls consist of a variety of derivatives frequently containing hydroxyl, epoxy, aldehyde, carboxylic acid, and keto groups. In addition, fatty acid esters of hydroxylated carotenoids are also widely found in nature. To date, over 700 carotenoid structures have been identified and compiled [235]. Furthermore, when the *cis* (*Z*) and *trans* (*E*) geometric isomers or *R* and *S* enantiomers are considered, many more configurations are possible. An exhaustive listing of carotenoids and their structures (in addition to UV/visible spectra, MS, NMR and other characterizing data) can be found in the *Carotenoids Handbook* [235].

β-ionone ring

β-carotene ($C_{40}H_{56}$)

β-ionone ring

lutein ($C_{40}H_{56}O_2$)

β-ionone ring

α-carotene ($C_{40}H_{56}$)

zeaxanthin ($C_{40}H_{56}O_2$)

β-cryptoxanthin ($C_{40}H_{56}O$)

lycopene ($C_{40}H_{56}$)

astaxanthin ($C_{40}H_{52}O_2$)

violaxanthin ($C_{40}H_{56}O_4$)

capsanthin ($C_{40}H_{56}O_3$)

bixin ($C_{25}H_{30}O_4$)

canthaxanthin ($C_{40}H_{52}O_2$)

β-apo-8′-carotenal ($C_{30}H_{40}O$)

FIGURE 10.14 Structures and formulas of carotenoids and apocarotenoids commonly acting as colorants in food and feed.

$CH_2{=}C(CH_3){-}CH{=}CH_2$

Isoprene

Head to tail

Tail to tail

Lycopene

FIGURE 10.15 Joining of eight isoprenoid units to form lycopene. (From Fraser, P.D. and Bramley, P.M., *Prog. Lipid Res.*, 43, 228, 2004.)

Carotenoids are biosynthesized in plants via a mevalonic acid–independent pathway called the methylerythritol 4-phosphate pathway [51]. Complete reviews on carotenoid biosynthesis can be found elsewhere [49,73]. The basic carotenoid structural backbone consists of 8 isoprene units linked covalently in either a head-to-tail or a tail-to-tail fashion to create a symmetrical molecule (Figure 10.15). Carotenoids are derived from this primary structure of 40 carbons. Some structures contain cyclic end groups (e.g., β-carotene, Figure 10.14) while others possess either one or no cyclized groups (e.g., lycopene, the prominent red pigment in tomatoes). Other compounds may have skeletons shorter than 40 carbons and are known as apocarotenoids (e.g., bixin, apo-8′-carotenal). Although rules exist for naming and numbering all carotenoids [101,102], the trivial names are commonly used and presented in this chapter.

The most widespread carotenoid found in plant tissues consumed by humans is β-carotene. This carotenoid is also used widely as a colorant in foods. Both the naturally derived and synthetic forms can be added to food products. Some carotenoids found in plants, or carotenoids commonly used as colorants in either foods or feed, are shown in Figure 10.14. This list includes β-carotene (ubiquitous in plants), α-carotene (carrots), β-cryptoxanthin (tangerines, papaya), astaxanthin (salmon, shrimp), capsanthin (red peppers, paprika), canthaxanthin (eggs from hens fed canthaxanthin-supplemented diets), lutein (corn, leafy greens, marigolds), zeaxanthin (goji berries), lycopene (tomatoes), violaxanthin (leafy greens), bixin (annatto seed), and β-apo-8′-carotenal (used as a color additive). Each item in parenthesis is an example of a major source of the carotenoid although these pigments can be found elsewhere as well.

Recently, it has been found that pea aphids have developed the ability to synthesize carotenoid pigments through genes acquired from fungi associated with these insects, the first animals shown to do so [150]. However, animals are generally unable to synthesize carotenoids and therefore derive these pigments by consumption of carotenoid-containing plant materials. For example, the pink color of salmon flesh is due mainly to the presence of astaxanthin, which is obtained by ingestion of carotenoid-containing marine plants. It is also well known that some carotenoids in both plants and animals are bound to or associated with proteins. The red astaxanthin pigment of shrimp and lobster exoskeletons is blue in color when complexed with proteins. Heating denatures crustacyanin (the astaxanthin protein complex),

releasing the carotenoid, and altering the spectroscopic and visual properties of the pigment, causing a hypsochromic shift from blue to red [39]. Other examples of carotenoid–chlorophyll–protein complexes are ovoverdin [231], the green pigment found in lobster eggs, and the carotenoid–chlorophyll–protein complexes in plant chloroplasts [85]. Other unique structures include carotenoid glycosides, some of which are found in bacteria and other microorganisms. Carotenoids can also exist as carotenoid glycosides in plants. A notable example is crocin, the glycoside of crocetin found in the stamens of *Crocus sativus* flowers, which provides the orange-yellow color to saffron [166].

10.2.3.2 Occurrence and Distribution

Edible plant tissues contain a wide variety of carotenoids [86]. Many red, orange, and yellow fruits, root crops, and vegetables are rich in carotenoids. All green leafy vegetables (and other nonedible green leaves) contain carotenoids, but their color is masked by the green chlorophylls. Generally, the highest concentrations of carotenoids exist in those tissues with the greatest amount of chlorophyll pigments. For example, spinach and kale are rich in carotenoids, and peas, green beans, and asparagus also contain significant concentrations. Table 10.8 provides data on the carotenoid content of selected foods in a western diet as reported in the USDA Nutrient Database. Many factors influence the carotenoid content of plants. In some fruits, ripening may bring about dramatic changes in carotenoids. For example, in tomatoes, the carotenoid content, especially lycopene, increases significantly during the ripening process. Thus, carotenoid concentrations differ wildly depending on the stage of plant maturity. Even after harvest, tomato carotenoids continue to be synthesized. Since light stimulates biosynthesis of carotenoids, the extent of light exposure is known to affect their concentration. Other factors that alter carotenoid occurrence or concentration include growing climate, pesticide and fertilizer use, and soil type [86].

TABLE 10.8
Carotenoid Content in Commonly Consumed Foods

Food	Weight (g)	β-Carotene	α-Carotene	β-Cryptoxanthin	Lycopene	Lutein + Zeaxanthin
Broccoli, raw	91	0.33	0.02	0	0	1.28
Cantaloupe, raw	177	3.58	0.03	0.03	0	0.05
Carrots, raw	128	10.61	4.45	0	0	0.33
Egg, hard-boiled	136	0.02	0	0.01	0	0.48
Grapefruit, pink	230	1.58	0.01	0.01	3.26	0.012
Kale, cooked	130	10.62	0	0	0	23.72
Mandarin oranges, canned	189	0.56	0.39	1.47	0	0.46
Peas, frozen	134	1.64	0.09	0	0	3.15
Pumpkin, canned	245	17.00	11.75	0	0	0
Red (sweet) peppers, raw	149	2.42	0.03	0.73	0	0.08
Spinach, raw	30	1.69	0	0	0	3.66
Squash, summer (yellow and green)	180	1.21	0	0	0	2.07
Squash, winter	205	5.73	1.40	0	0	2.90
Sweet potato, boiled	328	30.98	0	0	0	0
Tomato sauce[a]	132	0.52	0	0	16.72	0.25
Tomatoes, raw	149	0.67	0.15	0	3.83	0.18

Note: Content is given in mg carotenoid/serving, where 1 serving = 1 cup[a] and 1 cup measures in grams are given. Carotenoid data are selected from USDA Standard Reference 26.

[a] Tomato sauce serving = 0.5 cup.

10.2.3.3 Physical Properties, Extraction, and Analysis

All classes of carotenoids (both the hydrocarbon carotenes and the oxygenated xanthophylls) are lipophilic compounds and soluble in oils and organic solvents. They are moderately heat stable within a food matrix and are subject to loss of color by oxidation. Carotenoids can be easily isomerized by heat, acid, or light. Since they range in color from yellow to red, detection wavelengths for monitoring carotenoids typically range from approximately 400 to 480 nm. The higher wavelengths are usually used for some xanthophylls to prevent interference from chlorophylls in spectrophotometric methods. Many carotenoids exhibit spectral shifts after reaction with various reagents, and these spectral changes are useful to assist in identification.

The complex nature and diversity of carotenoid compounds present in plant foods necessitate chromatographic separation for accurate identification and quantitation [117]. Extraction procedures for quantitative removal of carotenoids from tissue utilize organic solvents that must penetrate a hydrophilic matrix. Mixtures of hexane and acetone are commonly employed for this purpose, but special solvents and treatments are sometimes needed to achieve satisfactory extraction, depending on the polarity of the carotenoid of interest [115,117].

Many chromatographic procedures, including HPLC, have been developed for separating carotenoids [44,56,117]. Special analytical challenges occur when carotenoid esters, *cis/trans* isomers, and optical isomers need to be separated and identified [117].

Carotenoids exist in red, orange, and yellow fruits in chromoplasts while existing in chloroplasts in green plant tissue [185,187]. Carotenoids can occur in several forms in fresh plant foods, including in carotenoid–protein complexes in chloroplasts, crystalline form inside chromoplasts, or lipid-dissolved droplets called plastoglobuli [217]. Crystalline structures are difficult to solubilize, while carotenoids associated with lipids may be more bioaccessible. These lipid-dissolved carotenoids may be more easily removed from the food matrix and thus, theoretically, more available for absorption by the enterocyte [22,23,186]. The physical state of carotenoids in chromoplasts accounts for the relatively low bioavailability in raw, green leafy vegetables like spinach; this is a function of carotenoids being tightly bound to protein complexes within plant cells [24,25]. Lycopene in red tomatoes is stored as crystals [90]. Conversely, lycopene from the unique, *cis*-lycopene-containing *tangerine* tomato, stores carotenoids as lipid-dissolved droplets. This difference in structure is thought to be responsible for the marked increase in lycopene bioavailability from *tangerine* tomatoes compared to red tomatoes [43]. Consequently it is also hypothesized to be the reason why lycopene from *tangerine* tomatoes is more susceptible to degradation and isomerization from thermal processing [43b]. The physical state of carotenoids in plants varies widely within the same plant species and even within different parts of the same plant [198], imparting large heterogeneity in carotenoid storage within plants [34]. It is important to keep in mind that the physical state in which carotenoids are stored in a plant can greatly influence carotenoid bioavailability *in vivo* [165].

10.2.3.4 Chemical Properties

10.2.3.4.1 Oxidation

Carotenoids are easily oxidized because of their large number of conjugated double bonds. Such reactions can cause color loss of carotenoids in foods and are a major degradation pathway. The susceptibility of a particular pigment to oxidation is highly dependent on its environment. Within plant tissues, pigments are often compartmentalized separately from degradative enzymes and therefore maintain some protection from oxidation. However, physical damage to the tissue or extraction of the carotenoids increases their susceptibility to oxidation. Storage of carotenoid pigments in organic solvents will often accelerate decomposition. Because of the highly conjugated, unsaturated structure of carotenoids, the products of their degradation are very complex. The characterization of these products in both foods and in human and animal blood and tissues represents an active area of research [64,114,118,219]. During oxidation, epoxides and carbonyl compounds are initially formed. Further

oxidation results in formation of short-chain mono- and dioxygenated compounds including epoxy-β-ionone. Generally, epoxides form within terminal rings, while oxidative scission can occur at a variety of sites along the chain. For provitamin A carotenoids, epoxide formation in the ring results in loss of the provitamin activity. Extensive autoxidation will result in bleaching of the carotenoid pigments and loss in color. Oxidative destruction of β-carotene is intensified in the presence of sulfite and metal ions [162].

Enzymatic activity, particularly lipoxygenase, hastens oxidative degradation of carotenoid pigments. This occurs by indirect mechanisms. Lipoxygenase first catalyzes oxidation of unsaturated or polyunsaturated fatty acids to produce peroxides, and these in turn readily react with carotenoid pigments. In fact, this coupled reaction scheme is quite efficient: the loss of carotene color and decreased absorbance in solution are often used as indicators of lipoxygenase activity [11].

10.2.3.4.2 Antioxidant Activity

Because carotenoids can be readily oxidized, it is not surprising that they have antioxidant properties. In addition to cellular and *in vitro* protection against singlet oxygen, carotenoids, at low oxygen partial pressures, inhibit lipid peroxidation [30]. At high oxygen partial pressures, β-carotene has prooxidant properties [31]. In the presence of molecular oxygen, photosensitizers (e.g., chlorophyll), and light, singlet oxygen may be produced, which is a highly reactive oxygen species. Carotenoids are known to quench singlet oxygen and thereby protect against cellular oxidative damage. Not all carotenoids are equally effective as photochemical protectors. For example, lycopene is known to be especially efficient in quenching singlet oxygen relative to other carotenoid pigments [142,194].

It has been proposed that the antioxidant functions of carotenoids play a role in reducing the risk of cancer, cataracts, atherosclerosis, and the processes of aging although this has not been definitively shown [35]. A detailed overview of the antioxidant role of carotenoid compounds is beyond the scope of this discussion, and the reader is referred to several excellent reviews [31,119,157,230].

10.2.3.4.3 Cis/Trans Isomerization

In general, the conjugated double bonds of carotenoids exist in the all-*trans* configuration. The *cis* isomers of a few carotenoids can be found naturally in few plant tissues although they generally have great consequence. The alga *Dunaliella salina* accumulates high concentration of *cis*-β-carotene and these preparations are often used in supplements. However, 9-*cis*-β-carotene is poorly converted to vitamin A when compared to all-*trans*-β-carotene and does not accumulate in plasma to any great extent [76,152,232]. This suggests that *cis*-β-carotene may be a less desirable form in which to administer this nutrient. Conversely, the *tangerine* tomato, an orange-colored tomato that lacks a functional copy of the enzyme carotenoid isomerase and is therefore unable to produce all-*trans*-lycopene and instead accumulates tetra-*cis*-lycopene (i.e. prolycopene). Lycopene from *tangerine* tomatoes have been shown to be better absorbed than lycopene from red tomatoes [43], in part because *cis*-lycopene isomers have been shown to be more bioavailable [216].

Isomerization reactions can be induced by thermal treatments, exposure to organic solvents, treatment with acids, ozonolysis, and illumination of solutions (particularly with iodine present). Iodine-catalyzed isomerization is a useful means in the study of photoisomerization because an equilibrium mixture of isomeric configurations is formed [74]. Theoretically, large numbers of possible geometrical configurations could result from isomerization because of the extensive number of double bonds present in carotenoids. For example, lycopene has potentially 1056 different *cis* forms as of a function of its 11 symmetrical double bonds. However, because of steric constraints, only a limited number of *cis* isomers occur in reality [233]. Due to the complexity of various *cis*/*trans* isomers within a single carotenoid, significant efforts have been made to develop accurate methods to identify and quantify these compounds in foods [117]. Isomerization from *trans* to *cis* can affect the provitamin A activity but not the color of carotenoids. The provitamin A activity of β-carotene *cis* isomers ranges, depending on the isomeric form, from 13% to 53% as compared to that of all-*trans*-β-carotene [234].

10.2.3.5 Stability during Processing

Carotenoids are relatively stable during typical storage and handling of most fruits and vegetables. Freezing causes little change in carotene content. However, blanching is known to influence the level of carotenoids. Often, blanched plant products exhibit an apparent increase in carotenoid content relative to raw tissues. Mild heat treatments traditionally used during blanching may disrupt cell structure and thus enhance the efficiency of extraction of the pigments relative to fresh tissue. Severe physical homogenization and thermal treatments also increase extraction [149] and bioavailability when consumed [114,143]. Lye peeling, which is commonly used for sweet potatoes and tomatoes, causes little destruction or isomerization of carotenoids. Additionally, inactivation of lipoxygenase, can also prevent oxidative decomposition of carotenoids.

Although carotenes were historically regarded as fairly stable during heating, it is now known that heat sterilization can induce *cis/trans* isomerization reactions. To lessen excessive isomerization, the severity of thermal treatments should be minimized when possible. In the case of extrusion cooking and high-temperature heating in oils, not only will carotenoids isomerize but thermal degradation will also occur. Very high temperatures can yield fragmentation products that are volatile. Products arising from severe heating of β-carotene in the presence of air are similar to those arising from β-carotene oxidation. In contrast, air dehydration exposes carotenoids to oxygen, which can cause extensive degradation of carotenoids. Dehydrated products that have large surface-to-mass ratios, such as carrot or sweet potato flakes, are especially susceptible to oxidative decomposition during drying, exposure to light, and storage in air.

When *cis* isomers are created, only slight hypsochromic shifts of 3–5 nm occur and thus color of the product is mostly unaffected; however, a decrease in provitamin A activity can occur. These reactions have important nutritional effects that should be considered when selecting analytical measurements for provitamin A. The older and AOAC methods for vitamin A determination in foods do not account for the differences in the provitamin A activity of individual carotenoids or their isomeric forms [237,238]. Therefore, older nutritional data for foods are in error, especially for foods that contain high proportions of provitamin A carotenoids other than β-carotene and those that contain a significant amount of *cis* isomers. Additional information about the provitamin A activity of carotenoids can be found in Chapter 8.

10.2.4 Anthocyanins and Other Phenols

10.2.4.1 Anthocyanins

Phenolic compounds comprise a large group of organic substances, and flavonoids are an important subgroup. The flavonoid subgroup contains the anthocyanins, one of the most broadly distributed pigment groups in the plant world. Anthocyanins are responsible for a wide range of colors in plants, including blue, purple, violet, magenta, red, and orange. The word anthocyanin is derived from two Greek words: "anthos," flower, and "kyanos," blue. These compounds have attracted the attention of chemists and botanists for over a century. However, interest on anthocyanins has greatly increased over the last few decades because of their potential health benefits and their potential use as food colorants [91].

10.2.4.1.1 Structure

Anthocyanins belong to the flavonoid group because of their characteristic $C_6C_3C_6$ carbon skeleton. The basic chemical structure of flavonoids and their relationship to anthocyanins are shown in Figure 10.16. Within each group, there are many different compounds with their color depending on the presence and number of substituents attached to the molecule.

The base structure of anthocyanins are polyhydroxy and/or polymethoxy derivatives 2-phenylbenzopyrylium of flavylium salt (Figure 10.17). Anthocyanins differ in the number of hydroxyl

Basic $C_6C_3C_6$ structure

Chalcone

Flavanones

Flavones

Flavanonols

Flavanols

Isoflavones

Flavan-3-ols

Anthocyanidins

Flavan-3,4-diols

Aurones

FIGURE 10.16 Carbon skeleton of some important flavonoids, classified by their C-3 chain structure.

and/or methoxy groups present; the types, numbers, and sites of attachment of sugars to the molecule; and the types and numbers of aliphatic or aromatic acids that are attached to the sugars in the molecule [79]. With this structural diversity, it is not surprising that more than 700 different anthocyanins have been identified in the plant world [5].

An anthocyanin free of sugar substitutions is known as an anthocyanidin (the aglycone portion). There are 27 different naturally occurring anthocyanidins that share the same $C_6C_3C_6$ skeleton [5] but close to 90% are derived from six aglycones, that is, cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin, and occur commonly in foods (Figure 10.18).

FIGURE 10.17 The flavylium cation. R_1 and R_2 = –H, –OH, or –OCH_3, R_3 = –glycosyl, R_4 = –H or –glycosyl.

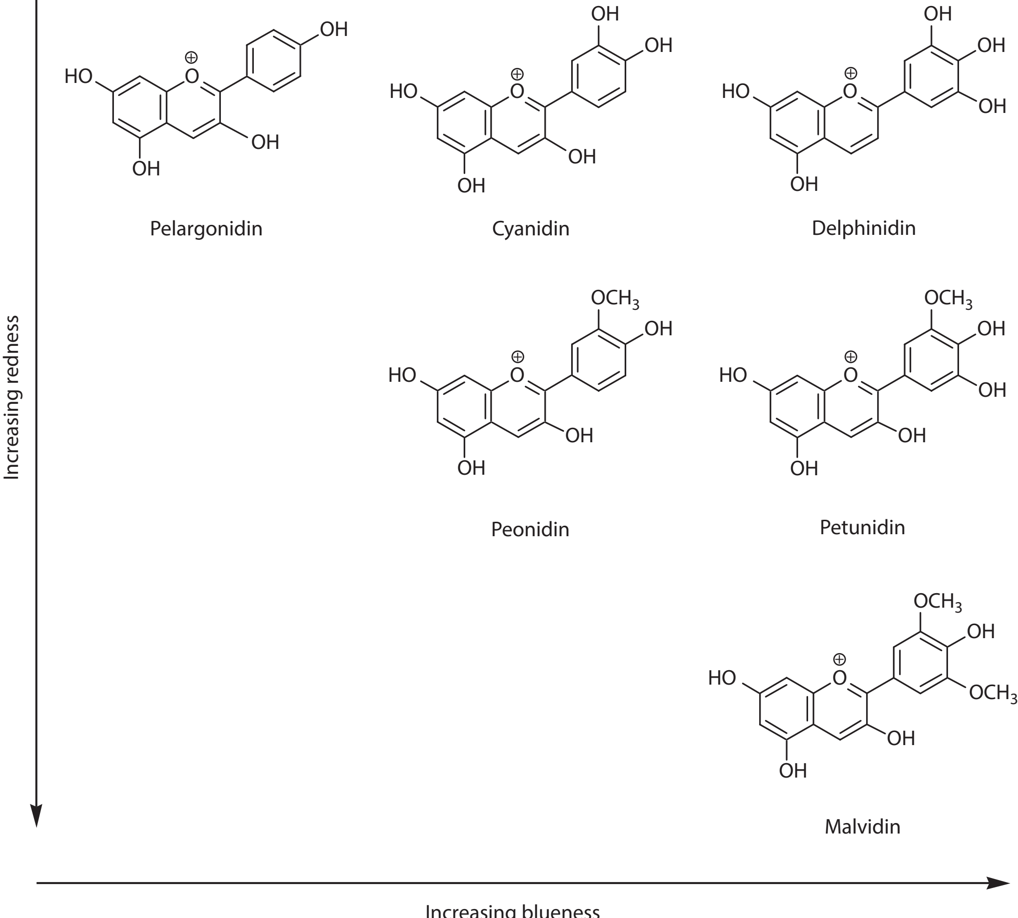


FIGURE 10.18 The most common anthocyanidins in foods, arranged in increasing redness and blueness.

Anthocyanidins are less water soluble than their corresponding glycosides (anthocyanins) and tend to be highly unstable. The free 3-hydroxyl group in the anthocyanidin molecule destabilizes the chromophore; therefore, anthocyanins are almost always glycosylated. Only 3-deoxyanthocyanidins, which are yellow, are found as aglycones in nature. Therefore, when only one sugar is present, this sugar is typically *O*-glycosylated on position C-3, and diglycosylated anthocyanins are typically glycosylated at positions C-3 and C-5. Additional glycosylation can also occur at C-7, -3′, -4′, and/or -5′ hydroxyl group (Figure 10.17).

Steric hindrance precludes glycosylation at both C-3′ and C-4′ [23]. However, on rare cases anthocyanin *C*-glycosylations have also been reported [173].

The most common sugar substitutions are glucose, followed by galactose, rhamnose, arabinose, xylose, and homogenous or heterogeneous di- and trisaccharides formed as glycosides of these sugars.

More than 65% of all anthocyanins identified from plants are acylated [5]. Acids most commonly involved in anthocyanin acylation are aromatic acids including *p*-coumaric, caffeic, ferulic, sinapic, gallic, *p*-hydroxybenzoic acids and/or aliphatic acids such as malonic, acetic, malic, succinic, or oxalic acids. These acyl substituents are commonly bound to the C-3 sugar, esterified to the 6-OH or less frequently to the 4-OH group of the sugars [91]. However, anthocyanins containing rather complicated acylation patterns attached on different sugar moieties have been reported [192,211,226].

10.2.4.1.2 Color and Stability of Anthocyanins

The color of anthocyanins and anthocyanidins results from excitation of a molecule by visible light. The ease with which a molecule is excited depends on the relative electron mobility in the structure. Double bonds, which are abundant in anthocyanins and anthocyanidins, are excited very easily, and their presence is essential for color. It should be noted that increasing substitutions on the B-ring (top, Figure 10.16) of the molecule results in a deeper hue. The deepening of hue is the result of a bathochromic shift (i.e. the light absorption band in the visible spectrum shifts from a shorter wavelength to a longer wavelength, with a resulting change in color from orange/red to purple at acidic pH). An opposite change is referred to as a hypsochromic shift. Bathochromic effects can be caused by auxochrome groups, groups that by themselves have no chromophoric properties but cause deepening in the hue when attached to the molecule. Auxochrome groups are electron-donating groups, and in the case of anthocyanidins they are typically the hydroxyl and methoxy groups. The methoxy groups cause a greater bathochromic shift than hydroxyl groups because their electron-donating capacity is greater than that of hydroxyl groups. The effect of the number of hydroxyl and methoxy groups on color is illustrated in Figure 10.18. In anthocyanins, the type and number of sugar substitutions and acylation patterns also play an important role on the color characteristics, as well as several other factors, such as responses to change in pH, metal complex formation, and copigmentation.

Plants not only contain mixtures of anthocyanins but the relative concentrations vary among cultivars and with maturity. Total anthocyanin content varies among plants and ranges from 0 to as high as a few g/100 g. Higher concentrations of pigments typically result on deeper coloration, although the color exhibited by anthocyanins can also be greatly affected by the microenvironment where they are located.

Anthocyanin pigments are relatively unstable, with the greatest stability occurring under acidic conditions. Both the color characteristics (hue and chroma) of the pigment and its stability are greatly impacted by the different substituents on the molecule. Degradation of anthocyanins can occur not only during extraction from plant tissues but also during processing and storage of food tissues.

Knowledge of the chemistry of anthocyanins can be used to minimize degradation by proper selection of processes and by selection of anthocyanin pigments that are most suitable for the intended application. Major factors governing degradation of anthocyanins include intrinsic factors, such as their chemical structure and intramolecular copigmentation, as well as extrinsic factors such as pH, temperature, and the composition of the matrix. Matrix components that can affect anthocyanin stability include the presence of degradative enzymes, ascorbic acid, sulfur dioxide, metal ions, and sugars. In addition, protein, fats, and other compounds in the matrix may affect or appear to affect the degradation rate.

10.2.4.1.3 Anthocyanin Chemical Structure

Degradation rates vary greatly among anthocyanins because of their diverse chemical structures. Generally, increased anthocyanin hydroxylation decreases stability, while increased methoxylation

increases stability. The color of foods containing anthocyanins that are rich in pelargonidin, cyanidin, or delphinidin aglycones is usually less stable than that of foods containing anthocyanins that are rich in petunidin or malvidin aglycones. The increased stability of the latter group occurs because reactive hydroxyl groups are blocked. Glycosylation also can increase stability. An anthocyanidin aglycone is highly unstable, and addition to one sugar at position C-3 greatly increases stability as well as solubility, in part by forming an intramolecular H-bonding network within the anthocyanin molecule [18]. The role of additional glycosylating groups on anthocyanin stability is not as clear, and they may or may not increase stability, depending on the anthocyanin and the conditions of the matrix or environment [215]. It has also been shown that the type of sugar moiety influences stability. Starr and Francis [200] found that cranberry anthocyanins that contained galactose were more stable during storage than those containing arabinose. Cyanidin 3-(2 glucosylrutinoside) at pH 3.5 and at 50°C, has a half-life of 26 h compared to 16 h for cyanidin-3-rutinoside [53]. The type of sugar substitution has a critical impact on stability when the degradation is enzyme mediated, as enzymatic activity tends to be very selective. These examples illustrate that substituents have a marked effect on anthocyanin stability even if they themselves do not react.

10.2.4.1.4 Structural Transformations and pH

In an aqueous medium, including foods, anthocyanins reversibly undergo transformations among four predominant structural forms depending on pH (Figure 10.19): the blue quinonoidal base (A), the red flavylium cation (AH^+), the colorless carbinol pseudo base (B), and the colorless chalcone (C) [24]. The equilibrium distributions of these four forms in the pH range 0–6 for malvidin-3-glucoside, dihydroxyflavylium chloride, and 4 methoxy-4-methyl-7-hydroxyflavylium chloride (Figure 10.19, panels II, III, IV, respectively). For each pigment, only two of the four species are important over this pH range. In a solution of malvidin-3-glucoside at low pH the flavylium structure dominates. As the pH increases from 3 to 6, rapid hydration of the flavylium cation occurs at the C-2 position to generate the colorless carbinol pseudobase. A similar situation exists with 4′,7-hydroxyflavylium except the equilibrium mixture consists mainly of the flavylium and the chalcone structure. Thus, as the pH approaches 6 the solution becomes colorless. Further pH increases will favor formation of the quinonoidal bases and many anthocyanins will exhibit blue colorations [2]. As the pH increases above 8, the quinonoidal base can be ionized to carry one or two negative charges [7].

Interestingly, at the same pH levels and under similar conditions, an anthocyanin 3,5-di-glucoside tends to have less proportion of the cation form than the corresponding 3-mono-glu, whereas acylated anthocyanins will exhibit larger proportions of flavylium cations, particularly at pH levels above 4 [45]. This is one of the reasons why acylated anthocyanins seem to be better candidates as food colorants for a larger number of applications, since they may retain color better at a larger pH range [79].

To further demonstrate the effect of pH on the color of anthocyanins, the spectra for acylated and nonacylated cyanidin-3-diglucoside-5-glucoside in buffer solutions at pH levels between 1 and 8 are shown in Figure 10.20. Between pH 1 and 6, the wavelength of absorption maximum shows little change; however, the intensity of absorption decreases drastically with increasing pH. Once the pH is raised again from 6 to 8, the color intensity increases again, which is shown by the bathochromic and hyperchromic shifts observed. Interestingly, the acylated anthocyanins show the same general behavior as its nonacylated counterpart; however, it does not seem to completely lose its color at any pH. Color changes in a mixture of chokeberry anthocyanins as a function of pH are illustrated in Figure 10.21. In buffer solutions, as in juices or cocktails, changes in pH can cause major changes in color. Anthocyanins show their greatest tinctorial strength at pH below 3, when the pigment molecules are mostly in the ionized form. At pH 4.5 anthocyanins in fruit juices are nearly colorless, particularly the nonacylated anthocyanins, and as the pH is further increased, the bluish quinonoidal forms appear.

10.2.4.1.5 Temperature

Anthocyanin stability in foods is greatly affected by temperature. Anthocyanin degradation typically follows first-order kinetics [4,171]. Rates of thermally induced degradation are also influenced

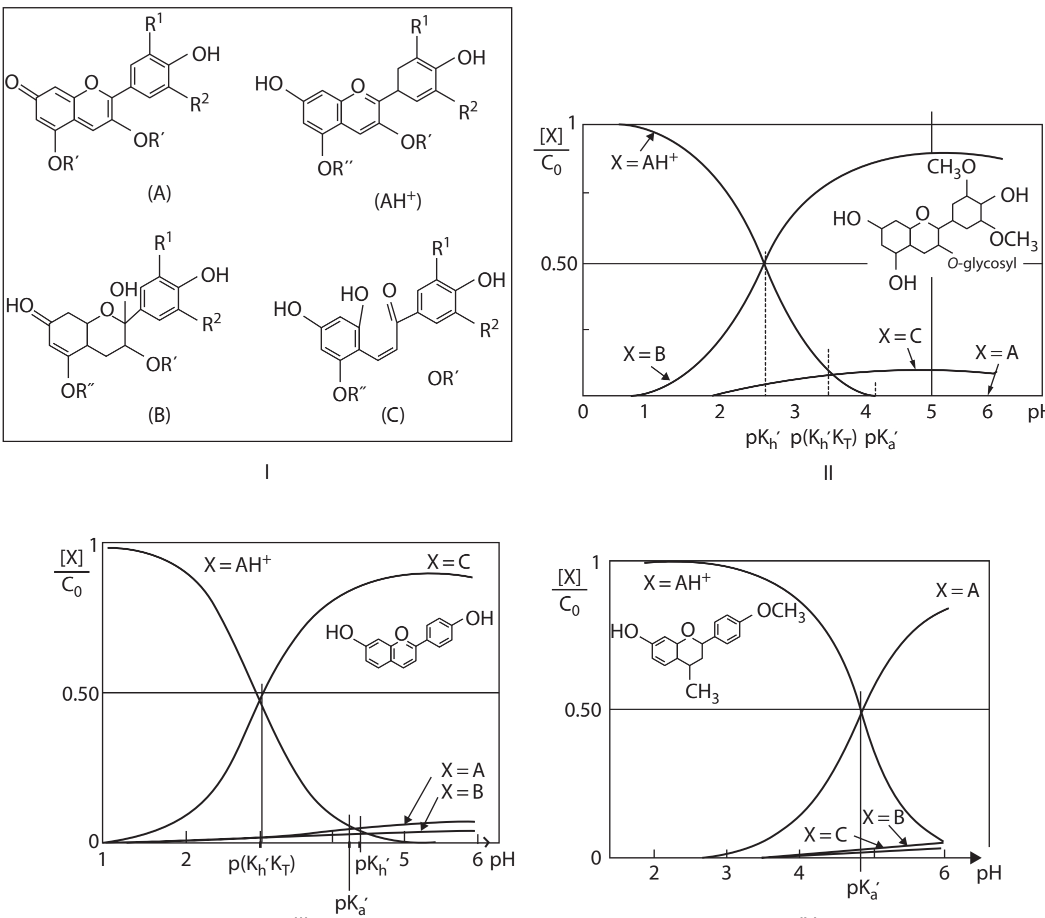


FIGURE 10.19 (I) The four anthocyanin structures present in aqueous acidic solution at room temperatures: A, quinonoidal base (blue); (AH^+) flavylium salt (red); B, pseudobase or carbinol (colorless); C, chalcone (colorless). (II–IV) Equilibrium distribution at 25°C of AH^+, A, B, and C as a function of pH: (II) for malvidin 3-glucoside; (III) for 4′,7-hydroxyflavylium chloride; and (IV) 4′-methoxyl-4-methyl-7-hydroxy flavylium chloride. (From Brouillard, R., Chemical structures of anthocyanins, in: Markakis, P., ed., *Anthocyanins as Food Colors*, Academic Press, New York, 1982, pp. 1–40.)

by the presence or absence of oxygen and other compounds in the matrix that could interact with anthocyanins and, as already pointed out, by pH and structural conformation. In general, structural features that lead to increased pH stability also lead to increased thermal stability. Highly hydroxylated anthocyanidins are less stable than methoxylated, glycosylated, or acylated anthocyanidins. For example, the half-life of 3,4′,5,5′,7-pentahydroxyflavylium at pH 2.8 is 0.5 days compared to 6 days for the 3,4′,5,5′,7-pentamethoxyflavylium [143]. Under similar conditions, the half-life for cyanidin-3-rutinoside is 65 days compared to 12 h for cyanidin [139]. It should be noted that comparisons of published data for pigment stability is difficult because of differing experimental conditions used. One of the errors in published data involves a failure to consider the equilibrium reactions among the four known anthocyanin structures (Figure 10.19).

Heating shifts the equilibria toward the chalcone form and the reverse reaction is slower than the forward reaction. It takes, for example, 12 h for the chalcone of a 3,5-diglycoside to reach equilibrium. Since determination of the amount of pigment remaining is generally based on measurement of the flavylium salt, an error is introduced if insufficient time is allowed for equilibrium to be attained [139].

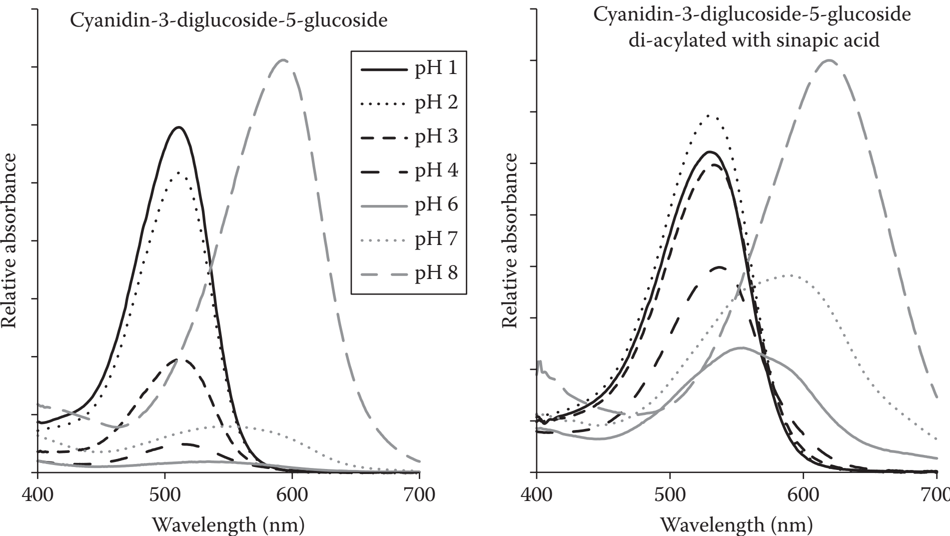


FIGURE 10.20 Absorbance spectra of acylated and nonacylated cyanidin-derivatives from red cabbage in buffers pH 1–8. Pigment concentration of 100 μM in 0.25 M KCl (pH 1), 0.1 M citrate (pH 2–4), and 0.1 M phosphate buffers (pH 6–8). (From Ahmadiani, N. and Giusti, M., 2015, Unpublished data.)

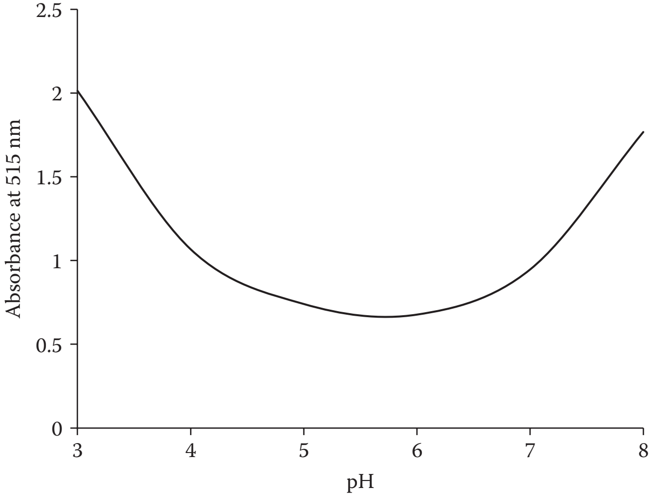


FIGURE 10.21 Changes on absorption intensity of chokeberry anthocyanins with changes in pH. Pigment concentration of 50 μM in 0.5 M sodium acetate (pH 3–6) or 0.5 M sodium phosphate buffers (pH 7–8). (From Sigurdson, G. and Giusti, M., 2015, Unpublished data.)

The exact mechanism of thermal degradation of anthocyanins has not been fully elucidated, but a review of current knowledge in the area was made by Patras and coworkers [160]. Heat-mediated anthocyanin degradation will depend on the temperature and time of the treatment and is primarily caused by oxidation and cleavage of covalent bonds. Three pathways have been suggested (Figure 10.22). In path (a), the flavylium cation is first transformed to the quinonoidal base, then to several intermediates, and finally to the coumarin derivative and a compound corresponding to the B-ring. In path (b), the flavylium cation is first transformed to the colorless carbinol base, then to the chalcone, and finally to brown degradation products. Path (c) is similar to (b) except that the

FIGURE 10.22 Mechanisms of degradation for anthocyanidin-3,5-diglycoside and for anthocyanidin 3-diglucosides via (a) quinoidal base, (b) carbinol base, and (c) deglycosylation. R_3', R_5' = –OH, –H, $–OCH_3$ or –OGL; GL = glycosyl group. (From Fulcrand, H. et al., *Phytochemistry*, 47, 1401, 1998.)

first step of degradation involves deglycosylation of the molecule. Anthocyanin hydrolysis can be favored by heat, resulting on the loss of the glycosidic bond and formation of the unstable anthocyanidins. The degradation products are brown to yellow in color, unsuitable as natural colorants [78]. In all proposed mechanisms, the thermal degradation of anthocyanins will be dependent on the type of anthocyanin involved with acylated anthocyanins showing greater stability to heat than their nonacylated counterparts.

10.2.4.1.6 Oxygen and Ascorbic Acid

The unsaturated nature of the anthocyanidin structure makes it susceptible to reaction with molecular oxygen. It has been known for many years that when grape juice is hot filled into bottles, complete filling of the bottles will delay degradation of the color from purple to dull brown. Similar observations have been made with other anthocyanin-containing juices. The positive effect of oxygen removal on retention of anthocyanin color has been further demonstrated by processing anthocyanin-pigmented fruit juices under nitrogen or vacuum [46,200]. Also, the stability of pigments from Concord grape juice in a dry beverage is greatly enhanced when the product is packaged in a nitrogen atmosphere. Anthocyanin stability was found to be greatest at a_w values in the range of 0.63–0.79 (Table 10.9).

Ascorbic acid can be present in a variety of fruits and vegetables and is also added to a variety of foods as acidulant and to enhance the nutritional value of a product. It is known that anthocyanins degrade faster in the presence of ascorbic acid, suggesting some direct interaction between the two molecules. However, another proposed mechanism is that ascorbic acid–induced degradation of anthocyanin results indirectly from hydrogen peroxide that forms during oxidation of ascorbic acid [103]. The latter reaction can be accelerated by the presence of copper and inhibited by the presence of certain flavonols such as quercetin and quercitrin [193] or catechins [38]. Conditions that do not favor formation of H_2O_2 during oxidation of ascorbic acid therefore account for anthocyanin stability in some fruit juices. H_2O_2 cleavage of the pyrylium ring by a nucleophilic attack at the C-2 position of the anthocyanin produces colorless esters and coumarin derivatives. These breakdown products may further degrade or polymerize and ultimately lead to a brown precipitate that is often observed in fruit juices.

10.2.4.1.7 Light

Light exposure of plants is an important factor that induces anthocyanin production and accumulation. However, light accelerates degradation of anthocyanins in foods after plant tissues have been disrupted. This adverse effect has been demonstrated in several fruit juices and red wine. In wine it has been determined that acylated, methoxylated diglycosides are more stable than nonacylated diglycosides,

TABLE 10.9
Effect of a_w on Color Stability of Anthocyanins[a] during Heating as Measured by Absorbance

	Absorbance at Water Activities						
Holding Time at 43°C (min)	**1.00**	**0.95**	**0.87**	**0.74**	**0.63**	**0.47**	**0.37**
0	0.84	0.85	0.86	0.91	0.92	0.96	1.03
60	0.78	0.82	0.82	0.88	0.88	0.89	0.90
90	0.76	0.81	0.81	0.85	0.86	0.87	0.89
160	0.74	0.76	0.78	0.84	0.85	0.86	0.87
Percent change in absorbance (0–160 min)	11.9	10.5	9.3	7.6	7.6	10.4	15.5

Source: Kearsley, M.W. and Rodriguez, N., *J. Food Technol.*, 16, 421, 1981.
[a] Concentration 700 mg/100 mL (1 g commercially dried pigment powder).

FIGURE 10.23 Molecular complex between anthocyanin and a polyhydroxyflavone sulfonate. (From Sweeny, J. et al., *J. Agric. Food Chem.*, 29, 563, 1981.)

which are more stable than monoglycosides [29]. Copigmentation (anthocyanin condensation with themselves or other organic compound) can either accelerate or retard degradation, depending on the circumstances. Polyhydroxylated flavone, isoflavone, and aurone sulfonates exert a protective effect against photodegradation [208]. The protective effect is attributable to the formation of intermolecular ring interactions between the negatively charged sulfonate and the positively charged flavylium ion (Figure 10.23). Anthocyanins substituted at the C-5 hydroxyl groups are more susceptible to photodegradation than those unsubstituted at this position. Unsubstituted or monosubstituted anthocyanins are susceptible to nucleophilic attack at the C-2 and/or C-4 positions. Other forms of radiant energy such as ionizing radiation can also result in anthocyanin degradation [140].

10.2.4.1.8 Sugars and Their Degradation Products

Sugars at high concentrations, as found in fruit preserves, stabilize anthocyanins. This effect is believed to result from a lowering of water activity (Table 10.9). Nucleophilic attack of the flavylium cation by water occurs at the C-2 position, forming the colorless carbinol base. When sugars are present at concentrations sufficiently low to have little effect on a_w, they or their degradation products sometimes can accelerate anthocyanin degradation. At low concentrations, fructose, arabinose, lactose, and sorbose have a greater degradative effect on anthocyanins than do glucose, sucrose, and maltose. The rate of anthocyanin degradation follows the rate of sugar degradation to furfural. Furfural, which is derived from aldopentoses, and hydroxylmethylfurfural, which is derived from ketohexoses, result from the Maillard reaction or from oxidation of ascorbic acid. These compounds readily condense with anthocyanins, forming brown compounds. The mechanism of this reaction is unknown. The reaction is temperature dependent, is hastened by the presence of oxygen, and causes noticeable changes in fruit juice color.

10.2.4.1.9 Metals

Metal complexes of anthocyanins are common in the plant world and they extend the color spectrum of flowers. Many of the beautiful blue colors of flowers are due to the complexation of anthocyanins and metals. Coated metal cans have long been found to be essential for retaining typical colors of anthocyanins of fruits and vegetables during commercial sterilization. Anthocyanins with vicinal, phenolic hydroxyl groups can sequester several multivalent metals. Complexation produces a bathochromic shift toward the blue. Addition of $AlCl_3$ to anthocyanin solutions has been used as an analytical tool to differentiate cyanidin, petunidin, and delphinidin from pelargonidin, peonidin, and malvidin. The latter group of anthocyanidins does not possess vicinal phenolic hydroxyls and will not react with Al^{3+} (Figure 10.18). Some studies have shown that metal complexation stabilizes the color of anthocyanin-containing foods. Ca, Fe, Al, and Sn ions have been shown to offer some protection to anthocyanins in cranberry juice; however, association with metals will also result in a bathochromic shift that may be desirable or undesirable depending on the application [72].

For example, ferric ion–treated anthocyanins exhibited greater bathochromic shifts than Al^{3+}-treated pigments. These bathochromic shifts can be as high as 100 nm or more. The degree of the bathochromic shift increases as the number and availability of free of hydroxyl groups increased [27].

Blue color formation was possible when Al^{3+} or Fe^{3+} salts were added to acylated cyanidin and delphinidin derivatives [196]. The pH and the composition of the solution were critical factors, and at pH 4–6 a strong hyperchromic effect was reported in addition to the bathochromic shifts leading to more intense and bluer colors.

A fruit discoloration problem referred to as "pinking" has been attributed to formation of metal–anthocyanin complexes. This type of discoloration has been reported in pears, peaches, and lychees. It is generally believed that pinking is caused by heat-induced conversion of colorless proanthocyanidins to anthocyanins under acid conditions, followed by complex formation with metals [134].

10.2.4.1.10 Sulfur Dioxide

One step in the production of maraschino, candied, and glacé cherries involves bleaching of anthocyanins by SO_2 at high concentrations (0.8%–1.5%). Fruits containing anthocyanins are preserved by holding them in a solution containing 500–2000 ppm SO_2, resulting on the formation of a colorless complex. This reaction has been extensively studied, and it is believed that the reaction involves attachment of SO_2 at position C-4 (Figure 10.24). The reason for suggesting involvement of the 4 position is that SO_2 in this position disrupts the conjugated double bond system, which results in loss of color. The apparent constant (k_s) for the discoloration reaction of cyanidin 3-glucoside by SO_2 has been calculated as 25,700/μA at pH 3.24 [213].

The large rate constant means that a small amount of SO_2 can quickly decolorize a significant amount of anthocyanin. Anthocyanins that are resistant to SO_2 bleaching either have the C-4 position blocked or exist as dimers linked through their 4 position [21]. The sulfur-mediated discoloration of anthocyanins can be reversible to a certain extent if the sulfite is removed. However, the bleaching that occurs during long incubation of cherries in sulfur dioxide in the production of maraschino or candied cherries is irreversible. The color has to be later restored to the cherries by addition of a colorant, most typically synthetic colorants, such as FD&C Red No. 40.

Oddly, sulfur dioxide or its equivalents such as bisulfite or metabisulfite have also been used to increase the extraction efficiency of anthocyanins from plant materials. Extracts obtained with aqueous bisulfite have produced more pure and intense and stable colors as compared to aqueous extracts [105].

10.2.4.1.11 Copigmentation

Anthocyanins are known to condense with themselves (self-association, also known as intramolecular copigmentation) and other organic compounds (extramolecular copigmentation). Weak complexes can be formed with proteins, tannins, other flavonoids, and polysaccharides. Although most of these compounds themselves are not colored, they may modulate the color of anthocyanins by causing bathochromic shifts and increased light absorption at the wavelength of maximum light absorption. These complexes also tend to be more stable during processing and storage. During winemaking, anthocyanins undergo a series of reactions to form more stable, complex wine pigments. The stable color of wine is believed to result from covalent self-association of anthocyanins.

FIGURE 10.24 Colorless anthocyanin–sulfate ($-SO_2$) complex. GL = glucose.

FIGURE 10.25 Colorless 4-substituted flav-2-enes resulting from the condensation of flavylium with (a) ethylglycine, (b) phloroglucinol, (c) catechin, and (d) ascorbic acid. (From Markakis, P., Stability of anthocyanins in foods, in: Markakis, P., ed., *Anthocyanins as Food Colors*, Academic Press, New York, 1982, pp. 163–180.)

Such polymers are less pH sensitive and, because the association occurs through the 4 position, are resistant to discoloration by SO_2. In addition, anthocyanin-derived pigments (vitisin A and B) have been found in wine [13,75] as a result of the reaction between malvidin and pyruvic acid or acetaldehyde, respectively. This reaction causes a hypsochromic shift on its visible wavelength of absorption, producing a more orange/red hue as compared to the typical bluish purple of malvidin. However, the contribution of vitisin to total wine color may be minor [184].

Adsorption of the flavylium cation and/or the quinonoidal base to a suitable substrate, such as pectins or starches, can stabilize anthocyanins. This stabilization should enhance their utility as potential food color additives. Other condensation reactions can lead to color loss. Certain nucleophiles, such as amino acids, phloroglucinol, and catechin, can condense with flavylium cations to yield colorless 4-substituted flav-2-enes [139]. Proposed structures are shown in Figure 10.25.

10.2.4.1.12 Enzyme Reactions

Enzymes have been implicated in the decolorization of anthocyanins. Two groups have been identified: glycosidases and polyphenol oxidases. Together they are generally referred to as anthocyanases. Glycosidases, as the name implies, hydrolyze glycosidic linkages, yielding sugars(s) and agylcone. Although anthocyanidins are also colored, loss of color can result rather quickly due to the decreased stability of the anthocyanidins and their transformation to colorless products. Polyphenol oxidases act in the presence of *o*-diphenols and oxygen to oxidize anthocyanins. The enzyme first oxidizes the *o*-diphenol to *o*-benzoquinone, which in turn reacts with the anthocyanins by a nonenzymatic mechanism to form oxidized anthocyanins and degradation products (Figure 10.26) [139].

Although blanching of fruits is not a general practice, anthocyanin-destroying enzymes can be inactivated by a short blanch treatment (45–60 s at 90°C–100°C). This has been suggested for sour cherries before freezing. Very low concentrations of SO_2 (30 ppm) have been reported to inhibit enzymatic degradation of anthocyanin in cherries [81]. Similarly, a heat-stabilization effect on

O_2 + catechol (OH, OH) —Enzyme→ H_2O + o-quinone (O, O)

o-quinone + Anthocyanin → Oxidized anthocyanin + Degradation products

FIGURE 10.26 Proposed mechanisms of anthocyanin degradation by polyphenol oxidase. (From Peng, C. and Markakis, P., *Nature*, 199, 597, 1963.)

anthocyanin has been noted when Na_2SO_3 is present [1]. Alternative approaches to avoid enzymatic degradation of anthocyanins are the use of acidified conditions that denature the enzymes and prevent them from destroying the pigments. Additionally, some enzyme such as macerating enzymes used to facilitate fruit pressing and to improve juice yields may also contain glycosidase activities. It is recommended to screen enzyme preparations for glycosidase activities to avoid pigment deglycosylation and color loss [225].

10.2.4.1.13 Anthocyanins as Natural Food Colorants

Interest in anthocyanins as potential alternatives to synthetic dyes has greatly increased over the last decades. Discovery of acylated anthocyanins with high stability have raised the possibility that these pigments may be used to impart desirable color and stability for a wide variety of commercial food products [79]. Examples of edible sources of such anthocyanins with desirable color and stability are radishes, red potatoes, red cabbage, black carrots, purple corn, and purple sweet potatoes. Among these, radishes and red potatoes stand out as potential alternatives for the use of FD&C Red No. 40 (allura red). Typical applications would be juices or water-based systems with pH below 3. However, other foods have been successfully colored with anthocyanin-based colorants; maraschino cherries (pH 3.5) with bright, attractive, and stable red color were prepared using anthocyanin-rich radish extract [80] demonstrating that radish pigments could function as suitable alternatives to allura red. Additional potential applications for acylated anthocyanins include other challenging systems such as low acid or neutral pH products, such as dairy products [68,79], including yogurt and milk. The unusual 3-deoxyanthocyanins from sorghum are also being investigated as potential alternatives to the use of artificial colorants [10]. These pigments are significantly more stable to pH changes, storage and processing conditions, and provide colors ranging from yellow-orange to red. The increased stability of these pigments together with their added value due to their potential health benefits provides new opportunities for their use in a variety of food applications. New applications of anthocyanins are also being explored in foods with near-neutral pH, where the quinonoidal bases are formed and colors are produced in the blue region of the visible spectra. In general, much less information is available about the chemistry and stability of anthocyanins in these pH ranges compared to acidic pH, but some studies suggest that it may be possible to use anthocyanins to impart colors other than the traditional reds and expand the applications of anthocyanins in selected foods [2,196].

Although the purpose of food colorants is to provide color, anthocyanins can also be considered as colorants with added value, since they are also potent antioxidants and have been associated with a number of health benefits. The topic of health benefits of anthocyanins is beyond the scope of this chapter, and readers interested in this topic can refer to an extensive compilation of the work provided in *Anthocyanins in Health and Disease* edited by Wallace and Giusti [239].

10.2.4.2 Other Flavonoids

Anthocyanins, as previously mentioned, are the most prevalent flavonoids. However, there are over 6000 different flavonoids characterized from plants, and some of them provide valuable contribution to color. Although most yellow colors in food are attributable to the presence of carotenoids, some

are attributable to the presence of nonanthocyanin (NA)-type flavonoids. In addition, flavonoids also account for some of the whiteness of plant materials, and the oxidation products of those containing phenolic groups contribute to the browns and blacks found in nature. The term anthoxanthin (Greek "anthos," flower; "xanthos," yellow) is also sometimes used to designate certain groups of yellow flavonoids. Differences among classes of flavonoids relate to the state of oxidation of the 3-carbon link (Figure 10.16). Structures commonly found in nature vary from flavan-3-ols (catechins) to flavonols (3-hydroxyflavones) and anthocyanins. The flavonoids also include flavanones, flavononols or dihydroflavonols, and flavan-3,4-diols (proanthocyanidin). In addition, there are five classes of compounds that do not possess the basic flavonoid skeleton, but are chemically related, and therefore are generally included in the flavonoid group. These are the dihydrochalcones, chalcones, isoflavones, neoflavones, and aurones. Individual compounds within this group are distinguished, as with anthocyanins, by the number of hydroxyl, methoxyl, and other substituents on the two benzene rings. Many flavonoid compounds carry a name related to the first source from which they were isolated, rather than being named according to the substituents of the respective aglycone. This inconsistent nomenclature has brought about confusion in assigning compounds to various classes.

10.2.4.2.1 Physical Properties

The light absorption characteristics of flavonoid classes clearly demonstrates the relationship of color and unsaturation within a molecule and the impact of auxochromes (groups present in a molecule that deepens the color). In the hydroxy-substituted flavans catechin and proanthocyanin, the unsaturation is interrupted between the two benzene rings, and therefore, the absorption spectra are similar to that of phenols, which exhibit maximum light absorption between 275 and 280 nm (Figure 10.27a). In the flavanone naringenin, the hydroxyl groups only occur in conjunction with the carbonyl group at C-4 and therefore do not exert their auxochromic characteristics (Figure 10.27b). Therefore, its light absorption is similar to that of flavans. In the case of the flavone luteolin (Figure 10.27c), the hydroxyl groups associated with both benzene rings exert their auxochromic characteristics through the conjugation of C-4. Light absorption of longer wavelength (350 nm) is associated with the B-ring, while that of shorter wavelength is associated with the A-ring. The hydroxyl group at C-3 in the flavonol quercetin causes a further shift to a still longer wavelength (380 nm) for maximum light absorption, compared to that of the flavones. The flavonols therefore appear yellow if present at high enough concentration. Acylation and/or glycosylation results in further shifts in spectral characteristics.

As previously mentioned, flavonoids can become involved in copigmentation, and this occurrence has a major impact on many hues in nature. In addition, flavonoids, like anthocyanins, are chelators of metals. Chelation with iron or aluminum increases the yellow saturation. Luteolin when chelated with aluminum is an attractive yellow (390 nm).

10.2.4.2.2 Importance in Foods

NA flavonoids make some contribution to color in foods; however, the paleness of most NA flavonoids generally restricts their overall contribution. The whiteness of vegetables such as cauliflower, onion, and potato is attributable largely to NA flavonoids, but their contribution to color through copigmentation is more important. The chelation characteristics of these compounds can contribute either positively or negatively to the color of foods. For example, rutin (3-rutinoside of quercetin) causes a greenish-black discoloration in canned asparagus when it complexes with Fe^{3+}. The addition of a chelator such as ethylenediaminetetraacetic acid (EDTA) will inhibit this undesirable color. The tin complex of rutin has a very attractive yellow color, which contributed greatly to the acceptance of yellow wax beans until the practice of canning wax beans in plain tin cans was eliminated. The Sn^{3+}-rutin complex is more stable than the Fe^{3+} complex; thus, the addition or availability of only very small amounts of tin would favor formation of the tin complex.

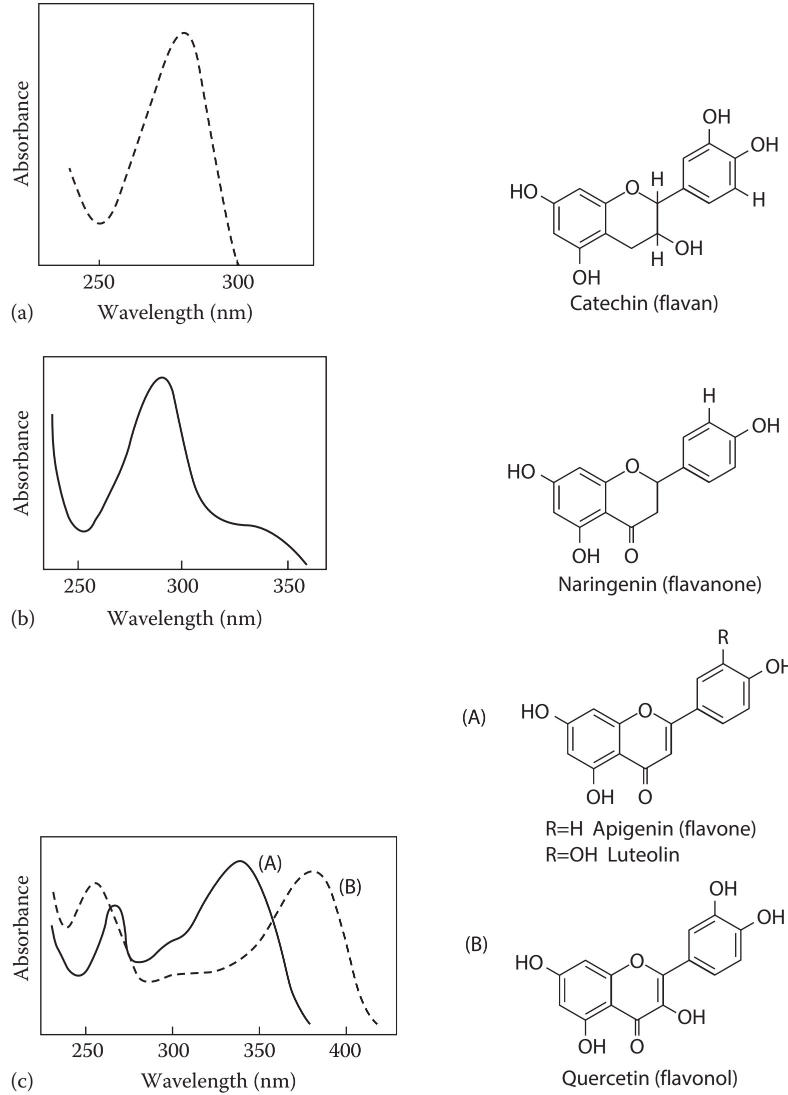


FIGURE 10.27 Absorption spectra of specific flavonoids.

The color of black ripe olives is due in part to the oxidative products of flavonoids. One of the flavonoids involved is luteolin-7-glucoside. Oxidation of this compound and formation of the black color occur during fermentation and subsequent storage [19]. Other very important functions of flavonoids in foods are their antioxidant properties and their contribution to flavors, particularly bitterness.

10.2.4.2.3 Proanthocyanidins

Consideration of proanthocyanidins under the general topic of anthocyanins is appropriate. Although these compounds are colorless they have structural similarities with anthocyanidins. They can be converted to colored products during food processing. Proanthocyanidins are also referred to as leucoanthocyanidins or leucoanthocyanins. Other terms used to describe these colorless compounds are anthoxanthin, anthocyanogens, flavolans, flavylans, and flaylogens. The term leucoanthocyanidin is appropriate if it is used to designate the monomeric flaven-3,4-diol (Figure 10.28), which is

FIGURE 10.28 Basic building block of proanthocyanidins.

the basic building block of proanthocyanidins. The latter can occur as dimers, trimers, or higher polymers. The intermonomer linkage is generally through carbons 4 and 8 or 4 and 6.

Proanthocyanidins were first found in cocoa beans, where upon heating under acidic conditions they hydrolyze into cyanidin and (–)-epicatechin (Figure 10.29) [67]. Dimeric proanthocyanidins have been found in apples, pears, kola nuts, and other fruits. These compounds are known to degrade in air or under light to red-brown stable derivatives. They contribute significantly to the color of apple juice and other fruit juices and to astringency in some foods. To produce astringency, proanthocyanidins of two to eight units interact with proteins. Other proanthocyanidins found in nature will yield on hydrolysis common anthocyanidins, including pelargonidin, petunidin, or delphinidin.

10.2.4.2.4 Tannins

A rigorous definition of tannins does not exist, and many substances varying in structure are included under this name. Tannins are special phenolic compounds and are given this name simply by virtue of their ability to combine with proteins and other polymers such as polysaccharides, rather than their exact chemical nature. They are functionally defined, therefore, as water-soluble polyphenolic compounds with molecular weights between 500 and 3000 that have the ability to precipitate alkaloids, gelatin, and other proteins. They occur in the bark of oak trees and in fruits. The chemistry of tannins is complex. They are generally considered as two groups: (1) proanthocyanidins, also referred to as “condensed tannins” (previously discussed), and (2) glucose polyesters of gallic acid of hexahydroxydiphenic acids (Figure 10.30). The latter group is also known as hydrolyzable tannins, because they consist of a glucose molecule bonded to several phenolic moieties.

FIGURE 10.29 Mechanism of acid hydrolysis of proanthocyanidin. (From Forsyth, W. and Roberts, J., *Biochem. J.*, 74, 374, 1960.)

Proanthocyanidin

Pentagalloyl glucose

FIGURE 10.30 Structure of tannins.

The most important example is glucose bonded to gallic acid and the lactone of its dimer, ellagic acid. Tannins range in color from yellowish white to light brown and contribute to astringency in foods. They contribute to the color of black teas when catechins are converted to theaflavins and thearubigins during fermentation. Their ability to precipitate proteins makes them valuable as clarifying agents.

10.2.4.3 Quinoids and Xanthones

Quinones are phenolic compounds varying in molecular weight from a monomer, such as 1,4-benzoquinone, to a dimer, 1,4-naphthaquinone, to a trimer, 9,10-anthraquinone, and finally polymers represented by hypericin (Figure 10.31). They are widely distributed in plants, specifically trees, where they contribute to the color of wood. Most quinones are bitter in taste. They contribute to

1,4-Benzoquinone

1,4-Napthoquinone

9,10-Anthraquinone

Hypericin

FIGURE 10.31 Structure of quinones.

FIGURE 10.32 Structure of mangiferin.

some of the darker colors; to the yellows, oranges, and browns of certain fungi and lichens; and to the reds, blues, and purples of sea lilies and coccid insects. Compounds with complex substituents such as naphthaquinone and anthraquinones occur in plants, and these have deep purple to black hues. Further color changes can occur in vitro under alkaline conditions by the addition of hydroxyl groups. Xanthone pigments are yellow, phenolic pigments and they are often confused with quinones and flavones because of their structural characteristics. The xanthone mangiferin (Figure 10.32) occurs as a glucoside in mangoes. They are easily distinguishable from quinones by their spectral characteristics.

10.2.5 Betalains

10.2.5.1 Structure

Betalains are a class of nitrogen-containing pigments made up of two structural subgroups, betacyanins (red/violet) and betaxanthins (yellow/orange). Plants containing betalains have colors similar to plants containing anthocyanins, but their color is less affected by changes in pH. Betalains are water soluble and exist as internal salts (zwitterions) in the vacuoles of plant cells. Plants containing these pigments are restricted to 10 families of the order Caryophyllales. The presence of betalains in plants is mutually exclusive of the occurrence of anthocyanins (i.e., betalains and anthocyanins do not exist together in the same plant) [199]. Sources of betalains include red beet, amaranth, cactus fruits, Swiss chard, yellow beet, and purple pitaya. Amaranth is either consumed fresh as greens or at the mature state as grain. The most well-studied betalains are those of the red beet.

Approximately 55 different betalain structures have been identified to date [201]. The general structure of betalains (Figure 10.33a) comes from the condensation of a primary or secondary amine with betalamic acid (BA) (Figure 10.33b). All betalain pigments can be described as a

FIGURE 10.33 General structures of betalains and their building blocks.

FIGURE 10.34 Structures of select betacyanins.

resonance-stabilized 1,2,4,7,7-pentasubstituted 1,7-diazaheptamethin system (Figure 10.33c). When R′ does not extend conjugation of the 1,7-diazaheptamethin system, the compound exhibits maximum light absorption at about 480 nm, characteristic of yellow-orange betaxanthins. If the conjugation is extended at R′, the maximum light absorption shifts to approximately 540 nm, characteristic of red-violet betacyanins.

Betacyanins are optically active because of the two chiral carbons C-2 and C-15 (Figure 10.34). Hydrolysis of betacyanins leads to either betanidin (Figure 10.34a), the C-15 epimer isobetanidin (Figure 10.34f), or a mixture of the two isomeric aglycones. These aglycones are comprised of BA conjugated to *cyclo*-Dopa and are shared by all betacyanins. Differences between betacyanins are found in their substituents at the C-5 and C-6 positions. Reported betacyanin substituents include glucose, glutamic acid, and apiose, which can be further modified through esterification with acids, such as malonic, 3-hydroxy-3-methyl-glutaric, caffeic, *p*-coumaric, and ferulic acid [204]. The first betacyanin isolated and characterized was betanin (betanidin 5-*O*-β-glucoside) from red beet [228]. Betanin and isobetanin (Figure 10.34b and g) are the predominant betacyanins in red beet, while amaranthin and isoamaranthin (Figure 10.34c and h) are the predominant betacyanins in amaranth.

Betaxanthins are structurally similar to betacyanins, but differ in that BA is conjugated to an amino acid or amine rather than *cyclo*-Dopa. The first betaxanthin isolated and characterized was indicaxanthin from cactus pear [168] (Figure 10.35a). Indicaxanthin is BA conjugated to the amino acid proline. Two betaxanthins have been isolated from beet, vulgaxanthin I and II (Figure 10.35b, c). They differ from indicaxanthin in that the proline has been replaced by glutamine or glutamic acid, respectively. Although only a small number of betaxanthins have been characterized to date, considering the number of amino acids available, it is likely many more betaxanthins exist.

10.2.5.2 Physical Properties

Betalains are water-soluble pigments and exhibit even greater hydrophilicity than anthocyanins. They can be extracted from plant material using water, but extraction with methanol will allow for the precipitation of potentially interfering proteins. Like other plant pigments, betalains absorb light strongly in the visible region. The $A^{1\%}{}_{1cm}$ values are 1120 for betanin and 750 for vulgaxanthin, suggesting high tinctorial strength in the pure state. Maximum absorption in the visible region depends on the structure of the betalain substituents but generally occurs around 535–538 nm for betacyanins [202] and between 460 and 477 nm for betaxanthins [203]. The observed color in nature is a function of the ratio of betacyanins to betaxanthins present in the plant [201].

Unlike anthocyanins, betalains are relatively pH stable between 4.0 and 7.0. Below pH 4.0, the absorption maximum shifts toward a slightly shorter wavelength (535 nm at pH 2.0 for betanin).

(a) Indicaxanthin

(b) Vulgaxanthin-I, R = NH_2
(c) Vulgaxanthin-II, R = OH

FIGURE 10.35 Structures of select betaxanthins.

Above pH 7.0, the absorption maximum shifts toward a longer wavelength (544 nm at pH 9.0 for betanin). Spectrophotometric, HPLC, MS, and NMR methods have been developed and used for the identification and structural elucidation of betalains. These methods of analysis have been reviewed elsewhere [201,204].

10.2.5.3 Chemical Properties

Like other natural pigments, betalains are affected by several environmental factors.

10.2.5.3.1 Heat and/or pH

Under mild alkaline conditions betanin is hydrolyzed at the aldimine bond to BA and *cyclo*-Dopa 5-*O*-glucoside (CDG) (Figure 10.36). As BA has an absorption maximum around 430 nm, the solution turns from red to yellow following betanin hydrolysis. This reaction also takes place during heating of acidic betanin solutions or during thermal processing of products

Betanin

$+H_2O$ $-H_2O$

cyclo-Dopa-5-*O*-glucoside

Betalamic acid

FIGURE 10.36 Degradation reaction of betanin.

TABLE 10.10
Effect of Oxygen and pH on the Half-Life Values of Betanin in Aqueous Solution at 90°C

	Half-Life Values of Betanin (min)	
pH	**Without O_2**	**With O_2**
3.0	56 ± 6	11.3 ± 0.7
4.0	115 ± 10	23.3 ± 1.5
5.0	106 ± 8	22.6 ± 1.0
6.0	41 ± 4	12.6 ± 0.8
7.0	4.8 ± 0.8	3.6 ± 0.3

Source: Adapted from Huang, A. and von Elbe, J., *J. Food Sci.*, 52, 1689, 1987.

containing beet root, but more slowly [182]. Hydrolysis is pH dependent (Table 10.10), and the greatest stability of betanin is in the pH range of 4.0–5.0 [96]. It should also be noted that the hydrolysis reaction requires water; thus, when water is unavailable or limited, betanin is very stable. It follows that a decrease in water activity (a_w) causes a decrease in the degradation rate of betanin [159]. An a_w of 0.12 and moisture content of 2% (dry weight basis) has been recommended for optimal storage stability of the pigments in beet powder [42]. The greatest degradation of betanin in encapsulated beetroot has been reported at a_w 0.64, suggesting that an intermediate water activity may be more detrimental to betalains than a high water activity [191].

Degradation of betanin to BA and CDG is reversible, and therefore, partial regeneration of the pigment occurs following heating. The mechanism proposed for regeneration involves a the condensation of the aldehyde group of BA and the nucleophilic amine of CDG to form a Schiff base (Figure 10.36). Regeneration of betanin is maximized at an intermediate pH range (4.0–5.0) [96]. It is because of this reverse reaction that canners have traditionally examined canned beets several hours after processing to evaluate color.

Betacyanins, because of the C-15 chiral center (Figure 10.34), exist in two epimeric forms. Epimerization is brought about by either acid or heat. It would therefore be expected that during heating of a food containing betanin, the ratio of isobetanin to betanin would increase. However, epimerization does not affect the absorption spectrum of the compound so the color remains the same.

While thermal degradation of betanin occurs mainly through hydrolytic cleavage, it has been shown that decarboxylation and dehydrogenation can also occur. When betanin in aqueous solution is heated, decarboxylation can occur to form red-orange decarboxy-betanins (505 nm). Evidence for this transformation is the generation of CO_2 and loss of the chiral center. The rate of decarboxylation increases with increasing acidity [96]. Betanin can also undergo dehydrogenation to form orange neobetanin (477 nm). Degradation reactions of betanin under acid and/or heat are summarized in Figure 10.37. For some acylated betacyanins, such as phyllocactin (malonyl-betanin) (Figure 10.34d) and hylocerenin (3″-hydroxy-3″-methyl-glutaryl-betanin) (Figure 10.34e) from purple pitaya, decarboxylation and dehydrogenation are the predominant reactions responsible for pigment degradation [93].

The stability of betaxanthins has not been studied extensively, but as both betacyanins and betaxanthins possess the same general structure, they are likely to have similar

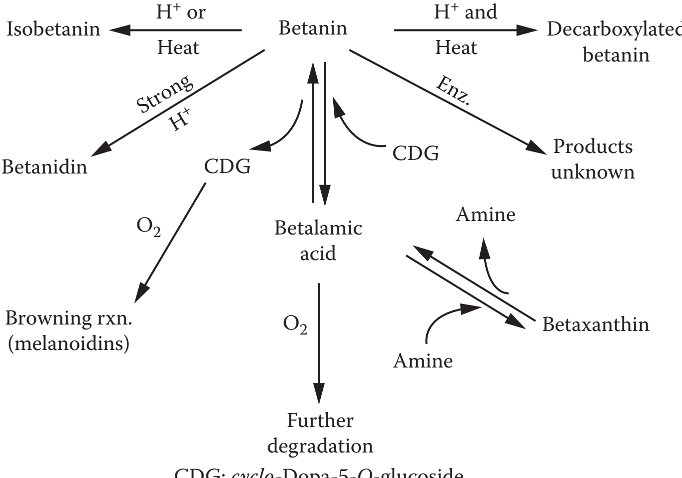


FIGURE 10.37 Degradation of betanin under acid and/or heat.

degradation mechanisms. Indicaxanthin from cactus pear juice has been shown to isomerize to form isoindicaxanthin under heat. As with betanin, regeneration of indicaxanthin has also been observed following thermal treatment and cold storage [151]. Similar to betacyanins, betaxanthins in solution are reported to be most stable at pH 5.5 [33]. While both subgroups are more stable dried than in an aqueous solution, betaxanthins appear to be better retained during cold storage in the absence of light and oxygen [32].

10.2.5.3.2 Oxygen and Light

Another major factor that contributes to degradation of betalains is the presence of oxygen. Oxygen in the headspace of canned beets has long been known to accelerate pigment loss. In solutions containing a molar excess of oxygen over betanin, betanin loss follows apparent first-order kinetics. Betanin degradation deviates from first-order kinetics when the molar oxygen concentration is reduced to near that of betanin. In the absence of oxygen, stability is increased. Molecular oxygen has been implicated as the active agent in oxidative degradation of betanin. Because betalains are susceptible to oxidation, these compounds are also effective antioxidants [224]. Glycosylation is also a factor as betanin has been shown to have a longer half-life than its aglycone betanidin when exposed to molecular oxygen. This corresponds with a lower redox potential for betanidin compared to betanin [52].

Oxidation of betalains accelerates in the presence of light. In a model system, light was shown to increase betanin degradation by 27%, 83%, and 212% at 55°C, 40°C, and 25°C, respectively [8]. The lesser impact at higher temperatures was explained by the dominance of heat-induced chemical degradation over photochemical oxidation. Similar effects of light have also been observed in betalain-rich foods, such as purple pitaya juice [92].

The presence of antioxidants, such as ascorbic acid and isoascorbic acid, improves betalain stability. Because metal cations catalyze oxidation of ascorbic acid by molecular oxygen, they detract from the effectiveness of ascorbic acid as a protector of betalains. The presence of metal chelators (EDTA or citric acid) greatly improves the effectiveness of ascorbic acid as a stabilizer of betalains [9,16]. Several antioxidants, including butylated hydroxyanisole, butylated hydroxytoluene, catechin, quercetin, nordihydroguaiartic acid, chlorogenic acid, and alpha-tocopherol, inhibit free-radical chain autoxidation. Since free-radical oxidation does not seem to be involved

in betalain oxidation, these antioxidants are, not surprisingly, ineffective stabilizers of betanin [9]. Similarly, sulfur-containing antioxidants such as sodium sulfite and sodium metabisulfite are not only ineffective stabilizers, they hasten loss of color. Sodium thiosulfite, a poor oxygen scavenger, has no effect on betanin stability. Thiopropionic acid and cysteine also are ineffective as stabilizers of betanin. These observations confirm that betanin does not degrade by a free-radical mechanism. The susceptibility of betalains to oxidation has limited their use as food colorants.

10.2.5.3.3 Enzymatic

Betalains are susceptible to enzymatic degradation. Peroxidases are present in red beets and can catalyze the oxidative degradation of betalains. Peroxidases have been shown to degrade betacyanins at a faster rate than betaxanthins [220]. In the presence of peroxidase from red beet root, BA and CDG polymers are the observed oxidation products of betanin, while betanidin quinone is the observed oxidation product of betanidin [141].

Polyphenol oxidases are also present in red beets and can catalyze the degradation of betalains. Polyphenol oxidase is a copper-containing enzyme responsible for browning in many fruits and vegetables. In beet root extract, polyphenol oxidase activity was greatest at pH 7, while peroxidase activity was greatest at pH 6 [100]. Peroxidases and polyphenol oxidases from beet root can be inactivated at temperatures above 70°C and 80°C, respectively, [100] as well as high-pressure carbon dioxide treatment [128].

10.2.5.3.4 Conversion and Stability of Betalains

In 1965, it was shown that the betaxanthin indicaxanthin could be formed from the betacyanin betanin and an excess of proline in the presence of 0.6 N ammonium hydroxide under vacuum [229]. This was the first conclusive evidence of a structural relationship between betacyanin and betaxanthin. It was further demonstrated that formation of betaxanthin from betanin involved condensation of the betanin hydrolysis product BA and an amino acid (Figure 10.38) [94,167,169].

Shown in Figure 10.39 are the heat stability differences between the betacyanin betanin and the betaxanthin vulgaxanthin under similar experimental conditions. The mechanism in Figure 10.38 suggests that an excess of the appropriate amino acid will shift the equilibrium toward the corresponding betaxanthin and will reduce the quantity of BA in solution. An excess of an amino acid increases the stability of the betaxanthin formed by reducing the amount of BA available for degradation. This effect is illustrated in the two upper curves of Figure 10.39. Conversion of betacyanin to betaxanthin can account for some of the loss of red color in protein-rich foods colored with betalains.

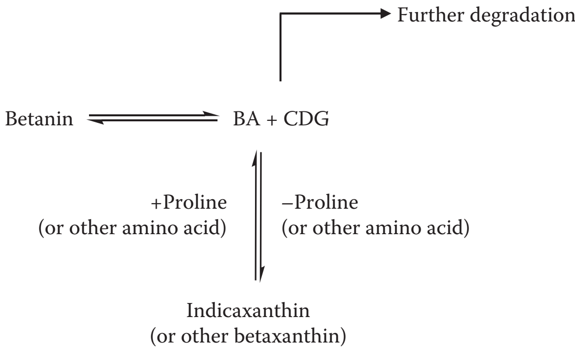


FIGURE 10.38 Formation of indicaxanthin from betanin in excess of proline. (From Wyler, H. et al., *Helv. Chim. Acta*, 48, 361, 1965.)

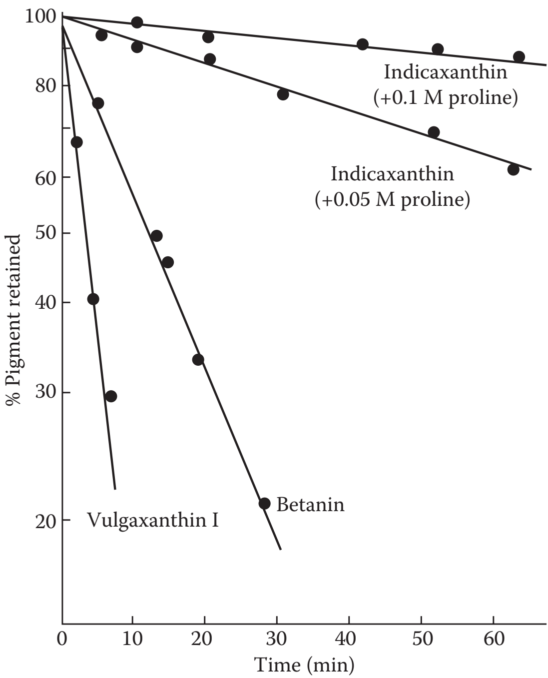


FIGURE 10.39 Stability comparison of betanin, vulgaxanthin I, and indicaxanthin with 0.05 or 0.1 M proline, in solution at pH 5.0, 90°C, under atmospheric conditions.

10.3 FOOD COLORANTS

10.3.1 Regulatory Aspects

Since ancient times, colors have been added to foods to make them more appealing, to increase uniformity, or to enhance or restore color lost through processing. Color additives obtained from vegetable sources such as paprika or turmeric and mineral sources such as iron oxides and copper sulfate are just some examples. In 1856, the first synthetic organic dye, called mauve, was discovered by W.H. Perkin, and many more soon followed [14]. However, some of the color additives were being used to hide defects in foods and some were even hazardous, containing poisonous materials such as lead, arsenic, and mercury. It became evident that careful regulation of the use of color additives was necessary to protect consumers, and ensure the safety of the foods.

From the modern ages, countries have developed different regulations to control the use of coloring agents in foods, added directly or indirectly. Early regulations dealt with adulteration, and addition of toxic substances, and expanded through the years to assure the safety of any additives for food use.

In a global market, keeping up to date with color regulations can be a challenge, as certain countries and areas of the world may allow the use of different materials under different conditions of use. Color regulations can be described as fluid and dynamic given their changes in response to new scientific evidence and consumer pressures, and they are expected to continue to change.

In this section, we will cover major regulatory issues that govern the use of food colorants in the United States and around the world. However, food processors interested in the application of colorants in foods are advised to check for the most up-to-date information for the specific region of the world where their product is intended to be sold.

10.3.1.1 United States

In the United States, colorant use is controlled by the 1960 Color Additive Amendment to the U.S. Food, Drug, and Cosmetic Act of 1938. The amendment classifies colorants into two

categories: certified colors and colors exempt from certification. Certified colors are synthetic dyes and are not found in nature. These colorants include the FD&C colors (e.g., FD&C Red No. 40 and Yellow No. 5). Certification means that the dye meets specific government quality standards. Samples of each production batch must be submitted to an FDA laboratory for determination of compliance. If the batch is in compliance it is assigned an official lot number. Certified dyes are further classified as either permanently or provisionally listed. A *provisionally* approved certified dye can be legally used pending completion of all scientific investigations needed for determination of permanent approval. The same considerations apply to lakes. Colorants exempt from certification are considered safe and are either pigments obtained from natural sources or specific synthetic dyes that are nature identical. An example of the latter is β-carotene, which is widely distributed in nature but also can be synthesized to achieve a *nature-identical* substance.

The Color Additive Amendment includes a simplified nomenclature for certified dyes. Rather than the use of long and difficult common names, certified dyes are referred to by a number and the abbreviation FD&C, D&C, or Ext. (external) D&C. FD&C stands for food, drugs, and cosmetics and colorants labeled as such may be used in all three goods D&C and Ext. D&C dyes can be used only in drugs and cosmetics. For example the certified dye sunset yellow FCF has the designation FD&C Yellow No. 6 indicating it is approved for use in foods, drugs, and cosmetics. The current list of certified dyes permitted in foods contains seven colorants for general use (Table 10.11). Two additional dyes, Orange B and Citrus Red No. 2, may be used; however, their use is restricted to specific applications. Orange B may be used only for coloring the casings or surfaces of frankfurters and sausages, and its use in these applications is restricted to no more than 150 ppm by weight of the finished product. Citrus Red No. 2 may be used only for coloring the skins of oranges not intended or used for processing, and its use in this application is restricted to no more than 2 ppm based on the whole fruit weight.

Adoption of the Nutritional Labeling and Education Act of 1990, which became effective in 1994, makes mandatory the individual listing of certified colors, by their abbreviated names. Colors exempt from certification must be declared, but they can be listed generically as "artificial color," "color added," and "colored with (name of color)" or using any other specific or generic name for the colorant. Despite the fact that most consumers and the food industry refer to colorants from natural sources as natural colors, the use of the term "natural" referred to color additives is prohibited in the United States. This is because it may lead the consumer to believe that the color is derived from the food itself. Color additives currently exempt from certification are listed in Table 10.12.

TABLE 10.11
Certified Color Additives Currently Permitted for General Use in Foods and Their Corresponding Nomenclature according to the European Union

Name	Status		Common Name	E Number[a]
	Dye	Lake		
FD&C Blue No. 1	Permanent	Provisional	Brilliant blue	E133
FD&C Blue No. 2	Permanent	Provisional	Indigotine	E132
FD&C Green No. 3	Permanent	Provisional	Fast green	NA[b]
FD&C Red No. 3	Permanent	Banned	Erythrosine	E123
FD&C Red No. 40	Permanent	Provisional	Allura red	E129
FD&C Yellow No. 5	Permanent	Provisional	Tartrazine	E102
FD&C Yellow No. 6	Permanent	Provisional	Sunset yellow	E110

Source: Code of Federal Regulations, Title 21, Chapter 74, revised as of April 2015.
[a] Numbers listed in the European Economic Community.
[b] Use is banned in the EU.

TABLE 10.12
U.S. Color Additives Currently Exempt from Certification, Color Use Limitation, and Their Corresponding Nomenclature according to the European Union[a]

Section	Color Additive	U.S. Food Use Limit	E Numbers[b]
73.30	Annatto extract	GMP	E160b
73.35	Astaxanthin	<80 mg/kg fish feed	E161j
73.37	Astaxanthin dimethyldisuccinate	<110 mg/kg fish feed	E161j
73.40	Dehydrated beets (beet powder)	GMP	E162
73.50	Ultramarine blue	Salt for animal feed	NA
73.75	Canthaxanthin	<30 mg/lb of solid/semisolid food or pint of liquid <4.41 mg/kg chicken feed	E161g
73.85	Caramel	GMP	E150a-d
73.90	β-Apo-8′-carotenal	<15 mg/lb solid/semisolid food or 15mg/pint liquid food	E160e
73.95	β-Carotene	GMP	E160a
73.100	Cochineal extract; carmine	GMP	E120
73.125	Sodium copper chlorophyllin	<0.2% for dry mix citrus-based beverages	E141
73.140	Toasted partially defatted cooked cottonseed flour	GMP	NA
73.160	Ferrous gluconate	GMP for ripe olives	NA
73.165	Ferrous lactate	GMP for ripe olives	NA
73.169	Grape color extract	GMP for nonbeverage foods	E163
73.170	Grape skin extract (enocianina)	GMP for beverages	E163
73.185	*Haematococcus* algae meal	<80 mg/kg salmonid fish feed	NA
73.200	Synthetic iron oxide	Pet food up to 0.25%	E172
73.250	Fruit juice	GMP	NA
73.260	Vegetable juice	GMP	NA
73.275	Dried algae meal	GMP for chicken feed	NA
73.295	Tagetes (Aztec marigold) meal and extract	GMP for chicken feed	NA
73.300	Carrot oil	GMP	NA
73.315	Corn endosperm oil	GMP for chicken feed	NA
73.340	Paprika	GMP	E160c
73.345	Paprika oleoresin	GMP	E160c
73.350	Mica-based pearlescent pigments	<1.25% in cereals, confectionary <0.07% in spirits of 18%–23% alcohol	NA
73.352	*Paracoccus* pigment	<80 mg/kg salmonid fish feed	NA
73.355	*Phaffia* yeast	<80 mg/kg salmonid fish feed	NA
73.450	Riboflavin	GMP	E101
73.500	Saffron	GMP	E164
73.530	Spirulina extract	GMP for candy & chewing gum	NA
73.575	Titanium dioxide	<1% by weight of food	E171
73.585	Tomato lycopene extract; tomato lycopene concentrate	GMP	E160d
73.600	Turmeric	GMP	E100
73.615	Turmeric oleoresin	GMP	E100

NA, not applicable; does not have an associated E number.
GMP, Good Manufacturing Practices.
[a] *Note:* From Code of Federal Regulations, Title 21, Chapter 1, Part 73, revised as of April 2015.
[b] *E numbers:* Numbers listed in the European Union (EU). In addition, the EU allows the use of anthocyanins/juice concentrates (E163), beet pigments (E162), and chlorophylls (E140).

Over the last decade, five colorants have been added to the exempt from certified color list. Complete information about the color additives permitted in the United States and the requirements of purity and usage levels are listed in the U.S. Code of Federal Regulations (http://www.ecfr.gov/), which is updated on a yearly basis.

10.3.1.2 International

Colors are added to foods in all countries around the world, but the type of colorants permitted for use varies greatly among countries, or regions of the world. Unfortunately, a worldwide list of permitted color additives does not exist; therefore, color additives have, in some instances, become trade barriers for foods. In the United States, for example, FD&C Red No. 40 is permitted for food use, whereas FD&C Red No. 2, since 1976, is no longer permitted. At the other extreme, Norway prohibits the use of any synthetic dye in the manufacturing of foods. Legislative authorities of the EU have attempted to achieve uniform color additive legislation for their member countries, currently at 28, with 5 more possible members. Each permitted color additive, regardless of their nature, has been assigned an E number (E = Europe). Listed in Table 10.11 are the FD&C colorants, their common names, and the equivalent E numbers, when they exist. Similar information for both U.S. and EU colorants exempt from certification is given in Table 10.12. In reviewing these tables, it must be remembered that use of a colorant may be restricted to one or more specific products. Additionally, an EU general-use colorant may not be approved by every country in the EU. In general, it is apparent that greater latitude of use of both synthetic and nature sourced colorants is currently allowed among EU countries than in the United States and Canada. Historically, Japan had a very strict policy on the use of colorants in foods and synthetic dyes were banned. However, over a decade ago Japan expanded its list of designated additives, which includes food coloring agents. As of December 2004, the list included not only the nonchemically synthesized food additives but also a list of 12 different

TABLE 10.13
Acceptable Daily Intake of Some Synthetic and Natural Colorants[a]

Pigment	E-Number	ADI (JEFCA) (mg/kg body weight)
Curcumin	E100	0.1
Riboflavin[b]	E101	0.5
Tartrazine	E102	7.5
Carmines	E120	5.0
Erythrosine	E127	0.1
Brilliant blue FCF	E133	12.5
Chlorophylls[b]	E140	NS
Caramel	E150	200
β-Carotene	E160a	5.0
Annatto	E160b	0.065
Paprika	E160c	NS
Beetroot red	E162	NS
Anthocyanins	E163	NS
Grape skin extract[b]	E163	2.5

[a] Modified from Henry, B.S., Natural food colours, In Hendry, G.A.F. and Houghton, J.D. (eds.), *Natural Food Colorants*, pp. 40–79, Springer-Science+Business Media, London, UK, 1996.
[b] From Francis, F. J. (1999). *Colorants*, Eagan Press Handbook Series, St Paul, Minnesota, USA.
NS, not specified.

synthetic dyes and many corresponding lakes, all with restricted uses. Among the seven FD&C dyes and lakes approved by the FDA, all but FD&C Yellow No. 6 were included in the Japanese list of designated additives under the food sanitation law [70].

The FAO of the United Nations and WHO have also attempted to harmonize food regulations among countries through their Codex Alimentarius. FAO and WHO formed the Joint WHO/FAO Expert Committee on Food Additives (JEFCA) to work on a global basis to assess the safety of food additives. The JEFCA has devised "acceptable daily intakes" for food additives, including colorants (Table 10.13) and recommend specifications for the identity and purity of the different color additives. The JEFCA feeds into the Codex Alimentarius that sets the General Standard for Food Additives (GSFA). These standards define food categories and food additives, as well as conditions for use of those additives, including food colors. Under the GSFA each color additive is assigned an INS number, which often matches the E number. A color additive such as allura red, may be listed as FD&C Red No. 40 under the U.S. regulations, as E129 under EU regulations and as INS129 under GSFA standards. Worldwide efforts toward establishing safety of colorants should eventually lead to internationally accepted regulations for colorant use in foods. In the meantime, use of food colorants in food products should follow the current regulations in the specific country where the product will be sold. For additional up-to-date information on the regulatory status of different food colorants in different regions of the world, readers can access the following websites:

- www.fda.gov (the Code of Federal Regulations, for U.S. regulations)
- http://laws.justice.gc.ca/en/F-27/C.R.C.-c.870/index.html (for Canadian regulations)
- https://webgate.ec.europa.eu/sanco_foods/main/?event=display (Food Additives Database Users Guide for EU regulations, updated in 2011)
- http://ec.europa.eu/food/food/fAEF/additives/index_en.htm (European Food Safety website)
- www.codexalimentarius.net/gsfaonline/index.html (guidelines provided by the JEFCA, GSFA (CODEX))
- http://www.mhlw.go.jp/ (regulations in Japan)
- http://www.foodstandards.govt.nz/thecode/foodstandardscode.cfm (regulations in Australia/New Zealand)

10.3.2 Properties of Certified Dyes

Certified dyes fall into four basic chemical classes: azo-, triphenylmethane-, xanthine-, or indigo-type dyes. Listed in Table 10.14 are the FD&C dyes, their chemical class, and some of their properties. The structures are shown in Figure 10.40. Listed in Table 10.15 are solubility and stability data for EU dyes.

A simplified sequence for chemical synthesis of FD&C Green No. 3, a triphenylmethane dye, is given in Figure 10.41. In manufacturing any dye, it is necessary to meet the specifications of purity given for certification in the United States (Code of Federal Regulations, Title 21, Part 70–83).

The pure dye content of a typical certified colorant is 86%–96%. Variation of 2%–3% in total dye content of a basic color is of little practical significance since such variation has no significant effect on the ultimate color of a product. The moisture content of dye powders is between 4% and 5%. The salt (ash) content of the dye powder is approximately 5%. The high ash content comes from the salt used to crystallize (salt out) the colorant. Although it is technically possible to remove the sodium chloride used, such steps would be costly and would have minimal benefit.

All water-soluble FD&C azo dyes are acidic, and their physical properties are quite similar. Chemically, they are reduced easily by strong reducing agents and susceptible to oxidation FD&C triphenylmethane dyes (FD&C Green No. 3 and FD&C Blue No. 1) are similar in structure, differing only in one –OH group. Differences in solubility and stability are therefore minor. Substitution

TABLE 10.14
Certified Colorants and Their Chemical and Physical Properties

		Solubility (g/100 mL)								Stability									
Common Name and FD&C		Water		Propolyne Glycol		Alcohol		Glycerine		At pH:							250 ppm	1% Ascorbic	1% Sodium
Number	Dye type	25°C	60°C	25°C	60°C	25°C	60°C	25°C	60°C	3.0	5.0	7.0	8.0	Light	10% AcOH	10% NaOH	SO_2	Acid	Benzoate
FDC Blud No. 1	Triphenylmethane	20.0	20.0	20.0	20.0	0.35	0.20	20.0	20.0	4	5	5	5	3	5	4	5	4	6
FDC Blue No. 2	Indigo	1.6	2.2	0.1	0.1	In	0.007	20.0	20.0	3	3	2	1	1	1	2[b2]	1	2	4
FDC Green No. 3	Triphenylmethane	20.0	20.0	20.0	20.0	0.01	0.03	1.0	1.3	4	4	4	4[b1]	3	5	2[b1]	5	4	6
FDC Red No. 3	Xanthine	9.0	17.0	20.0	20.0	In	0.01	20.0	20.0	In	In	6	6	2	In	2	In	In	5
FDC Red No. 40	Azo	22.0	26.0	1.5	1.7	0.001	0.113	3.0	8.0	6	6	6	6	5	5	3[b1]	6	6	6
FDC Yellow No. 5	Azo	20.0	20.0	7.0	7.0	In	0.201	18.0	18.0	6	6	6	6	5	5	4	3	3	6
FDC Yellow No. 6	Azo	19.0	20.0	2.2	2.0	In	0.001	20.0	20.0	6	6	6	6	3	5	5	3	2	6

[a] In, Insoluble.

[b] 1 = fades; 2 = considerable fade; 3 = appreciable fade; 4 = slight fade; 5 = very slight fade; 6 = no change; b1 = hue turns blue; b2 = hue turns yellow.

FD&C Blue No. 1

FD&C Blue No. 2

FD&C Green No. 3

FD&C Red No. 3

FD&C Red No. 40

FD&C Yellow No. 5

FD&C Yellow No. 6

FIGURE 10.40 Structures of certified color additives currently permitted for general use in the United States.

TABLE 10.15
Chemical and Physical Properties of Common EU Dyes

	Solubility (g/100 mL) at 16°C				Stability[a]				
								pH	
Name and EEC Number	Water	Propylene Glycol	Alcohol	Glycerine	Light	Heat	SO_2	3.5/4.0	8.0/9.0
Quinoline yellow, E104	14	<0.1	<0.1	<0.1	6	5	4	5	2
Ponceau 4R, E124	30	4	<0.1	0.5	4	5	3	4	1
Carmoisine, E122	8	1	<0.1	2.5	5	5	4	4	3
Amaranth, E123	5	0.4	<0.1	1.5	5	5	3	4	3
Patent blue, E131	6	2	<0.1	3.5	6	5	3	1	2
Green S, E142	5	2	0.2	1.5	3	5	4	4	3
Chocolate brown HT, E156	20	15	insoluble	5	5	5	3	4	4
Brilliant black BN, E151	5	1	<0.1	<0.5	6	1	1	3	4

[a] 1 = fades; 2 = considerable fade; 3 = appreciable fade; 4 = slight fade; 5 = very slight fade; 6 = no change.

FIGURE 10.41 Synthesis of FD&C Green No. 3 (fast green).

of a sulfonic acid group for a hydroxyl group in either of these dyes improves stability to light and resistance to alkali. Alkali decolorization of a triphenylmethane dye involves formation of a colorless carbinol base (Figure 10.42). The orthosubstituted sulfonic acid group sterically hinders access of the hydroxyl ion to the central carbon atom, thus preventing formation of the carbinol base.

FD&C Red No. 3 is the only approved xanthine-type dye. The structure of FD&C Red No. 3 suggests that the dye is insoluble in acids, quite stable to alkali, and exhibits strong fluorescence.

FIGURE 10.42 Formation of a colorless carbinol base from a triphenylmethane dye.

FIGURE 10.43 Structures of indigoid-type dyes.

The water-insoluble form (lake) of FD&C Red No. 3 is no longer permitted for use in foods because of toxicologic concerns. Although the dye in its water-soluble form is permanently listed, its long-term future is questionable. FD&C Blue No. 2 is the only indigoid-type dye. It is made from indigo, one of the oldest known and most extensively utilized natural pigments. The pigment is derived from various species of the indigo plant found in India. FD&C Blue No. 2 is made by sulfonating indigo, yielding 5,5′-indigotin disulfonate (Figure 10.43). The color is a deep blue, compared to the greenish blue of FD&C Blue No. 1. The dye has the lowest water solubility and poorest light resistance of any of the FD&C dyes, but is relatively resistant to reducing agents.

In general, conditions most likely to cause discoloration or precipitation of certified dyes are the presence of reducing agents or heavy metals, exposure to light, excessive heat, or exposure to acid or alkali. Many of the conditions causing failure of the dyes can be prevented in foods. Reducing agents are most troublesome. Reduction of the chromophores of azo and triphenylmethane dyes is shown in Figure 10.44. Azo dyes are reduced to the colorless hydrazoform or sometimes to the primary amine. Triphenylmethane dyes are reduced to the colorless leuco base. Common reducing agents in foods are monosaccharides (glucose, fructose), aldehydes, ketones, and ascorbic acid.

Free metals can combine chemically with many dyes causing loss of color. Of most concern are iron and copper. The presence of calcium and magnesium can result in the formation of insoluble salts and precipitates.

10.3.3 Use of Certified Colors

There are some practical advantages of the use of synthetic dyes. In general, they have high tinctorial power, so only small amounts are required to provide the desired color, resulting also in low cost. In addition, they have very high stability to processing and storage conditions as compared to their "natural" counterparts. Also, they are available in both water-soluble (dye) and water-insoluble (lake) forms. Better uniformity in incorporating a water-soluble dye in foods is achieved if the dye is first dissolved in water. Distilled water should be used to prevent precipitation. Liquid colors of various strengths can be purchased from manufacturers. Dye concentration in these preparations

Azo dye

$$R{-}N{=}N{-}R' \xrightarrow{[H]} R{-}N(H){-}N(H){-}R' \xrightarrow{[H]} RNH_2 + R'NH_2$$

Color Colorless

Triphenylmethane dye

Color $\underset{[O]}{\overset{[H]}{\rightleftharpoons}}$ Colorless

FIGURE 10.44 Reduction of azo or triphenylmethane dyes to colorless products.

usually does not exceed 3% to avoid overcoloring. Citric acid and sodium benzoate are commonly added to liquid preparations to prevent microbial spoilage.

Many foods contain low levels of moisture making it impossible to completely dissolve and uniformly distribute a dye. The result is a weak color and/or a speckled effect. This is a potential problem in hard candy that has a moisture content of <1%. The problem is averted by using solvents other than water, such as glycerol or propylene glycol (Tables 10.14 and 10.15). A second approach to overcoming problems of poor dispersion of dyes in foods with low moisture contents is the use of "lakes." Lakes exist as dispersions in food rather than in solution. They range in dye content from 1% to 40%. A large dye content does not necessarily lead to intense color. Particle size is of key importance—the smaller the particle size, the finer the dispersion and the more intense the color. Special grinding techniques used by color manufacturers have made it possible to prepare lakes with a mean particle size of less than 1 μm.

As with dyes, predispersion of lakes in glycerol, propylene glycol, or edible oils is often required. Predispersion helps prevent agglomeration of particles and thereby helps develop full color intensity and reduces the incidence of speckled products. Lake dispersions vary in dye content from 15% to 35%. A typical lake dispersion may contain 20% FD&C lake A, 20% FD&C lake B, 30% glycerol, and 30% propylene glycol, resulting in a final dye content of 16%.

Color manufacturers also prepare color pastes or solid cubes of dyes or lakes. A paste is made with the addition, for example, of glycerol as a solvent and powdered sugar to increase viscosity. Colorants in the form of cubes are achieved by adding gums and emulsifiers to color dispersions during the manufacturing process.

The safety of certified colors has received much public attention over the years. The root of the concern has been attributed in part to the unfortunate association of synthetic colors to the original term "coal tar" dyes, with a public perception of unsuitable for use in foods. The fact is that raw materials for synthesis of colors are highly purified before use. The final product is a specific chemical that bears little relationship to the term coal tar. In addition, synthetic food colorants have been increasingly linked with allergies, other concerns related to the presence of impurities or contaminants, and more recently to childhood behavioral problems.

A 2007 study by a group of researchers at Southampton University found a link between a combination of some synthetic food colorants and sodium benzoate (a preservative) in the diet and behavioral problems in children like hyperactivity [144]. As a consequence of this finding, since 2010 the EU has required foods that contain the synthetic colorants (azo dyes) included in that study to carry a label that warns consumers that the food "may have an adverse effect on activity and attention in children." In 2011 the FDA formed a Food Advisory Committee panel to review

FIGURE 10.45 Structure of carminic acid.

the scientific evidence on the link between artificial dyes and hyperactivity. The FDA reviewed the available evidence and concluded that it did not warrant further agency action. Nevertheless, the negative advertisement and more strict labeling regulations for synthetic colorants have resulted in an increasing trend toward the use of colorants from natural sources.

10.3.4 Colors Exempt from Certification

A brief description of each of the colorants listed in Table 10.12 follows:

Annatto extract is the extract prepared from annatto seed, *Bixa orellana* L. Several food-grade solvents can be used for extraction. Supercritical carbon dioxide extraction has been tested as an alternative to using standard organic solvents. This technology, however, has not yet been commercialized. The main pigment in annatto extract is the carotenoid bixin. Upon saponification of bixin (Figure 10.14), the methyl ester group is hydrolyzed and the resulting diacid is called norbixin. Bixin is more lipid soluble, while norbixin is more water soluble.

Dehydrated beet is obtained by dehydrating the juice of edible whole beets. The pigments in beet colorants are betalains including both betacyanins (red) and betaxanthins (yellow). The ratio of betacyanin/betaxanthin will vary depending on the cultivar and maturity of beets. Beet colorant can also be produced under the category of "vegetable juice." This type of beet colorant is obtained by concentrating beet juice under vacuum to a solid content sufficient to prevent spoilage (about 60% solids).

Canthaxanthin (β-carotene-4,4′-dione), *β-apo-8-carotenal*, and *β-carotene* are synthesized carotenoids and are regarded as *nature identical*. Structures of these compounds are shown in Figure 10.14. Both β-carotene and β-apo-8′-carotenal exhibit provitamin A activity.

Caramel is a dark-brown liquid produced by heat-induced caramelization of carbohydrates.

Cochineal extract is the concentrate produced from an aqueous-alcoholic extract of the cochineal insect, *Dactylopius coccus* L. Costa. The coloring is principally due to carminic acid, a red pigment (Figure 10.45). Cochineal extract contains about 2%–3% carminic acid. Colorants with carminic acid concentrations of up to 50% are also produced. These colorants are sold under the name carmine colors. Cochineal extract is neither vegetarian nor kosher.

Sodium copper chlorophyllin is a green to black powder prepared from chlorophyll by saponification and replacement of magnesium by copper (see Section 10.2.2 for more information). Chlorophyll is extracted from alfalfa using acetone, ethanol, and/or hexane. It may be safely used to color citrus-based dry beverage mixes in an amount not exceeding 0.2% in the dry mix.

Toasted, partially defatted, cooked cottonseed flour is prepared as follows: cottonseed is delineated and decorticated; the meats are screened, aspirated, and rolled; moisture is adjusted; the meats are heated and the oil is expressed; and the cooked meats are cooled, ground, and reheated to obtain a product varying in shade from light brown to dark brown.

Ferrous gluconate is a yellowish-gray powder with a slight odor resembling that of burnt sugar.

Ferrous lactate is a greenish-white powder used for the coloring of ripe olives.

Grape skin extract is a purplish-red liquid prepared from an aqueous extract of pomace remaining after grapes have been pressed to remove the juice. The coloring matter of the extract consists mainly of anthocyanins. It is sold under the name "enocianina" and is restricted for coloring noncarbonated and carbonated drinks, beverage bases, and alcoholic beverages.

Grape color extract is an aqueous solution of anthocyanin pigments made from Concord grapes or a dehydrated, water-soluble powder prepared from the aqueous solution. Grape color extract may be used for the coloring of nonbeverage foods as long as it does not affect their standard of identity.

Fruit and vegetable juices are acceptable color additives, and they can be used at single strength or as concentrated liquids. Depending on the source of the juice, pigments from many of the previously described classes can be involved. Beet and grape juice concentrates have been produced and marketed as colorants in this category. Grape juice concentrate, in contrast to grape skin extract, may be used in nonbeverage foods.

Carrot oil is produced by extracting edible carrots with hexane. The hexane is subsequently removed by vacuum distillation. The colorant is mainly β- and α-carotene with other minor carotenoids also present.

Paprika or paprika oleoresin is either the ground dried pods of paprika (*Capsicum annum* L.) or an extract of this plant. In the production of oleoresin, several solvents may be used. The main colorant in paprika is capsanthin (Figure 10.14), a carotenoid.

Mica-based pearlescent pigments are platelets of potassium aluminum silicate (mica) to which titanium dioxide have been deposited by chemical reaction. These pigments exhibit a pearlescent color effect that comes from a combination of partial transmittance, reflection, and interference of light through the platelets.

Riboflavin or vitamin B2 is an orange-yellow powder and is a native pigment in milk.

Saffron is the dried stigma of *C. sativus* L. Its yellow color is attributable to crocin, the digentiobioside of crocetin.

Spirulina extract. The color additive spirulina extract is prepared by the filtered aqueous extraction of the dried biomass of *Arthrospira platensis*, a cyanobacteria. The color additive contains phycocyanins as the principal coloring components and can be used to provide blue to green colors to confectionary products.

Titanium oxide is the whitest pigment known to date. It often contains silicon dioxide and/or aluminum oxide to aid dispersion in foods. These diluents may not exceed 2% of the total.

Tomato lycopene extract and *tomato lycopene concentrate*. These pigments are red to dark-brown pigments extracted with ethyl acetate from tomato pulp followed by removal of the solvent by evaporation. The resulting material can be used as a viscous oleoresin (lycopene extract) or in a powder form (lycopene concentrate). The main coloring component is red carotenoid pigment lycopene (Figure 10.14).

Turmeric and turmeric oleoresin are the ground rhizomes or an extract of turmeric (*Curcuma longa* L.). The coloring matter in turmeric is curcumin. Several organic solvents may be used in the production of turmeric oleoresin.

Other colorants exempt from certification (astaxanthin, ultramarine blue, *Haematococcus* spp. algae, iron oxide, dried algae meal [dried algae cells of the genus *Spongiococcum*], tagetes meal [dried ground flower petals of the Aztec marigold, *Tagetes erecta* L.], corn endosperm oil, Paracoccus pigment and *Phaffia* yeast) are of little interest here since these colorants are restricted to use in animal feeds. They can, however, indirectly affect the color of foods.

The labeling declaration of added colors that are exempt from certification is somewhat controversial. Although colors exempt from certification are obtained from natural sources or are nature

identical, they must be listed as *artificial color added*, or with a similar description. This is required because, in the vast majority of uses, the colorant added is not natural to the food product. Similar to certified colors, colors exempt from certification must be declared when used in foods in the United States.

10.3.5 Use of Colors Exempt from Certification

With the exception of synthetic nature-identical pigments, exempt-from-certification colorants are chemically crude preparations. They are either totally unpurified materials or crude plant or animal extracts. Because of their impurity, relatively large amounts are needed to achieve the desired color. This has caused some to suggest that these pigments lack tinctorial strength and contribute undesirable flavors to a product. Neither criticism is necessarily true. Many pure natural pigments have high tinctorial strengths. This can be illustrated by comparing the absorptivity values of a 1% solution of a natural pigment with that of a synthetic dye. At wavelengths of maximum light absorption, the $A^{1\%}{}_{1cm}$ values for FD&C Red No. 40 and Yellow No. 6 are 586 and 569, respectively, whereas the $A^{1\%}{}_{1cm}$ for betanin, the main pigment component in beet powder, and β-carotene are 1120 and 2400, respectively. Furthermore, most pure pigments do not contribute to product flavor. The lack of tinctorial strength and the possible contribution to flavor by the unpurified natural colorants can be overcome by applying available technologies of separation and purification. Unfortunately, some of these advances in technology have not been sanctioned.

The demand for healthier and more wholesome foods has also resulted in an increased demand of colorants from natural sources. Health benefits associated with many naturally occurring pigments make them attractive alternatives to synthetic colors. This, combined with legislative action and consumer awareness, has led to an increased interest in the use of colors from natural sources.

REFERENCES

1. Adams J (1973). Colour stability of red fruits. *Food Manuf 48*: 19–20.
2. Ahmadiani N (2012). Anthocyanin based blue colorants. MS thesis, The Ohio State University, Columbus, OH.
3. Ahmadiani N, Giusti M (2015). Unpublished data.
4. Ahmed J, Shivhare U, Raghavan G (2004). Thermal degradation kinetics of anthocyanin and visual color of plum puree. *Eur Food Res Technol 218*: 525–528.
5. Andersen O, Jordheim M (2014). Basic anthocyanin chemistry and dietary sources. In Wallace TC, Giusti MM (eds.), *Anthocyanins in Health and Disease*, pp. 13–113. CRC Press, Boca Raton, FL.
6. Aparicio-Ruiz R, Riedl KM, Schwartz SJ (2011). Identification and quantification of metallo-chlorophyll complexes in bright green table olives by high-performance liquid chromatography-mass spectrometry quadrupole/time-of-flight. *J Agric Food Chem 59*: 11100–11108.
7. Asenstorfer R, Iland P, Tate M, Jones G (2003). Charge equilibria and pKa of malvidin-3-glucoside by electrophoresis. *Anal Biochem 318*: 291–299.
8. Attoe EL, von Elbe J (1981). Photochemial degradation of betanine & selected anthocyanins. *J Food Sci 46*: 1934–1937.
9. Attoe EL, von Elbe JH (1985). Oxygen involvement in betanine degradation: effect of antioxidants. *J Food Sci 50*: 106–110.
10. Awika J, Rooney L, Waniska R (2004). Properties of 3-deoxyanthocyanins from sorghum. *J Agric Food Chem 52*: 4388–4394.
11. Ben Aziz A, Grossman S, Ascarelli I, Budowski P (1971). Carotene-bleaching activities of lipoxygenase and heme proteins as studied by a direct spectrophotometric method. *Phytochemistry 10*: 1445–1452.
12. Bacon MF, Holden M (1967). Changes in chlorophylls resulting from various chemical and physical treatments of leaves and leaf extracts. *Phytochemistry 6*: 193–210.
13. Bakker J, Timberlake C (1997). Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. *J Agric Food Chem 45*: 35–43.

14. Barrows J, Lipman A (2003). Color additives: FDA's regulatory process and historical perspectives. *Food Saf Mag* Volume: Oct/Nov 2003.
15. Bauernfeind J (1972). Carotenoid vitamin A precursors and analogs in food and feeds. *J Agric Food Chem 20*: 455–473.
16. Bilyk A, Kolodij MA, Sapers GM (1981). Stabilization of red beet pigments with isoascorbic. *J Food Sci 46*: 1616–1617.
17. Blair J (1940). Color stabilization of green vegetables. U.S. Patent 2,186,003, March 3, 1937.
18. Borkowski T, Szymusiak H, Gliszczynska-Swiglo A, Tyrakowska B (2005). The effect of 3-*O*-β-glucosylation on structural transformations of anthocyanidins. *Food Res Int 38*: 1031–1037.
19. Brenes P, Duran MG, Garrido A (1993). Concentration of phenolic compounds in storage brines or ripe olives. *J Food Sci 58*: 347–350.
20. Brewer S (2004). Irradiation effects on meat color—A review. *Meat Sci 68*: 1–17.
21. Bridle P, Scott K, Timberlake C (1973). Anthocyanins in Salix species. A new anthocyanin in *Salix purpurea* bark. *Phytochemistry 12*: 1103–1106.
22. Britton G, Weesie RJ, Askin D, Warburton JD, Gallardo-Guerrero L, Jansen FJ, de Groot HJM, Lugtenburg J, Cornard J-P, Merlin J-C (1997). Carotenoid blues: Structural studies on carotenoproteins. *Pure Appl Chem 69*: 2075–2084.
23. Brouillard R (1982). Chemical structures of anthocyanins. In Markakis P (ed.), *Anthocyanins as Food Colors*, pp. 1–40. Academic Press, New York.
24. Brouillard R, Delaporte B (1977). Chemistry of anthocyanin pigments. 2. Kinetic and thermodynamic study of proton transfer, hydration, and tautomeric reactions of malvidin 3-glucoside. *J Am Chem Soc 99*: 8461–8468.
25. Büchert AM, Civello PM, Martínez GA (2011). Chlorophyllase versus pheophytinase as candidates for chlorophyll dephytilation during senescence of broccoli. *J Plant Physiol 168*: 337–343.
26. Buchler J (1975) Static coordination chemistry of metalloporphyrins. In Smith KM (ed.), *Porphyrins and Metalloporphyrins*, pp. 157–278. Elsevier, Amsterdam, the Netherlands.
27. Buchweitz M, Carle R, Kammerer DR (2012). Bathochromic and stabilising effects of sugar beet pectin and an isolated pectic fraction on anthocyanins exhibiting pyrogallol and catechol moieties. *Food Chem 135*: 3010–3019.
28. Buckle K, Edwards R (1970). Chlorophyll, colour and pH changes in H.T.S.T. processed green pea puree. *Int J Food Sci Technol 5*: 173–186.
29. Van Buren J, Bertino J, Robinson W (1968). The stability of wine anthocyanins on exposure to heat and light. *Am J Enol Vitic 19*: 147–154.
30. Burton GW (1989). Antioxidant action of carotenoids. *J Nutr 119*: 109–111.
31. Burton G, Ingold K (1984). Beta-carotene: An unusual type of lipid antioxidant. *Science 224*: 569–573.
32. Cai Y, Sun M, Corke H (2005). Characterization and application of betalain pigments from plants of the Amaranthaceae. *Trends Food Sci Technol 16*: 370–376.
33. Cai Y, Sun M, Schliemann W, Corke H (2001). Chemical stability and colorant properties of betaxanthin pigments from *Celosia argentea*. *J Agric Food Chem 49*: 4429–4435.
34. Camara B, Hugueney P, Bouvier F, Kuntz M, Moneger R (1995). Biochemistry and molecular biology of chromoplast development. *Int Rev Cytol 163*: 175–247.
35. Canfield LM, Krinsky NI, Olson JA (1993). *Carotenoid in Human Health*, Vol. 691. New York Academy of Sciences, New York.
36. Canjura FL, Schawrtz SJ, Nunes RV (1991). Degradation kinetics of chlorophylls and chlorophyllides. *J Food Sci 56*: 1639–1643.
37. Castenmiller JJM, West CE, Linssen JPH, van het Hof KH, Voragen AGJ (1999). The food matrix of spinach is a limiting factor in determining the bioavailability of beta-carotene and to a lesser extent of lutein in humans. *J Nutr 129*: 349–355.
38. Chen L, Giusti M (2015). Effect of catechin copigmentation and ascorbic acid fortification on color stability of black carrot and chokeberry anthocyanins under heat treatment. In *Institute of Food Technologists Annual Meeting*, Chicago, IL.
39. Cianci M, Rizkallah PJ, Olczak A, Raftery J, Chayen NE, Zagalsky PF, Helliwell JR (2002). The molecular basis of the coloration mechanism in lobster shell: Beta-crustacyanin at 3.2-Å resolution. *Proc Natl Acad Sci 99*: 9795–9800.
40. Clydesdale F, Fleischmann D, Francis F (1970). Maintenance of color in processed green vegetables. *Food Prod Dev 4*: 127–138.
41. Clydesdale F, Lin Y, Francis F (1972). Formation of 2-pyrrolidone-5-carboxylic acid from glutamine during processing and storage of spinach puree. *J Food Sci 37*: 45–47.

42. Cohen E, Saguy I (1983). Effect of water activity and moisture content on the stability of beet powder pigments. *J Food Sci 48*: 703–707.
43. Cooperstone JL, Ralston RA, Riedl KM, Haufe TC, Schweiggert RM, King SA, Timmers CD et al. (2015). Enhanced bioavailability of lycopene when consumed as *cis*-isomers from tangerine compared to red tomato juice, a randomized, cross-over clinical trial. *Mol Nutr Food Res 59*: 658–669.
43a. Cooperstone JL, Schwartz SJ (2016). Recent insights into health benefits of carotenoids. In Carle, R, Schweiggert, RM (Eds.), *Handbook on Natural Pigments in Food and Beverages: Industrial Applications for Improving Food Color*. Woodhead Publishing. p. 473–497
43b. Cooperstone JL, Francis DM, Schwartz SJ (2016). Thermal processing differentially affects lycopene and other carotenoids in *cis*-lycopene containing, tangerine tomatoes. *Food Chem* 210: 466–472.
44. Craft NE (2005). Chromatographic techniques for carotenoid separation. In Wrolstad R, Acree E, Decker E, Penner M, Reid D, Schwartz S, Shoemaker C, Smith D, Sporns P (eds.), *Current Protocols in Food Analytical Chemistry*, Chapter F2.3. John Wiley & Sons, Hoboken, NJ.
45. Dangles O, Saito N, Brouillard R (1993). Anthocyanin intramolecular copigment effect. *Phytochemistry 34*: 119–142.
46. Daravingas G, Cain R (1965). Changes in anthocyanin pigments of raspberries during processing and storage. *J Food Sci 33*: 400–405.
47. Davidson J (1954). Procedures for the extraction, separation and estimation of the major fat-soluble pigments of hay. *J Sci Food Agric 5*: 1–7.
48. Davies W, Zhang J (1991). Root signals and the regulation of growth and development of plants in drying soil. *Annu Rev Plant Physiol Plant Mol Biol 42*: 55–76.
49. DellaPenna D, Pogson BJ (2006). Vitamin synthesis in plants: Tocopherols and carotenoids. *Annu Rev Plant Biol 57*: 711–738.
50. Downham A, Collins P (2000). Colouring our foods in the last and next millennium. *Int J Food Sci Technol 35*: 5–22.
51. Eisenreich W, Schwarz M, Cartayrade A, Arigoni D, Zenk MH, Bacherl A (1998). The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. *Chem Biol 5*: R221–R233.
52. Von Elbe J, Attoe E (1985). Oxygen involvement in betanine degradation measurement of active oxygen species and oxidation reduction potentials. *Food Chem 16*: 49–67.
53. Von Elbe J (1963). Factors affecting the color stability of cherry pigments and cherry juice. PhD thesis, University of Wisconsin, Madison, WI.
54. Von Elbe JH, Laborde LF (1989). Chemistry of color improvement in thermally processed green vegetables, ACS Symposium Series 405. In Jen JJ (ed.), *Quality Factors of Fruits and Vegetables*, pp. 12–28. American Chemical Society, Washington, DC.
55. Von Elbe JH, Huang AS, Attoe EL, Nank WK (1986). Pigment composition and color of conventional and Veri-Green canned beans. *J Agric Food Chem 34*: 52–54.
56. Emenhiser C, Sander LC, Schwartz SJ (1995). Capability of polymeric C30 stationary phase to resolve *cis-trans* carotenoid isomers in reversed-phase liquid chromatography. *J Chromatogr A 707*: 205–216.
57. Eugster CH (1995) History: 175 years of carotenoid chemistry. In Britton G, Liaaen-Jensen S, Pfander H (eds.), *Carotenoids*, Volume 1A: Isolation and Analysis, pp. 1–12. Birkhauser Verlag, Basel, Switzerland.
58. Falk J, Phillips J (1964). Physical and coordination chemistry of the tetrapyrrole pigments. In Dwyer F, Mellor D (eds.), *Chelating Agents and Metal Chelates*, pp. 441–490. Academic Press, New York.
59. Faustman C, Cassens RG, Schaefer DM, Buege DR, Williams SN, Scheller KK (1989). Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. *J Food Sci 54*: 858–862.
60. Faustman C, Cassens RG (1990). The biochemical basis for discoloration in fresh meat: A review. *J Muscle Foods 1*: 217–243.
61. Faustman C, Chan WKM, Schaefer DM, Havens A (1998). Beef color update: The role for Vitamin E. *J Anim Sci 76*: 1019–1026.
62. Ferruzzi MG, Schwartz SJ (2005). Chromatographic separation of chlorophylls. In Wrolstad RE, Acree TA, Decker EA, Penner M, Reid D, Schwartz SJ, Shoemaker C, Smith D, Sporns P (eds.), *Handbook of Food and Analytical Chemistry*, p. F4.4. Wiley, Hoboken, NJ.
63. Fishbach H (1943). Microdeterminations for organically combined metal in pigment of okra. *J Assoc Off Agric Chem 26*: 139–143.
64. Fleshman MK, Lester GE, Riedl KM, Kopec RE, Narayanasamy S, Curley RW, Schwartz SJ, Harrison EH (2011). Carotene and novel apocarotenoid concentrations in orange-fleshed *Cucumis melo* melons: Determinations of β-carotene bioaccessibility and bioavailability. *J Agric Food Chem 59*: 4448–4454.

65. Foote CS (1968). Mechanisms of photosensitized oxidation. *Science 162*: 963–970.
66. Forrest J, Aberle E, Hedrick H, Judge M, Merkel R (1975). *Principles of Meat Science*. W.H. Freeman, San Francisco, CA.
67. Forsyth W, Roberts J (1960). Cacao polyphenolic substances. 5. The structure of cacao 'leucocyanidin 1'. *Biochem J 74*: 374–378.
68. Fossen T, Cabrita L, Andersen O (1998). Colour and stability of pure anthocyanins influenced by pH including the alkaline region. *Food Chem 63*: 435–440.
69. Fox Jr J, Ackerman S (1968). Formation of nitric oxide myoglobin: Mechanisms of the reaction with various reductants. *J Food Sci 33*: 364–370.
70. Fox Jr. JB (1966). Chemistry of meat pigments. *J Agric Food Chem 14*: 207–210.
71. Francis FJ (2003). Color analysis. In Nielsen SS (ed.), *Food Analysis*, pp. 529–541. Kluwer Academic/Plenum Publishers, New York.
72. Francis F (1977). Anthocyanins. In Furia T (ed.), *Current Aspects of Food Colorants*, pp. 19–27. CRC Press, Cleveland, OH.
73. Fraser PD, Bramley PM (2004). The biosynthesis and nutritional uses of carotenoids. *Prog Lipid Res 43*: 228–265.
74. Frohlich K, Conrad J, Schmid A, Breithaupt DE, Bohm V (2007). Isolation and structural elucidation of different geometrical isomers of lycopene. *Int J Vitam Nutr Res 77*: 369.
75. Fulcrand H, Benabdeljalil C, Rigaud J, Cheynier V, Moutounet M (1998). A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry 47*: 1401–1407.
76. Gaziano JM, Johnson EJ, Russell RM, Manson JE, Stampfer MJ, Ridker PM, Frei B, Hennekens CH, Krinsky NI (1995). Discrimination in absorption or transport of beta-carotene isomers after oral supplementation with either all-*trans*- or 9-*cis*-beta-carotene. *Am J Clin Nutr 61*: 1248–1252.
77. Gieseker LF (1949). Art of preserving and maintaining color of green vegetables. U.S. Patent 2,473,747, June 21, 1949.
78. Giusti MM, Wallace TC (2009). Flavonoids as natural pigments. In Bechtold T, Mussak R (eds.), *Handbook of Natural Colorants*, pp. 257–275. John Wiley & Sons, West Sussex, U.K.
79. Giusti MM, Wrolstad RE (2003). Acylated anthocyanins from edible sources and their applications in food systems. *Biochem Eng J 14*: 217–225.
80. Giusti MM, Wrolstad RE (1996). Characterization of red radish anthocyanins. *J Food Sci 61*: 322–326.
81. Goodman L, Markakis P (1965). Sulfur dioxide inhibition of anthocyanin degradation by phenolase. *J Food Sci 30*(1): 135–137.
82. Goodwin T (1980). Functions of carotenoids. In Goodwin T (ed.), *The Biochemistry of Carotenoids*, Vol. 1, pp. 77–95. Chapman and Hall, New York.
83. Govindarajan S, Hultin H, Kotula AW (1977). Myoglobin oxidation in ground beef: Mechanistic studies. *J Food Sci 42*: 571–577.
84. Graham RT (1989). Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate, and cooking temperature. *J Food Sci 54*: 536–540.
85. Green B, Durnford D (1996). The chlorophyll-carotenoid proteins of oxygenic photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol 47*: 685–714.
86. Gross J (1991). *Pigments in Vegetables: Chlorophylls and Carotenoids*. Springer, New York.
87. Gupte S, El-Bisi HM, Francis F (1964). Kinetics of thermal degradation of chlorophyll in spinach puree. *J Food Sci 29*: 379–382.
88. Hagler L, Coppes Jr. RI, Herman RH (1979). Identification dependent and enzyme. *J Biol Chem 254*: 6505–6514.
89. Haisman D, Clarke M (1975). The interfacial factor in the heat-induced conversion of chlorophyll to pheophytin in green leaves. *J Sci Food Agric 26*: 1111–1126.
90. Harris WM, Spurr AR (1969). Chromoplasts of tomato fruits. II. The red tomato. *Am J Bot 56*: 380–389.
91. He J, Giusti MM (2010). Anthocyanins: Natural colorants with health-promoting properties. *Annu Rev Food S 1*: 163–187.
92. Herbach KM, Maier C, Stintzing FC, Carle R (2007). Effects of processing and storage on juice colour and betacyanin stability of purple pitaya (*Hylocereus polyrhizus*) juice. *Eur Food Res Technol 224*: 649–658.
93. Herbach KM, Stintzing FC, Carle R (2006). Betalain stability and degradation—Structural and chromatic aspects. *J Food Sci 71*: R41–R50.

94. Herbach KM, Stintzing FC, Carle R, Stintzing F, Carle R (2004). Quantitative and structural changes of betacyanins in red beet (*Beta vulgaris* L.) juice induced by thermal treatment. In Dufose L (ed.), *Pigments in Foods, More Than Colours*, pp. 103–105. Le Berre Imprimeur, Quimper, France.
95. Rabinowitch E, Govindjee (1969). Photosynthesis. John Wiley & Sons, New York, NY.
96. Huang A, von Elbe J (1987). Effect of pH on the degradation and regeneration of betanine. *J Food Sci 52*: 1689–1693.
97. Humphrey BB (1980). Chlorophyll. *Food Chem 5*: 57–67.
98. Hunt MC, Sorheim O, Slinde E (1999). Color and heat denaturation of myoglobin forms in ground beef. *J Food Sci 64*: 847–851.
99. Hyvärinen K, Hynninen P (1999). Liquid chromatographic separation and mass spectrometric identification of chlorophyll b allomers. *J Chromatogr A 837*: 107–116.
100. Im J-S, Parkin KL, von Elbe JH (1990). Endogenous polyphenoloxidase activity associated with the 'black ring' defect in canned beet (*Beta vulgaris* L.). *J Food Sci 55*: 1042–1059.
101. Isler O (1971). *Carotenoids*. Birkhauser Verlag, Basel, Switzerland.
102. IUPAC-IUB (1974). Nomenclature of carotenoids. *Pure Appl Chem 41*: 407–417.
103. Jackman R, Smith J (1996). Anthocyanins and betalains. In Hendry G, Houghton J (eds.), *Natural Food Colorants*, pp. 244–369. Chapman and Hall, New York.
104. Jackson A (1976). Structure, properties and distribution of chlorophyll. In Goodwin T (ed.), *Chemistry and Biochemistry of Plant Pigments*, pp. 1–63. Academic Press, New York.
105. Jahangiri Y, Ghahremani H, Toghabeh JA, Hassani S (2012). The effects of operational conditions on the total amount of anthocyanins extracted from Khorasan's native fig fruit 'Ficus carica'. *Ann Biol Res 3*: 2181–2186.
106. Jen JJ, Mackinney G (1970). On the photodecomposition of chlorophyll in vitro. II. Intermediates and breakdown products. *Photochem Photobiol 11*: 303–308.
107. Johnson J, Clydesdale FM (1982). Perceived sweetness and redness in colored sucrose solutions. *J Food Sci 47*: 3–8.
108. Jones I, White R, Gibbs E (1962). Some pigment changes in cucumbers during brining and brine storage. *Food Technol 16*: 96–102.
109. Jones I, White R, Gibbs E, Butler L, Nelson L (1977). Experimental formation of zinc and copper complexes of chlorophyll derivatives in vegetable tissue by thermal processing. *J Agric Food Chem 25*: 149–153.
110. Jones ID, White RC, Gibbs E, Denard C (1968). Absorption spectra of copper and zinc complexes of pheophytins and pheophorbides. *J Agric Food Chem 16*: 80–83.
111. Kearsley MW, Rodriguez N (1981). The stability and use of natural colours in foods: Anthocyanin, beta-carotene and riboflavin. *J Food Technol 16*: 421–431.
112. Kendrew J, Bodo G, Dintzis H, Parrish R, Wyckoff H (1958). A three-dimensional model of the myoglobin molecule obtained by x-ray analysis. *Nature 181*: 662–666.
113. Kevan P, Baker H (1983). Insects as flower visitors and pollinators. *Annu Rev Entomol 28*: 407–453.
114. Khachik F, Spangler C, Smith J, Canfield L, Steck A, Pfander H (1997). Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. *Anal Chem 69*: 1873–1881.
115. Khachik F, Beecher GR, Goli MB, Lusby WR (1991). Separation, identification, and quantification of carotenoids in fruits, vegetables and human plasma by high performance liquid chromatography. *Pure Appl Chem 63*: 71–80.
116. Killinger KM, Hunt MC, Campbell RE, Kropf DH (2000). Factors affecting premature browning during cooking of store-purchased ground beef. *J Food Sci 65*: 585–587.
117. Kopec RE, Cooperstone JL, Cichon MJ, Schwartz SJ (2012) Analysis methods of carotenoids. In Xu Z, Howard LR (eds.), *Analysis of Antioxidant Rich Phytochemicals*, pp. 105–148. Wiley-Blackwell, Oxford, U.K.
118. Kopec RE, Riedl KM, Harrison EH, Curley RW, Hruszkewycz DP, Clinton SK, Schwartz SJ (2010). Identification and quantification of apo-lycopenals in fruits, vegetables, and human plasma. *J Agric Food Chem 58*: 3290–3296.
119. Krinsky NI (1989). Antioxidant functions of carotenoids. *Free Radic Biol Med 7*: 617–635.
120. Krinsky NI, Mayne ST, Sies H (2004). *Carotenoids in Health and Disease*. Marcel Dekker, New York.
121. Kropf D (1980). Effects of retail display conditions on meat color. *Proc Reciprocal Meat Conf 33*: 15–32.

122. Kuronen P, Hyvarinen K, Hynninen PH, Kilpelainen I (1993). High-performance liquid chromatographic separation and isolation of the methanolic allomerization products of chlorophyll *a*. *J Chromatogr A 654*: 93–104.
123. LaBorde LF, von Elbe JH (1990). Zinc complex formation in heated vegetable purees. *J Agric Food Chem 38*: 484–487.
124. LaBorde LF, von Elbe JH (1994). Chlorophyll degradation and zinc complex formation with chlorophyll derivatives in heated green vegetables. *J Agric Food Chem 42*: 1100–1103.
125. LaBorde LF, von Elbe JH (1994). Effect of solutes on zinc complex formation in heated green vegetables. *J Agric Food Chem 42*: 1096–1099.
126. Lawrie R, Ledward D (2006). *Meat Science*. CRC Press, New York.
127. Liong EC, Dou Y, Scott EE, Olson JS, Phillips Jr. GN (2001). Waterproofing the heme pocket: Role of proximal amino acid side chains in preventing hemin loss from myoglobin. *J Biol Chem 276*: 9093–9100.
128. Liu X, Gao Y, Peng X, Yang B, Xu H, Zhao J (2008). Inactivation of peroxidase and polyphenol oxidase in red beet (*Beta vulgaris* L.) extract with high pressure carbon dioxide. *Innov Food Sci Emerg Technol 9*: 24–31.
129. Livingston D, Brown W (May 1981). The chemistry of myoglobin and its reactions [Meat pigments, food quality indices]. *Food Technol 35*(5): 238–252.
130. Llewellyn CA, Mantoura RFC, Brereton RG (1990). Products of chlorophyll photodegradation—1. Detection and separation. *Photochem Photobiol 52*: 1037–1041.
131. Llewellyn CA, Mantoura RFC, Brereton RG (1990). Products of chlorophyll photodegradation—2. Structural identification. *Photochem Photobiol 52*: 1043–1047.
132. Loef H, Thung S (1965). Ueber den Einfluss von Chlorophyllase auf die Farbe von Spinat waehrend und nach der Verwertung. *Z Leb Unters Forsch 126*: 401–406.
133. Van Loey A, Ooms V, Weemaes C, van den Broeck I, Ludikhuyze L, Denys S, Hendrickx M (1998). Thermal and pressure-temperature degradation of chlorophyll in broccoli (*Brassica oleracea* L. *italica*) juice: A kinetic study. *J Agric Food Chem 46*: 5289–5294.
134. Luh B, Leonard S, Patel D (1960). Pink discoloration in canned Bartlett pears. *Food Technol 14*: 53–56.
135. Luño M, Roncalés P, Djenane D, Beltrán JA (2000). Beef shelf life in low O_2 and high CO_2 atmospheres containing different low CO concentrations. *Meat Sci 55*: 413–419.
136. Madhavi DL, Carpenter CE (1993). Aging and processing affect color, metmyoglobin reductase and oxygen consumption of beef muscles. *J Food Sci 58*: 939–947.
137. Malecki G (1965). Blanching and canning process for green vegetables. U.S. Patent 3,183,102, May 11, 1965.
138. Malecki G (1978). Processing of green vegetables for color retention in canning. U.S. Patent 4,104,410, August 1, 1978.
139. Markakis P (1982) Stability of anthocyanins in foods. In Markakis P (ed.), *Anthocyanins as Food Colors*, pp. 163–180. Academic Press, New York.
140. Markakis P, Livingstone G, Fellers R (1957). Quantitative aspects of strawberry-pigment degradation. *Food Res 22*: 117–130.
141. Martinez-Parra J, Munoz R (2001). Characterization of betacyanin oxidation catalyzed by a peroxidase from *Beta vulgaris* L. roots. *J Agric Food Chem 49*: 4064–4068.
142. Di Mascio P, Kaiser S, Sies H (1989). Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys 274*: 532–538.
143. Mazza G, Miniati E (1993). *Anthocyanins in Fruits, Vegetables, and Grains*. CRC Press, Boca Raton, FL.
144. McCann D, Barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw K, Kitchin E et al. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: A randomised, double-blinded, placebo-controlled trial. *Lancet 370*: 1560–1567.
145. McFeeters RF (1975). Substrate specificity of chlorophyllase. *Plant Physiol 55*: 377–381.
146. Mendenhall VT (1989). Effect of pH and total pigment concentration on the internal color of cooked ground beef patties. *J Food Sci 54*: 1–2.
147. Merritt J, Loening K (1979). Nomenclature of tetrapyrroles. *Pure Appl Chem 51*: 2251–2304.
148. Mikkelsen A, Juncher D, Skibsted LH (1999). Metmyoglobin reductase activity in porcine *m. longissimus dorsi* muscle. *Meat Sci 51*: 155–161.
149. Mohr H (1980). Light and pigments. In Czygan F-C (ed.), *Pigments in Plants*, pp. 7–30. Gustav Fischer Verlag, Stuttgart, Germany.
150. Moran NA, Jarvik T (2010). Lateral transfer of genes from fungi underlies carotenoid production in aphids. *Science (80-) 328*: 624–627.

151. Moßhammer MR, Rohe M, Stintzing FC, Carle R (2007). Stability of yellow-orange cactus pear (*Opuntia ficus-indica* [L.] Mill. cv. 'Gialla') betalains as affected by the juice matrix and selected food additives. *Eur Food Res Technol 225*: 21–32.
152. Nagao A, Olson JA (1994). Enzymatic formation of 9-cis, 13-cis and all-trans retinals from isomers of beta-carotene. *FASEB J 8*: 968–973.
153. Nakatani H, Barber J, Forrester J (1978). Surface charges on chloroplast membranes as studied by particle electrophoresis. *Biochim Biophys Acta 504*: 215–225.
154. Okayama T (1987). Effect of modified gas atmosphere packaging after dip treatment on myoglobin and lipid oxidation of beef steaks. *Meat Sci 19*: 179–185.
155. Oram N, Laing DG, Hutchinson I, Owen J, Rose G, Freeman M, Newell G (1995). The influence of flavor and color on drink identification by children and adults. *Dev Psychobiol 28*: 239–246.
156. Osborn HM, Brown H, Adams JB, Ledward DA (2003). High temperature reduction of metmyoglobin in aqueous muscle extracts. *Meat Sci 65*: 631–637.
157. Palozza P, Krinsky NI (1992). Antioxidant effects of carotenoid in vivo and in vitro: An overview. *Methods Enzymol 213*: 403–420.
158. Parry AD, Horgan R (1992). Abscisic acid biosynthesis in roots. I. The identification of potential abscisic acid precursors, and other carotenoids. *Planta 187*: 185–191.
159. Pasch JH, von Elbe J (1975). Betanine as influenced degradation by water activity. *J Food Sci 40*: 1145–1146.
160. Patras A, Brunton NP, O'Donnell C, Tiwari B (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends Food Sci Technol 21*: 3–11.
161. Pegg R, Shahidi F, Fox Jr. J (1997). Unraveling the chemical identity of meat pigments. *Crit Rev Food Sci Nutr 37*: 561–589.
162. Peiser GD, Yang SF (1979). Sulfite-mediated destruction of beta-carotene. *J Agric Food Chem 27*: 446–449.
163. Peng C, Markakis P (1963). Effect of prenolase on anthocyanins. *Nature 199*: 597–598.
164. Pennington F, Strain H, Svec W, Katz JJ (1963). Preparation and properties of pyrochlorophyll *a*, methyl pyrochlorophyllide *a*, pyropheophytin *a*, and methyl pyropheophorbide *a* derived from chlorophyll by decarbomethoxylation. *J Am Chem Soc 3801*: 1418–1426.
165. Peto R, Doll R, Buckley J, Sporn M (1981). Can dietary beta-carotene materially reduce human cancer rates? *Nature 290*: 201–208.
166. Pfister S, Meyer P, Steck A, Pfander H (1996). Isolation and structure elucidation of carotenoid–glycosyl esters in gardenia fruits (*Gardenia jasminoides* Ellis) and saffron (*Crocus sativus* Linne). *J Agric Food Chem 44*: 2612–2615.
167. Piattelli M, Minale L, Nicolaus R (1965). Pigments of centrospermae—V. Betaxanthins from *Mirabilis jalapa* L. *Phytochemistry 4*: 817–823.
168. Piattelli M, Minale L, Prota G (1964). Isolation, structure and absolute configuration of indicaxanthin. *Tetrahedron 20*: 2325–2329.
169. Piattelli M, Minale L, Nicolaus R (1965). Pigments of centrospermae—III. Betaxanthins from *Beta vulgaris* L. *Phytochemistry 4*: 121–125.
170. Pumilia G, Cichon MJ, Cooperstone JL, Giuffrida D, Dugo G, Schwartz SJ (2014). Changes in chlorophylls, chlorophyll degradation products and lutein in pistachio kernels (*Pistacia vera* L.) during roasting. *Food Res Int 65*: 193–198.
171. Rhim J (2002). Kinetics of thermal degradation of anthocyanin pigment solutions driven from red flower cabbage. *Food Sci Biotechnol 11*: 361–364.
172. Ryan-Stoneham T, Tong CH (2000). Degradation kinetics of chlorophyll in peas as a function of pH. *J Food Sci 65*: 1296–1302.
173. Saito N, Tatsuzawa F, Miyoshi K, Shigihara A, Honda T (2003). The first isolation of C–glycosylanthocyanin from the flowers of *Tricyrtis formosana*. *Tetrahedron Lett 44*: 6821–6823.
174. Sante V, Renerre M, Lacourt A (1994). Effect of modified atmosphere packaging on color stability and on microbiology of turkey breast meat. *J Food Qual 17*: 177–195.
175. Schaber PM, Hunt JE, Fries R, Katz JJ (1984). High-performance liquid chromatographic study of the chlorophyll allomerization reaction. *J Chromatogr 316*: 25–41.
176. Schanderl SH, Chichester C, Marsh G (1962). Degradation of chlorophyll and several derivatives in acid solution. *J Org Chem 27*: 3865–3868.
177. Schanderl SH, Marsh G, Chichester C (1965). Color reversion in processed I. Studies on regreened vegetables pea purges. *J Food Sci 30*: 312–316.

178. Schelbert S, Aubry S, Burla B, Agne B, Kessler F, Krupinska K, Hörtensteiner S (2009). Pheophytin pheophorbide hydrolase (pheophytinase) is involved in chlorophyll breakdown during leaf senescence in *Arabidopsis*. *Plant Cell 21*: 767–785.
179. Schoefs B (2002). Chlorophyll and carotenoid analysis in food products. Properties of the pigments and methods of analysis. *Trends Food Sci Technol 13*: 361–371.
180. Schwartz SJ, von Elbe J (1983). Kinetics of chlorophyll degradation to pyropheophytin in vegetables. *J Food Sci 48*: 1303–1306.
181. Schwartz S, Lorenzo T (1991). Chlorophyll stability during continuous aseptic processing and storage. *J Food Sci 56*: 1059–1062.
182. Schwartz SJ, Elbe JH (1983). Identification of betanin degradation products. *Z Leb Unters Forsch 176*: 448–453.
183. Schwartz SJ, Woo SL, von Elbe JH (1981). High-performance liquid chromatography of chlorophylls and their derivatives in fresh and processed spinach. *J Agric Food Chem 29*: 533–535.
184. Schwarz M, Quast P, von Baer D, Winterhalter P (2003). Vitisin A content in Chilean wines from *Vitis vinifera* Cv. Cabernet Sauvignon and contribution to the color of aged red wines. *J Agric Food Chem 51*: 6261–6267.
185. Schweiggert RM, Carle R (2016). Carotenoid deposition in plant and animal foods and its impact on bioavailability. *Crit Rev Food Sci Nutr.* Epub ahead of print.
186. Schweiggert RM, Kopec RE, Villalobos-Gutierrez MG, Högel J, Quesada S, Esquivel P, Schwartz SJ, Carle R (2014). Carotenoids are more bioavailable from papaya than from tomato and carrot in humans: A randomised cross-over study. *Br J Nutr 111*: 490–498.
187. Schweiggert RM, Steingass CB, Heller A, Esquivel P, Carle R (2011). Characterization of chromoplasts and carotenoids of red- and yellow-fleshed papaya (*Carica papaya* L.). *Planta 234*: 1031–1044.
188. Seely G (1996). The structure and chemistry of functional groups. In Vernon L, Seely G (eds.), *The Chlorophylls*, pp. 67–109. Academic Press, New York.
189. Segner W, Ragusa T, Nank W, Hayle W (1984). Process for the preservation of green color in canned vegetables. European Patent 0112178 A2, June 27, 1984.
190. Seideman SC, Durland PR (1984). The utilization of modified gas atmosphere packaging for fresh meat: A review. *J Food Qual 6*: 239–252.
191. Serris GS, Biliaderis CG (2001). Degradation kinetics of beetroot pigment encapsulated in polymeric matrices. *J Sci Food Agric 81*: 691–700.
192. Shi Z, Lin M, Francis F (1992). Stability of anthocyanins from *Tradescantia pallida*. *J Food Sci 57*: 758–770.
193. Shrikhande A, Francis F (1974). Effect of flavonols on ascorbic acid and anthocyanin stability in model systems. *J Food Sci 39*: 904–906.
194. Sies H, Stahl W, Sundquist AR (1992). Antioxidant functions of vitamins. Vitamins E and C, beta-carotene, and other carotenoids. *Ann N Y Acad Sci 669*: 7–20.
195. Sigurdson G, Giusti M (2015). Unpublished data.
196. Sigurdson GT, Giusti MM (2014). Bathochromic and hyperchromic effects of aluminum salt complexation by anthocyanins from edible sources for blue color development. *J Agric Food Chem 62*: 6955–6965.
197. Simons MJP, Cohen AA, Verhulst S (2012). What does carotenoid-dependent coloration tell? Plasma carotenoid level signals immunocompetence and oxidative stress state in birds—A meta-analysis. *PLoS One 7*: e43088.
198. Sitte P, Falk H, Liedvogel B (1980). Chromoplasts. In Czygan F-C (ed.), *Pigments in Plants*, pp. 117–148. Gustav Fischer Verlag, Stuttgart, Germany.
199. Stafford HA (1994). Anthocyanins and betalains: Evolutions of the mutually exclusive pathways. *Plant Sci 101*: 91–98.
200. Starr M, Francis F (1968). Oxygen and ascorbic acid effect on the relative stability of four anthocyanins pigments in cranberry juice. *Food Technol 22*: 1293–1295.
201. Stintzing FC, Carle R (2007). Betalains—Emerging prospects for food scientists. *Trends Food Sci Technol 18*: 514–525.
202. Stintzing FC, Carle R (2004). Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends Food Sci Technol 15*: 19–38.
203. Stintzing FC, Schieber A, Carle R (2002). Identification of betalains from yellow beet (*Beta vulgaris* L.) and cactus pear [*Opuntia ficus-indica* (L.) Mill.] by high-performance liquid chromatography—Electrospray ionization mass spectrometry. *J Agric Food Chem 50*: 2302–2307.

204. Strack D, Vogt T, Schliemann W (2003). Recent advances in betalain research. *Phytochemistry 62*: 247–269.
205. Strain HH, Thomas MR, Katz JJ (1963). Spectra absorption properties of ordinary and fully deuteriated chlorophylls *a* and *b*. *Biochim Biophys Acta 75*: 306–311.
206. Struck A, Cmiel E, Schneider S, Scheer H (1918). Photochemical ring-opening in meso-chlorinated chlorophylls. *Photochem Photobiol 01*: 217–222.
207. Sweeney JP, Martin M (1958). Determination of chlorophyll and pheophytin in broccoli heated by various procedures. *J Food Sci 23*: 635–647.
208. Sweeny J, Wilkinson M, Iacobucci G (1981). Effect of flavonoid sulfonates on the photobleaching of anthocyanins in acid solution. *J Agric Food Chem 29*: 563–567.
209. Swirski MA, Allouf R, Guimard A, Cheftel H (1969). Water-soluble, stable green pigment, originating during processing of canned brussels sprouts picked before the first autumn frosts. *J Agric Food Chem 17*: 799–801.
210. Teng S, Chen B (1999). Formation of pyrochlorophylls and their derivatives in spinach leaves during heating. *Food Chem 65*: 367–373.
211. Terahara N, Oda M, Matsui T, Osajima Y, Saito N, Toki K, Honda T (1996). Five new anthocyanins, ternatins A3, B4, B3, B2, and D2, from *Clitoria ternatea* flowers. *J Nat Prod 59*: 139–144.
212. Tewari G, Jayas D. (2001). Prevention of transient discoloration of beef. *J Food Sci 66*: 506–510.
213. Timberlake CF, Bridle P (1967). Flavylium salts, anthocyanidins and anthocyanins. II. Reactions with sulphur dioxide. *J Sci Food Agric 18*: 479–485.
214. Tonucci LH, von Elbe JH (1992). Kinetics of the formation of zinc complexes of chlorophyll derivatives. *J Agric Food Chem 40*: 2341–2344.
215. Torskangerpoll K, Andersen OM (2005). Colour stability of anthocyanins in aqueous solutions at various pH values. *Food Chem 89*: 427–440.
216. Unlu NZ, Bohn T, Francis DM, Nagaraja HN, Clinton SK, Schwartz SJ (2007). Lycopene from heat-induced cis-isomer-rich tomato sauce is more bioavailable than from all-trans-rich tomato sauce in human subjects. *Br J Nutr 98*: 140–146.
217. Vasquez-Caicedo AL, Heller A, Neidhart S, Carle R (2006). Chromoplast morphology and beta-carotene accumulation during postharvest ripening of Mango Cv. 'Tommy Atkins'. *J Agric Food Chem 54*: 5769–5776.
218. Wakamatsu J, Nishimura T, Hattori A (2004). A Zn-porphyrin complex contributes to bright red color in Parma ham. *Meat Sci 67*: 95–100.
219. Wang X-D (2009) Biological activities of carotenoid metabolites. In Britton G, Liaaen-Jensen S, Pfander H (eds.), *Carotenoids Volume 5: Nutrition and Health*, pp. 383–408. Birkhauser Verlag, Basel, Switzerland.
220. Wasserman B, Eiberger L, Guilfoy M (1984). Effect of hydrogen peroxide and phenolic compounds on horseradish peroxidase-catalyzed decolorization of betalain pigments. *J Food Sci 49*: 536–538.
221. Watanabe T, Nakazato M, Mazaki H, Hongu A, Konno M, Saitoh S, Honda K (1985). Chlorophyll *a* epimer and pheophytin *a* in green leaves. *Biochim Biophys Acta 807*: 110–117.
222. Weast C, Mackinney G (1940). Chlorophyllase. *J Biol Chem 133*: 551–558.
223. Weemaes CA, Ooms V, van Loey AM, Hendrickx ME (1999). Kinetics of chlorophyll degradation and color loss in heated broccoli juice. *J Agric Food Chem 47*: 2404–2409.
224. Wettasinghe M, Bolling B, Plhak L, Xiao H, Parkin K (2002). Phase II enzyme-inducing and antioxidant activities of beetroot (*Beta vulgaris* L.) extracts from phenotypes of different pigmentation. *J Agric Food Chem 50*: 6704–6709.
225. Wightman J, Wrolstad R (1996). β-Glucosidase activity in juice-processing enzymes based on anthocyanin analysis. *J Food Sci 61*: 427–440.
226. Williams C, Greenham J, Harborne J, Kong J-M, Chia L-S, Goh N-K, Saito N, Toki K, Tatsuzawa F (2002). Acylated anthocyanins and flavonols from purple flowers of *Dendrobium* cv. 'Pompadour'. *Biochem Syst Ecol 30*: 667–675.
227. Woolley PS, Moir AJ, Hester RE, Keely BJ (1998). A comparative study of the allomerization reaction of chlorophyll *a* and bacteriochlorophyll *a*. *J Chem Soc Perkin Trans 2*: 1833–1840.
228. Wyler H, Mabry TJ, Dreiding AS (1963). Uber die Konstitution des Randenfarbstoffes Betanin. *Helv Chim Acta 23*: 1960–1963.
229. Wyler H, Wilcox M, Dreiding AS (1965). Urnwandlung eines Betacyans in ein Betaxanthin Synthese von Indicaxanthin aus Betanin. *Helv Chim Acta 48*: 361–366.
230. Young A, Phillip D, Lowe G (2004). Carotenoid antioxidant activity. In Krinsky NI, Mayne ST, Sies H (eds.), *Carotenoids in Health and Disease*, pp. 105–126. CRC Press, Boca Raton, FL.

231. Zagalsky P (1985). A study of the astaxanthin-lipovitellin, ovoverdin, isolated from the ovaries of the lobster, *Homarus gammarus* (L.). *Comp Biochem Physiol 8011*: 589–597.
232. Zechmeister L (1949). Stereoisomer provitamins A. In Harris RS, Thimann KV (eds.), *Vitamins and Hormones*, pp. 57–81. Academic Press, Inc., New York.
233. Zechmeister L (1944). *Cis–trans* isomerization and stereochemistry of carotenoids and diphenyl-polyenes. *Chem Rev 34*: 267–344.
234. Zechmeister L (1962). *Cis–Trans Isomeric Carotenoids, Vitamin A and Arylpolyenes*. Academic Press, Inc., New York.
235. Britton G, Liaaen-Jensen, S., Pfander H, eds. (2004). *Carotenoids Handbook*. Birkhauser Verlag, Basel, Switzerland.
236. Britton G, Liaaen-Jensen S, Pfander H, eds. (2009). *Carotenoids*, Vol. 5: Nutrition and Health. Birkhauser Verlag, Basel, Switzerland.
237. AOAC International (2012). Official method 970.64, Carotene and xanthophylls in dried plant materials and mixed feeds. In *Official Methods of Analysis of AOAC International*. AOAC International, Gaithersburg, MD.
238. AOAC International (2012). Official method 941.15, Carotene in fresh plant materials and silage. In *Official Methods of Analysis of AOAC International*. AOAC International, Gaithersburg, MD.
239. Wallace TC, Giusti MM, eds. (2014). *Anthocyanins in Health and Disease*. CRC Press, Boca Raton, FL.
240. Henry BS (1996) Natural food colours. In Hendry GAF, Houghton JD (eds.), *Natural Food Colorants*, pp 40–79. Springer-Science+Business Media, London, U.K.

FURTHER READING

Bauernfeind JB, ed. (1981). *Carotenoids as Colorants and Vitamin A Precursors*. Academic Press, New York.
Bechtold T, Mussak R, eds. (2009). *Handbook of Natural Colorants*. John Wiley & Sons, West Sussex, U.K.
Carle R, Schweiggert RM, eds. (2016). Handbook on Natural Pigments in Food and Beverages, Industrial Applications for Improving Food Color. Woodhead Publishing, Amsterdam, the Netherlands.
Counsell JN, ed. (1981). *Natural Colours for Food and Other Uses*, Applied Science, Essex, U.K.
Francis FJ (1999). *Colorants: Practical Guide for the Food Industry*. Eagan Press Handbook Series. Eagan Press, St. Paul, MN.
Griffiths JC (2005). Coloring foods and beverages. *Food Technol 59*(5): 38–44.
Houghton J, Hendry G, eds. (1996). *Natural Food Colorants*. Springer-Science+Business Media, Berlin, Germany.
Lauro GJ, Francis FJ, eds. (2000). *Natural Food Colorants*. IFT Basic Symposium Series. Marcel Dekker, Inc., New York.
Mazza G, Miniati E (1993). *Anthocyanins in Fruits, Vegetables and Grains*. CRC Press, Boca Raton, FL.
Schwartz SJ, Lorenzo TV (1990). *Chlorophylls in Foods. Food Science and Nutrition*. CRC Press, Boca Raton, FL.
Socaciu C, ed. (2008). *Food Colorants, Chemical and Functional Properties*. CRC Press, Boca Raton, FL.
Wallace TC, Giusti MM, eds. (2014). *Anthocyanins in Health and Disease*. CRC Press, Boca Raton, FL.
Wrolstad RE, Acree TE, Decker EA, Penner MH, Ried DS, Schwartz SJ, Shoemaker CF, Smith D, Sporns P, eds. (2005). Pigments and colorants. In *Handbook of Food Analytical Chemistry*, pp. 1–216. John Wiley & Sons, Hoboken, NJ.
Xu Z, Howard LR, eds. (2012). *Analysis of Antioxidant Rich Phytochemicals*. Wiley-Blackwell, West Sussex, U.K.

11 Flavors

Robert C. Lindsay

CONTENTS

11.1 Introduction 754
- 11.1.1 General Philosophy 754
- 11.1.2 Methods for Flavor Analysis 754
- 11.1.3 Sensory Assessment of Flavors 755
- 11.1.4 Molecular Mechanisms of Flavor Perception 756

11.2 Taste and Other Saporous Substances 757
- 11.2.1 Sweet Taste Substances 757
- 11.2.2 Bitter Taste Substances 759
- 11.2.3 Salty Taste Substances 765
- 11.2.4 Sour Taste Substances 766
- 11.2.5 Umami Taste Substances 766
- 11.2.6 Kokumi Taste Substances and Other Flavor Modifiers 767
- 11.2.7 Pungent Substances 769
- 11.2.8 Cooling Substances 770
- 11.2.9 Astringent Substances 771

11.3 Vegetable, Fruit, and Spice Flavors 771
- 11.3.1 Sulfur-Containing Volatiles in *Allium* sp. 772
- 11.3.2 Sulfur-Containing Volatiles in the Cruciferae 774
- 11.3.3 Unique Sulfur Compound in Shiitake Mushrooms 775
- 11.3.4 Methoxy Alkyl Pyrazine Volatiles in Vegetables 775
- 11.3.5 Enzymatically Derived Volatiles from Fatty Acids 776
  - 11.3.5.1 Lipoxygenase-Derived Flavors in Plants 776
  - 11.3.5.2 Volatiles from β-Oxidation of Long-Chain Fatty Acids 776
- 11.3.6 Volatiles from Branched-Chain Amino Acids 777
- 11.3.7 Flavors Derived from the Shikimic Acid Pathway 779
- 11.3.8 Volatile Terpenoids in Flavors 779
- 11.3.9 Citrus Flavors 780
- 11.3.10 Flavors of Herbs and Spices 782

11.4 Flavors from Lactic Acid–Ethanol Fermentations 782

11.5 Flavor Volatiles from Fats and Oils 785
- 11.5.1 Flavors from the Hydrolysis of Fats and Oils 785
- 11.5.2 Distinctive Flavors from Long-Chain Polyunsaturated Fatty Acids 785

11.6 Flavor Volatiles in Muscle Foods and Milk 787
- 11.6.1 Species-Related Flavors of Meats and Milk from Ruminants 787
- 11.6.2 Species-Related Flavors of Meats from Nonruminants 789
- 11.6.3 Volatiles in Fish and Seafood Flavors 790

11.7 Development of Process or Reaction Flavor Volatiles 791
- 11.7.1 Thermally Induced Process Flavors 792
- 11.7.2 Volatiles Derived from Oxidative Cleavages of Carotenoids 797

11.8 Future Directions of Flavor Chemistry and Technology 797

References 798

Further Reading 801

11.1 INTRODUCTION

11.1.1 General Philosophy

Knowledge of the chemistry of flavors is commonly perceived as a relatively recent development in food chemistry that evolved since the late 1950s with the advent of gas chromatography and fast-scan mass spectrometry. Although the availability of these instrumental tools has provided the means to definitively investigate the entire range of flavor substances, classic chemical techniques were elegantly applied in much earlier studies, especially for essential oils and spice extractives [28]. This extensive and somewhat separate focus of attention to perfumery, combined with a rapid, seemingly disorganized development about the chemistry of food flavors, has contributed to a slow evolution of a discipline-oriented identity for the field of flavors.

Although flavor substances represent an extremely wide range of chemical structures derived from almost every notable constituent of foods, the feature of "stimulating taste or aroma receptors or specialized nerves to produce an integrated psychological response known as flavor" remains as the only essential requirement for inclusion of a molecule into the flavor category of food chemistry. However, from a broader perspective, the term "flavor" has evolved to a usage that implies an overall integrated perception of all of the contributing senses (smell, taste, sight, feeling, and sound) at the time of food consumption. Thus, although the nonchemical or indirect senses (sight, sound, and feeling) often substantially influence the perception of flavors, and hence food acceptances, a discussion of these effects is beyond the scope of this chapter.

Attention in this chapter is directed toward chemicals that produce specialized odor- and/or taste-related responses, but a clear distinction between the meaning of these terms and that of flavor will not always be attempted. Included will be the chemistry of important flavor systems and character-impact compounds that have been selected to illustrate the chemistry of relevant food systems and the chemical basis for the existence of flavor compounds in foods. Where appropriate, and when information is available, structure–activity relationships for flavor compounds are noted.

Limited notice is given here to listings of profiles of flavor compounds present in various foods. Comprehensive lists of flavor compounds for foods are available elsewhere [48,83], as are tabulations of threshold concentrations for individual compounds [21]. Finally, a choice exists whether information pertaining to the flavor chemistry of major food constituents is dealt with here or in the chapters devoted to those major food constituents. It has been deemed appropriate to conduct these discussions in the major-constituent chapters. For example, many details for flavors deriving from the Maillard reaction are discussed in Chapter 3, and similarly those deriving from free radical oxidation of lipids are discussed in Chapter 4. Information on low-calorie sweeteners and on binding of flavors by macromolecules is, of necessity, partly covered here and partly in Chapters 3 and 5 (binding by macromolecules) and in Chapter 12 (low-calorie sweeteners).

11.1.2 Methods for Flavor Analysis [22,53,64]

As noted at the beginning of this chapter, flavor chemistry often has been equated with the analysis of volatile compounds using gas chromatography combined with fast-scan mass spectrometry, but this view is too restrictive as an extensive array of methods has evolved for the analysis of flavor compounds. However, only limited attention will be directed to flavor analysis here because extensive discussions can be found elsewhere [49,66,76].

Several factors make the analysis of flavors somewhat demanding, including their presence at low concentrations (ppm, 1×10^6; ppb, 1×10^9; ppt, 1×10^{12}), the complexity of mixtures (e.g., over 450 volatiles identified in coffee), the instability of some flavor compounds, and their sometimes extremely high volatility (high vapor pressures) or low volatility (low vapor pressures). Identification of flavor compounds typically requires initial isolation from the bulky constituents of foods combined with substantial concentration (e.g., distillation), but this should occur with minimal distortion

of the native composition, especially when flavor quality is being studied. Adsorption of equilibrium gas-phase flavor compounds over foods onto porous polymers followed by either thermal desorption or solvent elution has provided a means to minimize destruction of sensitive compounds during isolation. However, higher-boiling compounds and some compounds present in very low concentrations may still require distillation techniques to assure adequate recovery for analysis.

The identification of flavor-active compounds and associated precursor substances is a primary goal of flavor analysis, but accurate measurement of concentrations of these compounds in foods is an equally important goal. Quantitative information is especially valuable when correlations between occurrences of flavor compounds and resulting sensory perceptions are sought. Although extensive data have been accumulated on the quantitative presence of flavor substances in headspace gases over foods and variously obtained isolates from foods, because of missing compounds or distorted quantitative values, it is often difficult to reconstruct high-quality facsimile flavors from these data.

Additionally, attempts to duplicate flavors in nutritionally modified foods (e.g., low-fat formulations) by adapting flavor compositions used in unmodified foods (e.g., full-fat formulations) also generally have met with limited successes. In response to suspicions that differing relative rates of release of individual flavor compounds from the modified food matrix (e.g., low-fat formulation) in the mouth accounted for these difficulties, considerable efforts have been devoted to the application of atmospheric pressure ionization mass spectrometry techniques for the real-time measurement of release of flavor compounds in the oral cavity. While elegant experiments have validated the time–intensity release rate concept for individual compounds from foods in the mouth, the measured differences mostly relate to flavor intensity effects and do not seem to correlate with differences in perceived flavor qualities observed between unmodified and modified food matrix compositions. Because of these findings, attention has been shifting toward discovering flavor-modifying molecules that are present in unmodified foods (e.g., full-fat formulations) but that are excluded or omitted in modified foods (e.g., low-fat formulations).

Overall, while progress has been made in the development and application of methods that correlate objective flavor chemical data with subjective sensory information, routine assessment of flavors by purely analytical means remains limited. The development and commercialization of *electronic nose* devices [51] has been in response to the long-standing demands for rapid means to measure chemical parameters that provide reliable information about flavor intensity and quality of foods. While some successes in applications have been reported for these devices, especially in screening foods for deteriorative flavors (e.g., oxidative rancidity), they still are generally considered in early developmental stages.

11.1.3 Sensory Assessment of Flavors

Sensory assessments of flavor compounds and foods are essential for achieving objectives of flavor investigations regardless of ultimate goals. Some situations call for sensory characterization of samples by skilled individuals (experienced flavorists or researchers). However, in many instances, it is necessary to use formal sensory panels and statistical analysis to detect differences in flavors, obtain descriptive flavor information, or determine consumer flavor preferences. Excellent reviews and books are available on formal sensory analysis [1,2,61,71], and these should be consulted for detailed information on this extremely important aspect of flavor assessment.

Sensory assessments are used for the documentation of qualitative flavor characteristics of flavor and aroma chemicals, and both qualitative and quantitative flavor or aroma sensations for single or combinations of chemicals vary with the concentration. The determination of detection thresholds for flavor chemicals provides a measure of the potency of flavor provided by individual compounds, and detection threshold values are usually determined using an individual representative of the general population. A range of concentrations of a selected flavor compound in a defined medium (water, milk, air, etc.) is presented to sensory panelists, and each panelist indicates whether or not

the compound can be detected. The concentration range where at least half (sometimes greater) of the panelists can detect the compound is designated as the flavor threshold [21]. Compounds vary greatly in their flavor or odor potency, and thus, a minute amount of a compound having a very low threshold is perceived to have substantially greater influence on the flavor of a food than one that is quite abundant, but which possesses a high-flavor threshold.

The calculation of the odor units (OUs) involves dividing the concentration of the flavor compound by its flavor threshold (OU = concentration present/threshold concentration) and provides an estimate of the contribution by a flavor compound. More recently, aroma extract dilution analysis has been used extensively to identify the most potent odorants in foods [27], and this involves sensory detection of individual compounds (flavor dilution factor) in gas chromatographic effluents resulting from serial dilutions of aroma extracts from foods. Such methods provide quantitative information about the relative intensity or potency of flavor compounds present in foods and beverages. However, such determinations often exclude or grossly underestimate the qualitative flavor features of compounds. This is especially the case where individual compounds contribute the recognition, *characterizing*, or *character-impact* feature to a particular flavor. These most potent aroma compound methods are criticized also because they provide flavor potency data that are determined in the absence of influences of food matrices and interactive psychophysical effects of the perception of mixtures of flavor compounds. Therefore, extrapolation of such data to actual food systems is severely limited.

11.1.4 Molecular Mechanisms of Flavor Perception

Even though understanding the molecular basis of flavor perception has long been pursued as an important goal with many practical applications, it has only been in very recent times that research discoveries in this field have begun to replace theories with established facts and principles. Much of the recent progress in understanding flavor perception has been made with the use of techniques common to contemporary molecular biology investigations.

A strong driving force for pursuing basic studies on the mechanisms of flavor perception is provided by a desire to more extensively utilize structure–activity features of molecules (e.g., a defined structural feature yields a predictable aroma or taste) to guide the development of more useful and effective flavor compounds (such as intense sweeteners; see also Chapter 12). Similarly, high demands exist for substances that mask or cancel unpleasant flavors found in some food ingredients (e.g., soy protein derivatives), and especially unwanted bitterness that is inherent to some pharmaceuticals and nutraceutical ingredients (Chapter 13).

Specialized cells of the olfactory epithelium in the nasal cavity that have the ability to detect trace amounts of volatile odorants account for the nearly unlimited variations in intensity and quality of odors and flavors. Taste buds located on the tongue and back of the oral cavity enable humans to sense sweetness, sourness, saltiness, bitterness, and umami, and these sensations contribute the taste components of food flavors. Overall, the general process of odor and taste perception at the molecular level involves three successive stages that ultimately culminate in the sensory experience of the taster. These are reception, transduction, and neural processing or coding of electrical impulse information. Detailed overviews, including schematics and descriptions, of these biological processes can be found in recent reviews [44,73].

For odorants and some of the tastants (sweet, bitter, and umami), the initial perception event involves the selective binding (believed to comply with a structural lock-and-key conceptual model) of a flavor molecule to a specific receptor protein in the membrane of an appropriate receptor cell.

When the binding of the flavor molecule to the receptor protein occurs, the chemical energy is transduced into electrical energy via one of several very specific biochemical reaction cascades.

The initial binding between a receptor protein and a flavor molecule stimulates a G protein coupled receptor to activate enzymatic reactions yielding reaction cascade products (e.g., cyclic adenosine-5′-monophosphate [AMP] or inositol triphosphate) that interact with and open Na^+ or

Ca^{2+} ion channels in the receptor cell membrane. The resulting sudden flow of charged ions across the receptor cell membrane causes a depolarization of the cell and produces a unique series of electrical charges (action potentials or nerve impulses) that reflect the amount of odorant stimulating the cell and provide some information about the identity of the flavor molecule. The critical processes by which electrical information is coded are largely theory at this point, but genetic and physiologic evidence supports a position that this is achieved by the production of spatial maps (differing neural firing rates and intensities) in the olfactory bulb and other brain structures.

The molecular-level events involved in the perception of sour (H^+) and salt (Na^+) ionic tastants are different from those of odorants and sweet, bitter, and umami tastants, and they are also different from each other. However, both sour and salty ionic tastants interact directly with ionic channels in taste receptor cell membranes. For sour tastants, H^+ ions bind directly to ion channels causing their closure to Na^+ flow, which then results in membrane depolarization and a neural impulse. In contrast, saltiness perception is initiated by the direct entrance of Na^+ ions from the external environment into tastant receptor cells because the ion channels are permeable to the cation of salt (Na^+). Thus, when Na^+ ions enter receptor cells and change the electrical potential across the cell membrane, the cells depolarize and generate a neural impulse in response to the presence of salt (NaCl) in the external environment.

Some flavor molecules exhibit unique sensory properties, including heat or pungency, cooling, and tingling sensations, that greatly contribute to the flavors of certain foods and beverages. Because these sensations stem from influences on certain nerve fibers and lack the involvement of specific receptor (i.e., taste or olfactory) cells in their generation, in the past they commonly have been referred to as nonspecific saporous sensations. Such sensations in oral and nasal tissues appear parallel to those detected by the cutaneous (skin) chemosensory systems (irritation, pain, heat, cold, etc.). However, to distinguish these flavor-related sensations emanating from the innervating systems of the oral and nasal cavities (i.e., trigeminal, glossopharyngeal, and vagus), recently the term "chemesthesis" has been coined to collectively refer to these saporous sensations.

Other nonspecific, chemically induced, flavor-influencing sensations (fullness, complexity, etc.) apparently are sensed by the trigeminal neural system, but the compounds causing the effects are not widely known and the mechanisms of perception are not clearly understood.

Advances in unraveling the details of the intriguing process of flavor perception are still actively emerging, and reviews on the topic can be found elsewhere [50,73].

11.2 TASTE AND OTHER SAPOROUS SUBSTANCES

Frequently, although not always, substances responsible for these aspects of flavor perception are water soluble and relatively nonvolatile. As a general rule, they are also present at higher concentrations in foods than those responsible for aromas, and they have been often treated lightly in coverages of flavors. Because of their extremely influential role in the acceptance of foods, the chemistry of substances responsible for taste sensations as well as those responsible for some of the less defined flavor sensations is examined here.

11.2.1 Sweet Taste Substances

Sweet substances have been the focus of much attention because of interest in sugar alternatives and the desire to find suitable replacements for certain low-calorie sweeteners, including saccharin and cyclamate (see Chapter 12). Before modern sweetness theories were advanced, it was popular to deduce that sweetness was associated with hydroxyl (−OH) groups because sugar molecules are dominated by this feature. However, this view was soon subject to criticism because polyhydroxy compounds vary greatly in sweetness, and many amino acids, some metallic salts, and unrelated compounds, such as chloroform ($CHCl_3$) and saccharin (Chapter 12), are also sweet. Still, it was apparent that some common characteristics existed among sweet substances, and over the

past 75 years, a theory-relating molecular structure and sweet taste has evolved that satisfactorily explains why certain compounds exhibit sweetness.

Shallenberger and Acree [68] first proposed the AH/B theory for the saporous (taste eliciting) unit common to all compounds that cause a sweet sensation (Figure 11.1). The saporous unit was initially viewed as a combination of a covalently bound H-bonding proton and an electronegative orbital positioned at a distance of about 3 Å from the proton. Thus, vicinal electronegative atoms on a molecule are essential for sweetness. Further, one of the atoms must possess a H-bonding proton. Oxygen, nitrogen, and chlorine atoms frequently fulfill these roles in sweet molecules, and hydroxyl group oxygen atoms can serve either the AH or B function in a molecule. Simple AH/B relationships are shown for chloroform (I), saccharin (II), and glucose (III).

FIGURE 11.1 Schematic showing the relationship between AH/B and γ-sites in the saporous sweet unit for β-D-fructopyranose.

As indicated in Figure 11.1, however, stereochemical requirements are also imposed on the AH/B components of the saporous unit so that they will align suitably with the receptor site. The interaction between the active groups of the sweet molecule and the taste receptor is currently envisioned to occur through H-bonding of the AH/B components to similar structures in the taste receptor. A third feature also has been added to the theory to extend its validity to intensely sweet substances. This addition incorporates appropriate stereochemically arranged lipophilic regions of sweet molecules, usually designated as γ that are attracted to similar lipophilic regions of the taste receptor. Lipophilic portions of sweet molecules are frequently methylene ($-CH_2-$), methyl ($-CH_3$), or phenyl ($-C_6H_5$) groups. The complete sweet saporous structure is geometrically situated so that triangular contact of all active units (AH, B, and γ) with the receptor molecule occurs for intensely sweet substances, and this arrangement forms the rationale for the tripartite structure theory of sweetness.

The γ-site is an extremely important feature of intensely sweet substances, but plays a lesser role in sugar sweetness [8]. It appears to function through facilitating the accession of certain molecules to the taste receptor site and as such affects the perceived intensity of sweetness. Since sugars are largely hydrophilic, this feature comes into play in a limited sense only for some sugars, such as fructose. This component of the saporous sweet unit probably accounts for a substantial portion of the variation in sweetness quality that is observed between different sweet substances. Not only it is important in the time–intensity or temporal aspects of sweetness perception, but it also appears to relate to some of the interactions between sweet and bitter tastes observed for some compounds.

Sweet–bitter sugar structures possess features that apparently allow them to interact with either or both types of receptors, thus producing the combined taste sensation. Bitterness properties in structures depress sweetness even if the concentration in a test solution is below that for the bitter sensation. Bitterness in sugars appears to be imparted by a combination of effects involving the configuration of the anomeric center, the ring oxygen, the primary alcohol group of hexoses, and the nature of any substituents. Often, changes in the structure and stereochemistry of a sweet molecule lead to the loss or suppression of sweetness or the induction of bitterness.

11.2.2 Bitter Taste Substances [58,59]

The bitterness sensation appears to be closely related to sweetness from a molecular structure–receptor relationship. Bitterness resembles sweetness because of its dependence on the stereochemistry of stimulus molecules, and the two sensations are triggered by similar features in molecules, causing some molecules to yield both bitter and sweet sensations. Although sweet molecules must contain two polar groups that may be supplemented with a nonpolar group, bitter molecules appear to have a requirement for only one polar group and a hydrophobic group [9].

However, some [5,7,14] believe that most bitter substances possess an AH/B entity identical to that found in sweet molecules as well as the hydrophobic group. In this concept, the orientation of AH/B units within specific receptor sites, which are located on the flat bottom of receptor cavities, provides the discrimination between sweetness and bitterness for molecules possessing the required molecular features. Molecules that fit into sites that were oriented for bitter compounds give a bitter response; those fitting the orientation for sweetness elicit a sweet response. If the geometry of a molecule were such that it could orient in either direction, it would give bitter–sweet responses.

Such a model appears especially attractive for amino acids where D-isomers are sweet and L-isomers are bitter [39]. Since the hydrophobic or γ-site of the sweet receptor is nondirectionally lipophilic, it could participate in either sweet or bitter response. Molecular bulkiness factors serve to provide stereochemical selectivity to the receptor sites located in each receptor cavity. It can be concluded that there is a very broad structural basis for the bitter taste modality, but most empirical observations about bitterness and molecular structure can be explained by current theories.

Quinine is an alkaloid that is generally accepted as the standard for the bitter taste sensation. The detection threshold for quinine hydrochloride (IV) is about 10 ppm. In general, bitter substances have lower taste thresholds than other taste substances, and they also tend to be less soluble in water

than other taste-active materials. Quinine is permitted as an additive in beverages, such as soft drinks that also have tart–sweet attributes. The bitterness blends well with the other tastes and provides a refreshing gustatory stimulation in these beverages. The practice of mixing quinine into soft drink beverages apparently stems from efforts to suppress or mask the bitterness of quinine when it was prescribed as a drug for malaria.

(IV) Quinine hydrochloride

In addition to soft drinks, bitterness is an important flavor attribute of several other beverages consumed in large quantities, including coffee, cocoa, and tea. Caffeine (V) is moderately bitter at 150–200 ppm in water and is present in coffee, tea, and cola nuts. Theobromine (VI) is very similar to caffeine and is present most notably in cocoa, where it contributes to bitterness. Caffeine is added in concentrations up to 200 ppm to soft cola beverages, and much of the caffeine employed for this purpose is obtained from extractions of green coffee beans that are carried out in the preparation of decaffeinated coffee.

(V) Caffeine

(VIII) Phenylthiocarbamide (PTC)

(IX) 6-*n*-Propyl-2-thiouracil (PROP or PTU)

(VI) Theobromine

Large amounts of hops are employed in the brewing industry to provide unique flavors to beer. Bitterness, contributed by some unusual isoprenoid-derived compounds, is a very important aspect of hop flavor. These nonvolatile, bitter substances can generally be categorized as derivatives of humulone or lupulone, that is, α- or β-acids, respectively, as they are known in the brewing industry. Humulone is the most abundant substance, and it is converted during wort boiling to isohumulone by an isomerization reaction (Figure 11.2) [16].

FIGURE 11.2 Thermal isomerization of humulone to isohumulone occurring during wort boiling in the traditional brewing process.

Isohumulone is the precursor for the compound that causes the sunstruck or skunky flavor in beer exposed to light. In the presence of hydrogen sulfide from yeast fermentation, a photocatalyzed reaction occurs at the carbon adjacent to the keto group in the iso-hexenyl chain. This gives rise to 3-methyl-2-butene-l-thiol (prenylmercaptan) that has a skunky aroma. Selective reduction of the ketone in preisomerized hop extracts prevents this reaction and permits packaging of beer in clear glass without the development of the skunky or sunstruck flavor. Whether volatile hop aroma compounds survive the wort-boiling process was a controversial topic for a number of years. However, it is now well documented that influential compounds do survive the wort-boiling process and others are formed from hop bitter substances; together, they contribute to the kettle-hop aroma of beer.

Although bitterness is desirable in many foods and beverages, unwanted bitterness in some foods and beverages, including novel nutraceutical ingredients and pharmaceutical preparations, is frequently a problem. Extensive efforts have been expended to identify substances that mask bitter flavors, but to date these have met with limited successes. While certain gums or viscosity-enhancing polymers suppress bitter flavors, generally their use only partly alleviates the problem. However, recently it has been discovered that AMP (VII) that is associated with intermediary energy metabolism possesses potent bitter-blocking properties, and its application in bitter flavor suppressions appears promising.

(VII) Adenosine-5′-monophosphate
(AMP, a potent bitter blocker)

The development of excessive bitterness is a major problem of the citrus industry, especially in processed products. In the case of grapefruit, some bitterness is desirable and expected, but frequently the intensity of bitterness in both fresh and processed fruits exceeds that preferred by many consumers. Citrus fruits contain several of flavonone glycosides, and naringin is the predominant flavonone found in grapefruit and bitter orange (*Citrus aurantium*). Juices that contain

FIGURE 11.3 Reaction showing the enzymatic hydrolysis of naringin by naringinase that is used in the debittering of citrus products.

high levels of naringin are extremely bitter and are of little economic value except in instances where they can be extensively diluted with juices containing low bitterness levels. The bitterness of naringin is associated with the configuration of the molecule that develops from the 1 to 2-linkage between rhamnose and glucose. Naringinase is an enzyme that has been isolated from commercial citrus pectin preparations and from *Aspergillus* sp., and this enzyme hydrolyzes the 1 to 2-linkage (Figure 11.3) to yield nonbitter products. Immobilized enzyme systems have also been developed to debitter grapefruit juices containing excessive levels of naringin. Naringin has also been commercially recovered from grapefruit peels and is used instead of caffeine for bitterness in some food applications.

The principal bitter component in navel and Valencia oranges is a triterpenoid dilactone (A- and D-rings) called limonin, and it is also found as a bittering agent in grapefruit. Limonin is not present to any extent in intact fruits, but rather a flavorless limonin derivative produced by enzymic hydrolysis of limonin's D-ring lactone is the predominant form (Figure 11.4). After juice extraction, acidic conditions favor the closing of the D-ring to form limonin, and the phenomenon of delayed bitterness occurs, yielding serious economic consequences.

Methods for debittering orange juice have been developed using immobilized enzymes from *Arthrobacter* sp. and *Acinetobacter* sp. [34]. Enzymes that simply open the D-ring lactone provide only temporary solutions to the problem because the ring closes again under acidic conditions. However, the use of limonoate dehydrogenase to convert the open D-ring compound to nonbitter 17-dehydrolimonoate A-ring lactone (Figure 11.4) provides an irreversible means to debitter orange juice. Methods to debitter citrus juices also include the use of polymeric adsorbents that currently are the preferred methods for commercial processors [40].

Pronounced, undesirable bitterness is frequently encountered in protein hydrolysates and aged cheeses, and this effect is caused by the overall hydrophobicity of amino acid side chains in peptides. All peptides contain suitable numbers of AH-type polar groups that can fit the polar receptor site, but individual peptides vary greatly in the size and nature of their hydrophobic groupings and thus in the ability of these hydrophobic groups to interact with the essential hydrophobic sites of bitterness receptors. Ney [58] has shown that the bitter taste of peptides can be predicted by a calculation of a mean hydrophobicity value, termed *Q*. The ability of a protein to engage in hydrophobic associations is related to the sum of the individual hydrophobic contributions of the nonpolar,

FIGURE 11.4 Equilibrium reaction leading to the formation limonin and enzymatic debittering reactions that reverse bitterness development in citrus juices.

amino acid side chains, and these interactions contribute mainly to the free energy (ΔG) associated with protein unfolding. Thus, by summation of ΔG values for the individual amino acid side chains in a peptide, it is possible to calculate the mean hydrophobicity Q using Equation 11.1:

$$Q = \frac{\Sigma \Delta G}{n}, \tag{11.1}$$

where n is the number of amino acid residues. Individual ΔG values for amino acids have been determined from solubility data [75], and these are summarized in Table 11.1. Q-values above 5855 based on joules/mol (1400 based on calories/mol) indicate that the peptide will be bitter; values below 5436 based on joules/mol (1300 based on calories/mol) assure that the peptide will not be bitter. The molecular weight of a peptide also influences its ability to produce bitterness, and only those with molecular weights below 6000 have a potential for bitterness. Peptides larger than this apparently are denied access to receptor sites because of their bulkiness (see Chapter 5).

The peptide shown in Figure 11.5 is derived from the cleavage of α_{s1}-casein between residue 144–145 and residue 150–151 [58] and has a calculated Q-value of 9576 based on J/mol (2290 based on calories/mol). This peptide is very bitter and is illustrative of the strongly hydrophobic peptides that can be derived readily from α_{s1}-casein. Such peptides are responsible for bitterness that develops in aged cheeses.

Because of genetic differences in humans, individuals vary in their ability to perceive bitter substances. At a defined concentration, certain substances may be bitter, bitter–sweet, or tasteless depending on the individual. Saccharin is perceived as purely sweet by some individuals, but others find it to range from only slightly bitter and sweet to quite bitter and sweet. Many other compounds also show marked variations in the manner in which individuals perceive them and frequently either taste bitter or are not perceived at all.

Phenylthiocarbamide (PTC) (VIII) is one of the most notable compounds in this category [1], and it was discovered in the early 1930s [4] that for this compound about 40% of the Caucasian–American

TABLE 11.1
Calculated ΔG Values for Individual Amino Acids

Amino Acids	ΔG Value[a] (kJ/mol)
Glycine	0 (0)
Serine	167.3 (40)
Threonine	1839.9 (440)
Histidine	2090.8 (500)
Aspartic acid	2258.1 (540)
Glutamic acid	2299.9 (550)
Arginine	3052.6 (730)
Alanine	3052.6 (730)
Methionine	5436.1 (1300)
Lysine	6272.4 (1500)
Valine	7066.9 (1690)
Leucine	10119.5 (2420)
Proline	10955.8 (2620)
Phenylalanine	11081.2 (2650)
Tyrosine	12001.2 (2870)
Isoleucine	12419.4 (2970)
Tryptophan	12544.8 (3000)

Source: From Ney, K.H., Bitterness of peptides: Amino acid composition and chain length, in: Boudreau, J.C., ed., *Food Taste Chemistry*, American Chemical Society, Washington, DC, 1979, pp. 149–173.

[a] ΔG values in calories/mol are shown in parentheses; 1 calories = 4.1816 kJ. ΔG values represent free energy change for the transfer of amino acid side chains from ethanol to water. These values are slightly different from those obtained from transfer of amino acid side chains from octanol to water (see Table 5.3).

FIGURE 11.5 Reaction depicting the hydrolysis of α_{s1}-casein to form a bitter peptide (phe–tyr–pro–glu–leu–phe) that exhibits strong overall nonpolar features.

population taste is blind to the bitterness taste attribute that is perceived by the other 60% of the Caucasian–American population. Because the ability to perceive bitterness of PTC was so clearly genetically controlled, its use as a marker for exploring behavioral and metabolic differences between bitter tasters and nontasters soon followed. Because PTC also exhibits a sulfurous odor, more recently researchers have largely adopted 6-*n*-propyl-2-thio-uracil (PROP) (IX) that does not

have an inherent odor for continued studies [62]. For these two molecules, the N−C=S grouping is believed to be responsible for their bitter tastes.

(VIII) Phenylthiocarbamide (PTC)

(IX) 6-*n*-Propyl-2-thiouracil (PROP or PRU)

Recent studies have revealed that individuals who find PROP to be intensely bitter are genetically endowed with the ability to perceive many flavors in an intensified fashion, and these individuals have become known as “supertasters.” Currently, researchers are investigating many physiological and psychological aspects of both PROP-insensitive and PROP-sensitive individuals in hopes that underlying factors governing food intake and preference as well as certain pathological conditions and health risks in the general population can be discovered.

Although PTC and PROP are novel compounds that do not occur in foods, creatine (X) is a constituent of muscle foods that exhibits similar properties of varied bitter taste sensitivity in the population. Creatine may occur at levels up to about mg/g in lean meats [1], and this is adequate to make some soups taste bitter to sensitive individuals.

(X) Creatine

Bitterness occurs in salts, and this sensory property greatly hampers the substitution of alternate cations for sodium in foods compositionally modified to permit restricted sodium intake by consumers. The atomic (molecular) features causing bitterness in salts apparently are quite different from those that cause bitterness in organic compounds. Bitterness in salts seems to be related to the sum of the ionic diameters of the anion and cation components comprising the salt in question [5,6]. Salts with ionic diameters below 6.5 Å are purely salty in taste (LiCl = 4.98 Å, NaCl = 5.56 Å, KCl = 6.28 Å) although some individuals find KCl to be somewhat bitter. As the ionic diameter increases (CsCl = 6.96 Å, Csl = 7.74 Å), salts become increasingly bitter. Magnesium chloride (8.50 Å) is therefore quite bitter.

11.2.3 Salty Taste Substances

Classic, clean salty taste is provided by sodium chloride (NaCl), but it is also given by lithium chloride (LiCl), which cannot be used in foods because of its toxic properties. In general, salts exhibit complex tastes usually described as consisting of psychological mixtures of classic sweet, bitter, sour, and salty perceptual components. However, the tastes of salts often fall outside the traditional taste sensations [65] and are difficult to describe in the classic flavor terms. In some instances, other vague terms, such as chemical or soapy, often seem to more accurately describe the sensations produced by salts than do the classic terms.

The flavor effects of NaCl appear to extend well beyond those of the classic tastes, and in food applications NaCl clearly provides special flavor-enhancing properties. These effects can be easily demonstrated through the substantial reduction or omission of NaCl from standard food formulations (e.g., bread and bakery items).

It has been established that cations cause the basic salty taste and that anions modify the basic salt taste [3]. Sodium and lithium cations produce only salty taste, while potassium and other alkaline earth cations produce a combination of both salty and bitter tastes. Anions modify salt tastes by inhibiting the tastes of cations, and they also frequently contribute tastes of their own. Among the anions commonly found in foods, the chloride anion is least inhibitory to the salty taste, and the citrate anion is more inhibitory than orthophosphate anions. Furthermore, the chloride anion does not contribute a taste, and the citrate anion contributes less taste than the orthophosphate anion.

Anion taste effects impact the flavor of many foods, such as processed cheese, where citrate and phosphate anions contained in emulsifying salts (Chapter 12) suppress perceived saltiness contributed by sodium ions and also add anion tastes. Similarly, soapy tastes caused by sodium salts of long-chain fatty acids (XI) and detergents of long-chain sulfates (XII) result from specific tastes elicited by the anions, and these tastes can completely mask the taste of the cation.

(XI) Sodium laurate (XII) Sodium lauryl sulfate

National policies encouraging reduction in sodium consumption have stimulated interest in foods in which sodium salts have been replaced by alternative substances, particularly those containing potassium and ammonium ions. Since foods flavored with these substitutes have different, usually less desirable, tastes than those flavored with NaCl, renewed efforts are being expended to better understand the basic mechanisms of the salty taste and in the development of salt substitutes in the hope that low-sodium products with near normal salty taste can be devised.

11.2.4 Sour Taste Substances

Sour taste substances are acidic in nature and thus contain at least one proton that is dissociable in aqueous systems (Chapter 12). Although the initial molecular-level event in the perception of acidic, sour, or tart flavors involves the binding of protons (H^+) to receptor cell membrane ion channels that results in closure to Na^+ flow and depolarization, the qualitative aspects of the sour taste response are poorly understood. Contrary to popular belief, the acid strength in a solution does not appear to be the major determinant of the sour sensation; rather, other poorly understood molecular features appear to be of primary importance (e.g., molecular weight, size, and overall polarity), and prior empirical experience often determines the selection of acids for applications in foods.

11.2.5 Umami Taste Substances [39]

Compounds eliciting this taste sensation have been utilized by humans to improve flavors since the inception of food cooking and preparation. For many years, umami tastants, notably monosodium L-glutamate (MSG; XIII) and the 5′-ribonucleotides (inosine-5′-monophosphate [5′-IMP], XIV, and guanosine-5′-monophosphate), were scientifically relegated to the category of nonspecific taste responses because specific taste receptors for these substances had not yet been detected.

However, since the discovery of taste receptors for these compounds, umami is now widely accepted as a basic taste response [39].

(XIII) Monosodium L-glutamate (MSG, umami) (XIV) Inosine-5′-monophosphate (IMP, umami)

Umami substances contribute a delicious, mouthwatering taste to foods when used at levels in excess of their independent detection threshold, and they modify and enhance flavors at levels below their independent detection thresholds. Their effects are prominent and desirable in the flavors of vegetables, meats, poultry, fish, shellfish, and aged cheeses.

D-Glutamate and the 2′- or the 3′-ribonucleotides do not exhibit flavor-enhancing activity. Several synthetic derivatives of the 5′-ribonucleotides have strong flavor-enhancing properties [43]. Generally, these derivatives have substitutions on the purine moiety in the 2-position. A synergistic interaction occurs between MSG and the 5′-ribonucleotides in providing both the umami taste and in enhancing flavors, and mixtures of these substances are widely used commercially. There is some evidence to indicate that some of the flavor-enhancing properties of umami compounds result from their joint occupancy of receptor sites involved in perception of sweet, sour, salty, and bitter sensations.

Although MSG and 5′-IMP and 5′-guanosine monophosphate are the only flavor enhancers used commercially, 5′-xanthine monophosphate and a few natural amino acids, including L-ibotenic acid and L-tricholomic acid, are potential candidates for commercial use [88]. Much of the flavor contributed to foods by yeast hydrolysates results from the 5′-ribonucleotides present. Large amounts of purified flavor enhancers employed in the food industry are derived from microbial sources, including phosphorylated (*in vitro*) nucleosides derived from RNA [43]. Discussions on general flavor enhancement can be found in several reviews [43,88].

11.2.6 Kokumi Taste Substances and Other Flavor Modifiers

As mentioned in Section 11.2.3, common salt (NaCl) provides profound flavor-enhancing and flavor-modifying effects to many food flavors. Although salt is specifically sensed by specialized taste cells (Section 11.1.2), many believe that it also may provide flavor enhancing by modifying the functions of other basic taste receptor cells or through other sensations emanating from other neural systems (e.g., trigeminal nerve) in the oral cavity. Thus, salt likely possesses some properties similar to other flavor-enhancing substances whose overall taste-modifying functions remain poorly understood.

The Japanese have introduced a separate term, kokumi, to refer to at least part of these chemicals that do not elicit responses for the original four basic tastes or the umami response, but that enhance food palatability by providing what is best described as fullness, complexity, continuity, thickness, and body to food flavors [80]. For example, the principal precursors for the characterizing volatile aroma compounds in garlic and onions are *S*-substituted cysteine sulfoxide amino acids (Figure 11.6), and both of these compounds are readily water soluble, and they exhibit strong kokumi properties that distinctly influence palatability [80,81]. Thus, although flavors of foods containing garlic (e.g., pasta sauces, sauteed meats) may not exhibit readily distinguishable volatile

S-(1-Propenyl)-
L-cysteine sulfoxide
(in onions; kokumi)

S-(2-Propenyl)-
L-cysteine sulfoxide
(in garlic; kokumi)

Glutathione
(γ-glu–cys–gly; kokumi)

Sodium succinate
(Broth-like complexity)

FIGURE 11.6 Structures of some readily water-soluble, flavor-modifying compounds.

garlic flavor notes, their flavors are perceived as extremely complex, full, and palatable because of the presence of *S*-(2-propenyl)-L-cysteine sulfoxide.

Although readily water-soluble substances reported to provide kokumi flavors are not yet numerous, another cysteine-containing peptide, glutathione (Figure 11.6), also is kokumi active [82]. In a potentially related response, succinic acid (and its soluble salts, Figure 11.6) exhibits a distinct broth-like flavor characteristic in addition to a sour taste. Although the flavor of succinic acid has not been classified kokumi as yet, this substance is used commercially to provide a brothy complexity to savory flavors, especially in meat-type sauces.

A number of other descriptive terms are used to describe a variety of flavor modifications that appear to be kokumi-related influences, and these include velvety, richness, creaminess, and juiciness. A number of both natural and synthetic substances (Figure 11.7) possess the ability to provide

Maltol

Ethylmaltol

Vanillin

Ethylvanillin

m-Cresol

m-(*n*)-Propylphenol

FIGURE 11.7 Structures of some flavor-modifying compounds with limited water solubility.

such modifications to flavors, and some structural similarities occur among some of these substances. Of these, vanillin-type flavors comprise one of the most popular flavors worldwide, and the aromas of vanillin and ethylvanillin are perceived as extremely desirable by most. However, in addition to aroma contributions, vanillin-related substances also provide flavor-modifying effects that result in enhanced smoothness, richness, and creaminess flavor sensations, particularly in sweet, fat-containing foods such as ice cream.

Similarly, maltol and ethylmaltol (Figure 11.7) also have been used widely as commercial flavor enhancers for sweet goods and fruit-containing products. Although both of these substances possess pleasant, burnt caramel aromas at high concentrations, they often are marketed for the smooth, velvety sensation they impart to sweet goods and fruit juices and products at relatively low levels (ca 50 ppm) at which concentrations the caramelic notes are not distinguishable. Ethylmaltol is more effective as a sweetness enhancer than maltol, but maltol still generally lowers the detection threshold concentration for sucrose by a factor of 2.

Recently, some alkylphenols that occur naturally in the milk and meat of ruminants have been found to contribute mouth coating, richness enhancing, and juiciness sensations at very low concentrations (ng/g). *m*-Alkyl substitution on the aromatic ring provides the most influential flavor-modifying effects among the members of this group, and *m*-cresol and *m*-(*n*)-propylphenol (Figure 11.7) are the most important in bovine-derived products and ingredients [32].

11.2.7 Pungent Substances

Pungency is a chemesthetic property exhibited by a number of compounds found in spices and vegetables that cause characteristic hot, sharp, and stinging sensations [15]. Some pungent principles, such as those found in chili peppers, black pepper, and ginger, are not volatile and, unless made airborne via aerosol droplets, exert their effects principally on oral tissues. Other spices and vegetables contain pungent principles that are somewhat volatile and produce both pungency in the oral and nasal cavities and characteristic aromas. These include mustards, horseradish, vegetable radishes, watercress, onions, and the aromatic spice, clove, which contains eugenol as the active component.

All of these pungent spices and vegetables are used in foods to provide characteristic flavors or to generally enhance palatability. Usage at low concentrations in processed foods frequently provides a liveliness to flavors through subtle contributions that fill out the perceived flavors. Only the three major pungent spices, chili peppers, ginger, and pepper, are discussed in this section of the chapter, but others are mentioned later in discussions about plant-derived flavor systems (i.e., isothiocyanates, thiopropanal-*S*-oxide, and eugenol). Comprehensive reviews on pungent compounds are also available (cf. [24]).

Chili peppers (*Capsicum* sp.) contain a group of substances known as capsaicinoids, which are vanillylamides of monocarboxylic acids with varying chain length (C_8−C_{11}) and unsaturation. Capsaicin (XV) is representative of these pungent principles. Several capsaicinoids containing saturated straight-chain acid components are synthesized as substitutes for natural chili extractives or oleoresins. The total capsaicinoid content of world *Capsicum* sp. varies widely [24]; for example, red pepper contains 0.06%; cayenne red pepper, 0.2%; Sannam (India), 0.3%; and Uganda (Africa), 0.85%. Sweet paprika contains a very low concentration of pungent compounds and is used mainly for its coloring effects and subtle flavors in part derived from carotenoid oxidation. Chili peppers also contain a number of volatile aroma compounds that become part of the overall flavor of foods seasoned with them.

(XV) Capsaicin

FIGURE 11.8 Reactions altering gingerol that affect the relative pungency of ginger.

Black and white pepper are made from the berries of *Piper nigrum* and differ only in that black pepper prepared from immature, green berries and white pepper is made from more mature berries usually harvested at the time they are changing from green to yellow in color, but before they become red. The principal pungent compound in pepper is piperine (XVI), an amide. The *trans*-geometry of the alkyl unsaturation is necessary for strong pungency, and loss of pungency during exposure to light and storage is attributed mainly to isomerizations to *cis* forms of these double bonds [24]. Pepper also contains volatile compounds, including L-formylpiperidine and piperonal (heliotropin), which contribute to flavors of foods seasoned with pepper spice or oleoresins. Piperine is also synthesized for use in flavoring foods.

(XVI) Piperine

Ginger is a spice derived from the rhizome of a tuberous perennial, *Zingiber officinale* Roscoe, and it possesses pungent principles as well as some volatile aroma constituents. The pungency of fresh ginger is caused by a group of phenylalkyl ketones called gingerols, and [6]-gingerol (Figure 11.8) is the most active of these compounds. Gingerols vary in chain length (C_5-C_9) external to the hydroxyl-substituted C atom. During drying and storage, gingerols tend to dehydrate to form an external double bond that is in conjugation with the keto group. The reaction results in a group of compounds known as shogaols that are even more potent pungent compounds than the gingerols. Exposure of [6]-gingerol to substantially elevated temperatures can lead to cleavage of the alkyl chain external to the keto group, yielding a methyl ketone, zingerone, that exhibits only mild pungency.

11.2.8 Cooling Substances [84]

Cooling is another chemesthetic sensation that occurs when certain chemicals contact the nasal or oral tissues and stimulate the nonspecific neural systems (e.g., trigeminal nerve). These effects when caused by naturally occurring substances are most commonly associated with mint-like flavors, including peppermint, spearmint, and wintergreen. Several compounds cause the sensation, but (−)-menthol (XVII), in the natural form (L-isomer), is most commonly used in flavors. A number

of synthetic cooling compounds have been discovered, and both natural and synthetic compounds often also produce an accompanying camphoraceous aroma. Camphor (XVIII) is cited often as the model for camphoraceous group of compounds because it produces a distinctive odor in addition to a cooling sensation.

(XVII) (–)-Menthol (XVIII) D-Camphor

The cooling effect produced by the mint-related compounds is mechanistically different from the slight cooling sensation produced when polyol sweeteners (Chapters 3 and 12), such as xylitol, are tasted as crystalline materials. In the latter case, it is generally believed that an endothermic dissolution of the materials gives rise to the effect.

11.2.9 Astringent Substances

Astringency is a feeling-related phenomenon perceived as a dryness in the mouth along with a coarse puckering of the oral tissue [45]. Astringency usually results from the association of tannins or polyphenols (Chapter 10) with proteins in the saliva to form precipitates or aggregates. Additionally, sparingly soluble proteins, such as those found in certain dry milk powders, can also combine with proteins and mucopolysaccharides of saliva and cause astringency. Astringency is often confused with bitterness because many individuals do not clearly understand its nature, and many polyphenols or tannins cause both astringent and bitter sensations, which is the case for red wines [1].

The more astringent tannins are often condensed tannins resulting from oxidative reactions, and these molecules offer broad cross-sectional areas (Figure 11.9) suitable for hydrophobic associations with proteins. Tannins contain many phenolic groups that can convert to quinoid structures, and these in turn can cross-link chemically with proteins [57]. Such cross-links have been suggested as a possible contributor to astringency activity.

Astringency may be a desirable flavor property, such as in tea. However, the practice of adding milk or cream to tea suppresses astringency through binding of polyphenols with milk proteins. Red wine is a good example of a beverage that exhibits both astringency and bitterness caused by polyphenols or tannins. However, too much astringency is considered sensorily undesirable in wines, and means are often taken to reduce polyphenol tannins that are related to the anthocyanin pigments.

Astringency derived from polyphenols in unripe bananas can also lead to an undesirable taste in products to which such bananas have been added [23].

11.3 VEGETABLE, FRUIT, AND SPICE FLAVORS [11,63]

Categorization of vegetable and fruit flavors in a reasonably small number of distinctive groups is not easy since logical groupings are not necessarily available for vegetables and fruits. For example, some information on plant-derived flavors was presented in the section on pungency, and some are covered in the section dealing with the development of "reaction" flavors. Emphasis in this section is on the biogenesis and development of flavors in important vegetables and fruits. For information on other fruit and vegetable flavors, the reader is directed to the general references (cf. [48]).

FIGURE 11.9 Model reaction illustrating the formation of a procyanidin-type tannin with large planar hydrophobic areas capable of associating with proteins to cause astringency.

11.3.1 Sulfur-Containing Volatiles in *Allium* sp.

Plants in the genus *Allium* are characterized by strong, penetrating aromas, and important members are onions, garlic, leek, chives, and shallots. These plants lack the strong characterizing aroma unless the tissue is damaged and enzymes are decompartmentalized so that the flavor precursors can be converted to odorous volatiles. In the case of onions (*A. cepa* L.), the precursor of the sulfur compounds that are responsible for the volatile flavor and aroma of this vegetable is *S*-(1-propenyl)-L-cysteine sulfoxide [69,85], and this substance also possesses kokumi flavor properties (Section 11.2.6). This substituted cysteine sulfoxide precursor is also found in leek.

Rapid hydrolysis of *S*-(1-propenyl)-L-cysteine sulfoxide by alliinase in onions yields an unstable sulfenic acid intermediate along with ammonia and pyruvate (Figure 11.10). The sulfenic acid undergoes further rearrangements to yield the lachrymator thiopropanal-*S*-oxide that is also associated with the overall aroma of fresh onions. The pyruvic acid produced by the enzymatic conversion of the precursor compound is a stable product of the reaction and serves as a good indirect index of the flavor intensity of onion products. Part of the unstable sulfenic acid also rearranges and decomposes to a rather large number of compounds in the classes of mercaptans, disulfides, trisulfides, and thiophenes. More extensive formation of these compounds and other derivatives also comprises the flavor substances, which provide cooked onion flavors.

The flavor of garlic (*Allium sativum* L.) is formed by the same general type of mechanism that functions in onion, except that the precursor is *S*-(2-propenyl)-L-cysteine sulfoxide [69]. Diallyl thiosulfinate (allicin) (Figure 11.11) contributes to flavor of garlic, and an *S*-oxide lachrymator similar to that formed in onion is not formed. The thiosulfinate flavor compound of garlic decomposes and rearranges in much the same manner as indicated for the sulfenic acid of onion (Figure 11.10). This results in methyl, allyl, and diallyl disulfides and other principles in garlic oil and cooked garlic flavors.

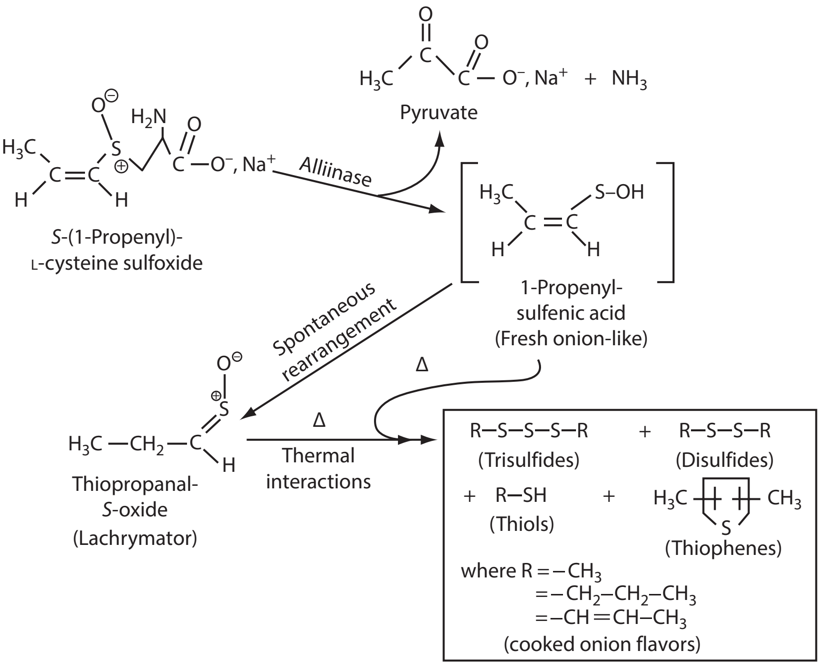


FIGURE 11.10 Reactions involved in the formation of onion flavors.

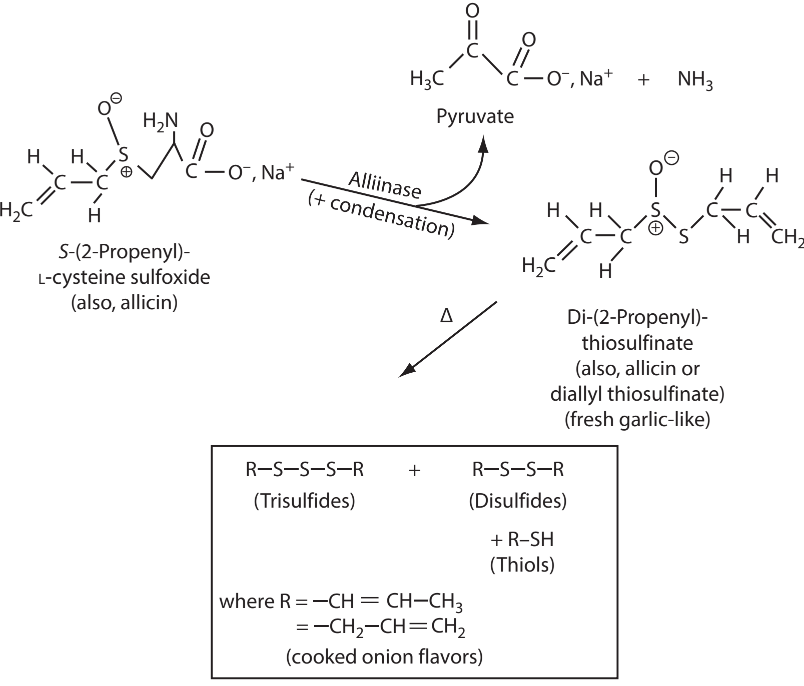


FIGURE 11.11 Reactions involved in the formation of garlic flavor compounds.

11.3.2 Sulfur-Containing Volatiles in the Cruciferae

The Cruciferae family contains *Brassica* plants such as cabbage (*Brassica oleracea* var. *capitata* L.), Brussels sprouts (*B. oleracea* var. *gemmifera* L.), turnips (*Brassica rapa* var. *rapa* L.), brown mustard (*Brassica juncea* Coss.), as well as water cress (*Nasturtium officinale* R. Br.), radishes (*Raphanus sativus* L.), and horseradish (*Armoracia lapathifolia* Gilib). As noted in the discussion about pungent compounds, the active pungent principles in the Cruciferae are also volatile and therefore contribute to characteristic aromas. Further, the pungent compounds in Cruciferae frequently cause pronounced irritation sensations, particularly in the nasal cavity, as well as lachrymatory effects. Flavor compounds in these plants are formed through enzymic processes in disrupted tissues and through cooking.

The fresh flavors of disrupted tissues are caused mainly by isothiocyanates resulting from the action of glucosinolates on thioglycoside precursors. The reaction shown in Figure 11.12 is illustrative of the flavor-forming mechanism in fresh Cruciferae. Allyl isothiocyanate is the main source of pungency and aroma in horseradish and black mustard [24].

Several glucosinolates (*S*-glycosides, Chapter 13) occur in the Cruciferae [69], and each gives rise to characteristic flavors. The mild pungency of radishes is caused by the aroma compound, 4-methylthio-3-*t*-butenylisothiocyanate (XIX). In addition to the isothiocyanates, glucosinolates also yield thiocyanates and nitriles.

(XIX) 4-Methylthio-3-*t*-butenylisothiocyanate (radish)

Although not usually distinctly pungent, fresh cabbage and Brussels sprouts contain the potential for both allyl isothiocyanate and allyl nitrile, and the concentration of each varies with the stage of growth, the location in the edible part, and the severity of processing encountered. Processing at temperatures well above ambient (cooking and dehydrating) tends to destroy the isothiocyanates and enhance the amount of nitriles and other sulfur-containing degradation and rearrangement compounds. Several aromatic isothiocyanates occur in Cruciferae; for example, 2-phenylethyl isothiocyanate is one of the main aroma compounds of watercress. This compound also contributes a tingling pungent sensation, which influences the flavors of salads containing watercress.

FIGURE 11.12 Reactions involved in the formation of Cruciferae flavors.

11.3.3 Unique Sulfur Compound in Shiitake Mushrooms

A novel C–S lyase flavor-related enzyme system occurs in Shiitake mushrooms (*Lentinus edodes*) that are prized in Japan and elsewhere for their unique desirable flavor. The precursor for the major flavor contributor, lentinic acid, is an *S*-substituted L-cysteine sulfoxide bound as a γ-glutamyl peptide [89]. The initial enzyme reaction in flavor development involves a γ-glutamyl transpeptidase, which releases a cysteine sulfoxide precursor (lentinic acid). Lentinic acid is then attacked by *S*-alkyl-L-cysteine sulfoxide lyase, which yields products that subsequently form lenthionine, the active flavor compound for Shiitake flavor (Figure 11.13). These reactions are initiated only after the tissue is disrupted, and the maximum flavor develops only after drying and rehydration or after holding freshly macerated tissue for a period of time. Other polythiepanes in addition to lenthionine are formed and contribute to the overall sulfurous flavor of flavor mushrooms [37,69].

11.3.4 Methoxy Alkyl Pyrazine Volatiles in Vegetables

Many fresh vegetables exhibit green-earthy aromas that contribute strongly to their recognition, and it has been found that the methoxy alkyl pyrazines are frequently responsible for this property [85]. Also, more recently, methoxy alkyl pyrazines have been associated with the flavors of certain varietal grape wines. These compounds have unusually potent and penetrating odors, and they provide strong identifying aromas. 2-Methoxy-3-isobutylpyrazine was the first of this class discovered, and it exhibits a powerful bell pepper aroma detectable at a threshold level of 0.002 ppb. Much of the aroma of raw potatoes, green peas, and pea pods is contributed by 2-methoxy-3-isopropylpyrazine, and 2-methoxy-3-*sec*-butylpyrazine contributes to the aroma of raw red beet roots. These compounds arise biosynthetically in plants, and some strains of microorganisms (*Pseudomonas perolens* and *Pseudomonas tetrolens*) also actively produce these unique substances [52]. Branched-chain amino acids serve as precursors for methoxy alkyl pyrazine volatiles, and the mechanistic scheme shown in Figure 11.14 has been proposed.

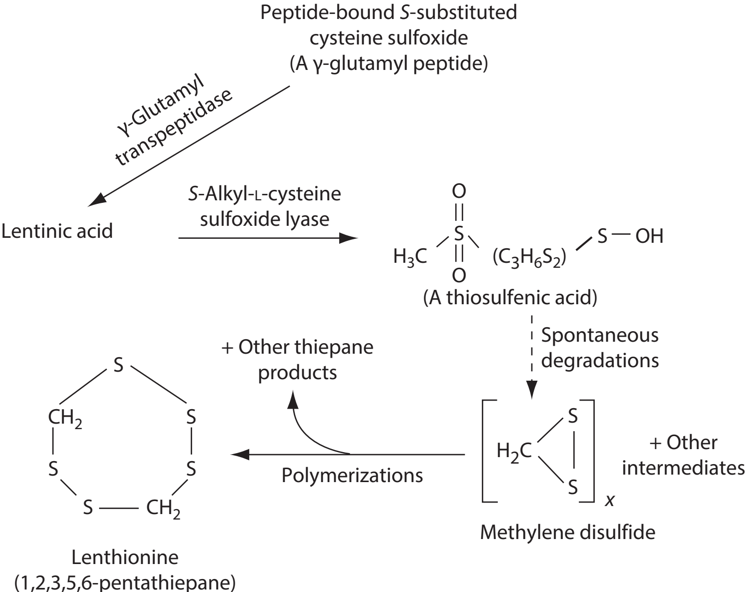


FIGURE 11.13 Reactions involved in the formation of lenthionine in Shiitake mushrooms.

FIGURE 11.14 Proposed enzymic scheme for the formation of methoxy alkyl pyrazines.

11.3.5 Enzymatically Derived Volatiles from Fatty Acids

Enzymically generated compounds derived from long-chain fatty acids play an extremely important role in the characteristic flavors of fruits and vegetables. In addition, these types of reactions can lead to important off-flavors, such as those associated with processed soybean proteins. Further information about these reactions can be found in the discussions about lipids (Chapter 4) and enzymes (Chapter 6).

11.3.5.1 Lipoxygenase-Derived Flavors in Plants

In plant tissues, enzyme-induced oxidative breakdown of unsaturated fatty acids occurs extensively, and this yields characteristic aromas associated with some ripening fruits and disrupted tissues [19]. In contrast to the random production of lipid-derived flavor compounds by purely autoxidizing systems, very distinctive flavors occur when the compounds produced are enzyme determined. The enzymic specificity for producing flavor compounds is illustrated in Figure 11.15, where 1-octene-3-one, *t*-2-*c*-6-nonadienal, and *t*-2-hexenal are formed from unsaturated fatty acids, and these compounds provide characterizing flavors to fresh mushrooms, cucumbers, and tomatoes, respectively. Site-specific peroxidations of liberated fatty acids are directed by specified lipoxygenases and subsequent lyase cleavage reactions. Upon cleavage of the fatty acid molecule, oxo-acids are also formed, but they do not appear to influence flavors.

Decompartmentalization of enzymes is required to initiate this and other reactions, and since successive reactions occur, overall flavors change with time. For example, the lipoxygenase-derived aldehydes and ketones are converted to corresponding alcohols (Figure 11.16) that usually have higher detection thresholds and heavier aromas than the parent carbonyl compounds. Additionally, *cis*–*trans* isomerases that are also present convert *cis*-3 bonds of aldehydes to *trans*-2 isomers (Figure 11.15), and these structural changes alter the aroma quality of the aldehydes. Generally, C_6 compounds yield green plantlike aroma-like fresh-cut grass, C_9 compounds, smell-like cucumbers and melons; and C_8 compounds, smell-like mushrooms or violet and geranium leaves [78]. The C_6 and C_9 compounds are primary alcohols and aldehydes; the C_8 compounds are secondary alcohols and ketones.

11.3.5.2 Volatiles from β-Oxidation of Long-Chain Fatty Acids

The development of pleasant, fruity aromas is associated with the ripening of pears, peaches, apricots, and other fruits, and these aromas are frequently dominated by medium-chain-length (C_6–C_{12}) volatiles derived from long-chain fatty acids by β-oxidation [79]. The formation of ethyl

FIGURE 11.15 Formation of lipoxygenase-directed carbonyl compounds from unsaturated fatty acids: (a) important in fresh mushrooms, (b) important in cucumbers, and (c) important in fresh tomatoes.

FIGURE 11.16 Conversion of an aldehyde to a corresponding alcohol resulting in a subtle flavor modification.

deca-2-*t*,4-*c*-dienoate by this means is illustrated in Figure 11.17. This ester is the impact or characterizing aroma compound in the Bartlett pear. Although not included in the figure, hydroxy acids (C_8–C_{12}) also may be formed as part of the overall enzymic process, and they readily cyclize to yield γ- and δ-lactones. Similar reactions occur during metabolism and biosynthesis of milk fat, and these reactions are discussed in more detail in Section 11.5. The C_8–C_{12} lactones possess distinct coconut and peach-like aromas characteristic of these respective fruits.

11.3.6 Volatiles from Branched-Chain Amino Acids

Branched-chain amino acids serve as important flavor precursors for the biosynthesis of compounds associated with some ripening fruits. Bananas and apples are particularly good examples for this process because much of the ripe flavor of each is caused by volatiles from amino acids [79]. The initial reaction involved in flavor formation (Figure 11.18) is sometimes referred to as enzymic Strecker degradation because transamination and decarboxylation occur that is parallel to those occurring during nonenzymatic browning. Several microorganisms, including baker's yeast and malty flavor-producing strains of *Lactococcus lactis*, can also modify most of the amino acids in a fashion similar to that shown in Figure 11.18. Plants can also produce similar derivatives from amino acids other than leucine, and the occurrence of 2-phenethanol with a rose- or lilac-like aroma in blossoms is attributed to these reactions.

FIGURE 11.17 Formation of a key aroma substance in ripened pears through β-oxidation of linoleic acid followed by esterification.

FIGURE 11.18 Enzymatic conversion of leucine to volatiles illustrating the aroma compounds formed from amino acids in ripening fruits.

Although the aldehydes, alcohols, and acids from these reactions contribute directly to the flavors of ripening fruits, the esters are the dominant character-impact compounds. It has long been known that isoamyl acetate (3-methylbutyl acetate) is important in banana flavor, but other compounds are also required to give full banana flavors. Ethyl 2-methylbutyrate is even more apple-like than ethyl 3-methylbutyrate and is the dominant note in the aroma of ripe, delicious variety apples.

11.3.7 Flavors Derived from the Shikimic Acid Pathway

In biosynthetic systems, the shikimic acid pathway provides the aromatic portion of compounds related to shikimic acid, and the pathway is best known in its role in the production of phenylalanine and other aromatic amino acids. In addition to flavor compounds derived from aromatic amino acids, the shikimic acid pathway provides other volatile compounds that are frequently associated with essential oils (Figure 11.19). It also provides the phenylpropanoid skeleton to lignin polymers that are the main structural elements of plants. As indicated in Figure 11.19, lignin yields many phenols during pyrolysis [86], and the characteristic aroma of smokes used in foods is largely caused by compounds developed from precursors in the shikimic acid pathway.

Also apparent from Figure 11.19 is that vanillin, the most important characterizing compound in vanilla extracts, can be obtained naturally via this pathway or as a lignin by-product during processing of wood pulp and paper. Vanillin is also biochemically synthesized in the vanilla bean where it is initially present largely as vanillin glucoside until the glycoside linkage is hydrolyzed during fermentation. The methoxylated aromatic rings of the pungent principles in ginger, pepper, and chili peppers, discussed in Section 11.2.7, also contain many of the essential features of the compounds shown in Figure 11.19. Cinnamyl alcohol is an aroma constituent of cinnamon spice, and eugenol is the principal aroma and pungency element in cloves.

11.3.8 Volatile Terpenoids in Flavors

Because of the abundance of terpenes in plant materials used in the essential oil and perfumery industries, their importance in other plant-associated flavors is sometimes underestimated. They are largely responsible, however, for the flavors of citrus fruits and many seasonings and herbs. Volatile terpenes are present in low concentrations in several fruits and are responsible for much of the flavor of raw carrot roots. Terpenes are biosynthesized through the isoprenoid (C_5) pathway (Figure 11.20), and monoterpenes contain 10 C atoms; the sesquiterpenes contain 15 C atoms. Sesquiterpenes are also important characterizing aroma compounds, and β-sinensal (XX) and nootkatone (XXI) serve as good examples because they provide characterizing flavors

FIGURE 11.19 Some important flavor compounds variously derived from shikimic acid pathway precursors.

FIGURE 11.20 Generalized isoprenoid scheme for the biosynthesis of monoterpenes.

to oranges and grapefruit, respectively. The diterpenes (C_{20}) are too large and nonvolatile to contribute directly to aromas.

Volatile terpenes frequently possess extremely strong character-impact aroma properties, and many can be identified easily by one experienced with natural product aromas. Optical isomers (i.e., structural mirror images) of terpenes, as well as optical isomers of other nonterpenoid compounds, can exhibit extremely different odor qualities [12,42,54]. In the case of terpenes, the carvones have been studied extensively from this perspective, and the aroma of *d*-carvone [4*S*-(+)-carvone] (XXII) has the characteristic aroma of caraway spice; *l*-carvone [4*R*-(−)-carvone] (XXIII) possesses a strong, characteristic spearmint aroma. Studies on such pairs of compounds are of special interest since they provide information on the fundamental process of olfaction and structure–activity relationships for molecules.

11.3.9 Citrus Flavors

Citrus flavors are among the most popular fresh fruits as well as flavors for beverages, and most information about the flavor chemistry of natural citrus flavors stems from research on processed juices, peel essential oils, essence oils, and aqueous essences used to flavor juice products. Several classes of flavor components serve as major contributors to citrus flavors, including terpenes,

TABLE 11.2
Some Volatile Compounds Considered Important in Citrus Flavors

Orange	Mandarin	Grapefruit	Lemon
Ethanol	Ethanol	Ethanol	Neral
Octanol	Octanol	Decanal	Geranial
Nonanal	Decanal	Ethyl acetate	β-Pinene
Citral	α-Sinensal	Methyl butanoate	Geraniol
Ethyl butanoate	γ-Terpinene	Ethyl butanoate	Geranyl acetate
d-Limonene	β-Pinene	*d*-Limonene	Neryl acetate
α-Pinene	Thymol	Nootkatone	Bergamotene
	Methyl-*N*-methyl-anthranilate	1-*p*-Methene-8-thiol	Caryophyllene
			Carvyl ethyl ether
			Linalyl ethyl ether
			Fenchyl ethyl ether
			Methyl epijasmonate

Source: From Shaw, P.E., Fruits II, in: Maarse, H., ed., *Volatile Compounds in Foods and Beverages*, Marcel Dekker, New York, 1991, pp. 305–327.

aldehydes, esters, and alcohols, and large numbers of volatile compounds have been identified in the various extracts from each citrus fruit [70]. However, the important flavor compounds, including character-impact compounds, for citrus fruits generally are limited to relatively few, and those considered important to some major citrus fruits are shown in Table 11.2.

The flavors of orange and mandarin (tangerine is used interchangeably with mandarin in the United States) are delicate and often are exchanged to various degrees in flavor applications. As can be noted from Table 11.2, relatively few aldehydes and terpenes are considered essential for these flavors, although a large number of other compounds are present. Both α- and β-sinensal (XX) are present in orange and mandarin flavors, and α-sinensal is considered especially important to providing a ripe orange citrus flavor to mandarin flavors. Grapefruit contains two character-impact compounds, nootkatone (XXI) and 1-*p*-methene-8-thiol, which provide much of the easily recognized flavor to this fruit. Nootkatone is used extensively to provide artificial grapefruit flavor, and *p*-methene-8-thiol is one of apparently few sulfur compounds that are influential in citrus flavors.

Lemon flavor requires contributions from a large number of important compounds, and lemon flavors benefit from the presence of several terpene ethers. Similarly, the number of essential compounds in lime flavor is large, and two types of lime oil are commonly used. The major lime oil in commerce is distilled Mexican lime oil that possesses a harsh, strong lime flavor that is popular in lemon–lime and cola soft drinks. Cold-pressed Persian lime oil and centrifuged Mexican lime oils are becoming more popular because they possess a more natural flavor. The less rigorous processing involved in cold-pressing and centrifugation, compared to distillation, results in the survival of some of the more sensitive, but important fresh lime flavor compounds. For example, citral, which has a desirable fresh-like aroma, is degraded under acidic distillation conditions to *p*-cymene and α-*p*-dimethylstyrene that have coarse flavors, and these conversions lead to the more harsh flavors of distilled lime oil [70].

Citrus essential oils, or flavor extracts containing terpenes, can be separated into nonoxygenated (hydrocarbon) and oxygenated fractions using silicic acid chromatography and nonpolar and polar solvent elutions, respectively. Terpeneless orange oil, for example, contains principally the oxygenated terpenes, aldehydes, and alcohols that have been recovered from orange oils. Because of their higher flavor quality, oxygenated terpene fractions are frequently more desired in flavors than nonoxygenated fractions.

11.3.10 Flavors of Herbs and Spices

Although some variations occur between industrial and the various domestic and international regulatory agencies regarding definitions, spices and herbs are known and regulated as spices and condiments that are natural vegetable products used for flavoring, seasoning, and imparting aroma to foods. The U.S. Food and Drug Administration excludes alliaceous products, such as onions and garlic, from classification as spices, but international and industrial spice classifications commonly include these materials. The term condiments has been retained in regulatory definitions and is defined as something to enhance the flavor of food, or a pungent seasoning. It has been argued that the term should be dropped from usage, but some flavor retention of the term for certain situations. However, the term generally does not provide a basis for inclusion in classification schemes for aromatic plant materials.

In some botanically based classification schemes, culinary herbs are separated from spices and include such aromatic, soft-stemmed plants as basil, marjoram, mints, oregano, rosemary, and thyme as well as aromatic shrub (sage) and tree (laurel) leaves. In such classifications, spices comprise all other aromatic plant materials used in the flavoring or seasoning of foods. Such spices generally lack chlorophyll and include rhizomes or roots (ginger), barks (cinnamon), flower buds (cloves), fruits (dill, pepper), and seeds (nutmeg, mustard).

Spices and herbs have been used since antiquity for adding savoriness, tanginess, and zestiness as well as characterizing flavors to foods and beverages. Some also have been used extensively in perfumery and for medicinal purposes, and many exhibit antioxidant or microbial inhibitory effects. While many herbs and spices exist throughout the world, some of which are used for perfumery and herbal medicines, only about 70 are officially recognized as useful ingredients for foods. However, the flavor characteristics often vary for a given spice depending on location of growth and genetic variations; thus, this group provides a wide range of flavors for foods. Here, only those spices and herbs that are commonly used in the food industry for flavoring are considered.

Spices are generally derived from tropical plants, while herbs are generally derived from subtropical or temperate plants. Spices also generally contain high concentrations of phenylpropanoids from the shikimic acid pathway (e.g., eugenol in cloves; see Figure 11.19); herbs generally contain higher concentrations of *p*-menthanoids from terpene biosynthesis (e.g., menthol in peppermint; see XVII).

Typically, spices and herbs contain a large number of volatile compounds, but in most instances certain compounds, either abundant or minor volatile constituents, provide character-impact aromas and flavors to the material. Important flavor compounds found in the principal herbs and spices used by the food industry are summarized in Tables 11.3 and 11.4 [12,63], respectively. Successful applications of herbs and spices in foods and beverages require either a personal knowledge of the materials or knowledge of the dominant and subtle flavor notes provided by flavor chemicals. Evaluation of the important flavor compounds listed in Tables 11.3 and 11.4 for spices and herbs shows which spices and herbs provide related flavors and also indicates the type of flavors to be expected from their usage. Still, it must be remembered that a large number of compounds of lesser influence are present and also contribute to the unique flavors of herbs and spices.

11.4 FLAVORS FROM LACTIC ACID–ETHANOL FERMENTATIONS

Involvement of microorganisms in flavor production is extensive, but often their specific or definitive role in the flavor chemistry of fermentations is not well known or the flavor compounds do not have great character impact. Much attention has been given to cheese flavor, but apart from the distinctive flavor properties given by methyl ketones and secondary alcohols to blue-mold cheeses and the moderate flavor properties given by certain sulfur compounds to surface-ripened cheeses, microbially derived flavor compounds cannot be classified in the character-impact category. Similarly, yeast fermentations, carried out extensively for beer, wines, spirits, and yeast-leavened breads, do not

TABLE 11.3
Important Flavor Compounds Found in Some Culinary Herbs Commonly Used for Flavoring in the Food Industry

Herbs	Plant Part	Important Flavor Compounds
Basil, sweet	Leaves	Methyl chavicol, linalool, methyl eugenol
Bay laurel	Leaves	1,8-Cineole
Marjoram	Leaves, flowers	*c*- and *t*-Sabinene hydrates, terpinen-4-ol
Oregano	Leaves, flowers	Carvacrol, thymol
Origanum	Leaves	Thymol, carvacrol
Rosemary	Leaves	Verbenone, 1,8-cineole, camphor, linalool
Sage, clary	Leaves	Salvial-4(14)-en-1-one, linalool
Sage, Dalmatian	Leaves	Thujone, 1,8-cineole, camphor
Sage, Spanish	Leaves	*c*- and *t*-Sabinyl acetate, 1,8-cineole, camphor
Savory	Leaves	Carvacrol
Tarragon	Leaves	Methyl chavicol, anethole
Thyme	Leaves	Thymol, carvacrol
Peppermint	Leaves	*l*-Menthol, menthone, menthofuran
Spearmint	Leaves	*l*-Carvone, carvone derivatives

Source: Boelens, M.H. et al. *Perfum. Flavor.*, 18, 1, 1993; Richard, H.M.J., Spices and condiments I, in: Maarse, H., ed., *Volatile Compounds in Foods and Beverages*, Marcel Dekker, New York, 1991, pp. 411–447.

appear to yield strong, distinctive character-impact flavor compounds. Ethanol in alcoholic beverages, however, should be considered as having character impact.

The primary fermentation products of heterofermentative lactic acid bacteria (e.g., *Leuconostoc citrovorum*) are summarized in Figure 11.21, and the combination of acetic acid, diacetyl, and acetaldehyde provides much of the characteristic aroma of cultured butter and buttermilk. Homofermentative lactic acid bacteria (e.g., *Lactococcus lactis*, *Streptococcus thermophilus*) produce only lactic acid, acetaldehyde, and ethanol in milk cultures. Acetaldehyde is the character-impact compound found in yoghurt, a product generally prepared by a homofermentative process. Diacetyl is the character-impact compound in most mixed-strain lactic fermentations and has become universally known as a dairy or butter-type flavorant. Acetoin, although essentially odorless, can undergo oxidation to diacetyl. Lactic acid is nonvolatile and contributes only sourness to cultured or fermented dairy products.

Viewed in general terms, lactic acid bacteria produce very little ethanol (parts per million levels), and they use pyruvate as the principal final H acceptor in metabolism. On the other hand, yeast produces ethanol as a major end product of metabolism. Malty strains of *Lactococcus lactis* and all brewer's yeasts (*Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis*) also actively convert amino acids to volatile compounds through transaminations and decarboxylations. In the example shown in Figure 11.22, phenylalanine yields a series of aromatic volatile compounds that generally are perceived as floral- or roselike. Aged Cheddar cheese containing excessive phenethyl alcohol production is often considered defective in part because cheeses with the roselike flavor in the past have been associated with uncontrolled lactic fermentations resulting from the use of incompletely cleaned dairy equipment.

The reactions shown for phenylalanine are analogous to those discussed for branched-chain amino acids in Section 11.3.6. However, these microorganisms tend to produce mainly the reduced forms of the derivatives (alcohols), although some oxidized compounds (aldehydes and acids) also appear. Wine and beer flavors, which can be ascribed directly to fermentations, involve complex

TABLE 11.4
Important Flavor Compounds Found in Some Spices Commonly Used for Flavoring in the Food Industry

Spice	Plant Part	Important Flavor Compounds
Allspice (pimento)	Berry, leaves	Eugenol, β-caryophyllene
Anise	Fruit	(*E*)-Anethole, methyl chavicol
Capsicum peppers	Fruit	Capsaicin, dihydrocapsaicin
Caraway	Fruit	*d*-Carvone, carvone derivatives
Cardamom	Fruit	α-Terpinylacetate, 1,8-cineole, linalool
Cinnamon, cassia	Bark, leaves	Cinnamaldehyde, eugenol
Clove	Flower bud	Eugenol, eugenylacetate
Coriander	Fruit	*d*-Linalool, C_{10}–C_{14} 2-alkenals
Cumin	Fruit	Cuminaldehyde, *p*-1,3-menthadienal
Dill	Fruit, leaves	Anethofuran, *d*-carvone
Fennel	Fennel	(*E*)-Anethole, fenchone
Ginger	Rhizome	Gingerol, shogaol, neral, geranial
Mace	Aril	α-Pinene, sabinene, 1-terpenin-4-ol
Mustard	Seed	Allyl isothiocyanate
Nutmeg	Seed	Sabinene, α-pinene, myristicin
Parsley	Leaves, seed	Apiol
Pepper	Fruit	Piperine, δ-3-carene, β-caryophyllene
Saffron	Stigma	Safranal
Turmeric	Rhizome	Turmerone, zingiberene, 1,8-cineole
Vanilla	Fruit, seed	Vanillin, *p*-OH-benzyl methyl ether

Source: From Boelens, M.H. et al., *Perfum. Flavor.*, 18, 1, 1993; Richard, H.M.J., Spices and condiments I, in: Maarse, H., ed., *Volatile Compounds in Foods and Beverages*, Marcel Dekker, New York, 1991, pp. 411–447.

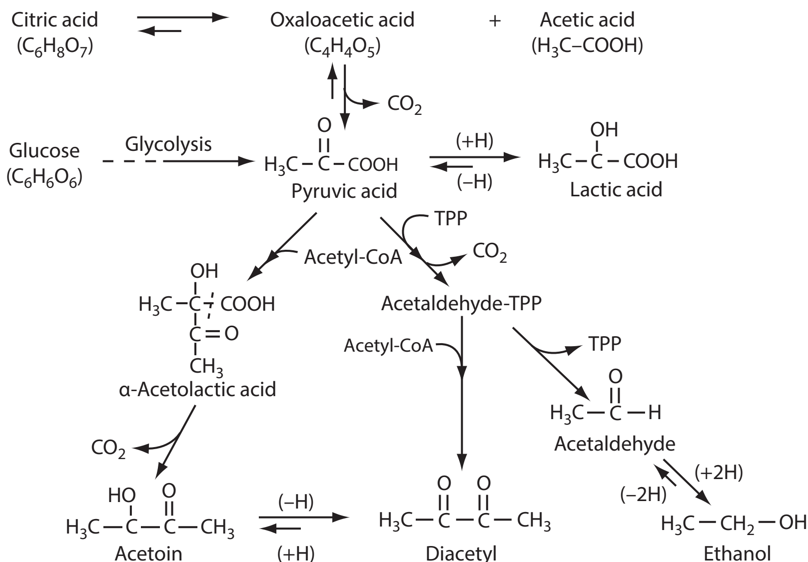


FIGURE 11.21 Formation of the principal flavor-related fermentation products in heterofermentative lactic acid bacterial metabolism (TPP, thiamin pyrophosphate).

FIGURE 11.22 Enzymatic formation of volatiles from amino acids by microorganisms using phenylalanine as a model precursor compound.

mixtures of these volatiles and interaction products of these compounds with ethanol, such as mixed esters and acetals. These mixtures give rise to familiar yeasty and fruity flavors associated with fermented beverages.

11.5 FLAVOR VOLATILES FROM FATS AND OILS

Fats and oils are notorious for their role in the development of off-flavors through autoxidation, and the chemistry of lipid-derived flavors has been well summarized [26,38]. Aldehydes and ketones are the main volatiles from autoxidation, and these compounds can cause painty, fatty, metallic, papery, and candle-like flavors in foods when their concentrations are sufficiently high. However, many of the desirable flavors of cooked and processed foods are derived from modest concentrations of these compounds. Details of lipid autoxidation mechanisms and other degradations of lipids are largely presented in Chapter 4.

11.5.1 Flavors from the Hydrolysis of Fats and Oils

Hydrolysis of plant acylglycerols and animal depot fats leads mainly to the formation of potentially soapy-tasting fatty acids. Milk fat, on the other hand, serves as rich source of volatile flavor compounds that are influential in the flavor of dairy products and foods prepared with milk fat or butter. The classes of volatiles obtained by hydrolysis of milk fat are shown in Figure 11.23, with specific compounds selected to illustrate each class. The even C-numbered, short-chain fatty acids (C_4–C_{12}) are extremely important in the flavor of cheese and other dairy products, with butyric acid being the most potent and influential of the group. Hydrolysis of hydroxy fatty acids leads to the formation of lactones, which provide desirable coconut- or peach-like flavor notes to baked goods, but cause staling in stored, sterile concentrated milks. Methyl ketones are produced from β-keto acids by heating after hydrolysis, and they contribute to the flavor of dairy products in much the same manner as the lactones. In blue-mold cheeses, however, methyl ketones are much more abundantly produced by the metabolic activities of *Penicillium roqueforti* on fatty acids than by the conversion of the keto acids that are bound in acylglycerols.

Although hydrolysis of fats other than milk fat in general does not yield distinct flavors as noted earlier, animal fats are believed to be inextricably involved in the species-related flavors of meats. The role of lipids in species-related aspects of meat flavors is discussed in the next section on muscle foods and milk (Section 11.6).

11.5.2 Distinctive Flavors from Long-Chain Polyunsaturated Fatty Acids

Many consider cooking with animal fats (i.e., lard, beef tallow) to yield foods with significantly different and better flavors than those cooked with any of the current plant-derived fats or oils.

FIGURE 11.23 Formation of influential volatile flavor compounds from milk fat obtained through hydrolytic cleavage of acylglycerols.

The reasons considered responsible for the different flavors have included differing bulk fatty acid compositions that yield different oxidation flavor compound profiles and differing minor components that are dissolved in the respective fats. However, the chemical basis for these differences is still debated.

Terrestrial plant-derived food fats and oils currently contain only polyunsaturated fatty acids with 18 C (mainly 18:2:ω6 and 18:3:ω3) or less. However, while animal-derived fats and products contain bulk polyunsaturated fatty acids similar to plant fats and oils, they also contain notable amounts of the long-chain polyunsaturated fatty acid, arachidonic acid (20:4:ω6). Recent research has clarified the primary flavorful oxidation products derived from arachidonic acid [10], and these findings provide some insights into the key flavor chemistry differences between plant and animal fats. Oxidizing pure arachidonic acid emanates distinctive, sometimes unpleasant, uncooked poultry- and animallike aromas, and the aromas are substantially caused by (*E*, *Z*, *Z*)-2,4,7-tridecatrienal that in the pure form exhibits potent uncooked egg- and poultry-like aromas (Figure 11.24). Since this unsaturated oxidation product of arachidonic acid is so potent and distinct, it is likely the

FIGURE 11.24 Formation of distinctive volatile flavor compounds from long-chain polyunsaturated fatty acids.

characterizing or character-impact compound responsible for the casual observation that "many white meats taste a lot like chicken."

Animal fats also contain ω3 long-chain polyunsaturated fatty acids although in notably less abundance than arachidonic acid, but fish oils contain relatively large amounts of these fatty acids.

Included are docosahexaenoic acid (22:6ω3; DHA) and eicosapentaenoic acid (EPA; 205:ω3) and oxidation of either yields, among other products, (*E*, *Z*, *Z*)-2,4,7-decatrienal, which possesses potent oxidized cod liver oil-like or stale fishlike flavors (Figure 11.24). In excessive concentrations, (*E*, *Z*, *Z*)-2,4,7-decatrienal causes extremely undesirable fishy flavors, but at acceptable levels, it imparts desirable characterizing or character-impact flavor properties to fish and seafoods. Thus, the products of ω3 long-chain polyunsaturated fatty acid oxidations provide a chemical basis for differentiating between the flavors of aquatic and terrestrial animal foods. However, it is also likely that the ω3 long-chain polyunsaturated fatty acids are also involved in the flavor differences resulting from plant and animal fats and oils. Notably, with commercial algal culturing and genetic engineering advances that have occurred recently, it is certain that the traditional differentiating flavor features between plant and animal fats and oils rapidly will become obscured.

11.6 FLAVOR VOLATILES IN MUSCLE FOODS AND MILK

The flavors of meats have attracted much attention, but in spite of considerable research, knowledge about the flavor compounds causing strong character impacts for meats of various species remains somewhat obscure [17,67]. Nevertheless, the concentrated research efforts on meat flavors have produced a wealth of information about compounds that contribute to cooked meat flavors. The somewhat distinctive flavor qualities of meat flavor compounds that are not species related are very valuable to the food and flavor industry, but chemical definitions of lightly cooked and species-related flavors are still eagerly sought. Some details of the chemistry relating to the flavors of well-cooked meat are discussed in Section 11.7.

11.6.1 Species-Related Flavors of Meats and Milk from Ruminants

The characterizing flavors of at least some meats are inextricably associated with the lipid fraction. Although long a subject of debate, significant advances in defining species-related flavors were initiated by Wong and coworkers [87] in relation to lamb and mutton flavors. These workers showed that a characteristic sweat-like flavor of mutton was closely associated with some volatile, medium-chain-length fatty acids of which some methyl-branched members are highly significant. The formation of one of the most important branched-chain fatty acids in lamb and mutton, 4-methyloctanoic acid, is shown in Figure 11.25.

Ruminal fermentations yield acetate, propionate, and butyrate, but most fatty acids are biosynthesized from acetate, which yields straight or nonbranched chains. However, some methyl branching occurs routinely because of the presence of propionate, but when dietary and other factors enhance the propionate concentrations in the rumen, greater methyl branching occurs [72]. Several medium-chain, methyl-branched fatty acids are important contributors to species-related flavors, including 4-methylnonanoic acid in lamb and mutton flavors. Additionally, 4-ethyloctanoic acid (threshold = 1.8 ppb in water) is particularly important for conveying very distinctive goatlike flavors to both meats and milk products, and it is formed when the initial primer fatty acid is butyric acid (butyryl-CoA) rather than propionic acid (propionyl-CoA, and it is coupled with a butyl-enzyme moiety as shown in Figure 11.25 [29–31].

Several alkylphenols (methylphenol isomers, ethylphenol isomers, *n*-propyl isomers, isopropylphenol isomers, and methyl-isopropylphenol isomers) contribute very characteristic beef- and sheeplike species-related flavors to meats and milks [31,32,47]. Additionally, some alkylphenols, especially *m*-substituted members, exhibit kokumi-like flavor properties (Section 11.2.6). Alkylphenols are present as free (flavorful) and conjugate-bound (flavorless) substances in meat and milk and are initially derived via rumen fermentation conversions of shikimic acid pathway

FIGURE 11.25 Reactions showing ruminant biosynthesis of methyl-branched, medium-chain fatty acids.

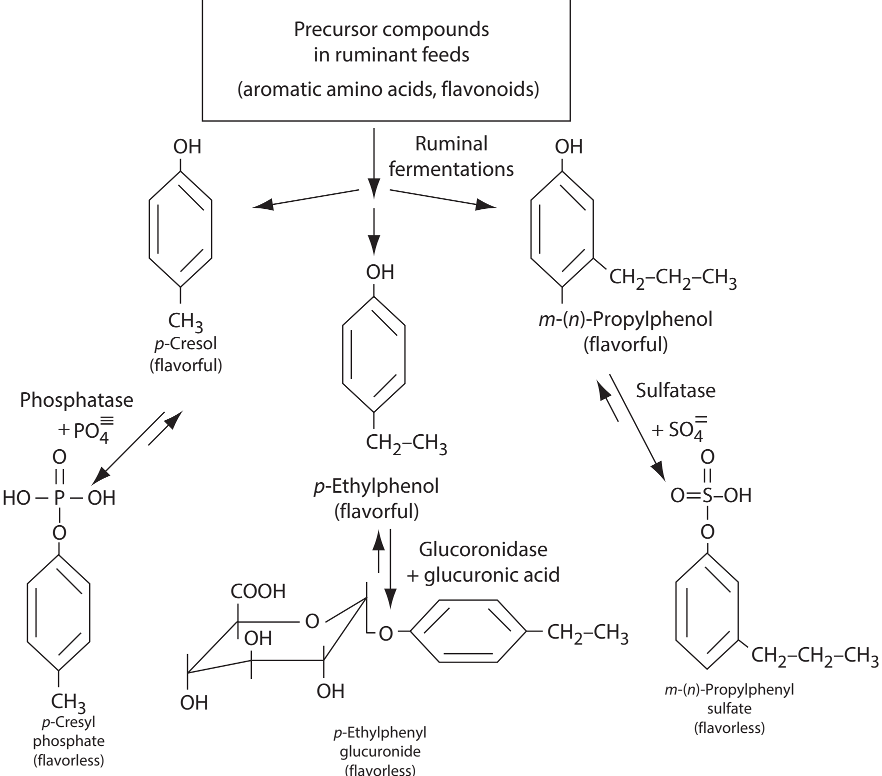


FIGURE 11.26 Formation of alkylphenols from feeds by ruminants.

biochemicals found in forages (Figure 11.26). Sulfate, phosphate, and glucuronide conjugates of alkylphenols are formed *in vivo* in ruminants to improve the water solubility of alkylphenols to enhance their elimination in the urine. Subsequently, both enzymic and thermal hydrolysis may release alkylphenols from conjugates and thereby enhance flavor development during fermentations and cooking of meat and dairy products.

11.6.2 Species-Related Flavors of Meats from Nonruminants

Species-related aspects of the flavor chemistry of nonruminant meats appear to remain somewhat incomplete. The distinct pork-like or piggy flavor, noticeable in lard or cracklings and in some pork, is caused in part by *p*-cresol and isovaleric acid that are produced from microbial conversions of corresponding amino acids in the lower gut of swine [29,31]. Similar formation of indole and skatole from tryptophan may also intensify unpleasant piggy flavors in pork. Excessive levels of (*E*, *Z*, *Z*)-2,4,7-tridecatrienal from arachidonic acid oxidation (Section 11.5.2) also accentuates piggy flavors, but acceptable concentrations (see Section 11.1.3) of this compound contribute desirably to pork flavors. Also, studies have shown that the γ-C_5, C_9, and C_{12} lactones are reasonably abundant in pork [17], and these compounds may contribute some of the sweet-like flavor of swine meat.

Much interest has centered on the aroma compounds responsible for the swine sex odor that causes serious off-flavors in pork. Two compounds that contribute much to the off-flavor are 5-α-androst-16-en-3α-one, which has a urinous aroma, and 5-α-androst-16-en-3α-ol, which has a musk-like aroma (Figure 11.27 [25]). The swine sex odor compounds are mainly associated with males, but may occur in castrated males and in females. These steroid compounds are particularly offensive to some individuals, especially women, and yet others are genetically odor blind to them. Since the compounds responsible for the swine sex odor have only been found to cause off-flavors in pork, they can be regarded as species-related flavor compounds for swine.

The distinctive flavors of poultry have also been the subject of many studies, and lipid oxidation yields the character-impact compounds for chicken. Early research [33] implicated the carbonyls, *c*-4-decenal, *t*-2-*c*-5-undecadienal, and *t*-2-*c*-4-*t*-7-tridecatrienal in the characteristic flavor of stewed chicken, and they were believed to be derived from linoleic and arachidonic acids. However, a recent detailed reevaluation [10] of the volatile products from the autoxidation of arachidonic acid (also see Section 11.5.2) has shown that (*E*, *Z*, *Z*)-2,4,7-tridecatrienal from the reaction is an extremely potent poultry-like substance and thus is a character-impact compound for chicken and other white meat flavors. Other factors, such as cooking method, also influence the characteristics

FIGURE 11.27 Formation of steroid compounds responsible for urine- and musk-like aromas associated with the swine sex odor defect of pork.

of cooked poultry flavors. One likely influential factor is that chickens accumulate α-tocopherol (an antioxidant), but turkeys do not, and during cooking, especially roasting, carbonyls are formed to a much greater extent in turkey than in chicken.

11.6.3 Volatiles in Fish and Seafood Flavors

Flavors in seafoods cover a somewhat broader range of flavor qualities than those occurring in other muscle foods. The broad range of animals involved (finfish, shellfish, and crustaceans) and the variable flavor and aroma qualities related to freshness each account for the different flavors that are encountered. Because the fresh flavors and aromas of seafoods frequently have been greatly diminished or lost from fresh-stored, frozen, and processed products available through commercial channels, many consumers associate secondarily developed stale and fishy flavors with all fish and seafoods. However, very fresh seafoods exhibit delicate aromas and flavors quite different from those usually evident in *commercially fresh* seafoods. One contributing factor is the accumulation and subsequent decline of the umami substance, inosine-5′-monophosphate (Section 11.2.5), in tissues that cause dramatic time-related changes in the flavors of refrigerated fish and seafoods.

A group of lipoxygenase-derived C_6, C_8, and C_9 aldehydes, ketones, and alcohols initially provide the characterizing and pleasant aroma and flavor of fresh fish [46]. Overall the flavor compounds produced in fish and seafoods are similar to those produced by plant lipoxygenases (Section 11.3.5). However, the lipoxygenases found in fish and seafoods perform enzymic oxidations related to leukotriene synthesis, and flavor compound production is a by-product of those reactions. For the example shown in Figure 11.28, peroxidation followed by disproportionation reactions apparently leads first to the alcohol and then to the corresponding carbonyl [74]. Collectively, these compounds provide melon-like, green plantlike notes to fresh fish aromas and contribute to the delicate flavors of very fresh cooked fish, either directly or as reactants that lead to new flavors during cooking.

The flavors of crustaceans and mollusks rely particularly heavily on nonvolatile taste substances in addition to contributions from volatiles. For example, the taste of cooked snow crab meat has been claimed to be largely duplicated with a mixture of 12 amino acids, nucleotides, and salt ions [41]. Good imitation crab flavors can be prepared from the taste substances just mentioned, along with some contributions from carbonyls and trimethylamine. Trimethylamine has long been associated with fish- and crablike aromas, and it alone exhibits an ammoniacal, fishy aroma. Trimethylamine and dimethylamine are produced through microbial or endogenous enzymic degradation of trimethylamine oxide (Figure 11.29) that is found in significant quantities only in saltwater species of fish and seafoods. Trimethylamine oxide serves as part of the osmolyte system in marine or saltwater fish species [36]. Since very fresh fish contain essentially no trimethylamine, this compound modifies and contributes to the aroma of staling fresh fish in which it enhances fish house-type aromas. Dimethylamine formation is most often associated with staling that occurs during frozen storage.

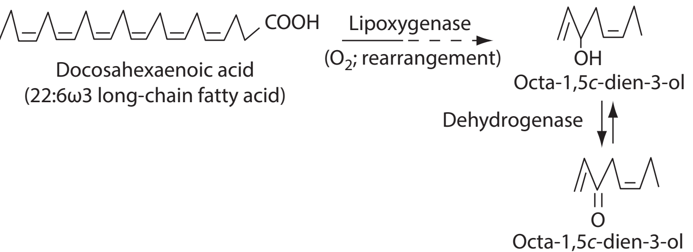


FIGURE 11.28 Enzymatic formation of influential volatiles in fresh fish aroma from long-chain ω3 unsaturated fatty acid.

FIGURE 11.29 Formation of principal volatile amines in muscle tissues of saltwater fish species.

FIGURE 11.30 Formation of dimethyl sulfide in seafoods.

The formaldehyde produced concurrently with dimethylamine is believed to facilitate protein cross-linking and thereby contributes to the toughening of fish muscle during frozen storage.

Other aromas and flavors associated with fish products are often characterized by such terms as "stale or oxidized fish" and "cod liver oil-like," and these are caused by certain carbonyl compounds that develop from the autoxidation of ω3 long-chain polyunsaturated fatty acids (Section 11.5.2). Of these, (*E*, *Z*, *Z*)-2,4,7-decatrienal (cf. Figure 11.24) provides an especially important character-impact fishy flavor quality, and *c*-4-heptenal potentiates its fishy character [46].

Some important characterizing flavors in fish and seafoods are derived from the environment, principally through the natural food chain for the harvested species. For example, dimethyl sulfide provides a characterizing top-note aroma to cooked clams and oysters, and it arises principally from the thermal degradation of dimethyl-β-propiothetin (Figure 11.30) present in ingested marine microflora [55] whose presence in the habitat is dependent on the environmental conditions.

The flavors of aquacultured shrimp and salmon are affected by their diets that differ substantially from those encountered by free-roaming wild counterparts [55]. One of the key differences in the corresponding flavors of these animals is caused by a general lack of bromophenols in aquaculture diets compared to those consumed by wild-capture counterparts. Bromophenols are produced metabolically (Figure 11.31) by a variety of lower-form marine or saltwater organisms, and they are passed up through the food chain where they provide character-impact flavors to both fish and other seafoods [13]. One of the most notable flavor effects of bromophenols is encountered in wild-capture shrimp where the flavor may vary from subtle sealike to distinctly iodine-like. On the other hand, the very low levels of bromophenols that occur in aquacultured shrimp result in indistinct or bland flavors that lack traditional marine- or sealike flavor notes.

11.7 DEVELOPMENT OF PROCESS OR REACTION FLAVOR VOLATILES

Many flavor compounds found in cooked or processed foods occur as the result of reactions common to all types of foods regardless of whether they are of animal, plant, or microbial derivation. These reactions take place when suitable reactants are present and appropriate conditions (heat, pH, light) exist. Process or reaction flavors are discussed separately in this section because of their broad importance to all foods and because they comprise a large volume of natural flavor

FIGURE 11.31 Formation of bromophenols in marine seafoods.

concentrates that are used widely in foods, especially when meat or savory flavors are desired. Related information can be found in discussions dealing with carbohydrates (Chapter 3), lipids (Chapter 4), and vitamins (Chapter 8).

11.7.1 Thermally Induced Process Flavors

Traditionally, these flavors have been broadly viewed as products from browning reactions because of early discoveries showing the role of reducing sugars and amino compounds in the induction of a process that ultimately leads to the formation of brown pigments (see Chapters 3 and 4 for details of Maillard browning reactions). Although browning reactions are almost always involved in the development of process flavors in foods, the interactions between products of the browning reaction and other food constituents are also important and extensive. By taking a broad approach to discussions of thermally induced flavors, the aforementioned interactions, as well as reactions that occur following heat treatment, can be appropriately considered.

Although many of the compounds associated with process flavors possess potent and pleasant aromas, relatively few of these compounds seem to provide truly distinguishing character-impact flavor effects. Instead, they often contribute general nutty, meaty, roasted, toasted, burnt, floral, plant, or caramel odors. Some process flavor compounds are acyclic, but many are heterocyclic, with nitrogen, sulfur, or oxygen substituents common (Figure 11.32). These process flavor compounds occur in many foods and beverages, such as roasted meats, boiled meats, coffee, roasted nuts, beer, bread, crackers, snack foods, cocoa, and most other processed foods. The distribution of individual compounds, however, depends on factors such as the availability of precursors, temperature, time, and water activity.

Production of process flavor concentrates is accomplished by selecting reaction mixtures and conditions so that those reactions occurring in normal food processing are duplicated. Selected ingredients (Table 11.5), usually including a reducing sugar, amino acids, and compounds with sulfur atoms, are processed under elevated temperatures to produce a distinctive profile of flavor compounds [35]. Thiamine is a popular ingredient because it provides both nitrogen and sulfur atoms already in ring structures (see Chapter 8).

Because of the large number of process flavor compounds produced during normal food processing or process simulation, it is unrealistic to cover the chemistry of their formation in depth.

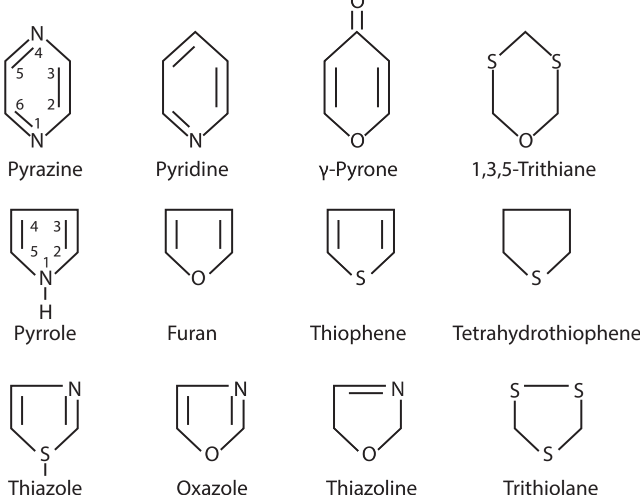


FIGURE 11.32 Some heterocyclic molecular skeletons found commonly in flavor compounds associated with thermally induced or browning flavors.

TABLE 11.5
Some Common Ingredients Used in Process Flavor Reaction Systems for the Development of Meat-Like Flavors

Hydrolyzed Vegetable Protein	Thiamine
Yeast autolysate	Cysteine
Beef extract	Glutathione
Specific animal fats	Glucose
Chicken egg solids	Arabinose
Glycerol	5′-Ribonucleotides
Monosodium glutamate	Methionine

Rather, examples are given to illustrate some of the more important flavor volatiles formed and the mechanisms of their formation. Alkyl pyrazines were among the first compounds to be recognized as important contributors to the flavors of all roasted, toasted, or similarly thermally processed foods. The most direct route to their formation results from the interaction of α-dicarbonyl compounds (intermediate products in the Maillard reaction) with amino acids through the Strecker degradation reaction (Figure 11.33). Transfer of the amino group to the dicarbonyl provides a means for integrating amino acid nitrogen into small compounds destined for any of the condensation reaction mechanisms envisioned in these reactions. Methionine has been selected as the amino acid involved in the Strecker degradation reaction because it contains a sulfur atom and it leads to formation of methional, which is an important character-impact compound in boiling potatoes and cheese–cracker flavors. Methional also readily decomposes further to yield methanethiol (methyl mercaptan) that oxidizes to dimethyl disulfide, thus providing a source of reactive, low molecular weight sulfur compounds that contribute to the overall system of flavor development.

Hydrogen sulfide and ammonia are very reactive ingredients in mixtures intended for the development of process flavors, and they are often included in model systems and assist in promoting

FIGURE 11.33 Formation of an alkyl pyrazine and small sulfur compounds through reactions occurring in the development of process flavors.

FIGURE 11.34 Formation of a thiazoline found in cooked beef through the reaction of fragments from cysteine and sugar-amino browning.

certain reaction mechanisms. Thermal degradation of cysteine (Figure 11.34) yields both ammonia and hydrogen sulfide and acetaldehyde. Subsequent reaction of acetaldehyde with a mercapto derivative of acetoin (from the Maillard reaction) gives rise to thiazoline that contributes to the flavor of boiled beef [56].

Some heterocyclic flavor compounds are quite reactive and tend to degrade or interact further with components of foods or reaction mixtures. An interesting example of flavor stability and carry

through in foods is provided by the compounds shown in Equation 11.2, both of which provide distinct, but different meat-like, aromas [20]. A roasting meat aroma is exhibited by

2-Methyl-3-furanthiol ⇌ (2-Methyl-3-furyl)-bis-disulfide (11.2)

2-methyl-3-furanthiol (reduced form), but upon oxidation to the disulfide form, the flavor becomes more characteristic of fully cooked meat that has been held for some time. Chemical reactions, such as the one just mentioned, are responsible for the subtle changes in meat flavor that occur because of the degree of cooking and the time interval after cooking.

During processing of complex systems, sulfur as such, or as thiols or polysulfides, can be incorporated in various compounds, resulting in the generation of new flavors. However, even though dimethyl sulfide is often found in processed foods, it does not react readily. In plant foods, dimethyl sulfide originates from biologically synthesized molecules, especially *S*-methylmethionine sulfonium salts (Figure 11.35). *S*-Methylmethionine salts are quite labile to heat, and dimethyl sulfide is readily released during cooking. In the case of plant-derived foods, dimethyl sulfide provides especially characterizing top notes to the flavors of freshly boiled and canned sweet corn as well as to tomato juice and other cooked tomato products.

Some of the most pleasant aromas derived from process reactions are provided by the compounds shown in Figure 11.36. These compounds exhibit caramellike aromas and have been found in many processed foods. Cyclotene is used widely as a synthesized maple syrup flavor substance, and maltol is used widely as a flavor enhancer for sweet foods and beverages (Section 11.2.6). 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol) is sometimes known as the "pineapple compound" because it was first isolated from processed pineapple, where it contributes strongly to its characteristic flavor. Furaneol is also produced in biological systems, and it contributes a very ripe strawberry note that can be detected over freshly picked fruit. 3-OH-4,5-Dimethyl-2,5-dihydrofuran-2-one is often referred as sugar furanone because its highly characterizing aroma can easily be detected in the gases above bulk refined sugars (sucrose). Additionally, furanones are found in cooked meats, such as boiled beef, where they apparently enhance meatiness.

The planar enol–cyclic–ketone structure for the compounds shown in Figure 11.36 is typically derived from the sugar precursors, and this structural component appears responsible for their

Methionine → Biosynthesis ($\sim\sim CH_3$) → *S*-Methylmethionine sulfonium salt → H_2O Δ → Dimethyl sulfide + Homoserine

FIGURE 11.35 Formation of dimethyl sulfide from thermal degradation of *S*-methylmethionine sulfonium salts.

FIGURE 11.36 Structures of some important caramellike flavor compounds derived from reactions occurring during processing.

caramellike aroma quality [60]. As shown in Equation 11.3 for maltol, the planar enolone form is largely favored over the cyclic diketone form because the enolone form allows strong intramolecular H-bonding to occur.

(11.3)

The flavor of chocolate and cocoa has received much attention because of the high demand for these flavors. After harvesting, cocoa beans are often fermented under somewhat poorly controlled conditions. The beans are then roasted, sometimes with an intervening alkali treatment that darkens the color and yields a less harsh flavor. The fermentation hydrolyzes sucrose to reducing sugars, frees amino acids, and oxidizes some polyphenols. During roasting, many pyrazines and other heterocyclics are formed, but the unique flavor of cocoa is derived from an interaction between aldehydes from the Strecker degradation reaction. The reaction shown in Figure 11.37 between phenylacetaldehyde (from phenylalanine) and 3-methylbutanal (from leucine) constitutes

FIGURE 11.37 Formation of an important cocoa aroma volatile through an aldol condensation of two Strecker reaction-derived aldehydes.

β-Carotene
+ O2 Free radical oxidation + O2
c-Theaspirane β-Ionone (Isomerization) β-Damascenone

FIGURE 11.38 Formation of some important tea flavor compounds through oxidative cleavages of carotenoids.

an important flavor-forming reaction in cocoa. The product of this aldol condensation, 5-methyl-2-phenyl-2-hexenal, exhibits a character-impact persistent chocolate aroma. This example also serves to show that reactions in the development of process flavors do not always yield heterocyclic aroma compounds.

11.7.2 Volatiles Derived from Oxidative Cleavages of Carotenoids

Oxidations focusing on triacylglycerols and fatty acids were discussed in previous sections, but some extremely important flavor compounds that are oxidatively derived from carotenoid precursors have not been covered and deserve mention here. Some of these reactions require singlet oxygen through chlorophyll sensitization; others are photooxidation processes. A large number of flavor compounds, derived from oxidizing carotenoids (or isoprenoids), have been identified in curing tobacco [18], and many of these are considered important for characterizing tobacco flavors. However, relatively few compounds in this category (three representative compounds are shown in Figure 11.38) are currently considered highly important as food flavors. Each of these compounds exhibits unique sweet, floral, and fruit-like characteristics that vary greatly with concentration. They also blend nicely with aromas of foods to produce subtle effects that may be highly desirable or very undesirable. β-Damascenone exerts very positive effects on fresh apple aromas. It also enhances the flavor of wines, but in beer this compound at only a few parts per billion results in a stale, raisin-like note. β-Ionone also exhibits a pleasant violet, floral aroma compatible with fruit-type flavors, but it is also the principal off-flavor compound present in oxidized, freeze-dried carrots. Furthermore, these compounds have been found in black tea, where they make positive contributions to the flavor. Theaspirane and related derivatives contribute importantly to the sweet, fruity, and earthy notes of tea aroma. Although usually present in low concentrations, these compounds and related ones appear to be widely distributed, and it is likely that they contribute to the full, well-blended flavors of many foods.

11.8 FUTURE DIRECTIONS OF FLAVOR CHEMISTRY AND TECHNOLOGY

Much has been accomplished over the past 45 years since the golden era of modern flavor chemistry and technology [77] was initiated with the development and ready availability of powerful gas chromatography and fast-scan mass spectrometry instruments. Overall, much of this period was

devoted to the systematic discovery of aroma-active substances, and aside from ferreting out details, focused efforts have largely accomplished the objectives of the task. Similarly, the knowledge base surrounding the mechanistic aspects involved in the formation and degradation of flavor compounds has become extensive, but many details of these processes still remain incomplete and available for further fruitful study. Certainly, the continued advancement of genetic engineering for specific flavor effects in various single-cell and higher organisms serves to provide motivations for additional efforts to more thoroughly understand the intricacies of mechanisms involved in the biosynthesis of flavor-related substances.

Nevertheless, it is likely that the focus of future flavor chemistry research will need to shift considerably to meet the most pressing demands facing the food industry. Substantial expansions of nutraceutical or functional foods will bring new challenges to mask or otherwise neutralize undesirable flavors that are inherent in ingredients employed to provide the functional properties. However, the most challenging problems reside in the successful reformulation of foods for health reasons to avoid excessive amounts of more traditional ingredients, particularly salt, fats, and refined carbohydrates. Initial attempts to develop what have been called "nutritionally appropriate" foods have met with many failures and frustrations, but public pressures are building to make the food industry overcome hurdles that stymied earlier attempts to provide these foods.

Recent flavor chemistry studies on rates of release of individual flavor chemicals from various matrixes were carried out in an attempt to understand and overcome problems encountered in providing high-quality flavors in reformulated foods whose compositions have been radically changed to meet a nutrition goal (e.g., low-fat foods). Still, to date, breakthrough findings have not emerged from flavor release studies, which suggest that other approaches also need to be vigorously explored. One of the most overlooked areas in this regard is that of flavor enhancers and modifiers (Section 11.2.6) whose roles in food palatability are not well recognized nor well understood, and the area is ripe for exploration. Such efforts, however, will require changes in traditional food and flavor chemistry thinking and necessarily also will require alterations in sensory analysis techniques to specifically detect and assess the subtle food palatability characteristics provided by flavor modifiers. The challenges are great, but the potential benefits to the health and well-being of the population also are great.

REFERENCES

1. Amerine, M. A., R. M. Pangborn, and E. B. Roessler (1965). *Principles of Sensory Evaluation of Food*, Academic Press, New York, p. 106.
2. ASTM (1968). *Manual on Sensory Testing Methods*, STP434, American Society for Testing Materials, Philadelphia, PA.
3. Bartoshuk, L. M. (1980). Sensory analysis in the taste of NaCl. In *Biological and Behaviorial Aspects of Salt Intake* (R. H. Cagan and M. R. Kare, eds.), Academic Press, New York, pp. 83–96.
4. Bartoshuk, L. M., K. Fast, D. Snyder, and V. B. Duffy (2004). Genetic differences in human oral perception. In *Genetic Variation in Taste Sensitivity* (J. Prescott and B. J. Tepper, eds.), Marcel Dekker, New York, pp. 1–41.
5. Beets, M. G. J. (1978). The sweet and bitter modalities. In *Structure–Activity Relationships in Human Chemoreception*, Applied Science Publishers, London, U.K., pp. 259–303.
6. Beets, M. G. J. (1978). The sour and salty modalities. In *Structure–Activity Relationships in Human Chemoreception*, Applied Science Publishers, London, U.K., pp. 348–362.
7. Belitz, H. D. and H. Wieser (1985). Bitter compounds: Occurrence and structure–activity relationships. *Food Rev. Int.* 1:271–354.
8. Birch, G. G. (1981). Basic tastes of sugar molecules. In *Criteria of Food Acceptance* (J. Solms and R. L. Hall, eds.), Forster Verlag, Zurich, Switzerland, pp. 282–291.
9. Birch, G. G., C. K. Lee, and A. Ray (1978). The chemical basis of bitterness in sugar derivatives. In *Sensory Properties of Foods* (G. G. Birch, J. G. Brennan, and K. J. Parker, eds.), Applied Science Publishers, London, U.K., pp. 101–111.

10. Blank, I., J. Lin, F. A. Vera, D. H. Welti, and L. B. Fay (2001). Identification of potent odorants formed by autoxidation of arachidonic acid: Structure elucidation and synthesis of (*EZZ*)-2,4,7-tridecatrienal. *J. Agric. Food Chem.* 49:2959–2965.
11. Boelens, M. H. (1991). Spices and condiments II. In *Volatile Compounds in Foods and Beverages* (H. Maarse, ed.), Marcel Dekker, New York, pp. 449–482.
12. Boelens, M. H., H. Boelens, and L. J. van Gemert (1993). Sensory properties of optical isomers. *Perfum. Flavor.* 18:1–16.
13. Boyle, J. L., R. C. Lindsay, and D. A. Stuiber (1993). Occurrence and properties of flavor-related bromophenols found in the marine environment: A review. *J. Aquat. Food Prod. Technol.* 2:75–112.
14. Brieskorn, C. H. (1990). Physiological and therapeutical aspects of bitter compounds. In *Bitterness in Foods and Beverages* (R. L. Rouseff, ed.), Elsevier, Amsterdam, the Netherlands, pp. 15–33.
15. Cliff, M. and H. Heymann (1992). Descriptive analysis of oral pungency. *J. Sens. Stud.* 7:279–290.
16. DeTaeye, L., D. DeKeukeleire, E. Siaeno, and M. Verzele (1977). Recent developments in hop chemistry. In *European Brewery Convention Proceedings, European Brewing Congress*, Amsterdam, the Netherlands, pp. 153–156.
17. Dwivedi, B. K. (1975). Meat flavor. *Crit. Rev. Food Technol.* 5:487–535.
18. Enzell, C. R. (1981). Influence of curing on the formation of tobacco flavour. In *Flavour '81* (P. Schreier, ed.), Walter de Gruyter, Berlin, Germany, pp. 449–478.
19. Eriksson, C. E. (1979). Review of biosynthesis of volatiles in fruits and vegetables since 1975. In *Progress in Flavour Research* (D. G. Land and H. E. Nursten, eds.), Applied Science Publishers, London, U.K., pp. 159–174.
20. Evers, W. J., H. H. Heinsohn, B. J. Mayers, and A. Sanderson (1976). Furans substituted at the three positions with sulfur. In *Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors* (G. Charalambous and I. Katz, eds.), American Chemical Society, Washington, DC, pp. 184–193.
21. Fazzalari, F. A. (ed.) (1978). *Compilation of Odor and Taste Threshold Values Data*, American Society for Testing Materials, Philadelphia, PA.
22. Forss, D. A. (1981). Sensory characterization. In *Flavor Research, Recent Advances* (R. Teranishi, R. A. Flath, and H. Sugisawa, eds.), Marcel Dekker, New York, pp. 125–174.
23. Forsyth, W. G. C. (1981). Tannins in solid foods. In *The Quality of Foods and Beverages*, Vol. 1, Chemistry and Technology (G. Charalambous and G. Inglett, eds.), Academic Press, New York, pp. 377–388.
24. Govindarajan, V. S. (1979). Pungency: the stimuli and their evaluation. In *Food Taste Chemistry* (J. C. Boudreau, ed.), American Chemical Society, Washington, DC, pp. 52–91.
25. Gower, D. B., M. R. Hancock, and L. H. Bannister (1981). Biochemical studies on the boar pheromones, 5α-androst-16-en-3-one and 5α-androst-16-en-3α-ol, and their metabolism by olfactory tissue. In *Biochemistry of Taste and Olfaction* (R. H. Cagan and M. R. Kare, eds.), Academic Press, New York, pp. 7–31.
26. Grosch, W. (1982). Lipid degradation products and flavour. In *Food Flavours, Part A, Introduction* (I. D. Morton and A. J. Macleod, eds.), Elsevier Scientific, Amsterdam, the Netherlands, pp. 325–398.
27. Grosch, W. (1994). Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). *Flavour Frag. J.* 9:147–158.
28. Guenther, E. (1948). *The Essential Oils*, Vols. 1–6, van Nostrand, New York.
29. Ha, J. K. and R. C. Lindsay (1990). Distribution of volatile branched-chain fatty acids in perinephric fats of various red meat species. *Lebensm. Wissen. Tech.* 23:433–439.
30. Ha, J. K. and R. C. Lindsay (1991). Contributions of cow, sheep, and goat milks to characterizing branched-chain fatty acid and phenolic flavors in varietal cheeses. *J. Dairy Sci.* 74:3267–3274.
31. Ha, J. K. and R. C. Lindsay (1991). Volatile alkylphenols and thiophenol in species-related characterizing flavors of red meats. *J. Food Sci.* 56:1197–1202.
32. Han, L.-H. A. (2000). Characterization and enhancement of contributions of alkylphenols to baked-butter and other flavors. PhD thesis, University of Wisconsin-Madison, Madison, WI, pp. 90–135.
33. Harkes. P. D. and W. J. Begemann (1974). Identification of some previously unknown aldehydes in cooked chicken. *J. Am. Oil Chem. Soc.* 51:356–359.
34. Hasegawa, S., M. N. Patel, and R. C. Snyder (1982). Reduction of limonin bitterness in navel orange juice serum with bacterial cells immobilized in acrylamide gel. *J. Agric. Food Chem.* 30:509–511.
35. Heath, H. B. (1981). *Source Book of Flavors*, AVI Publishing Co., Westport, CT, p. 110.
36. Hebard, C. E., G. J. Flick, and R. E. Martin (1982). Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In *Chemistry and Biochemistry of Marine Products* (R. E. Martin, G. J. Flick, and D. R. Ward, eds.), AVI Publishing Co., Westport, CT, pp. 149–304.

37. Hiraide, M., Y. Miyazaki, and Y. Shibata (2004). The smell and odorous components of dried shiitake mushroom, *Lentinula edodes* I: Relationship between sensory evaluations and amounts of odorous components. *J. Wood Sci.* 50:358–364.
38. Ho, C. T. and T. G. Hartman (eds.) (1994). *Lipids in Food Flavors,* American Chemical Society, Washington, DC, pp. 1–333.
39. Kawamura, Y. and M. R. Kare (eds.) (1987). *Umami: A Basic Taste*, Marcel Dekker, New York, pp. 1–649.
40. Kimball, D. A. and S. I. Norman (1990). Processing effects during commercial debittering of California navel orange juice. *J. Agric. Food Chem.* 38:1396–1400.
41. Konosu, S. (1979). The taste of fish and shellfish. In *Food Taste Chemistry* (J. C. Boudreau, ed.), American Chemical Society, Washington, DC, pp. 203–213.
42. Koppenhoefer, B., R. Behnisch, U. Epperlein, H. Holzschuh, and R. Bernreuther (1994). Enantiomeric odor differences and gas chromatographic properties of flavors and fragrances. *Perfum. Flavor.* 19:1–14.
43. Kuninaka, A. (1981). Taste and flavor enhancers. In *Flavor Research Recent Advances* (R. Teranishi, R. A. Flath, and H. Sugisawa, eds.), Marcel Dekker, New York, pp. 305–353.
44. Laing, D. G. and A. Jinks (1996). Favour perception mechanisms. *Trend Food Sci. Technol.* 7:387–389, 421–424.
45. Lee, C. B. and H. T. Lawless (1991). Time-course of astringent sensations. *Chem. Senses* 16:225–238.
46. Lindsay, R. C. (1990). Fish flavors. *Food Rev. Int.* 6:437–455.
47. Lopez, V. and R. C. Lindsay (1993). Metabolic conjugates as precursors for characterizing flavor compounds in ruminant milks. *J. Agric. Food Chem.* 41:446–454.
48. Maarse, H. (1991). *Volatile Compounds in Foods and Beverages*, Marcel Dekker, New York.
49. Martin, G., G. Remaud, and G. J. Martin (1993). Isotopic methods for control of natural flavours authenticity. *Flavour Frag. J.* 8:97–107.
50. McGregor, R. (2004). Taste modification in the biotech era. *Food Technol.* 58:24–30.
51. Mielle, P. (1996). "Electronic noses": Towards the objective instrumental characterization of food aroma. *Trend Food Sci. Technol.* 7:432–438.
52. Morgan, M. E., L. M. Libbey, and R. A. Scanlan (1972). Identity of the musty-potato aroma compound in milk cultures of *Pseudomonas taetrolens. J. Dairy Sci.* 55:666.
53. Morton, I. D. and A. J. Macleod (eds.) (1982). *Food Flavours, Part A, Introduction*, Elsevier Scientific, Amsterdam, the Netherlands.
54. Mosandl, A. (1988). Chirality in flavor chemistry—Recent developments in synthesis and analysis. *Food Rev. Int.* 4:1–43.
55. Motohito, T. (1962). Studies on the petroleum odor in canned chum salmon. *Mem. Fac. Fisheries Hokkaido Univ.* 10:1–5.
56. Mussinan, C. J., R. A. Wilson, I. Katz, A. Hruza, and M. H. Vock (1976). Identification and some flavor properties of some 3-oxazolines and 3-thiazolines isolated from cooked beef. In *Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors* (G. Charalambous and I. Katz, eds.), American Chemical Society, Washington, DC, pp. 133–145.
57. Neucere, N. J., T. J. Jacks, and G. Sumrell (1978). Interactions of globular proteins with simple polyphenols. *J. Agric. Food Chem.* 26:214–216.
58. Ney, K. H. (1979). Bitterness of peptides: Amino acid composition and chain length. In *Food Taste Chemistry* (J. C. Boudreau, ed.), American Chemical Society, Washington, DC, pp. 149–173.
59. Ney, K. H. (1979). Bitterness of lipids. *Fette Seifen Anstrichm.* 81:467–469.
60. Ohloff, G. (1981). Bifunctional unit concept in flavor chemistry. In *Flavour '81* (P. Schreier, ed.), Walter de Gruyter, Berlin, Germany, pp. 757–770.
61. O'Mahony, M. (1986). *Sensory Evaluation of Food: Statistical Methods and Procedures*, Marcel Dekker, New York.
62. Reed, D. R. (2004). Progress in human bitter phenylthiocarbamide genetics. In *Genetic Variation in Taste Sensitivity* (J. Prescott and B. J. Tepper, eds.), Marcel Dekker, New York, pp. 43–61.
63. Richard, H. M. J. (1991). Spices and condiments I. In *Volatile Compounds in Foods and Beverages* (H. Maarse, ed.), Marcel Dekker, New York, pp. 411–447.
64. Scanlan, R. A. (ed.) (1977). *Flavor Quality: Objective Measurement*, American Chemical Society, Washington, DC.
65. Schiffman, S. S. (1980). Contribution of the anion to the taste quality of sodium salts. In *Biological and Behavioral Aspects of Salt Intake* (R. H. Cagan and M. R. Kare, eds.), Academic Press, New York, pp. 99–114.
66. Schreier, P. (ed.) (1981). *Flavour '81*, Walter de Gruyter, Berlin, Germany.

67. Shahidi, F. (ed.) (1994). *Flavor of Meat and Meat Products*, Blackie Academic & Professional, London, U.K., pp. 1–298.
68. Shallenberger, R. S. and T. E. Acree (1967). Molecular theory of sweet taste. *Nature (London)* 216:480–482.
69. Shankaranarayana, M. L., B. Raghaven, K. O. Abraham, and C. P. Natarajan (1982). Sulphur compounds in flavours. In *Food Flavours, Part A, Introduction* (I. D. Morton and A. J. Macleod, eds.), Elsevier Scientific, Amsterdam, the Netherlands, pp. 169–281.
70. Shaw, P. E. (1991). Fruits II. In *Volatile Compounds in Foods and Beverages* (H. Maarse, ed.), Marcel Dekker, New York, pp. 305–327.
71. Stone, H. and J. L. Sidel (1985). *Sensory Evaluation Practices*, Academic Press, New York.
72. Smith, A. and W. R. H. Duncan (1979). Characterization of branched-chain lipids from fallow deer perinephric triacylglycerols by gas chromatography-mass spectrometry. *Lipids* 14:350–355.
73. Special Issue on Flavour Perception (1996). *Food Sci. Technol.* 7:457.
74. Swoboda, P. A. T. and K. E. Peers (1979). The significance of octa-1-*cis*-5-dien-3-one. In *Progress in Flavour Research* (D. G. Land and H. E. Nursten, eds.), Applied Science Publishers, London, U.K., pp. 275–280.
75. Tanford, C. (1960). Contribution of hydrophobic interactions to the stability of globular conformations of proteins. *J. Am. Chem. Soc.* 84:4240–4247.
76. Teranishi, R., R. A. Flath, and H. Sugisawa (eds.) (1981). *Flavor Research: Recent Advances*, Marcel Dekker, New York.
77. Teranishi, R., E. L. Wick, and I. Hornstein (eds.) (1999). *Flavor Chemistry: Thirty Years of Progress*, Kluwer Academic/Plenum Publishers, New York.
78. Tressl, R., D. Bahri, and K. H. Engel (1982). Formation of eight-carbon and ten-carbon components in mushrooms (*Agaricus campestris*). *J. Agric. Food Chem.* 30:89–93.
79. Tressl, R., M. Holzer, and M. Apetz (1975). Biogenesis of volatiles in fruit and vegetables. In *Aroma Research: Proceedings of the International Symposium on Aroma Research*, Zeist, the Netherlands (H. Maarse and P. J. Groenen, eds.), Centre for Agricultural Publishing and Documentation, PUDOC, Wageningen, the Netherlands, pp. 41–62.
80. Ueda, Y., M. Sakaguchi, and K. Hirayama (1990). Characteristic flavor constituents in water extract of garlic. *Agric. Biol. Chem.* 54:163–169.
81. Ueda, Y., T. Tsubuku, and R. Miyajima (1994). Composition of sulfur-containing components in onion and their flavor characters. *Biosci. Biotech. Biochem.* 58:108–110.
82. Ueda, Y., M. Yonemitsu, T. Tsubuku, and M. Sakaguchi (1997). Flavor characteristics of glutathione in raw and cooked foodstuffs. *Biosci. Biotech. Biochem.* 61:1977–1980.
83. van Straten, S., F. de Vrijer, and J. C. deBeauveser (eds.) (1977). *Volatile Compounds in Food*, 4th edn., Central Institute for Nutrition and Food Research, Zeist, the Netherlands.
84. Watson, H. R. (1978). Flavor characteristics of synthetic cooling compounds. In *Flavor: Its Chemical, Behavioral, and Commercial Aspects* (C. M. Apt, ed.), Westview Press, Boulder, CO, pp. 31–50.
85. Whitfield, F. B. and J. H. Last (1991). Vegetables. In *Volatile Compounds in Foods and Beverages* (H. Maarse, ed.), Marcel Dekker, New York, pp. 203–269.
86. Wittkowski, R., J. Ruther, H. Drinda, and F. Rafiei-Taghanaki (1992). Formation of smoke flavor compounds by thermal lignin degradation. In *Flavor Precursors: Thermal and Enzymatic Conversions* (R. Teranishi, G. R. Takeoka, and M. Guntert, eds.), American Chemical Society, Washington, DC, pp. 232–243.
87. Wong, E., L. N. Nixon, and C. B. Johnson (1975). Volatile medium chain fatty acids and mutton flavor. *J. Agric. Food Chem.* 23:495–498.
88. Yamaguchi, S. (1979). The umami taste. In *Food Taste Chemistry* (J. C. Boudreau, ed.), American Chemical Society, Washington, DC, pp. 33–51.
89. Yashimoto, K., K. Iwami, and H. Mitsuda (1971). A new sulfur-peptide from *Lentinus edodes* acting as a precursor for lenthionine. *Agric. Biol. Chem.* 35:2059–2069.

FURTHER READING

Amerine, M. A., R. M. Pangborn, and E. B. Roessler (1965). *Principles of Sensory Evaluation of Food*, Academic Press, New York.

Beets, M. G. J. (1978). *Structure–Activity Relationships in Human Chemoreception*, Applied Science Publishers, London, U.K.

Burdock, G. A. (2004). *Fenaroli's Handbook of Flavor Ingredients*, 5th edn., CRC Press, Boca Raton, FL.

Fazzalari, F. A. (ed.) (1978). *Compilation of Odor and Taste Threshold Values Data*, American Society for Testing Materials, Philadelphia, PA.

Ho, C. T. and T. G. Hartman (eds.) (1994). *Lipids in Food Flavors*, ACS Symposium Series 558, American Chemical Society, Washington, DC.

Maarse, H. (1991). *Volatile Compounds in Foods and Beverages*, Marcel Dekker, New York.

Morton, I. D. and A. J. Macleaod (eds.) (1982). *Food Flavors, Part A, Introduction*, Elsevier Scientific, New York.

O'Mahony, M. (1986). *Sensory Evaluation of Food: Statistical Methods and Procedures*, Marcel Dekker, New York.

Parliment, T. H., M. J. Morello, and R. J. McGorrin (1994). *Thermally Generated Flavors: Maillard, Microwave, and Extrusion Processes*, ACS Symposium Series 543, American Chemical Society, Washington, DC.

Rouseff, R. L. (ed.) (1990). *Bitterness in Foods and Beverages*, Elsevier, Amsterdam, the Netherlands.

Shahidi, F. (ed.) (1994). *Flavor of Meat and Meat Products*, Blackie Academic & Professional, London, U.K.

Stone, H. and J. L. Sidel (1985). *Sensory Evaluation Practices*, Academic Press, New York.

Teranishi, R., G. R. Takeoka, and M. Guntert (eds.) (1992). *Flavor Precursors: Thermal and Enzymatic Conversions*, ACS Symposium Series 490, American Chemical Society, Washington, DC.

12 Food Additives

Robert C. Lindsay

CONTENTS

12.1 Introduction 804
12.2 Acids 804
12.2.1 General Attributes 804
12.2.2 Chemical Leavening Systems 807
12.3 Bases 811
12.4 Buffer Systems and Salts 812
12.4.1 Buffers and pH Control in Foods 812
12.4.2 Salts in Processed Dairy Foods 813
12.4.3 Phosphates and Water Binding in Animal Tissues 814
12.5 Chelating Agents (Sequestrants) 814
12.6 Antioxidants 816
12.7 Antimicrobial Agents 818
12.7.1 Sulfites and Sulfur Dioxide 818
12.7.2 Nitrite and Nitrate Salts 820
12.7.3 Acetic Acid 821
12.7.4 Propionic Acid 821
12.7.5 Sorbic Acid and Other Medium-Chain Fatty Acids 821
12.7.6 Glyceryl Esters 824
12.7.7 Lauric Arginate 824
12.7.8 Natamycin 825
12.7.9 Benzoic Acid 825
12.7.10 *p*-Hydroxybenzoate Alkyl Esters 826
12.7.11 Epoxides 826
12.7.12 Antibiotics 827
12.7.13 Diethyl Pyrocarbonate 828
12.8 Intensely Sweet Nonnutritive and Low-Calorie Sweeteners 829
12.8.1 Sulfoamides: Cyclamate, Saccharin, and Acesulfame K 830
12.8.2 Peptides: Aspartame, Neotame, and Alitame 831
12.8.3 Chlorosaccharides: Sucralose 833
12.8.4 Other Intensely Sweet Nonnutritive or Low-Calorie Sweeteners 834
12.9 Polyols: Sweeteners, Texturizers, and Emulsifiers 836
12.10 Stabilizers and Thickeners 841
12.11 Fat Replacers 841
12.11.1 Carbohydrate Fat Mimetics 842
12.11.2 Protein Fat Mimetics 842
12.11.3 Reduced-Calorie Synthetic Triacylglycerol Fat Substitutes 842
12.11.4 Synthetic Fat Replacers 843
12.11.4.1 Polydextrose 843
12.11.4.2 Sucrose Polyesters 844
12.12 Masticatory Substances 845
12.13 Firming Texturizers 845
12.14 Appearance Control and Clarifying Agents 846

12.15 Flour Bleaching Agents and Bread Improvers 849
12.16 Anticaking Agents 851
12.17 Gases and Propellants 852
12.17.1 Protection from Oxygen 852
12.17.2 Carbonation 852
12.17.3 Propellants 853
12.18 Summary 853
References 861
Further Reading 863

12.1 INTRODUCTION

Many substances are incorporated into foods for functional purposes, and in many cases these ingredients can also be found occurring naturally in some food. However, when they are used in processed foods, these chemicals have become known as "food additives." From a regulatory standpoint, each of the food additives must provide some useful and acceptable function or attribute to justify its usage. Generally, improved keeping quality, enhanced nutritional value, functional property provision and improvement, processing facilitation, and enhanced consumer acceptance are considered acceptable functions for food additives. The use of food additives to conceal damage or spoilage to foods or to deceive consumers is expressly forbidden by regulations governing the use of these substances in foods. Additionally, food additive usages are discouraged where similar effects can be obtained by economical, good manufacturing practices.

The breadth of food constituents that can be considered to be of suitable importance for specific attention in food chemistry textbooks has expanded in recent years, and some of these constituents can be handled in a manner similar to traditional food additives. However, increased understandings of health benefits provided by individual or groups of food constituents have prompted commercial introductions of a broad range of new food ingredients. Many of these ingredients have been developed to meet formulation challenges presented by specific needs, such as fat calorie reductions, while others have emerged as ingredients intended to provide specific health benefits, for example, plant sterols for blood cholesterol reduction. While many of these ingredients and substances clearly fall into the food additives category, a movement is under way to disassociate them from the unfavorable image of food additives.

Some blurring of traditional terminology already has occurred. Notable is the recent widespread acceptance of the terms "functional foods" and "functional food ingredients" to designate foods and ingredients containing health-related constituents in greater abundance than usual counterparts. As a result of this usage, the utility of the terms "functional purposes" and "functional food ingredients," as they have been applied traditionally to food additive performances, has been diminished, and alternative terminology needs to be explored.

Nevertheless, natural counterparts exist for many food additives, and increasingly new ingredients themselves are derived commercially from natural sources. This chapter focuses on the role of both natural and synthetic substances that are added to foods and provides an integrating view of their functionalities. Further discussions about the chemistry of specific food components often can be found in the appropriate primary constituent chapters of this book. For example, natural substances sometimes used as food additives are discussed in Chapters 4 (Section 4.11.4), 8 through 11, and 13.

12.2 ACIDS

12.2.1 General Attributes

Both organic and inorganic acids occur extensively in natural systems where they function in a variety of roles ranging from intermediary metabolites to components of buffer systems. Acids are added for numerous purposes in foods and food processing where they provide the benefits of many

of their natural actions. One of the most important functions of acids in foods is participation in buffering systems, and this aspect is discussed in the following section. The use of acids and acid salts in chemical leavening systems, the role of specific acidic microbial inhibitors (e.g., sorbic acid, benzoic acid) in food preservation, and the function of acids as chelating agents are also discussed in subsequent sections of this chapter. Acids are important in the setting of pectin gels (Chapter 3), they serve as defoaming agents and emulsifiers, and they induce coagulation of milk proteins (Chapters 5 and 14) in the production of cheese and cultured dairy products such as sour cream. In natural culturing processes, lactic acid (CH_3–CHOH–COOH) produced by streptococci and lactobacilli causes coagulation by lowering the pH to near the isoelectric point of casein. Cheeses can be produced by adding rennet and acidulants such as citric acid and hydrochloric acid to cold milk (4°C–8°C). Subsequent warming of the milk (to 35°C) produces a uniform gel structure. The addition of acid to warm milk results in a protein precipitate rather than a gel.

δ-Gluconolactone that is derived from glucose via a process involving fermentation can also be used for slow acid production in cultured dairy products and chemical leavening systems because it slowly hydrolyzes in aqueous systems to form gluconic acid (Figure 12.1). Dehydration of lactic acid yields lactide, a cylic dilactone (Figure 12.2), that also can be used as a slow-release acid

FIGURE 12.1 Reactions for the formation of gluconic acid via fermentative oxidation of glucose followed by thermal dehydration to form δ-gluconolactone; the latter reaction is reversed on contact of δ-gluconolactone, with water providing a means for slow-release acidification of foods.

FIGURE 12.2 Equilibrium reaction showing the formation of lactic acid from the hydrolysis of lactide.

in aqueous systems. The dehydration reaction occurs under conditions of low water activity and elevated temperature. The introduction of lactide into foods with high water activity causes a reversal of the process with the production of 2 mol of lactic acid.

Acids such as citric are added to some moderately acidic fruits and vegetables to lower the pH to a value below 4.5. In canned foods, this permits sterilization to be achieved under less severe thermal conditions than is necessary for less acidic products and has the added advantage of precluding the growth of hazardous microorganisms (i.e., *Clostridium botulinum*).

Acids, such as potassium acid tartrate (HOOC−CHOH−CHOH−COOK), are employed in the manufacture of fondant and fudge to induce limited hydrolysis (inversion) of sucrose (Chapter 3). Inversion of sucrose yields fructose and glucose, which improve texture through inhibition of excessive growth of sucrose crystals. Monosaccharides inhibit sucrose crystallization by contributing to the complexity of the syrup and by lowering its water activity and corresponding equilibrium relative humidity.

One of the most important contributions of acids to foods is their ability to produce a sour or tart taste ([22]; Chapter 11). Acids also have the ability to modify and intensify the taste perception of other flavoring agents. The hydrogen ion or hydronium ion (H_3O^+) is involved in the generation of the sour taste response. Furthermore, short-chain free fatty acids (C_2−C_{12}) contribute significantly to the aroma of foods. For example, acetic acid dominates the aroma and taste (flavor) of vinegar. Butyric acid, at relatively high concentrations, contributes strongly to the characteristic flavor of hydrolytic rancidity in dairy products, but at lower concentrations contributes to the typical flavor of foods such as cheese and butter.

The ability to influence the pH of foods obviously also is an important consideration in the selection of an acid for a specific application, and this is governed largely by the extent of dissociation experienced by the acid functional group in aqueous systems. Dissociation constants for some acids used in foods are shown in Table 12.1 [59]. Some of the more commonly used organic acids for food applications are acetic (CH_3COOH), lactic (CH_3−CHOH−COOH),

TABLE 12.1
Dissociation Constants at 25°C for Some Acids Used in Foods

Acid	Step	pK_a	Acid	Step	pK_a
Organic acids					
Acetic		4.75	Propionic		4.87
Adipic	1	4.43	Succinic	1	4.16
	2	5.41		2	5.61
Benzoic		4.19	Tartaric	1	3.22
n-Butyric		4.81		2	4.82
Citric	1	3.14	**Inorganic acids**		
	2	4.77	Carbonic	1	6.37
	3	6.39		2	10.25
Formic		3.75	*o*-Phosphoric	1	2.12
Fumaric	1	3.03		2	7.21
	2	4.44		3	12.67
Hexanoic		4.88	Pyrophosphoric	1	0.85
Lactic		3.08		2	1.49
Malic	1	3.40		3	5.77
	2	5.10		4	8.22
			Sulfuric	2	1.92

Source: From Weast, R.C., ed., *Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL, 1988, pp. D161–D163.

citric ($HOOC{-}CH_2{-}COH(COOH){-}CH_2{-}COOH$), malic ($HOOC{-}CHOH{-}CH_2{-}COOH$), fumaric ($HOOC{-}CH{=}CH{-}COOH$), succinic ($HOOC{-}CH_2{-}CH_2{-}COOH$), and tartaric ($HOOC{-}CHOH{-}CHOH{-}COOH$). Phosphoric acid ($H_3PO_4$) is the most widely used inorganic acid food acidulant, and it is employed extensively in flavored carbonated beverages, particularly in colas and root beer.

The strong mineral acids (e.g., HCl, H_2SO_4) are often too highly dissociable for general food acidulation, and their direct introduction may lead to problems with quality attributes of foods. However, the highly dissociable mineral acids are useful and economic in the manufacture of some intermediate ingredients, such as protein hydrolysates. Sodium acid sulfate (e.g., sodium bisulfate [$NaHSO_4$]) or half-neutralized sulfuric acid provides another ingredient option and has recently received U.S. generally recognized as safe (GRAS) approval. Sodium acid sulfate is manufactured in a granular powder form, and its pK_a allows it to provide acidulation properties similar to phosphoric acid.

12.2.2 Chemical Leavening Systems

Chemical leavening systems are composed of compounds that react to release gas in a dough or batter under appropriate conditions of moisture and temperature. During baking, this gas release, along with expansion of entrapped air and moisture vapor, imparts a characteristic porous, cellular structure to finished goods. Chemical leavening systems are found in self-rising flours, prepared baking mixes, household and commercial baking powders, and refrigerated dough products [25].

Carbon dioxide is the only gas generated from currently used chemical leavening systems, and it is derived from a carbonate or bicarbonate salt. The most common leavening salt is sodium bicarbonate ($NaHCO_3$), although ammonium carbonate [$(NH_4)_2CO_3$] and bicarbonate (NH_4HCO_3) are sometimes used in cookies. Both of the ammonium salts decompose at baking temperatures and thus do not require, as does sodium bicarbonate, an added leavening acid for functionality. Potassium bicarbonate ($KHCO_3$) has been employed as a component of leavening systems in reduced-sodium diets, but its application is somewhat limited because of its hygroscopic nature and slight bitter flavor.

Sodium bicarbonate is quite soluble in water (619 g/100 mL) and ionizes completely (Equations 12.1 through 12.3):

$$NaHCO_3 \rightleftarrows Na^+ + HCO_3^- \tag{12.1}$$

$$HCO_3^- + H_2O \rightleftarrows H_2CO_3 + OH^- \tag{12.2}$$

$$HCO_3^- \rightleftarrows CO_3^{2-} + H^+ \tag{12.3}$$

These reactions, of course, apply only to simple water solutions. In dough systems, the ionic distribution becomes much more complex since proteins and other naturally occurring ionic species are available to participate in the reactions. In the presence of hydrogen ions provided mainly by leavening acids and, to some extent, by the dough, sodium bicarbonate reacts to release carbon dioxide (Equation 12.4, showing a carboxyl acid):

$$R{-}COO^-, H^+ + NaHCO_3 \rightleftarrows R{-}COO^-, Na^+ + H_2O + CO_2\uparrow \tag{12.4}$$

The proper balance of acid and sodium bicarbonate is essential because excess sodium bicarbonate imparts a soapy taste to bakery products; an excess of acid leads to tartness and sometimes bitterness. The neutralizing power of leavening acids is not uniform, and the relative activity of an acid is given by its neutralizing value, which is determined by calculating the parts by weight of sodium bicarbonate that will neutralize 100 parts by weight of the leavening acid [53]. However, in the presence of natural flour ingredients, the amount of leavening acid required to give neutrality or any

other desired pH in a baked product may be quite different from the theoretical amount determined for a simple system. Still, neutralizing values are useful in determining initial formulations for leavening systems. Residual salts from a properly balanced leavening process help stabilize the pH of finished products.

Leavening acids are often not easily recognized as acids in the usual sense, yet they must provide hydrogen ions to release carbon dioxide. The phosphates and potassium acid tartrate are salts of partially neutralized acids; sodium aluminum sulfate reacts with water to yield sulfuric acid (Equation 12.5). As mentioned earlier, δ-gluconolactone is an intramolecular ester (or lactone) that hydrolyzes slowly in aqueous systems to yield gluconic acid:

$$Na_2SO_4 \cdot Al_2(SO_4)_3 + 6H_2O \rightleftarrows Na_2SO_4 + 2Al(OH)_3 + 3H_2SO_4 \quad (12.5)$$

Leavening acids generally exhibit limited water solubility at room temperature, but some are less soluble than others. This difference in solubility or availability accounts for the initial rate of carbon dioxide release at room temperature and is the basis for classifying leavening acids according to speed. For example, if the compound is moderately soluble, carbon dioxide is rapidly evolved and the acid is referred to as fast acting. Conversely, if the acid dissolves slowly, it is a slow-acting leavening acid. Leavening acids usually release a portion of the carbon dioxide before baking and the remainder under elevated temperatures of the baking process.

General patterns of carbon dioxide release at 27°C for fast-acting monocalcium phosphate monohydrate [$Ca(H_2PO_4)_2 \cdot H_2O$] and slow-acting 1–3–8 sodium aluminum phosphate [$NaH_{14}Al_3(PO_4)_8 \cdot 4H_2O$] are shown in Figure 12.3 [53]. Over 60% of the carbon dioxide is released very quickly from the more soluble monocalcium phosphate monohydrate; only 20% of the potential carbon dioxide is released from the slow-acting 1–3–8 sodium aluminum phosphate during a 10 min reaction period. Because of a hydrated alumina coating, the latter leavening acid reacts to only a small extent until activated by heat. Also shown in Figure 12.3 is the low temperature release pattern of carbon dioxide from coated anhydrous monocalcium phosphate [$Ca(H_2PO_4)_2$]. The crystals of this leavening acid were coated with compounds of slightly soluble alkali metal phosphates.

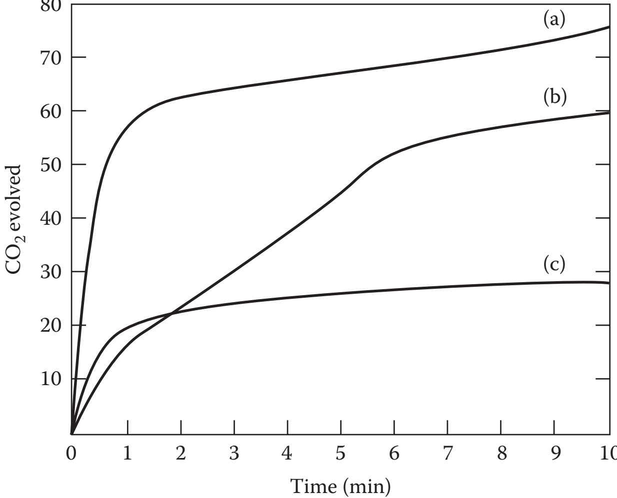


FIGURE 12.3 Carbon dioxide production at 27°C from the reaction of $NaHCO_3$ with (a) monocalcium phosphate · H_2O, (b) coated anhydrous monocalcium phosphate, and (c) 1–3–8 sodium aluminum phosphate. (Data from Stahl, J.E. and Ellinger, R.H., The use of phosphates in the baking industry, in *Symposium: Phosphates in Food Processing*, Deman, J.M. and Melnychyn, P., eds., AVI Publishing Co., Westport, CT, 1971, pp. 194–212.)

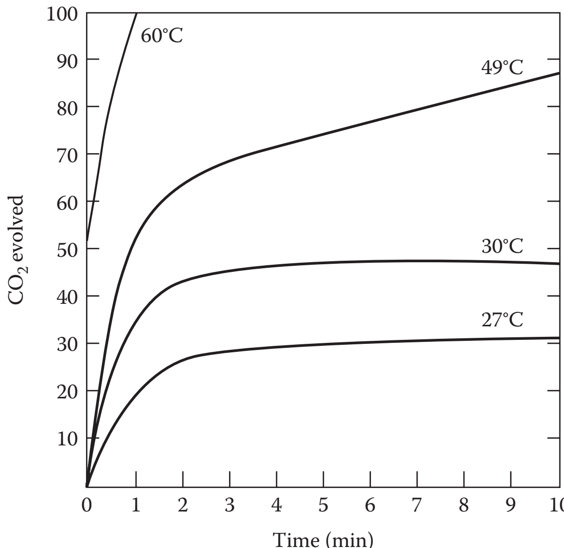


FIGURE 12.4 Effect of temperature on the rate of carbon dioxide evolution from the reaction of $NaHCO_3$ and slow-speed acid pyrophosphate. (Reprinted from Stahl, J.E. and Ellinger, R.H., The use of phosphates in the baking industry, in *Symposium: Phosphates in Food Processing*, Deman, J.M. and Melnychyn, P., eds., AVI Publishing Co., Westport, CT, 1971, p. 201.)

The gradual release of carbon dioxide over the 10 min reaction period corresponds to the time required for water to penetrate the coating. This behavior is very desirable in some products that encounter a delay prior to baking.

The release of the remainder of the carbon dioxide from leavening systems during baking provides the final modifying action on texture. In most leavening systems, the rate at which carbon dioxide is released greatly accelerates as the temperature is elevated. The effect of elevated temperatures on the release rate of carbon dioxide from slow-acting sodium acid pyrophosphate ($Na_2H_2P_2O_7$) is presented in Figure 12.4. Even a slight increase in temperature (from 27°C to 30°C) noticeably accelerates gas production. Temperatures near 60°C cause a complete release of carbon dioxide within 1 min. Some leavening acids are less sensitive to high temperatures and do not exhibit vigorous activity until temperatures near the maximum baking temperature are obtained. Dicalcium phosphate ($CaHPO_4$) is unreactive at room temperature because it forms a slightly alkaline solution at this temperature. However, upon heating above approximately 60°C, hydrogen ions are released, thereby activating the leavening process. This slow action confines its use to products requiring long baking times, such as some types of cakes. Formulations of leavening acids employing one or more acidic components are common, and systems are often tailored for specific dough or batter applications.

Leavening acids currently employed include potassium acid tartrate, sodium aluminum sulfate, δ-gluconolactone, and ortho- and pyrophosphates. The phosphates include calcium phosphate, sodium aluminum phosphate, and sodium acid pyrophosphate. Some general properties of commonly used leavening acids are given in Table 12.2. It must be remembered that these are only examples and that an extensive technology has developed for modification and control of the phosphate leavening acids [53].

Baking powders account for a large part of the chemical leaveners used both in the home and in bakeries. These preparations include sodium bicarbonate, suitable leavening acids, and starch and other extenders. Federal standards for baking powder require that the formula must yield at least 12% by weight of available carbon dioxide, and most contain 26%–30% by weight

TABLE 12.2
Some Properties of Common Leavening Acids

Acid	Formula	Neutralizing Value[a]	Relative Reaction Rate at Room Temperature[b]
Sodium aluminum sulfate	$Na_2SO_4 \cdot Al_2(SO_4)_3$	100	Slow
Dicalcium phosphate dihydrate	$CaHPO_4 \cdot 2H_2O$	33	None
Monocalcium phosphate monohydrate	$Ca(H_2PO4)_2 \cdot H_2O$	80	Fast
1–3–8 Sodium aluminum phosphate	$NaH_{14}Al_3(PO_4)_8 \cdot 4H_2O$	100	Slow
Sodium acid pyrophosphate (slow type)	$Na_2H_2P_2O_7$	72	Slow
Potassium acid tartrate	$KHC_4H_4O_6$	50	Medium
δ-Gluconolactone	$C_6H_{10}O_6$	55	Slow

Source: From Stahl, J.E. and Ellinger, R.H., The use of phosphates in the baking industry, in *Symposium: Phosphates in Food Processing*, Deman, J.M. and Melnychyn, P., eds., AVI Publishing Co., Westport, CT, 1971, pp. 194–212.

[a] In simple model systems; parts by weight of $NaHCO_3$ that will neutralize 100 parts by weight of the leavening acid.

[b] Rate of CO_2 evolution in the presence of $NaHCO_3$.

of sodium bicarbonate. Traditional baking powders of the potassium acid tartrate type have been largely replaced by double-acting preparations. In addition to $NaHCO_3$ and starch, these baking powders usually contain monocalcium phosphate monohydrate [$Ca(H_2PO_4)_2 \cdot H_2O$], which provides rapid action during the mixing stage, and sodium aluminum sulfate [$Na_2SO_4 \cdot Al_2(SO_4)_3$], which does not react appreciably until the temperature increases during baking.

The increase in convenience foods has stimulated sales of prepared baking mixes and refrigerated dough products. In white and yellow cake mixes, the most widely used blend of leavening acids contains anhydrous monocalcium phosphate [$Ca(H_2PO_4)_2$] and sodium aluminum phosphate [$NaH_{14}Al_3(PO_4)_8 \cdot 4H_2O$]; chocolate cake mixes usually contain anhydrous monocalcium phosphate and sodium acid pyrophosphate ($Na_2H_2P_2O_7$) [53]. Typical blends of acids contain 10%–20% fast-acting anhydrous monophosphate and 80%–90% of the slower-acting sodium aluminum phosphate or sodium acid pyrophosphate compounds. The leavening acids in prepared biscuit mixes usually consist of 30%–50% anhydrous monocalcium phosphate and 50%–70% sodium aluminum phosphate or sodium acid pyrophosphate. The earliest self-rising flours and corn meal mixes contained monocalcium phosphate monohydrate [$Ca(H_2PO_4)_2 \cdot H_2O$], but coated anhydrous monocalcium phosphate and sodium aluminum phosphate are in common use [53].

Refrigerated doughs for biscuit and roll products require limited initial carbon dioxide release during preparation and packaging and considerable gas release during baking. Formulations for biscuits usually contain from 1.0% to 1.5% sodium bicarbonate and 1.4% to 2.0% slow-acting leavening acids, such as coated monocalcium phosphate and sodium acid pyrophosphate, based on total dough weight. The pyrophosphates are useful in dough because they can be manufactured with a wide range of reactivities. For example, pyrophosphatase in flour is capable of hydrolyzing sodium acid pyrophosphate to orthophosphate (Figure 12.5), and the reaction of sodium bicarbonate and

$$Na^+, {}^-O-\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}-O-\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}-O^-, Na^+ \xrightleftharpoons[]{\text{Pyrophosphatase} +H_2O} 2Na^+, {}^-O-\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}-OH$$

Sodium acid pyrophosphate (SAAP) — Monosodium *o*-phosphate

FIGURE 12.5 Enzymic hydrolysis of sodium acid pyrophosphate.

pyrophosphate yields some trisodium monohydrogen pyrophosphate, which also can be hydrolyzed to orthophosphates. This enzymatic action leads to gas production that assists in sealing packages of refrigerated dough, but it can also lead to formation of large crystals of orthophosphates that may be mistaken for broken glass by the consumer.

12.3 BASES

Basic or alkaline substances are used in a variety of applications in foods and food processing. Although the majority of applications involve buffering and pH adjustments, other functions include carbon dioxide evolution, enhancement of color and flavor, solubilization of proteins, and chemical peeling. The role of carbonate and bicarbonate salts in carbon dioxide production during baking has been discussed previously.

Alkali treatments are imposed on several food products for the purpose of color and flavor improvement. Ripe olives are treated with solutions of sodium hydroxide (0.25%–2.0%) to aid in the removal of the bitter principal and to develop a darker color. Pretzels are dipped in a solution of 1.25% sodium hydroxide at 87°C–88°C (186°F–190°F) before baking to alter proteins and starch so that the surface becomes smooth and develops a deep-brown color during baking. It is believed that the NaOH treatment used to prepare hominy and tortilla dough destroys disulfide bonds that are base labile and improves the flavor. Soy proteins are solubilized through alkali processing, and concern has been expressed about alkaline-induced racemization of amino acids (Chapter 5) and losses of other nutrients. Small amounts of sodium bicarbonate are used in the manufacture of peanut brittle candy to enhance caramelization and browning and to provide, through release of carbon dioxide, a somewhat porous structure. Bases, usually potassium carbonate, are also used in cocoa processing for the production of dark (Dutched) chocolate [41]. The elevated pH enhances sugar–amino browning reactions and polymerization of flavonoids (Chapter 10) resulting in a smoother, less acid, and less bitter chocolate flavor, a darker color, and a slightly improved solubility.

Food systems sometimes require adjustment to higher pH values to achieve more stable or more desirable characteristics. For example, alkaline salts, such as disodium phosphate, trisodium phosphate, and trisodium citrate, are used in the preparation of processed cheese (1.5%–3%) to increase the pH (from 5.7 to 6.3) and to effect protein (casein) dispersion. This salt–protein interaction improves the emulsifying and water-binding capabilities of the cheese proteins [48] because the salts bind the calcium components of the casein micelles forming chelates.

Instant milk–gel puddings are prepared by combining dry mixes containing pregelatinized starch with cold milk and allowing them to stand for a short time at refrigerator temperatures. Alkaline salts, such as tetrasodium pyrophosphate ($Na_4P_2O_7$) and disodium phosphate (Na_2HPO_4), in the presence of calcium ions in milk cause milk proteins to gel in combination with the pregelatinized starch. The optimum pH for acceptable puddings falls between 7.5 and 8.0. Although some of the necessary alkalinity is contributed by alkaline phosphate salts, other alkalizing agents are often added [13].

The addition of phosphates and citrates changes the salt balance in fluid milk products by forming complexes with calcium and magnesium ions from casein. The mechanism is incompletely understood, but depending on the type and concentration of salt added, the milk protein system can undergo stabilization, gelation, or destabilization.

Alkaline agents are used to neutralize excess acid in the production of such foods as cultured butter. Before churning, the cream is fermented by lactic acid bacteria so that it contains about 0.75% titratable acidity expressed as lactic acid. Alkalis are then added to achieve a titratable acidity of approximately 0.25%. The reduction in acidity improves churning efficiency and retards the development of oxidative off-flavors. Several materials, including sodium bicarbonate ($NaHCO_3$), sodium carbonate (Na_2CO_3), magnesium carbonate ($MgCO_3$), magnesium oxide (MgO), calcium hydroxide ($Ca(OH)_2$), and sodium hydroxide (NaOH), are utilized alone or in combination as neutralizers for foods. Solubility, foaming as a result of carbon dioxide release, and strength of the base influence

the selection of the alkaline agent. The use of alkaline agents or bases in excessive amounts leads to soapy or neutralizer flavors, especially when substantial quantities of fatty acids are present.

Strong bases are employed for peeling various fruits and vegetables. Exposure of the product to hot solutions 60°C–82°C (140°F–180°F) of sodium hydroxide (about 3%), with subsequent mild abrasion, effects peel removal with substantial reductions in plant wastewater as compared with other conventional peeling techniques. Differential solubilization of cell and tissue constituents (pectic substances in the middle lamella are particularly soluble) provides the basis for caustic peeling processes.

12.4 BUFFER SYSTEMS AND SALTS

12.4.1 Buffers and pH Control in Foods

Since most foods are complex materials of biological origin, they contain many substances that can participate in pH control and buffering systems—included are proteins, organic acids, and weak inorganic acid phosphate salts. Lactic acid and phosphate salts, along with proteins, are important for pH control in animal tissue; polycarboxylic acids, phosphate salts, and proteins are important in plant tissues. The buffering effects of amino acids and proteins and the influence of pH and salts on their functionalities are discussed in Chapter 5. In plants, buffering systems containing citric acid (lemons, tomatoes, rhubarb), malic acid (apples, tomatoes, lettuce), oxalic acid (rhubarb, lettuce), and tartaric acid (grapes, pineapple) are common, and they usually function in conjunction with phosphate salts in maintaining pH control. Milk acts as a complex buffer because of its content of carbon dioxide, proteins, phosphate, citrate, and several other minor constituents.

In situations where the pH must be altered, it is usually desirable to stabilize the pH at the desired level through a buffer system. This is accomplished naturally when lactic acid is produced in cheese and pickle fermentations. Also, in some instances where substantial amounts of acids are used in foods and beverages, it is desirable to reduce the sharpness of acid tastes and obtain smoother product flavors without inducing neutralization flavors. This usually can be accomplished by establishing a buffer system in which the salt of a weak organic acid is dominant. The common ion effect is the basis for obtaining pH control in these systems, and the system develops when the added salt contains an ion that is already present in an existing weak acid. The added salt immediately ionizes resulting in repressed ionization of the acid with reduced acidity and a more stable pH. The efficiency of a buffer depends on the concentration of the buffering substances. Since there is a pool of undissociated acid and dissociated salt, buffers resist changes in pH. For example, relatively large additions of a strong acid, such as hydrochloric acid, to an acetic acid–sodium acetate system causes hydrogen ions to react with the acetate-ion pool to increase the concentration of slightly ionized acetic acid, and the pH remains relatively stable. In a similar manner, an addition of sodium hydroxide causes hydroxyl ions to react with hydrogen ions to form undissociated water molecules.

Titration of buffered systems and resulting titration curves (i.e., pH vs. volume of base added) reveal their resistance to pH change. If a weak acid buffer is titrated with a base, there is a gradual but steady increase in the pH as the system approaches neutralization; that is, the change in pH per mL of added base is small. Weak acids are only slightly dissociated at the beginning of the titration. However, the addition of hydroxyl ions shifts the equilibrium to the dissociated species and eventually the buffering capacity is overcome.

In general, for an acid (HA) in equilibrium with ions (H^+) and (A^-), the equilibrium is expressed as in Equation 12.6:

$$HA \rightleftarrows H^+ + A^- \tag{12.6}$$

$$K_a^1 = \frac{[H^+ + A^-]}{[HA]} \tag{12.7}$$

The constant K_a^1 is the apparent dissociation constant (Equation 12.7) and is characteristic of the particular acid. The apparent dissociation constant K_a^1 becomes equal to the hydrogen ion concentration $[H^+]$ when the anion concentration $[A^-]$ becomes equal to the concentration of undissociated acid [HA]. This situation gives rise to an inflection point on a titration curve, and the pH corresponding to this point is referred to as the pK_a^1 of the acid. Therefore, for a weak acid, pH is equal to pK_a^1 when the concentrations of the acid and conjugate base are equal (Equation 12.8):

$$pH = pK_a^1 = -\log_{10}[H^+] \quad (12.8)$$

A convenient method for calculating the approximate pH of a buffer mixture, given the pK_a^1 of the acid, is provided by Equation 12.11. This equation is arrived at first by solving Equation 12.7 for $[H^+]$ to yield Equation 12.9:

$$[H^+] = K_a^1 \frac{[HA]}{[A^-]} = K_a^1 \frac{[acid]}{[salt]} \quad (12.9)$$

Since the salt in solution is almost completely dissociated, it is assumed equal to the concentration of the conjugate base $[A^-]$. The negative logarithms of the terms yield Equation 12.10. By substituting pH for $-\log\ [H^+]$ and pK_a^1 for $-\log K_a^1$, Equation 12.11 is obtained. The pH of a buffer system derived from any weak acid that dissociates to H^+ and A^- can be calculated by using Equation 12.11:

$$-\log[H^+] = -\log K_a^1 - \log \frac{[acid]}{[salt]} \quad (12.10)$$

$$pH = pK_a^1 + \log \frac{[salt]}{[acid]} = pK_a^1 + \log \frac{[A^-]}{[HA]} \quad (12.11)$$

In calculating the pH values of buffer solutions, it is important to recognize that the apparent dissociation constant K_a^1 differs from K_a, the true dissociation constant. However, for any buffer, the value of K_a^1 remains constant as the pH is varied, provided that the total ionic strength of the solution remains unchanged.

The sodium salts of gluconic, acetic, citric, and phosphoric acids are commonly used for pH control and tartness modification in the food industry. The citrates are usually preferred over phosphates for tartness modification since they yield smoother sour flavors. When low sodium products are required, potassium buffer salts may be substituted for sodium salts. In general, calcium salts are not used because of their limited solubilities and incompatibilities with other components in the system. The effective buffering ranges for combinations of common acids and salts are pH 2.1–4.7 for citric acid–sodium citrate, pH 3.6–5.6 for acetic acid–sodium acetate, and pH 2.0–3.0, 5.5–7.5, and 10–12, respectively, for the three ortho- and pyrophosphate anions.

12.4.2 Salts in Processed Dairy Foods

Salts are used extensively in processed cheeses and imitation cheeses to promote a uniform, smooth texture. These additives are sometimes referred to as emulsifying salts because of their ability to aid in dispersion of fat. Although the emulsifying mechanism remains somewhat less than fully defined, anions from the salts when added to processed cheese combine with and remove calcium from the *para-casein* complex, and this causes rearrangement and exposure of both polar and nonpolar regions of the cheese proteins. It is also believed that the anions of these salts participate in ionic bridges between protein molecules and thereby provide a stabilized matrix that entraps the

fat in processed cheese [48]. Salts used for cheese processing include mono-, di-, and trisodium phosphate, dipotassium phosphate, sodium hexametaphosphate, sodium acid pyrophosphate, tetrasodium pyrophosphate, sodium aluminum phosphate, and other condensed phosphates, trisodium citrate, tripotassium citrate, sodium tartrate, and sodium potassium tartrate.

The addition of certain phosphates, such as trisodium phosphate, to evaporated milk prevents separation of the butterfat and aqueous phases. The amount required varies with the season of the year and the source of milk. Concentrated milk that is sterilized by a high-temperature short-time method frequently gels upon storage. The addition of polyphosphates, such as sodium hexametaphosphate and sodium tripolyphosphate, prevents gel formation through a protein denaturation and solubilization mechanism involving complexation of calcium and magnesium by phosphates.

12.4.3 Phosphates and Water Binding in Animal Tissues

The addition of appropriate phosphates increases the water holding capacity of raw and cooked meats [27], and these phosphates are used to make sausages, to cure ham, to decrease drip losses in poultry and seafoods, and more recently to provide succulence to prepackaged fresh pork and beef. Sodium tripolyphosphate ($Na_5P_3P_{10}$) is the most common phosphate added to processed meat, poultry, and seafoods. It is often used in blends with sodium hexametaphosphate [$(Na\ PO_3)_n$, $n = 10–15$] to increase tolerance to calcium ions that exist in brines used in meat curing. Ortho- and pyrophosphates often precipitate if used in brines containing substantial amounts of calcium, but calcium can be complexed with hexametaphosphate, which remains soluble and prevents calcium phosphate precipitation.

The mechanism by which alkaline phosphates and polyphosphates enhance meat hydration is not clearly understood despite extensive studies. The action may involve the influence of pH changes (Chapter 15), effects of ionic strength, and specific interactions of phosphate anions with divalent cations and myofibrillar proteins. Many believe that calcium complexing and a resulting loosening of the tissue structure is a major function of polyphosphates. It is also believed that binding of polyphosphate anions to proteins and simultaneous cleavage of cross-linkages between actin and myosin result in increased electrostatic repulsion between peptide chains and a swelling of the muscle system. If exterior water is available, it can be then taken up in an immobilized state within the loosened protein network. Further, because the ionic strength has been increased, the interaction between proteins is perhaps reduced to a point where part of the myofibrillar proteins form a colloidal solution. In comminuted meat products, such as bologna and sausage, the addition of sodium chloride (2.5%–4.0%) and polyphosphate (0.35%–0.5%) contributes to a more stable emulsion and after cooking to a cohesive network of coagulated proteins.

If the phosphate-induced solubilization occurs primarily on the surface of tissues, as is the case with polyphosphate-dipped (6%–12% solution with 0.35%–0.5% retention) fish fillets, shellfish, and poultry, a layer of coagulated protein is formed during cooking and this improves moisture retention [38].

12.5 CHELATING AGENTS (SEQUESTRANTS)

Chelating agents or sequestrants play a significant role in food stabilization through reactions with metallic and alkaline earth ions to form complexes that alter the properties of the ions and their effects in foods. Many of the chelating agents employed in the food industry are natural substances, such as polycarboxylic acids (citric, malic, tartaric, oxalic, and succinic), polyphosphoric acids (adenosine triphosphate and pyrophosphate), and macromolecules (porphyrins and proteins). Many metals exist in a naturally chelated state. Examples include magnesium in chlorophyll; copper, zinc, and manganese in various enzymes; iron in proteins such as ferritin; and iron in the porphyrin ring of myoglobin and hemoglobin. When these ions are released by hydrolytic or other degradative reactions, they are free to participate in reactions that lead to discoloration, oxidative rancidity,

FIGURE 12.6 Schematic representation of chelation of cupric cation (a lipid oxidation catalyst) by disodium ethylenediaminetetraacetic acid.

turbidity, and flavor changes in foods. Chelating agents are sometimes added to form complexes with these metal ions and thereby stabilize the foods.

Any molecule or ion with an unshared electron pair can coordinate or form complexes with metal ions. Therefore, compounds containing two or more functional groups, such as −OH, −SH, −COOH, $-PO_3H_2$, C=O, $-NR_2$, −S−, and −O−, in proper geometrical relation to each other, can chelate metals in a favorable physical environment. Citric acid and its derivatives, various phosphates, and salts of ethylenediaminetetraacetic acid (EDTA) are the most popular chelating agents used in foods. Usually, the ability of a chelating agent (ligand) to form a five- or six-membered ring with a metal is necessary for stable chelation. For example, EDTA forms chelates of high stability with cupric cations because of an initial coordination involving the electron pairs of its nitrogen atoms and the free electron pairs of the anionic oxygen atoms of two of the four carboxyl groups (Figure 12.6). The spatial configuration of the cupric cation–EDTA complex is such that it allows additional coordination of the cupric ion with the free electron pairs of the anionic oxygen atoms of the remaining two carboxyl groups, and this results in an extremely stable complex utilizing all six electron donor groups.

In addition to steric and electronic considerations, factors such as pH influence the formation of strong metal chelates. The nonionized carboxylic acid group is not an efficient donor group, but the carboxylate ion functions effectively. Judicious raising of the pH allows dissociation of the carboxyl group and enhances chelating efficiency. In some instances, hydroxyl ions compete for metal ions and reduce the effectiveness of chelating agents. Metal ions exist in solution as hydrated complexes ($metal \cdot H_2O^{M+}$), and the rate at which these complexes are disrupted influences the rate at which they can be complexed with chelating agents. The relative attraction of chelating agents for different ions can be determined from stability or equilibrium constants (K = [metal · chelating agent]/[metal][chelating agent]) (Chapter 9). For example, for calcium the stability constant (expressed as log K) is 10.7 with EDTA, 5.0 with pyrophosphate, and 3.5 with citric acid [20]. As the stability constant (K) increases, more of the metal is complexed, leaving less metal in the noncomplexed cation form (i.e., the metal in the complex is more tightly bound).

Chelating agents are not antioxidants in the sense that they arrest oxidation by chain termination or serve as oxygen scavengers. They are, however, valuable antioxidant synergists since they remove metal ions that catalyze oxidation (Chapter 4). When selecting a chelating agent for an antioxidant synergist role, its solubility must be considered. Citric acid and citrate esters (20–200 ppm) in propylene glycol solution are solubilized by fats and oils and thus are effective synergists in all-lipid systems. On the other hand, Na_2EDTA and Na_2Ca-EDTA dissolve to only a limited extent and are not effective in pure fat systems. The EDTA salts (to 500 ppm), however, are very effective antioxidants in emulsion systems, such as salad dressings, mayonnaise, and margarine, because they can function in the aqueous phase, and especially at the interface between the aqueous and fat phases.

Polyphosphates and EDTA are used in canned seafoods to prevent the formation of glassy crystals of struvite or magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$). Seafoods contain substantial amounts of magnesium ions that sometimes react with ammonium phosphate during storage of canned seafoods to give crystals that may be mistaken as glass contamination. Chelating agents complex magnesium and minimize struvite formation. Chelating agents also can be used to complex iron, copper, and zinc in seafoods to prevent reactions, particularly with sulfides, that lead to product discoloration. The addition of chelating agents to vegetables prior to blanching can inhibit metal-induced discolorations and can remove calcium from pectic substances in cell walls and thereby promote tenderness.

Although citric and phosphoric acids are employed as acidulants in soft drink beverages, they also chelate metals that otherwise could promote oxidation of flavor compounds, such as terpenes, and catalyze discoloration reactions. Chelating agents also stabilize fermented malt beverages by complexing copper. Free copper catalyzes oxidation of polyphenolic compounds that subsequently interact with proteins to form permanent hazes or turbidity.

The extremely efficient chelating abilities of some agents, notably synthetic EDTA and natural phytic acid (hexaphosphoinositol), have caused speculation that excessive usage or occurrence in foods could lead to the depletion of calcium and other cationic minerals in the body. To deal with this concern, levels and applications of EDTA are regulated, and in some instances calcium is added to food systems through the use of the Na_2Ca salt of EDTA rather than the all-sodium (Na, Na_2, Na_3, or Na_4 EDTA) or acid forms. However, most current scientific thinking embraces the view that there is little dietary concern about these chelators in the amounts permitted or encountered considering the natural concentrations of calcium and other divalent cations that are present in foods.

12.6 ANTIOXIDANTS

Oxidation occurs when electrons are removed from an atom or group of atoms. Simultaneously, there is a corresponding reduction reaction that involves the addition of electrons to a different atom or group of atoms. Oxidation reactions may or may not involve the addition of oxygen atoms or the removal of hydrogen atoms from the substance being oxidized. Oxidation–reduction reactions are common in biological systems and are also common in foods. Although some oxidation reactions are beneficial in foods, others can lead to detrimental effects including degradation of lipids (Chapter 4), vitamins (Chapter 8), and pigments (Chapter 10) with loss of nutritional value and development of off-flavors. Control of undesirable oxidation reactions in foods is usually achieved by employing processing and packaging techniques that exclude oxygen or involve the addition of appropriate chemical agents.

Before the development of specific chemical technology for the control of free radical–mediated lipid oxidation, the term "antioxidant" was applied to all substances that inhibited oxidation reactions regardless of the mechanism. For example, ascorbic acid was considered an antioxidant and was employed to prevent enzymic browning of the cut surfaces of fruits and vegetables (Chapter 6). In this application, ascorbic acid functions as a reducing agent by transferring hydrogen atoms back to quinones that are formed by enzymic oxidation of phenolic compounds. In closed systems, ascorbic acid reacts readily with oxygen and thereby serves as an oxygen scavenger. Likewise, sulfites are readily oxidized in food systems to sulfonates and sulfates and thereby function as effective antioxidants in foods such as dried fruits (Section 12.7.1). The most commonly employed food antioxidants are phenolic substances. More recently, the term "food antioxidants" often has been applied to those compounds that interrupt the free radical chain reaction involved in lipid oxidation and those that scavenge singlet oxygen; however, the term probably should not be used in such a narrow sense.

Antioxidants often exhibit variable degrees of efficiency in protecting food systems, and combinations often provide greater overall protection than can be accounted for through the simple additive effects [55]. Thus, mixed antioxidants sometimes have a synergistic action, the mechanisms for which are not completely understood. It is believed, for example, that ascorbic acid can

regenerate phenolic antioxidants by supplying hydrogen atoms to the phenoxy radicals that form when the phenolic antioxidants yield hydrogen atoms to the lipid oxidation chain reaction. In order to achieve this action in lipids, ascorbic acid must be made less polar so it will dissolve in fat. This is done by esterification to fatty acids to form compounds, such as ascorbyl palmitate.

The presence of transition-state metal ions, particularly copper and iron, promotes lipid oxidation through catalytic actions (Chapters 4 and 9). These metallic prooxidants are frequently inactivated by adding chelating agents, such as citric acid or EDTA (Section 12.5). In this role chelating agents are also referred to as synergists since they greatly enhance the action of the phenolic antioxidants. However, they are often ineffective as antioxidants when employed alone.

Many naturally occurring substances possess antioxidant capabilities, and the tocopherols are noted examples that are widely employed (Chapter 4). Recently, extractives of spices containing polyphenolic substances, particularly from rosemary, also have been successfully commercially exploited as natural antioxidants. Gossypol that occurs naturally in cottonseed is an antioxidant, but it has toxic properties. Other naturally occurring antioxidants are coniferyl alcohol (found in plants) and guiaconic and guiacic acid (from gum guaiac). All of these are structurally related to butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, and di-*t*-butylhydroquinone, which are synthetic phenolic antioxidants currently approved for use in foods (Chapter 4). Nordihydroguaiaretic acid, a compound related to some of the constituents of gum guaiac, is an effective antioxidant, but its use directly in foods has been suspended because of toxic effects. All of these phenolic substances serve as oxidation terminators by participating in the reactions through resonance stabilized free radical forms [55], but they also are believed to serve as singlet oxygen scavengers. On the other hand, β-carotene is considered to function more efficiently as a singlet oxygen scavenger than phenolic substances.

Thiodipropionic acid and dilauryl thiodipropionate remain as approved food antioxidants, even though removal of these compounds from the approved list has been proposed because they were not being used in foods. The presence of a sulfur atom in the thiodipropionates has led to speculation that they could cause off-flavors, but this view is unfounded. A more compelling reason that the thiodipropionates have not been used in foods is their failure to inhibit lipid oxidation in foods, as measured by peroxide value, when used at permitted levels (to 200 ppm) [32]. The classic role of thiodipropionates is as secondary antioxidants, where at high concentrations (>1000 ppm), they degrade hydroperoxides formed during olefin oxidation to relatively stable end products, and used as such, they are useful in stabilizing synthetic polyolefins.

Although thiodipropionates, at levels allowed in foods, are ineffective in reducing peroxide values, they are highly effective in decomposing peracids (Figure 12.7) found in oxidizing lipids [32]. Peracids are very efficient substances for mediating the oxidation of double bonds to epoxides, and in the presence of water, epoxides formed by this reaction readily hydrolyze to form diols. When these reactions occur with cholesterol, both cholesterol epoxide and the cholesterol-triol derivative are formed, and these cholesterol oxides are widely considered potentially mutagenic and atherogenic, respectively, to humans [42]. Because the thiodipropionates readily inhibit the accumulation of peracids, they have been retained as approved antioxidants by the U.S. Food and Drug Administration (FDA).

A chemical structure similar to that in the thiodipropionates occurs in methionine (Chapter 5) and accounts, presumably by an analogous mechanism, for some of the antioxidant properties

$$2\,R{-}C({=}O){-}OOH + S(CH_2{-}CH_2{-}COOH)_2 \longrightarrow O_2S(CH_2{-}CH_2{-}COOH)_2 + 2\,R{-}C({=}O){-}OH$$

Alkyl peracid; Thiodipropionic acid; Thiodipropionic acid sulfone; Alkyl acid

FIGURE 12.7 Mechanism for peracid destruction in oxidizing lipids by thiodipropionic acid.

shown by proteins. Reaction of a sulfide (i.e., thioether) with one peracid or hydroperoxide yields a sulfoxide, while reaction with two peracids or hydroperoxides yields a sulfone.

12.7 ANTIMICROBIAL AGENTS

Chemical preservatives with antimicrobial properties play an important role in preventing spoilage and assuring safety of many foods. Some of these are discussed in the following section.

12.7.1 Sulfites and Sulfur Dioxide

Sulfur dioxide (SO_2) and its derivatives have long been used in foods as general food preservatives. They are added to food to inhibit nonenzymic browning, to inhibit enzyme-catalyzed reactions, to inhibit and control microorganisms, and to act as an antioxidant and a reducing agent. Generally, SO_2 and its derivatives are metabolized to sulfate and excreted in the urine without any obvious pathologic results [60]. However, because of somewhat recently recognized severe reactions to sulfur dioxide and its derivatives by some sensitive asthmatics, their use in foods is currently regulated and subject to rigorous labeling restrictions. Nonetheless, these preservatives serve key roles in contemporary foods.

The commonly used forms in foods include SO_2 gas and the sodium, potassium, or calcium salts of sulfite (SO_3^{2-}), bisulfite (HSO_3^-), or metabisulfite ($S_2O_5^{2-}$). The most frequently used sulfiting agents are the sodium and potassium metabisulfites because they exhibit good stability toward autoxidation in the solid phase. However, gaseous sulfur dioxide is employed where leaching of solids causes problems or where the gas may also serve as an acid for the control of pH.

Although the traditional names for the anions of these salts are still widely used (sulfites, bisulfites, and metabisulfites), they have been designated by IUPAC as the sulfur (IV) oxoanions, sulfites (SO_3^{2-}), hydrogen sulfites (HSO_3^-), and disulfites ($S_2O_5^{2-}$), respectively. The oxoacids, H_2SO_3 and $H_2S_3O_5$, are designated as sulfurous and disulfurous acids, respectively [60].

Widely held views also have changed somewhat on the existence of sulfurous acid in aqueous solutions. Earlier, it was presumed that when sulfur dioxide was dissolved in water, it formed sulfurous acid, because the salts of simple oxoanions of sulfur (IV) (valence +4) are salts of this acid (H_2SO_3; sulfurous acid). However, evidence for the existence of free sulfurous acid has not been found, and it has been estimated that it accounts for less than 3% of nondissociated dissolved SO_2. Instead, solution of SO_2 yields only weak interactions with water that results in a nondissociated complex, which is particularly abundant below pH 2. This complex has been denoted $SO_2 \cdot H_2O$, and a distinction between this complex and sulfurous acid is not generally made [60] (Equation 12.12).

Since the acidity of solutions of SO_2 is significant and free sulfurous acid is not found, it is argued that the strong acid, $HSO_2(OH)$, exists in small amounts and its dissociation leads predominantly to the HSO_3^- (bisulfite) ion rather than the $SO_2(OH)^-$ ion that has been estimated to occur to an extent of only 2.5% of the hydrogen sulfite species (Equation 12.13). The HSO_3^- ion predominates from pH 3 to 7, but above pH 7, the SO_3^{2-} ion is most abundant (Equation 12.14). The pK_a for the first dissociation of *sulfurous acid* is 1.86, and the second dissociation has pK_a of 7.18. In dilute solutions of HSO_3^- (10^{-2} M), little metabisulfite (disulfite) ion exists, but as the concentration of bisulfite increases, the proportion increases rapidly (Equation 12.15). Thus, the relative proportion of each form depends on the pH of the solution, the ionic strength of the sulfur (IV) oxospecies, and the concentration of neutral salts [60]:

$$SO_2 + H_2O \leftrightarrows SO_2 \cdot H_2O \quad (12.12)$$

$$SO_2 \cdot H_2O \leftrightarrows HSO_2(OH) \leftrightarrows HSO_3^- + H^+ \quad (12.13)$$

$$HSO_3^- \leftrightarrows H^+ + SO_3^{2-} \quad (12.14)$$

$$2HSO_3^- \leftrightarrows S_2O_5^{2-} + H_2O \quad (12.15)$$

Sulfur dioxide is most effective as an antimicrobial agent in acid media, and this effect may result from conditions that permit undissociated compounds to penetrate the cell wall. At high pH, it has been noted that the HSO_3^- ion is effective against bacteria, but not against yeasts. Sulfur dioxide acts as both a biocidal and biostatic agents and is more active against bacteria than molds and yeasts. Also, it is more effective against Gram-negative bacteria than Gram-positive bacteria.

The nucleophilicity of the sulfite ion is believed to be responsible for much of the effectiveness of sulfur dioxide as a food preservative in both microbial and chemical applications [61]. Some evidence has accumulated that the interaction of sulfur (IV) oxospecies with nucleic acids causes the biostatic and biocidal effects [60]. Other postulated mechanisms by which sulfur (IV) oxospecies inhibit microorganisms include the reaction of bisulfite with acetaldehyde in the cell, the reduction of essential disulfide linkages in enzymes, and the formation of bisulfite addition compounds that interfere with respiratory reactions involving nicotinamide dinucleotide.

Of the known inhibitors of nonenzymic browning in foods (Chapter 3), sulfur dioxide is one of the most effective. Multiple chemical mechanisms are involved in sulfur dioxide inhibition of nonenzymic browning (Figure 12.8), but one of the most important involves the reaction of sulfur (IV) oxoanions (bisulfite) with carbonyl groups of reducing sugars and other compounds participating

Glucose + Glycine → 3-Deoxy-glucosone ⇌ ($+SO_3^-$) 3-Deoxy-glucosone-dihydroxy-1,2-disulfonate

Glucose ⇌ ($+SO_3^-$) Glucose hydroxy-sulfonate

3-Deoxy-glucosone → Hydroxymethyl furfural (HMF) ⇌ ($+SO_3^-$) Hydroxymethyl furfuryl hydroxy-sulfonate

3-Deoxy-glucosone ⇌ ($+SO_3^-$) 3-Deoxyglucosone-4-sulfonate

FIGURE 12.8 Mechanisms of inhibition of Maillard (carbonylamino) browning by some sulfur (IV) oxoanions (bisulfite, sulfite).

in browning. These reversible bisulfite addition compounds, thus, bind carbonyl groups to retard the browning process, but it also has been proposed that the reaction removes carbonyl chromophores in melanoidin structures that leads to a bleaching effect on the pigment. Sulfur (IV) oxoanions also irreversibly react with hydroxyl groups, especially those in the 4-position, on sugar and ascorbic acid intermediates in browning reactions to yield sulfonates ($R-CHSO_3^--CH_2-R'$). The formation of relatively stable sulfonate derivatives retards the overall reaction and interferes with pathways that are particularly prone to producing colored pigments [60].

Sulfur dioxide also inhibits certain enzyme-catalyzed reactions, notably enzymic browning, that are important in food preservation. The production of brown pigments by enzyme-catalyzed oxidation of phenolic compounds can lead to a serious quality problem during the handling of some fresh fruits and vegetables (Chapter 6). However, the use of sulfite or metabisulfite sprays or dips with or without added citric acid provides effective control of enzymic browning in prepeeled and presliced potatoes, carrots, and apples.

Sulfur dioxide also functions as an antioxidant in a variety of food systems, but it is not usually employed for this purpose. When it is added to beer, the development of oxidized flavors is inhibited significantly during storage. The red color of fresh meat also can be effectively maintained by the presence of sulfur dioxide. However, this practice is not permitted because of the potential for masking deterioration in abused meat products.

When added during manufacture of wheat flour doughs, sulfur dioxide effects a reversible cleavage of protein disulfide bonds. In the instance of cookie manufacture, the addition of sodium bisulfite reduces mixing time and the elasticity of the dough that facilitates dough sheeting, and it also reduces variations caused by different lots of flour [60]. Before drying of fruits, gaseous sulfur dioxide is often applied, and this is sometimes done in the presence of buffering agents (i.e., $NaHCO_3$). This treatment prevents browning and induces a reversible bleaching of anthocyanin pigments. The resulting properties are desired in products, such as those used to make white wines and maraschino cherries. Levels of sulfur dioxide encountered in dried fruits immediately following processing sometimes approach 2000 ppm. However, much lower amounts are found in most other foods because concentrations above 500 ppm give noticeably disagreeable flavors and because sulfites tend to volatilize and/or react during storage and cooking.

12.7.2 Nitrite and Nitrate Salts

The potassium and sodium salts of nitrite and nitrate are commonly used in curing mixtures for meats to develop and fix the color, to inhibit microorganisms, and to develop characteristic flavors [50]. Nitrite rather than nitrate is apparently the functional constituent. Nitrites in meat form nitric oxide, which reacts with heme compounds to form nitrosomyoglobin, the pigment responsible for the pink color of cured meats (Chapter 10). Sensory evaluations also indicate that nitrite contributes to cured meat flavor apparently through an antioxidant role, but the details of this chemistry are poorly understood [46]. Furthermore, nitrites (150–200 ppm) inhibit clostridia in canned-comminuted and cured meats. In this regard, nitrite is more effective at pH 5.0–5.5 than it is at higher pH values. The antimicrobial mechanism of nitrite is unknown, but it has been suggested that nitrite reacts with sulfhydryl groups to create compounds that are not metabolized by microorganisms under anaerobic conditions.

Nitrites have been shown to be involved in the formation of low, but possibly toxic levels of nitrosamines in certain cured meats. The chemistry and health implications of nitrosamines are discussed in Chapter 13. Nitrate salts also occur naturally in many foods, including vegetables such as spinach. The accumulation of large amounts of nitrate in plant tissues grown on heavily fertilized soils is of concern, particularly in infant foods prepared from these tissues. The reduction of nitrate to nitrite in the intestine, with subsequent absorption, could lead to cyanosis due to metmyoglobin formation. For these reasons, the use of nitrites and nitrates in foods has been questioned. The antimicrobial capability of nitrite provides some justification for its use in cured meats,

especially where growth of *C. botulinum* is possible. However, in preserved products where botulism does not present a hazard, there appears to be little justification for adding nitrates and nitrites.

12.7.3 Acetic Acid

The preservation of foods with acetic acid (CH_3COOH) in the form of vinegar dates to antiquity. In addition to vinegar (4% acetic acid) and acetic acid, also used in food are sodium acetate (CH_3COONa), potassium acetate (CH_3COOK), calcium acetate [$(CH_3{-}COO)_2Ca$], and sodium diacetate ($CH_3COONa \cdot CH_3{-}COOH \cdot \frac{1}{2}H_2O$). The salts are used in bread and other baked goods (0.1%–0.4%) to prevent ropiness and the growth of molds without interfering with yeast [8]. Vinegar and acetic acid are used in pickled meats and fish products. If fermentable carbohydrates are present, at least 3.6% acid must be present to prevent growth of lactic acid bacilli and yeasts. Acetic acid is also used in foods such as catsup, mayonnaise, and pickles where it serves a dual function of inhibiting microorganisms and contributing to flavor. The antimicrobial activity of acetic acid increases as the pH is decreased, a property analogous to that found for other aliphatic fatty acids.

12.7.4 Propionic Acid

Propionic acid ($CH_3{-}CH_2{-}COOH$) and its sodium and calcium salts exert antimicrobial activity against molds and a few bacteria. This compound occurs naturally in Swiss cheese (up to 1% by weight), where it is produced by *Propionibacterium shermanii* [8]. Propionic acid has found extensive use in the bakery field where it not only inhibits molds effectively but also is active against the ropy bread organism *Bacillus mesentericus*. Levels of use generally range up to 0.3% by weight. As with other carboxylic acid antimicrobial agents, the undissociated form of propionic acid is active, and the range of effectiveness extends up to pH 5.0 in most applications. The toxicity of propionic acid to molds and certain bacteria is related to the inability of the affected organisms to metabolize the three carbon skeleton. In mammals, propionic acid is metabolized in a manner similar to that of other fatty acids, and it has not been shown to cause any toxic effects at the levels encountered in foods.

12.7.5 Sorbic Acid and Other Medium-Chain Fatty Acids

Medium-chain and longer, monocarboxylic, aliphatic fatty acids exhibit antimicrobial, especially antimycotic, activities. The α-unsaturated fatty acid analogs are especially effective. Sorbic acid ($C{-}C{=}C{-}C{=}C{-}COOH$) and its sodium and potassium salts are widely used to inhibit mold and yeasts in a wide variety of foods including cheese, baked products, fruit juices, wine, and pickles. Sorbic acid is found in nature, most notably in the berries of the mountain ash. Commercial sorbate salts employed in the food industry are synthesized industrially and are comprised largely of *trans* fatty acid isomers. Since sorbic acid is chemically a fatty substance, from a labeling perspective, its use contributes to the *trans* fat content of a food.

Sorbic acid is particularly effective in preventing mold growth, and it contributes little flavor at the concentrations employed (up to 0.3% by weight). The method of application may involve direct incorporation, surface coatings, or incorporation in a wrapping material. The activity of sorbic acid increases as the pH decreases, indicating that the undissociated form is more inhibitory than the dissociated form. In general, sorbic acid is effective up to pH 6.5, which is considerably above the effective pH ranges for propionic and benzoic acids.

The antimycotic action of sorbic acid appears to arise because molds are unable to metabolize the α-unsaturated diene system of its aliphatic chain. It has been suggested that the diene structure of sorbic acid interferes with cellular dehydrogenases that normally dehydrogenate fatty acids as the first step in oxidation.

FIGURE 12.9 Mold-mediated detoxification of a fatty acid (octanoic acid) via f-oxidation followed by decarboxylation to yield a methyl ketone (heptanone).

Saturated fatty acids (C_2–C_{12}) are also moderately inhibitory to many molds, such as *Penicillium roqueforti*. However, some of these molds are capable of mediating β-oxidation of saturated fatty acids to corresponding β-keto acids, especially when the concentration of the acid is only marginally inhibitory. Decarboxylation of the resulting β-keto acid yields the corresponding methyl ketone (Figure 12.9), which does not exhibit antimicrobial properties. On the other hand, this mechanism for the formation of methyl ketones accounts for a major portion of the characteristic flavor of mold-ripened blue cheeses. A few molds have also been shown to metabolize sorbic acid, and it has been suggested that this metabolism proceeds through β-oxidation, similar to that in mammals. All evidence indicates that animals and humans metabolize sorbic acid in much the same way as they do other naturally occurring fatty acids.

Although sorbic acid might at first appear quite stable and unreactive, it is quite often microbiologically or chemically altered in foods. Two other mechanisms for deactivating the antimicrobial properties of sorbic acid are shown in Figure 12.10. The reaction labeled "a" in Figure 12.10 has been demonstrated in molds, especially *P. roqueforti*. This involves direct decarboxylation of sorbic acid to yield the hydrocarbon, 1,3-pentadiene. The intense aroma of this compound can cause gasoline or hydrocarbon-like off-flavors when mold growth occurs in foods containing sorbic acid, especially on the surface of cheese treated with sorbate.

If wine containing sorbic acid undergoes spoilage in the bottle by lactic acid bacteria, an off-flavor described as geranium-like develops [11]. Lactic acid bacteria reduce sorbic acid to sorbyl alcohol and then, because of the acid conditions they have created, cause a rearrangement to a secondary alcohol (Figure 12.10, labeled as "b"). The final reaction involves the formation of an ethoxylated hexadiene that has a pronounced, easily recognized aroma of geranium leaves.

Sorbic acid is sometimes used in combination with sulfur dioxide, and this leads to reactions that deplete both sorbic acid and sulfur (IV) oxoanions (Figure 12.11) [23]. Under aerobic conditions, especially in the presence of light, SO_3^- radicals are formed, and these radicals sulfonate olefin bonds as well as promote oxidation of sorbic acid. This reaction, uniquely involving sorbic acid, is not noticeably affected by the presence of conventional antioxidants, and aerobically held foods containing sulfur dioxide and sorbic acid are very susceptible to autoxidation. Under anaerobic conditions, the combination of sorbic acid and sulfur dioxide in foods results in a much slower nucleophilic reaction of the sulfite ion (SO_3^-) with the diene (1,4-addition) in sorbic acid to yield 5-sulfo-3-hexenoic acid (Figure 12.11).

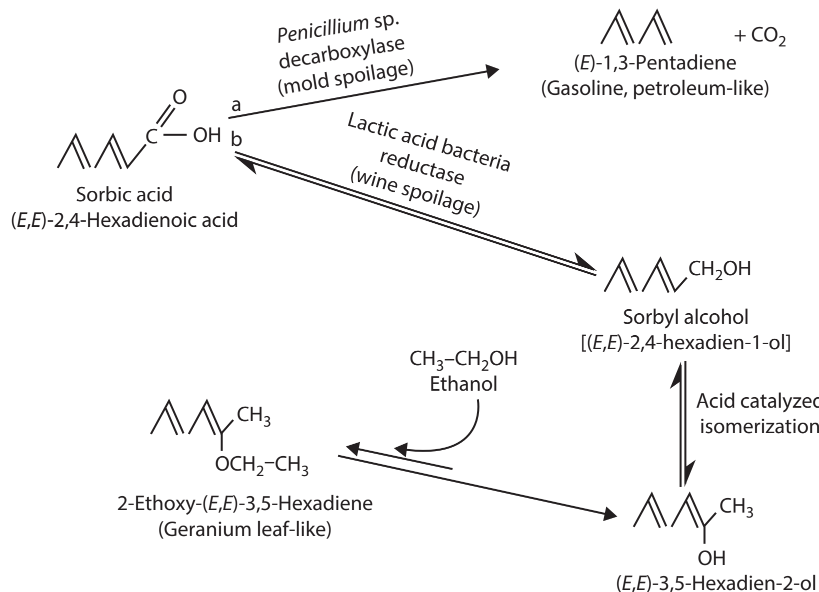


FIGURE 12.10 Enzymic conversions destroying the antimicrobial properties of sorbic acid: (a) decarboxylation carried out by *Penicillium* sp. and (b) formation of ethoxylated diene hydrocarbon in wine resulting from a reduction of the carboxyl group followed by rearrangement and development of an ether.

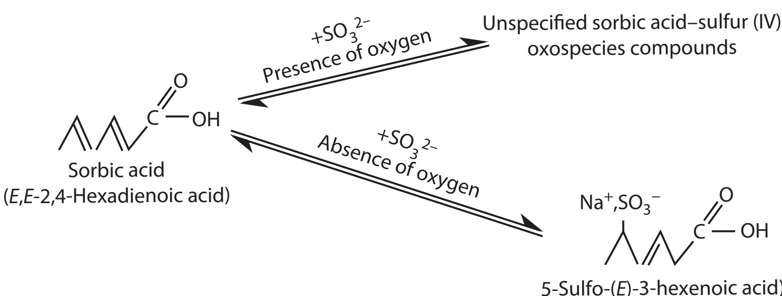


FIGURE 12.11 Reactions of sorbic acid with sulfur dioxide [sulfur (IV) anions].

Reactions between sorbic acid and proteins occur when sorbic acid is used in certain foods, such as wheat dough, whose proteins contain substantial amounts of oxidized or reduced thiol groups (R−S−S−R in cystine and R−SH in cysteine, respectively). The thiol groups (R−SH) dissociate to thiolate ions ($R{-}S^-$) that are reactive nucleophiles, and they react mainly by 1,6-addition to the conjugated diene of sorbic acid. This reaction, which readily occurs at higher pH (>5) and elevated temperatures, binds the protein to the sorbic acid (e.g., during bread baking). Although the reaction is reversible under very acidic conditions (pH < 1), the usual consequence at the higher pH values of foods is the loss of the preservative action of sorbic acid [33].

While sorbic acid and potassium sorbate have gained wide recognition as antimycotics, more recent research has established that sorbate has broad antimicrobial activity that extends to yeasts and many bacterial species that are involved in spoilage of fresh poultry, fish, and meats. It is especially effective in retarding toxigenesis of *C. botulinum* in bacon and refrigerated fresh fish packaged in modified atmospheres.

12.7.6 Glyceryl Esters

Many fatty acids and monoglycerides show pronounced antimicrobial activity against Gram-positive bacteria and some yeasts [31]. Unsaturated members, especially those with 18-carbon atoms, show strong activity as fatty acids; the medium-chain members (12-carbon atoms) are most inhibitory when esterified to glycerol. Glyceryl monolaurate (I), also known under the trade name of Monolaurin, is inhibitory against several potentially pathogenic *Staphylococcus* and *Streptococcus* when present at concentrations of 15–250 ppm. It is commonly used in cosmetics and because of its lipid nature can be used in some foods.

Monolauren (I)

Lipophilic agents of this kind also exhibit inhibitory activity against *C. botulinum*, and glyceryl monolaurate, serving this function, may find applications in cured meats and in refrigerated, packaged fresh fish. The inhibitory effect of lipophilic glyceride derivatives apparently relates to their ability to facilitate the conduction of protons through the cell membranes that effectively destroys the proton motive force that is needed for substrate transport [18]. Cell-killing effects are observed only at high concentrations of the compounds, and death apparently results from the generation of holes in cell membranes.

12.7.7 Lauric Arginate

Lauric arginate (ethyl-*N*-dodecanoyl-L-arginate hydrochloride; II) is a newly available antimicrobial that, like glyceryl monolaurate, contains a medium-chain fatty acid moiety.

Lauric arginate (II)

Lauric arginate has received U.S. GRAS status approval, and it exhibits broad-spectrum antimicrobial efficacy that is anticipated to be useful in the control of food pathogens (*Campylobacter*,

Salmonella, *Clostridium*, *Escherichia*, and *Staphylococcus*). The mode of action of lauric arginate involves the disruption of the plasma membrane lipid bilayer, which leads to interferences with metabolic processes and cell cycling [3].

12.7.8 Natamycin

Natamycin or pimaricin (CAS Reg. No. 768-93-8) is a polyene macrolide antimycotic (III) that has been approved in the United States for use against molds on cured cheeses.

Natamycin (III)

This mold inhibitor is highly effective when applied to surfaces of foods exposed directly to air where mold has a tendency to proliferate. The mechanism of action of natamycin is believed to result from a binding of natamycin to sterol molecules in fungal cell membranes that alters the permeability of the membranes and thereby causes disruption of cell processes. Natamycin is especially attractive for application on fermented foods, such as cured cheeses, because it selectively inhibits molds while allowing normal growth and metabolism of ripening bacteria.

12.7.9 Benzoic Acid

Benzoic acid (C_6H_5COOH) has been widely employed as an antimicrobial agent in foods [5], and it occurs naturally in cranberries, prunes, cinnamon, and cloves. The undissociated acid is the form with antimicrobial activity, and it exhibits optimum activity in the pH range of 2.5–4.0, making it well suited for use in acid foods such as fruit juices, carbonated beverages, pickles, and sauerkraut. Benzoates exhibit very little antimicrobial activity in foods with pH values above 5.2–5.5. Sodium and potassium salts of benzoic acid are generally used because they are more readily dispersible in aqueous foods than the acid form. Once in a suitably acidic product, much of a benzoate salt converts to the active protonated form that is most effective against yeasts and bacteria and least effective against molds. Often, benzoic acid is used in combination with sorbic acid or parabens, and levels of use usually range from 0.05% to 0.1% by weight.

The mode of antimicrobial activity of benzoic acid has not been clearly established. However, the lipophilic character of protonated benzoic acid is believed to facilitate entry of the molecule into cell membranes and interiors. Depending on the microbial type, evidence indicates that multiple modes of activity may be involved, including both disruption of proton motive forces and inhibition of key metabolic enzymes. Benzoic acid has been found to cause no deleterious effects in humans when used in small amounts. It is readily eliminated from the body primarily after conjugation with glycine (Figure 12.12) to form hippuric acid (benzoyl glycine). This detoxification step precludes accumulation of benzoic acid in the body.

Benzoic acid + Glycine ⇌ Hippuric acid

FIGURE 12.12 Metabolic conjugation of the preservative benzoic acid with glycine to facilitate excretion.

12.7.10 *p*-Hydroxybenzoate Alkyl Esters

The parabens are a group of alkyl esters of *p*-hydroxybenzoic acid that have been used widely as antimicrobial agents in foods, pharmaceutical products, and cosmetics. The methyl (IV), propyl (V), and heptyl (VI) esters are used domestically, and in some other countries the ethyl and butyl esters are used as well.

Methyl paraben (IV) (Methyl-*p*-hydroxybenzoate)

Propyl paraben (V) (Propyl-*p*-hydroxybenzoate)

Heptyl paraben (VI) (Heptyl-*p*-hydroxybenzoate)

Parabens are used as microbial preservatives in baked goods, soft drinks, beer, olives, pickles, jams, jellies, and syrups. They have little effect on flavor, are effective inhibitors of molds and yeasts (0.050.1% by weight), and are relatively ineffective against bacteria, especially Gram-negative bacteria [8]. The antimicrobial activity of parabens increases, and their solubility in water decreases with increases in the length of the alkyl chain. The shorter-chain members often are used because of their solubility characteristics. In contrast to other antimycotic agents, the parabens are active at pH 7 and higher, apparently because of their ability to remain undissociated at these pH values. The phenolic group provides a weak acid character to the molecule. The ester linkage is stable to hydrolysis even at temperatures used for sterilization. The parabens have many properties in common with benzoic acid, and they are often used together. Parabens exhibit a low order of toxicity to humans and are excreted in the urine after hydrolysis of the ester group and subsequent metabolic conjugation.

12.7.11 Epoxides

Most antimicrobial agents used in foods exhibit inhibitory rather than lethal effects at the concentrations employed. However, exceptions occur with ethylene (VII) and propylene (VIII) oxides.

Ethylene oxide (VII)

Propylene oxide (VIII)

These chemical sterilants are used to treat certain low-moisture foods and to sterilize aseptic packaging materials. In order to achieve intimate contact with microorganisms, the epoxides

H_2C—CH_2 (O)
Ethylene oxide
$+H_2O$
$+H^+, Cl^-$
H_2C—CH_2 (OH, OH)
Ethylene glycol
H_2C—CH_2 (Cl, OH)
Ethylene chlorohydrin

FIGURE 12.13 Reactions of ethylene oxide with water and chloride ion, respectively.

are used in a vapor state, and after adequate exposure, most of the residual unreacted epoxide is removed by flushing and evacuation.

The epoxides are reactive cyclic ethers that destroy all forms of microorganisms, including spores and even viruses, but the mechanism of action of epoxides is poorly understood. In the case of ethylene oxide, it has been proposed that alkylation of essential intermediary metabolites having a hydroethyl group ($-CH_2-CH_2-OH$) could account for the lethal results [8]. The site of attack would be any labile hydrogen in the metabolic system. The epoxides also react with water to form corresponding glycols (Figure 12.13). However, the toxicity of the glycols is low and, therefore, cannot account for the inhibitory effect.

Since the majority of the active epoxide is removed from the treated food, and the glycols formed are of low toxicity, it might appear that these gaseous sterilants would be used extensively. Their use, however, is limited to dry items, such as nutmeats and spices, because reaction with water rapidly depletes the concentration of epoxides in high-moisture foods. Spices often contain high microbial loads and are destined for incorporation into perishable foods. Thermal sterilization of spices is unsuitable because important flavor compounds are volatile and the product is generally unstable to heat. Thus, treatment with epoxides is a suitable method for reducing the microbial load.

The potential formation of relatively toxic chlorohydrins as a result of reactions between epoxides and inorganic chlorides (Figure 12.13) is a point of some concern. However, there are reports that dietary chlorohydrin in low concentrations causes no ill effect [62]. Another consideration in the use of epoxides is their possible adverse effects on vitamins, including riboflavin, niacin, and pyridoxine.

Ethylene oxide (boiling point, 13.2°C) is more reactive than propylene oxide and is also more volatile and flammable. For safety purposes, ethylene oxide is often supplied as a mixture consisting of 10% ethylene oxide and 90% carbon dioxide. The product to be sterilized is placed in a closed chamber; the chamber is evacuated and then pressurized to 30 lb with the ethylene oxide–carbon dioxide mixture. This pressure is needed to provide a concentration of epoxide sufficient to kill microorganisms in a reasonable time. When propylene oxide (boiling point, 34.3°C) is used, sufficient heat must be applied to maintain the epoxide in a gaseous state.

12.7.12 Antibiotics

Antibiotics comprise a large group of antimicrobial agents produced naturally by a variety of microorganisms. They exhibit selective antimicrobial activity, and their applications in medicine have contributed significantly to the field of chemotherapy. The successes of antibiotics in controlling pathogenic microorganisms in living animals have led to extensive investigations into

their potential applications in food preservation. However, because of the fear that routine use of antibiotics will cause resistant organisms to evolve, their application to foods, with one exception (nisin), is not currently permitted in the United States. The development of resistant strains of organisms would be of particular concern if an antibiotic proposed for use in food is also used in a medical application.

Nisin, a polypeptide antibiotic, is produced by lactic streptococci, and in the United States, it is now permitted in high-moisture processed cheese products where it is used to prevent potential outgrowth of *C. botulinum*. Nisin has been explored extensively for applications in food preservation. It is active against Gram-positive organisms, especially in preventing the outgrowth of spores [49], and it is not used in medical applications. Nisin is also used in other parts of the world for prevention of spoilage of dairy products, such as processed cheese and condensed milk. Nisin is not effective against Gram-negative spoilage organisms, and some strains of clostridia are resistant. However, nisin is essentially nontoxic to humans, does not lead to cross-resistance with medical antibiotics, and is degraded harmlessly in the intestinal tract.

Some other countries allow limited use of relatively few other antibiotics. These include chlortetracycline and oxytetracycline [8]. Most actual or proposed applications of antibiotics in foods involve their use as adjuncts to other methods of food preservation. Notably, this includes delaying spoilage of refrigerated, perishable foods and reducing the severity of thermal processes. Fresh meats, fish, and poultry comprise a group of perishable products that could benefit from the action of broad-spectrum antibiotics. In fact, many years ago, the U.S. FDA permitted dipping whole poultry carcasses into solutions of chlortetracycline or oxytetracycline. This increased the shelf life of the poultry, and residual antibiotics were destroyed by usual cooking methods.

The biochemical modes of actions for antibiotics are just coming into focus, with research efforts emphasizing molecular mechanisms. In addition, there is a continuing search for natural preservatives that hopefully will be suitable for application to foods. However, the necessarily stringent requirements placed on substances for food applications indicate that acceptable substances will be difficult to find.

12.7.13 Diethyl Pyrocarbonate

Diethyl pyrocarbonate has been used as an antimicrobial food additive for beverages such as fruit juices, wine, and beer. The advantage of diethyl pyrocarbonate is that it can be used in a cold pasteurization process for aqueous solutions, following which it readily hydrolyzes to ethanol and carbon dioxide (Figure 12.14). Usage levels between 120 and 300 ppm in acid beverages (below pH 4.0) cause complete destruction of yeasts in about 60 min. Other organisms, such as lactic acid bacteria,

$H_5C_2-O-C(=O)-O-C(=O)-O-C_2H_5$
Diethyl pyrocarbonate

$+2NH_3$ → $2\ H_5C_2-O-C(=O)-NH_2$
Urethane
(Ethyl carbamate)

$+2H_3O$ → $2\ C_2H_5OH + CO_2$
Ethanol

FIGURE 12.14 Reactions showing the hydrolysis and amidization of diethyl pyrocarbonate.

are more resistant, and sterilization is achieved only when the microbial load is low (<500 mL^{-1}) and the pH is below 4.0. The low pH retards the rate of diethyl pyrocarbonate decomposition and intensifies its effectiveness.

Concentrated diethyl pyrocarbonate is an irritant. However, since hydrolysis is essentially complete within 24 h in acid beverages, there is little concern for direct toxicity. Unfortunately, diethyl pyrocarbonate reacts with a variety of compounds to form carbethoxy derivatives and ethyl esters. Specifically, diethyl pyrocarbonate reacts readily with ammonia to yield urethane (ethyl carbamate; Figure 12.14). Ostensibly, this reaction was considered responsible for urethane found in foods treated with diethyl pyrocarbonate, and a ban on the use of diethyl pyrocarbonate was issued in 1972 because urethane is a known carcinogen. Since ammonia is ubiquitous in plant and animal tissues, it seemed reasonable that foods treated with diethyl pyrocarbonate will contain some urethane.

However, it was shown later that urethane occurs intrinsically in fermented foods and beverages, and it is usually present below 10 ppb in most fermented foods, including bread, wine, and beer [26]. It has been suggested that the major pathway for its production in these foods is the reaction of urea, from arginine metabolism, with ethanol. Alcoholic beverages contain much higher levels of urethane than nonalcoholic foods, and levels up to 10 ppm have been reported in stone fruit brandies. In spite of these natural occurrences of urethane, addition of diethyl pyrocarbonate is no longer permitted in foods in the United States because of the potential for elevating levels of a carcinogen in foods.

12.8 INTENSELY SWEET NONNUTRITIVE AND LOW-CALORIE SWEETENERS

Nonnutritive and low-calorie sweeteners encompass a broad group of substances that evoke a sweet taste or enhance the perception of sweet tastes (see Chapter 11). The ban on the use of cyclamates in the United States along with questions raised about the safety of saccharin initiated an intensive search for alternative low-calorie sweeteners to meet the demand for low-calorie foods and beverages. This has led to the discovery of many new sweet molecules, and the number of viable potentially commercially useful, low-calorie sweeteners continues to grow. The relative sweetness values for some of these substances are given in Table 12.3.

TABLE 12.3
Relative Sweetness of Some Intensely Sweet Substances

Substance	Relative Sweetness Values[a] (Sucrose = 1, wt. Basis)
Acesulfame K	200
Alitame	2,000
Aspartame	180–200
Cyclamate	30
Glycyrrhizin	50–100
Monellin	3,000
Neohesperidin dihydrochalcone	1,600–2,000
Neotame	7,000–13,000
Saccharin	300–400
Stevioside	300
Sucralose	600–800
Thaumatin	1,600–2,000

[a] Commonly cited relative sweetness values are listed; however, the concentration and the food or beverage matrix may greatly influence actual relative sweetness values for sweeteners.

12.8.1 Sulfoamides: Cyclamate, Saccharin, and Acesulfame K

The sulfoamide sweeteners are substances that are structurally related through the sulfamic acid grouping (IX), and commercially available members include acesulfame K (X), cyclamate (XI), and saccharin (XII).

Sulfamic acid (IX)

Acesulfame K (X)

Sodium cyclamate (XI)
(Sodium *N*-cyclohexylsulfamate)

Sodium saccharin (XII)

Cyclamate (cyclohexyl sulfamic acid; cyclamic acid; XI) was approved as a food additive in the United States in 1949, and before their use was prohibited by the U.S. FDA in late 1969, the sodium and calcium salts and the acid form of cyclamic acid were widely employed as sweeteners. Cyclamates are about 30 times sweeter than sucrose, taste much like sucrose without significant interfering taste sensations, and are heat stable. Cyclamate sweetness has a slow onset and persists for a period of time that is longer than that for sucrose.

Some early experimental evidence with rodents had suggested that cyclamate and its hydrolysis product, cyclohexylamine (Figure 12.15), caused bladder cancer [5,45]. However, extensive testing subsequently has not substantiated the early reports, and petitions have been filed in the United States for reinstatement of cyclamate as an approved sweetener [40]. Currently, cyclamate is permitted for use in low-calorie foods in some 40 countries and Canada. Still, for various reasons, even though extensive data support the conclusion that neither cyclamate nor cyclohexylamine is carcinogenic or genotoxic [4], the U.S. FDA has not reapproved cyclamates for use in foods.

The calcium and sodium salts and free acid form of saccharin (3-oxo-2,3-dihydro-1,2-benzisothiazole-1, 1-dioxide; XII) are available as nonnutritive sweeteners. The commonly accepted rule of thumb is that saccharin is about 300 times as stweet as sucrose in concentrations up to the equivalent of a

Sodium cyclamate — Hydrolase + H_2O → Cyclohexylamine + $HO-SO_2-O^-, Na^+$

FIGURE 12.15 Formation of cyclohexylamine by the hydrolysis of cyclamate.

10% sucrose solution, but ranges from 200 to 700 times the sweetness of sucrose depending on the concentration and the food matrix [47]. Saccharin exhibits a bitter, metallic aftertaste, especially to some individuals, and this effect becomes more evident with increasing concentration.

The safety of saccharin has been under investigation for over 50 years, and it has been found to cause a low incidence of carcinogenesis in laboratory animals. However, many scientists argue that the animal data are not relevant to humans. In humans, saccharin is rapidly absorbed and then is rapidly excreted in the urine. Although current regulations in the United States prohibit the use of food additives that cause cancer in any experimental animals, a ban on saccharin in the United States, proposed by the FDA in 1977, had been stayed by congressional legislation pending further research. In 2000, saccharin was delisted as a human carcinogen in the United States, which negated the health warning statement required on packages of saccharin-containing foods. Saccharin is approved for use in more than 90 countries around the world.

Acesulfame K [6-methyl-1,2,3-oxathiazine-4(3*H*)-one-2,2-dioxide] (X) was discovered in Germany and was first approved for use as a nonnutritive sweetener in the United States in 1988. The complex chemical name of this substance led to the creation of the trademarked common name Acesulfame K, which is based on relationships to acetoacetic acid and sulfamic acid employed in its synthesis and to its potassium salt nature.

Acesulfame K is about 200 times as sweet as sucrose at a 3% concentration in solution, and it exhibits a sweetness quality between that of cyclamates and saccharin. Since Acesulfame K possesses some metallic and bitter taste notes at higher concentrations, it is especially useful when blended with other low-calorie sweeteners, such as aspartame. Acesulfame K is exceptionally stable at elevated temperatures encountered in baking, and it is also stable in acidic products, such as carbonated soft drinks. Acesulfame K is not metabolized in the body, thus providing no calories, and is excreted through the kidneys unchanged. Extensive testing has shown no toxic effects in animals and exceptional stability in food applications.

12.8.2 Peptides: Aspartame, Neotame, and Alitame

The peptide sweeteners have emerged in response to demands for sweetening ingredients to lower the caloric content of foods and beverages. Although the component amino acids of the peptide sweeteners become calorically available during digestive processes, their intense sweetness allows functionality to be achieved at very low levels that provide insignificant calories. Aspartame (XIII), neotame (XIV), and alitame (XV) comprise the group of peptide sweeteners that are permitted in foods in at least some countries.

Aspartame (XIII)
(L-Aspartyl-L-phenylalanine methyl ester)

Neotame (XIV)
(L-Phenylalanine, *N*-{*N*-(3,3-dimethylbutyl)-L-α-aspartyl}-,1-methyl ester)

Alitame (XV)
[L-α-Aspartyl-*N*-(2,2,4,4-tetramethyl-3-thietanyl)-D-alaninamide]

Aspartame (L-aspartyl-L-phenylalanine methyl ester; XIII) with a sweetening power of 180–200 times that of sucrose was first approved in the United States in 1981 and now is approved for use in over 75 countries where it is used in numerous products. It is noted for a clean, sweet taste although it lacks some of the sweetness qualities of sucrose. Two technical disadvantages of aspartame are its

FIGURE 12.16 Reactions involved in the degradation of aspartame.

instability under acid conditions and its rapid degradation when exposed to elevated temperatures. Under acidic conditions, such as carbonated soft drinks, the rate of loss of sweetness is gradual and depends on the temperature and pH. The peptide nature of aspartame makes it susceptible to hydrolysis (Figure 12.16), and this feature also permits other chemical interactions and microbial degradations. In addition to loss of sweetness resulting from hydrolysis of either the methyl ester on phenylalanine or the peptide bond between the two amino acids, aspartame readily undergoes an intramolecular condensation, especially at elevated temperatures, to yield the diketopiperazine (5-benzyl-3,6-dioxo-2-piperazine acetic acid) shown in Figure 12.16. This reaction is especially favored at neutral and alkaline pH values because nonprotonated amine groups on the molecule are more available for reaction under these conditions. Similarly, alkaline pH values promote carbonylamino reactions, and aspartame has been shown to react readily with glucose and vanillin under such conditions. With the glucose reaction, loss of aspartame's sweetness during storage is the principal concern while loss of vanilla flavor is the main concern in the latter case.

Even though aspartame is composed of naturally occurring amino acids and its daily intake is projected to be very small (0.8 g/person), concern has been expressed about its potential safety as a food additive. Aspartame-sweetened products must be labeled prominently about their phenylalanine content to allow avoidance of consumption by phenylketonuric individuals who lack 4-monooxygenase that is involved in the metabolism of phenylalanine. Concern also has been expressed by some about the potential long-term toxicological effects of the methanol that is released via hydrolysis of the methyl ester. This health issue relates to the potential for metabolic conversion of methanol to formaldehyde. However, substantial methanol that is released from pectin polymers in plant-based foods (Chapter 3) also is consumed without apparent

toxic effects. Similarly, consumption of aspartame by the normal population is not associated with adverse health effects. Although criticized by some, extensive testing has similarly shown that the diketopiperazine from aspartame does not pose risks to humans at concentrations potentially encountered in foods [29].

Neotame (L-phenylalanine, *N*-{*N*-(3,3-dimethylbutyl)-L-α-aspartyl}-L,1-methyl ester; XIV] is structurally related to aspartame (XIII) and was approved for use in foods in the United States in 2002. Neotame was developed as an ingredient because it exhibits increased stability to conditions encountered in food preparation and its very high sweetening power (7,000–13,000 times that of sucrose) that permits usage without the necessity for cautionary labeling for phenylketonurics. The intense sweetness of neotame compared to that of aspartame is derived in a large part from the addition of the 3,3-dimethylbutyl substituent to the amino group of the aspartic acid moiety of aspartic acid. This supplements the γ-grouping of aspartame with a strongly hydrophobic component that promotes intense sweetness (Chapter 11). Because usage of very low levels of neotame often exhibits beneficial effects on the flavors of some foods, it is also marketed as a flavor enhancer (Chapter 11).

Alitame [L-α-aspartyl-*N*-(2,2,4,4-tetramethyl-3-thietanyl)-D-alaninamide; XV] is an amino acid–based sweetener that possesses a sweetening power of about 2000 times that of sucrose, and it exhibits a clean sweet taste similar to sucrose. It is highly soluble in water and has good thermal stability and shelf life, but prolonged storage in some acidic solutions may result in off-flavors. Generally, alitame has the potential for use in most foods where sweeteners are employed, including baked goods.

Alitame is synthesized from the amino acids, L-aspartic acid and D-alanine, and a novel amine. The alanine amide moiety of alitame apparently passes through the body with minimal metabolic changes. Extensive testing indicates that alitame is safe for human consumption, and a petition for its use in foods was filed in 1986 with the U.S. FDA. Although not yet approved for use in foods in the United States, alitame has been approved in Australia, New Zealand, China, and Mexico.

12.8.3 Chlorosaccharides: Sucralose

Chlorosaccharides are synthesized by combining selective chlorination of saccharide (sugar) molecules with other synthetic strategies, such as directed condensations, to yield molecules that may possess intense sweetness. The chlorosaccharide, sucralose (1,6-dichloro-1,6-dideoxy-β-fructofuranosyl-4-chloro-α-D-galactopyranoside), was approved in 1998 and 1999 for broad food uses in the United States and currently has also been approved for use in more than 40 countries.

Sucralose is about 600 times sweeter than sucrose and possesses a sweetness time–intensity profile similar to sucrose with no bitterness or other unpleasant aftertastes; it exhibits a high degree of crystallinity, high water solubility, and very good stability at high temperatures. It is also quite stable at the pH of carbonated soft drinks, and only limited hydrolysis to monosaccharide units occurs during usual handling and storage of these products.

The sucralose molecule is designed to resist digestive and metabolic attack by providing molecular features that are not easily recognized by constitutive hydrolytic enzymes. The molecular features contributing to this stability are shown in Figure 12.17 where they are compared with those of the naturally occurring counterpart saccharides, sucrose, and lactose (Chapter 3).

In addition to the replacement of three hydroxyl groups with chlorine atoms on the sucralose molecule, it also possesses a glycosidic β-linkage between its galactose and fructose moieties (Figure 12.17a). Comparison of counterpart features in sucrose (Figure 12.17b) and lactose (Figure 12.17c) reveals a mixing of the two basic structures in sucralose that preclude recognition by usual digestive and metabolic enzymes. However, some hydrolysis of the sucralose molecule has been reported to occur during digestion, mediated either by acid-catalyzed or microbial enzymic processes (Figure 12.18).

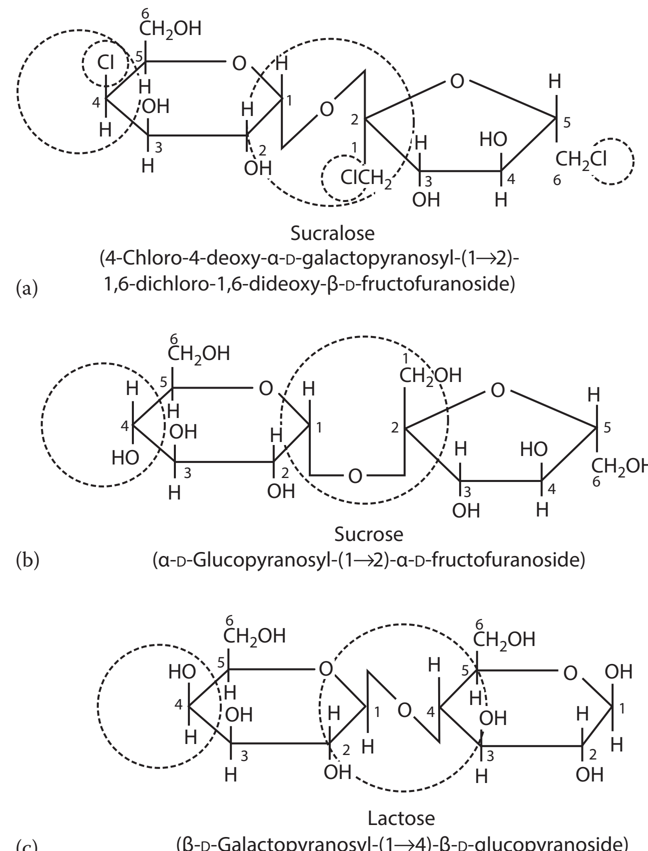


FIGURE 12.17 Comparison of structural features of (a) sucralose, (b) sucrose, and (c) lactose.

Extensive studies have been conducted on the safety of sucralose, and these have generally demonstrated that the substance is safe at the expected usage levels. However, some have criticized the approval of sucralose as premature because it contains structural components of harmful substances that might be formed, especially upon exposure to thermal degradative conditions (Figure 12.19).

12.8.4 Other Intensely Sweet Nonnutritive or Low-Calorie Sweeteners

The intensive search for alternative sweeteners over the past two decades has led to the discovery of a large number of new sweet compounds, and some of these are undergoing further development and safety studies to determine if they are suitable for future commercialization. These compounds join a substantial list of less well-known, but emerging intensely sweet compounds, and some of the latter group are discussed here.

Glycyrrhizin (glycyrrhizic acid) is a triterpene saponin that is found in licorice root and is 50,100 times sweeter than sucrose. Glycyrrhizin is a glycoside that on hydrolysis yields two moles of glucuronic acid and one mole of glycyrrhetinic acid, a triterpene related to oleanolic acid. Ammonium glycyrrhizin, the fully ammoniated salt of glycyrrhizic acid, is commercially available and is approved for use only as a flavor and as a surfactant, but not as a sweetener. Glycyrrhizic acid is used primarily in tobacco products and to some extent in foods and beverages. Its licorice-like flavor influences its suitability for some applications.

Sucralose
(4-Chloro-4-deoxy-α-D-galactopyranosyl-(1→2)-
1,6-dichloro-1,6-dideoxy-β-D-fructofuranoside)

Hydrolysis; + H_2O

4-Chloro-4-deoxy-
α-D-galactopyranose

\+

1,6-Dichloro-1,6-dideoxy-
β-D-fructofuranose

FIGURE 12.18 Reaction showing the hydrolysis products of sucralose.

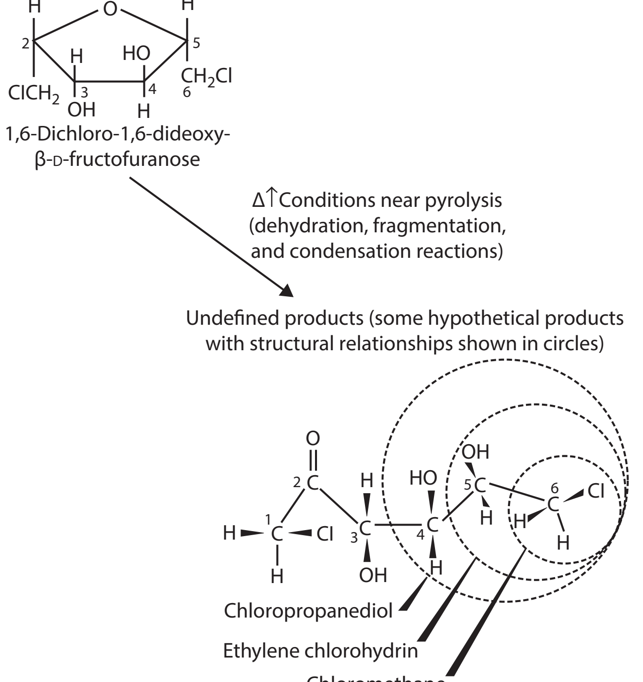


FIGURE 12.19 Structures of potentially harmful substances contained within the overall structure of 1,6-dichloro-1,6-dideoxy-β-D-fructofuranose.

A mixture of glycosides found in the leaves of the South American plant *Stevia rebaudiana* Bertoni is the source of stevioside and rebaudiosides. Pure stevioside is about 300 times as sweet as sucrose. Stevioside exhibits some bitterness and undesirable aftertastes at higher concentrations, and rebaudioside A exhibits the best taste profile of the mixture. However, extracts produced from *S. rebaudiana* are used as commercial forms of this sweetener, and they are employed extensively in Japan. Extensive safety and toxicological testing have indicated that the extracts are safe for human consumption, but they are not approved in the United States.

Neohesperidin dihydrochalcone is a nonnutritive sweetener that is 1500–2000 times as sweet as sucrose, and it is derived from the bitter flavonones of citrus fruit. Neohesperidin dihydrochalcone exhibits a slow onset in sweetness and a lingering sweet aftertaste, but it decreases the perception of concurrent bitterness. This intensely sweet substance as well as other similar compounds are produced by hydrogenation of (1) naringin to yield naringin dihydrochalcone, (2) neohesperidin to yield neohesperidin dihydrochalcone, or (3) hesperidin to yield hesperidin dihydrochalcone 4′–*O*-glucoside [44]. Neohesperidin dihydrochalcone has been extensively tested for safety, and the studies have generally confirmed its safety. It is approved for use in Belgium and Argentina, but the U.S. FDA has requested additional toxicology testing.

Several sweet proteins are now known, and thaumatin I and II obtained from the tropical African fruit katemfe (*Thaumatococcus daniellii*) have been well characterized. Thaumatin I and II are alkaline proteins, each with a molecular weight of about 20,000 [58], and on a mass basis they are about 1600–2000 times as sweet as sucrose. An extract of katemfe fruit is marketed under the trade name of Talin in the United Kingdom, and its use as a sweetener and flavor enhancer has been approved in Japan and Great Britain. It is also permitted as a flavor enhancer in chewing gum in the United States. Talin exhibits long-lasting sweetness with a slight licorice-like taste that limits its use along with its high costs.

Monellin is a sweet protein obtained from the serendipity berry, and it has a molecular weight of about 11,500. Monellin is about 3000 times as sweet as sucrose on a mass basis, and the sweetness of natural monellin is destroyed by boiling. The potential use of sweet proteins is limited because the compounds are expensive, unstable to heat, and lose sweetness when held in solution below pH 2 at room temperature.

Brazzein is a sweet plant protein (54 amino acid residues) that was initially discovered in the fruits of the African vine *Pentadiplandra brazzeana*. Field corn varieties have been genetically engineered to produce the sweet protein, and efforts are underway to commercially produce the sweetener for food uses by extracting it from the germ of corn seeds. It is reported to be quite stable and possess both sweetness and desirable mouthfeel characteristics.

Another basic protein, miraculin, has been isolated from miracle fruit (*Richadella dulcifica*), and this protein is tasteless. However, it has the peculiar property of changing sour taste into sweet taste, that is, it makes lemons taste sweet. Miraculin is a glycoprotein with a molecular weight of 42,000 [58], and similar to other protein sweeteners, miraculin is heat labile and inactivated at low pH values. The sweetness induced by 0.1 M citric acid after tasting 1 mM miraculin solution is equivalent to a 0.4 M sucrose solution; thus, the sweetness of a miraculin solution induced by 0.1 M citric acid solution has been calculated to be 400,000 times that of a sucrose solution. The taste effects of miraculin persist for over 24 h after placing it in the mouth, and this limits its potential use. In the 1970s, miraculin was introduced in the United States as a sweetening aid for diabetics, but it was banned by the U.S. FDA because of insufficient safety data.

12.9 POLYOLS: SWEETENERS, TEXTURIZERS, AND EMULSIFIERS

Simple polyols or polyhydric alcohols are structural analogs of carbohydrates that contain only hydroxyl groups as functional groups (Chapter 3). Thus, simple sugars and polyhydric alcohols (sugar alcohols) are structurally similar, except that sugars also contain aldo or keto groups (free or bound) that may adversely affect their chemical stability, especially at high temperatures.

Polyols are generally quite water-soluble, hygroscopic materials that exhibit moderate viscosities at high concentrations in water, and the polyhydroxy structures of these compounds provide water-binding properties that have been exploited in foods. Specific functions of polyhydric alcohols include control of viscosity and texture, addition of bulk, retention of moisture, reduction of water activity, control of crystallization, improvement or retention of softness, improvement of rehydration properties of dehydrated foods, and use as a solvent for flavor compounds [24]. Many applications of polyhydric alcohols in foods rely on concurrent contributions of functional properties from sugars, proteins, starches, and gums.

Some simple polyhydric alcohols occur naturally, but because of their limited concentrations, they often do not contribute functional roles in food. For example, free glycerol exists in wine and beer as a result of fermentation, and sorbitol occurs in fruits such as pears, apples, and prunes. While the number of available simple polyhydric alcohols is substantial, relatively few have been important in food applications (Figure 12.20).

Simple polyols (sugar alcohols) generally are sweet, but less so than sucrose (Table 12.4). Very short-chain members, such as glycerol, are slightly bitter at high concentrations. When used in the dry form, simple polyols often contribute a pleasant cooling sensation because of their negative heat of solution. Notably, the usage of some polyhydric alcohols is growing because of demands for their reduced-calorie sweetener properties. Historically, the energy value of simple polyols derived from sugars, like sugars, has been considered to be 16.7 kJ (4 kcal)/g (Joules = calories × 4.1816) for labeling purposes in the United States. However, this view has changed very recently following a 1990 European Union lead of assigning an energy value of 10 kJ (2.4 kcal)/g to polyols as a group. The U.S. FDA has accepted caloric contents ranging from 6.7 to 12.5 kJ (1.6–3.0 kcal)/g for the various commercially available polyols (Table 12.4). This has markedly changed the positioning of polyols as food ingredients, and it can be anticipated that their presence in low-calorie, reduced-fat, and sugar-free foods will continue to increase in the future. Although there is some lingering controversy relating to the influence of polyols on diabetics, the currently accepted general philosophy is that they are suitable in diets of these individuals.

Xylitol, sorbitol, mannitol, maltitol, and lactitol are manufactured by hydrogenation of xylose, glucose (Figure 12.21), mannose, maltose, and lactose, respectively. Hydrogenated starch

FIGURE 12.20 Comparative structures of simple polyhydric alcohols used as food ingredients.

TABLE 12.4
Relative Sweetness and Energy Values of Some Relatively Simple Polyols and Sugars

Substance	Relative Sweetness[a] (Sucrose = 1, wt. Basis)	Energy Value[b] (kJ/g)
Simple polyols		
Erythritol	0.7	0.84
Mannitol	0.6	6.69
Lactitol	0.3	8.36
Isomalt	0.4–0.6	8.36
Xylitol	1.0	10.03
Sorbitol	0.5	10.87
Maltitol	0.8	12.54
Hydrogenated corn syrup	0.3–0.75	12.54
Sugars		
Xylose	0.7	16.72
Glucose	0.5–0.8	16.72
Fructose	1.2–1.5	16.72
Galactose	0.6	16.72
Mannose	0.4	16.72
Lactose	0.2	16.72
Maltose	0.5	16.72
Sucrose	1.0	16.72

[a] Commonly cited relative sweetness values are listed; however, the concentration and the food or beverage matrix may greatly influence actual relative sweetness values for sweeteners.
[b] Energy values accepted by the U.S. FDA; 1 kcal = 4.1816 kJ.

FIGURE 12.21 Reaction showing the hydrogenation of glucose for the manufacture of sorbitol.

hydrolysates also are employed as food ingredients, especially in confections, and these contain sorbitol from glucose, maltitol from maltose, and various polymeric polyols (hydrogenated maltodextrins) from oligosaccharides. Isomalt is derived from sucrose using a multiple-step process (Figure 12.22). The 1- to 2-glycosidic linkage of sucrose is first enzymatically isomerized to 1–6 linkages between the glucose and fructose moieties, respectively. Subsequent hydrogenation of this intermediate results in an equimolar mixture of two disaccharide polyols, gluco-mannitol and gluco-sorbitol.

Sucrose
(α-D-Glucopyranosyl-(1→2)-α-D-fructofuranoside)

Enzymic (1→2) to (1→6)
linkage isomerization

Fructose moiety

Isomaltulose
(α-D-Glucopyranosyl-(1→6)-D-fructofuranoside)

Carbonyl hydrogenation
with Raney nickel

Isomalt
(An equimolar mixture)

(α-D-Glucopyranosyl-(1→6)-D-sorbitol) (α-D-Glucopyranosyl-(1→6)-D-mannitol)

FIGURE 12.22 Reactions utilized in the manufacture of isomalt.

Simple polyols may also provide the starting materials for the manufacture of other food ingredients, such as emulsifiers (Chapter 7). An example of this is the use of sorbitol as a reactant in the manufacture of Spans and Tweens (Figure 12.23). Sorbitol is first converted to a sorbitan and then is esterified to a fatty acid (stearic acid in this case) to provide an amphiphilic property to the resulting sorbitan monostearate (Span) molecule. The remaining hydroxyl groups on sorbitan monostearate can then provide reaction sites for adding repeating ether linked moieties from ethylene oxide to yield polysorbate (Tween) emulsifiers.

High-molecular-weight polymeric forms of polyhydric alcohols have also been developed for food applications. Whereas ethylene glycol ($CH_2OH{-}CH_2OH$) is toxic, polyethylene glycol 6000 is allowed in some food coating and plasticizing applications. Polyglycerol [$CH_2OH{-}CHOH{-}CH_2{-}(O{-}CH_2CHOH{-}CH_2)_n{-}O{-}CH_2{-}CHOH{-}CH_2OH$], formed from glycerol through an alkaline catalyzed polymerization, also exhibits useful properties. It can be further modified by esterification with fatty acids to yield materials with lipid-like characteristics. These polyglycerol materials have been approved for food use because the hydrolysis products, glycerol and fatty acids, are metabolized normally.

Intermediate moisture (IM) foods deserve some discussion since polyhydric alcohols can make an important contribution to the stability of these products. IM foods contain substantial

Sorbitol

($\Delta\uparrow$; Dehydration) H_2O

A sorbitan
(1,4-Anhydrosorbitol isomer)

+ Fatty acid: lauric, stearic or oleic; (esterification; H^+)

Sorbitan monostearate
(A span)

+ H_2C—CH_2 (O)
Ethylene oxide
(condensations)

Polyoxyethylene sorbitan monostearate
(A polysorbate; also a tween)

FIGURE 12.23 Reactions showing the use of sorbitol in the manufacture of Span and Tween (polysorbate) emulsifiers.

moisture (15%–30%), yet are stable to microbiological deterioration without refrigeration. Several familiar foods, including dried fruits, jams, jellies, marshmallows, fruit cake, and jerky, owe their stability to IM characteristics [35]. Some of these items may be rehydrated before consumption, but all possess a plastic texture and can be consumed directly. Although moist shelf-stable pet foods have found ready acceptance, new forms of IM foods for human consumption have not as yet become popular. Nevertheless, meat, vegetable, fruit, and combination prepared dishes are underdevelopment and may eventually become important forms of preserved foods.

Most IM foods possess water activities of 0.70–0.85, and those containing humectants contain moisture contents of about 20 g of water/100 g of solids (82% H_2O by weight). If IM foods with a water activity of about 0.85 are prepared by desorption, they are still susceptible to attack by molds and yeasts. To overcome this problem, the ingredients can be heated during preparation and an antimycotic agent, such as sorbic acid, can be added.

To obtain the desired water activity, it is usually necessary to add a humectant that binds water and maintains a soft palatable texture. Relatively few substances, mainly glycerol, sucrose,

glucose, propylene glycol, and sodium chloride, are sufficiently effective in lowering the water activity while being tolerable organoleptically to be of value in preparing IM foods. On the other hand, the formulation technology for most contemporary sugar-free candies relies on fundamental IM principles.

12.10 STABILIZERS AND THICKENERS

Many hydrocolloid materials are widely used for their unique textural, structural, and functional characteristics in foods where they provide stabilization for emulsions, suspensions, and foams and for general thickening properties. Most of these materials, sometimes classed as gums, are derived from natural sources although some are chemically modified to achieve desired characteristics. Many stabilizers and thickeners are polysaccharides, such as gum arabic, guar gum, carboxymethylcellulose, carrageenan, agar, starch, and pectin. The chemical properties of these and related carbohydrates are discussed in Chapter 3. Gelatin, a protein derived from collagen, is one of the few noncarbohydrate stabilizers used extensively, and it is discussed in Chapter 5. All effective stabilizers and thickeners are hydrophilic and are dispersed in solution as colloids, which leads to the designation hydrocolloid. General properties of useful hydrocolloids include significant solubility in water, a capability to increase viscosity, and in some cases an ability to form gels (Chapter 3). Some specific functions of hydrocolloids include improvement of texture, inhibition of crystallization (sugar and ice), stabilization of emulsions and foams, improvement (reduced stickiness) of icings on baked goods, and encapsulation of flavors [31]. Hydrocolloids are generally used at concentrations of about 2% or less because many exhibit limited dispersibility, and the desired functionality is provided at these levels. The efficacy of hydrocolloids in many applications is directly dependent on their ability to increase viscosity. For example, this is the mechanism by which hydrocolloids stabilize oil-in-water emulsions. They cannot function as true emulsifiers since they lack the necessary combination of strong hydrophilic and lipophilic properties within individual molecules.

12.11 FAT REPLACERS

Although fat is an essential dietary component, too much fat in the diet has been linked with a higher risk of coronary heart disease and certain types of cancer. Consumers are being advised to eat lean meats, especially fish and skinless poultry, and low-fat dairy products and to restrict their consumption of fried foods, high-fat baked goods, and sauces and dressings. However, consumers want substantially reduced-calorie foods that possess the sensory properties of traditional high-fat foods.

While the increasing availability of complex prepared foods has contributed to the overabundance of fat in the diets of developed countries, it also has provided an opportunity to develop the complex technologies required for the manufacture and mass marketing of reduced-fat foods that simulate full-fat counterparts. Over the past two decades, a great deal of progress has been made in the adaptation and development of ingredients for use in reduced-fat foods. The types of ingredients suggested for various reduced-fat food applications vary widely and are derived from several chemical groups, including carbohydrates, proteins, lipids, and purely synthetic compounds.

When fat is either partially or completely omitted from foods, the properties of the foods are altered, and it is necessary to replace it by some other ingredients or components. Hence, the term “fat replacers” has been spawned to broadly indicate the ingredients that are functionally used in this capacity. When the substances provide identical physical and sensory properties to fats, but without calories, they are designated “fat substitutes.” These ingredients convey both fatlike sensory properties in foods and perform physically in various applications, such as in frying foods.

Other ingredients that do not possess full functional equivalency to fats are termed "fat mimetics," because they can be made to mimic the effects of fat in certain applications. An example of this is the simulation of the pseudo-moistness provided by fat to certain high-fat bakery products. Certain substances, such as specially modified starches, can be used to provide the desired simulated fat properties by contributing to sensory properties arising from bulking and moisture retention.

12.11.1 Carbohydrate Fat Mimetics

Modestly processed starches, gums, hemicelluloses, and cellulose are used in many forms for providing partial fat functionality in reduced-fat foods, and the chemistry of these substances is discussed in Chapter 3 and in Sections 12.9 and 12.10. Additional information about their applications in reduced-fat foods can also be found in reviews [2,37]. Generally, some carbohydrate fat mimetics provide essentially no calories (e.g., gums, celluloses), while others provide up to 16.7 kJ (4 kcal)/g (e.g., modified starches) rather than the 37.6 kJ (9 kcal)/g of traditional fats. These substances mimic the smoothness or creaminess of fats in foods primarily by moisture retention and bulkiness of their solids that assist in providing fatlike sensations, such as moistness in baked goods and the textural bite of ice cream.

12.11.2 Protein Fat Mimetics

Several attempts have been made to exploit selected proteins (Chapter 5) as fat mimetics [39], and they generally have U.S. GRAS approval. However, the functionality of these proteins as fat mimetics is limited because they do not perform like fats at highly elevated temperatures, such as is required in frying applications. Nevertheless, some of these protein (16.7 kJ [4 kcal]/g) ingredients may be useful for replacing fat in foods, especially in oil-in-water emulsions. For these applications, they can be variously prepared into microparticulates (<3 μm diameter) where they simulate the physical nature of fats through a means that has been described as being similar to flexible ball bearings. Proteins in solution also provide thickening, lubricity, and mouth-coating effects. Gelatin is quite functional in reduced-fat, solid products, such as margarine, where it provides thermally reversible gelation during manufacture, and subsequently it provides thickness to the margarine mass.

The manufacture of protein-based fat mimetics involves several strategies that each utilize soluble proteins as the starting materials. Particulate proteins are obtained from soluble proteins by inducing one of the following events: (1) hydrophobic interactions, (2) isoelectric precipitation, (3) heat denaturation and/or coagulation, (4) protein–protein complex formation, or (5) protein–polysaccharide complex formation [39]. These processes are often accompanied by physical shearing action that assures the formation of microparticles.

12.11.3 Reduced-Calorie Synthetic Triacylglycerol Fat Substitutes

Attempts have been made to take advantage of certain triglycerides (triacylglycerols, Chapter 4), which because of unique structural features do not yield full caloric value when consumed by humans and other monogastrics. These triglycerides are variously synthesized utilizing hydrogenation and directed esterifications or interesterifications. One member of this group of lipids is the medium-chain triglycerides (MCTs), which have long been used in the treatment of certain lipid metabolism disorders. MCTs are composed of saturated fatty acids with chain lengths of C_6–C_{12}, and they provide about 34.7 (8.3 kcal)/g compared with regular triglycerides that contain 37.6 kJ (9 kcal)/g [34].

The incorporation of saturated short-chain fatty acids (C_2–C_5) along with a long-chain saturated fatty acid (C_{14}–C_{24}) in a triglyceride molecule is another strategy, and this greatly reduces the caloric value. The caloric reduction results in part because short-chain fatty acids provide fewer calories per unit weight than long-chain fatty acids. In addition, the position of the long-chain fatty acid on the glycerol molecule greatly influences the absorption of the long-chain fatty acid. In some positional combinations of short- and long-chain saturated fatty acids, the absorption of the long-chain fatty acid may be reduced by over half (see Chapter 4).

A family of triglycerides based on the earlier-mentioned principles, trade named Salatrim (*S*hort *a*nd *l*ong *a*cyl*tri*glyceride *m*olecule), has been developed [52]. Salatrim (XVI) is a mixture of triglycerides composed of mainly stearic acid (C18) as the long-chain fatty acid obtained from hydrogenated vegetable fats and various proportions of acetic, propionic, and butyric acids (C_2, C_3, and C_4, respectively) as the short-chain fatty acids. Humans realize between 19.6 and 21.3 kJ (4.7 and 5.1 kcal)/g for various Salatrim products, and the fatty acid composition can be controlled to provide the desired physical properties, such as melting points.

Salatrim isomer (XVI)
(1-Propionyl-2-butyrl-3-stearoyl-*sn*-glycerol)

Caprenin is the trade name of a similarly synthesized, reduced-calorie triglyceride (about 20.9 kJ [5 kcal]/g) product that contains the medium-chain fatty acids, caprylic (C_6) and capric (Ci_0) acids, along with the long-chain fatty acid, behenic acid (C_{22}). Caprenin has been used in candy bars. Caprylic and capric acids are obtained from coconut and palm oils, and behenic acid can be obtained from hydrogenated marine oils, hydrogenated rapeseed oil, and peanut oil. Peanut oil contains about 3% behenic acid, whereas rapeseed oil contains about 35% erucic acid ($C_{22:1}$), which is converted to behenic acid by hydrogenation. Marine oils often contain over 10% of docosahexaenoic acid, which is also converted to behenic acid by hydrogenation.

12.11.4 Synthetic Fat Replacers

A great number of synthetic compounds have been found to provide either fat mimetic or fat substitute properties [1,2]. Many of them contain triacylglycerol-like structural and functional groups, such as the trialkoxycarballates, which in effect have the ester groups reversed compared to conventional fats (i.e., a tricarboxylic acid is esterified to saturated alcohols rather than glycerol being esterified to fatty acids). Owing to their synthetic nature, these compounds are resistant to enzymic hydrolysis and are largely undigested in the gut. U.S. FDA approval for many of these substances is proving to be difficult to obtain, and it remains to be seen whether some of these compounds will find an ultimate role in the food supply.

12.11.4.1 Polydextrose

Although principally used as a reduced-calorie, carbohydrate bulking ingredient, polydextrose (XVII) behaves as a fat mimetic in some applications. Since polydextrose yields only 4.18 kJ (1 kcal)/g, it is especially attractive as a dual purpose ingredient that reduces calories from carbohydrates as well as fats. Contemporary polydextrose (trade name Litesse) is manufactured by randomly polymerizing glucose (minimum 90%), sorbitol (maximum 2%), and citric acid, and it

contains minor amounts of glucose monomer and 1,6-anhydroglucose [2]. To maintain suitable water solubility, the molecular weight of polydextrose polymers is controlled below 22,000.

Polydextrose (XVII)

12.11.4.2 Sucrose Polyesters

Sucrose polyesters (XVIII) comprise a family of substances [1] that are formed through esterification of two or more of the eight available hydroxyl groups on the sucrose molecule. Some sucrose polyesters are found in nature, such as in the waxy coatings on leaves of some plants. Sucrose polyesters are readily commercially manufactured by esterification of sucrose with fatty acids obtained from natural sources, and resulting polarity and melting point properties depend both on selection of fatty acids and degree of esterification. Sucrose polyesters exhibiting lower degrees of esterification possess amphiphilic properties that permit their use in emulsifier applications. Complete esterification of sucrose molecules, especially with longer-chain fatty acids, yields lipophilic, nondigestible, and nonabsorbable sucrose octaesters that possess physical and chemical properties of conventional fats.

A sucrose polyester isomer (XVII)

Although not used widely, sucrose polyester emulsifiers were approved for use in foods in the United States in 1983 with very little debate about health issues. This resulted because only small amounts of sucrose polyesters are employed in emulsifier applications, and they are readily digested because of their low degree of esterification. On the other hand, sucrose polyester fat replacers (octaesters, trade names Olestra and Olean) were deemed safe and approved for limited use as an industrial frying medium for snackfoods (e.g., potato and corn chips) by the U.S. FDA only in 1996 after over two decades of health and safety studies. The main health issues that caused the limited approval of sucrose polyester fat substitutes were much debated concerns about interferences with absorption of fat-soluble vitamins and micronutrients as well as diarrhea and other disturbances caused by passage of excessive amounts of fatlike sucrose polyesters through the digestive tract.

12.12 MASTICATORY SUBSTANCES

Masticatory substances are employed to provide the long-lasting, pliable properties of chewing gum. These substances are either natural products or the result of organic synthesis, and both kinds are quite resistant to degradation. Synthetic masticatory substances are prepared by the Fischer–Tropsch process involving carbon monoxide, hydrogen, and a catalyst, and after further processing to remove low-molecular-weight compounds, the product is hydrogenated to yield synthetic paraffin [10]. Chemically modified masticatory substances are prepared by partially hydrogenating wood rosin, which is largely composed of diterpenes, and then esterifying the products with pentaerythritol or glycerol. Other polymers similar to synthetic rubbers have also been prepared for use as masticatory substances, and these substances are prepared from ethylene, butadiene, or vinyl monomers.

Much of the masticatory base employed in chewing gum is derived directly from plant gums. These gums are purified by extensive treatments involving heating, centrifuging, and filtering. Chicle from plants in the Sapotaceae (Sapodilla) family, gums from Gutta Katiau from *Palaquium* sp., and latex solids (natural rubber) from *Hevea brasiliensis* are the widely used, naturally derived, masticatory substances.

12.13 FIRMING TEXTURIZERS

Thermal processing or freezing of plant tissues usually causes softening because the cellular structure is modified. Stability and integrity of these tissues are dependent on maintenance of intact cells and firm molecular bonding between constituents of cell walls. The pectic substances (Chapters 3 and 16) are extensively involved in structure stabilization through cross-linking of their free carboxyl groups via polyvalent cations. Although considerable amounts of polyvalent cations are naturally present, calcium salts (0.1%–0.25% as calcium) are frequently added. This increases firmness since the enhanced cross-linking results in increased amounts of relatively insoluble calcium pectinate and pectate. These stabilized structures support the tissue mass, and integrity is maintained even through heat processing. Fruits, including tomatoes, berries, and apple slices, are commonly firmed by adding one or more calcium salts before canning or freezing. The most commonly used salts include calcium chloride, calcium citrate, calcium sulfate, calcium lactate, and monocalcium phosphate. Most calcium salts are sparingly soluble, and some contribute a bitter flavor at higher concentrations.

Acidic alum salts, sodium aluminum sulfate [$NaAl(SO_4)_2 \cdot 12H_2O$], potassium aluminum sulfate, ammonium aluminum sulfate, and aluminum sulfate [$Al_2(SO_4)_3 \cdot 18H_2O$] are added to fermented, salt-brined pickles to make cucumber products that are crisper and firmer than those prepared without these salts. The trivalent aluminum ion is believed to be involved in the crisping process through the formation of complexes with pectin substances. However, some investigations have demonstrated that aluminum sulfate has a softening effect on fresh-pack or pasteurized pickles and should not be included in these products [14]. The reasons for the softening are not understood,

but the presence of aluminum sulfate counteracts the firming effects normally provided by adjusting the pH to near 3.8 with acetic or lactic acids.

The firmness and texture of some vegetables and fruits can be manipulated during processing without the use of direct additives. For example, an enzyme, pectin methylesterase, is activated during low temperature blanching (70°C–82°C for 3–15 min) rather than inactivated as is the case during usual blanching (88°C–100°C for 3 min). The degree of firmness produced following low temperature blanching can be controlled by the holding time before retorting [57]. Pectin methylesterase hydrolyzes esterified methanol (sometimes referred to as methoxyl groups) from carboxyl groups on pectin to yield pectinic and pectic acids. Pectin, having relatively few free carboxyl groups, is not strongly bound, and because it is water soluble, it is free to migrate from the cell wall. On the other hand, pectinic acid and pectic acid possess large numbers of free carboxyl groups, and they are relatively insoluble, especially in the presence of endogenous or added calcium ions. As a result, they remain in the cell wall during processing and produce firm textures. Firming effects through activation of pectin methylesterase have been obtained for snap beans, potatoes, cauliflower, and sour cherries as well as for a number of other fruits and vegetables. Addition of calcium ions in conjunction with enzyme activation leads to additional firming effects.

12.14 APPEARANCE CONTROL AND CLARIFYING AGENTS

The appearance of beverage foods is critical to consumer acceptance, and maintenance of dispersions of colloidal particles or other entities is a key consideration for these products. In some instances, the physical appearance may be suitably maintained by slowing the settling of solids through ionic associations and viscosity enhancement. Such is the case when carrageenan (Chapter 3) is added to milk in the manufacture of chocolate milk that has been flavored

Vegetable oil component
[1-Oleolyl-2-linoleolyl-3-oleoyl-*sn*-glycerol]

$+2Br_2$

Partially brominated vegetable oil component
[1-(9,10-Dibromo)-stereolyl-2-(12,13-dibromo)-oleolyl-3-oleoyl-*sn*-glycerol]

FIGURE 12.24 Reactions employed in the manufacture of brominated vegetable oils.

with cocoa solids. In other cases, however, it is not practical or adequate to simply enhance the viscosity to stabilize the appearance of a fluid food or beverage. In such cases, alteration of the density of a dispersed phase may provide a convenient method to stabilize the appearance of the product.

Maintenance of the cloud provided by dispersed flavoring oils in soft drinks, particularly those composed of citrus oils (terpenes, Chapter 11), is achieved by increasing the density of the citrus oil phase (sp. gr. 0.85–0.90 g/cm^3) to near that of supporting sugar–water bulk phase (sp. gr. 1.04–1.05 g/cm^3). Historically, this has been achieved by dissolving a small amount of brominated vegetable oil (sp. gr. 1.23–1.33 g/cm^3) into the citrus oil flavoring employed. Brominated vegetable oils are prepared by reacting unsaturated vegetable oils with bromine (Figure 12.24). However, because of toxicological implications, alternative weighting agents are often substituted for brominated vegetable oils in citrus flavoring oil applications. Alternatives include the damar gums (sp. gr. about 1.05 g/cm^3) that are natural exudates obtained from shrubs of the Caesalpiniaceae and Dipterocarpaceae families and ester gums, such as glyceryl triabietate (sp. gr. about 1.05 g/cm^3; XIX), that are manufactured from wood rosin. Sucrose diacetate hexaisobutyrate (sp. gr. 1.10–1.14 g/cm^3; XX) that is a synthesized sucrose polyester is also widely used. For comparison, when incorporated into soybean oil and dispersed in a standardized oil-in-water emulsion, isodensity (with water) concentrations (weight %) of weighting agents in soybean oil have been found to be 25, 45, 55, and 55 for brominated vegetable oil, sucrose diacetate hexaisobutyrate, damar gum, and ester gum, respectively [7].

Glyceryl triabietate (XIX)

Sucrose diacetate hexaisobutyrate (XX)

In beer, wine, and many fruit juices, the formation of hazes or sediments and oxidative deterioration have been long-standing problems. Natural phenolic substances are involved in these phenomena. The chemistry of this important group, including anthocyanins, flavonoids, proanthocyanidins, and tannins, is discussed in Chapter 10. Proteins and pectic substances participate with polyphenols in the formation of haze-forming colloids. Specific enzymes have been utilized to partially hydrolyze high-molecular-weight proteins (Chapter 6) and thereby reduce the tendency toward haze formation. However, in some instances excess enzymic activity can adversely affect other desirable properties, such as foam formation in beer.

An important means of manipulating polyphenolic composition to control both its desirable and undesirable effects is to use various clarifying (*fining*) agents and adsorbants. Preformed haze can be at least partially removed by filter aids, such as diatomaceous earth. Many of the clarifying agents that have been used are nonselective, and they affect the polyphenolic content more or

less incidentally. Adsorption is usually maximal when solubility of the adsorbate is minimal, and suspended or nearly insoluble materials such as a tannin–protein complex tend to collect at any interface. As the activity of the adsorbent increases, the less soluble substances still tend to be adsorbed preferentially, but more soluble compounds are also adsorbed.

Bentonite, a montmorillonite clay, is representative of many similar and moderately effective minerals that have been employed as clarifying agents. Montmorillonite is a complex hydrated aluminum silicate with exchangeable cations, frequently sodium ions. In aqueous suspension, bentonite behaves as small platelets of insoluble silicate. The bentonite platelets have a negative charge and a very large surface area of about 750 m^2/g. Bentonite is a rather selective adsorbent for protein, and evidently this adsorption results from an attraction between the positive charges of the protein and the negative charges of the silicate. A particle of bentonite covered with adsorbed protein will adsorb some phenolic tannins on or along with the protein [51]. Bentonite is used as a clarifying or fining agent for wines to preclude protein precipitation. Doses of the order of a few pounds per thousand gallons usually reduce the protein content of wine from 50 to 100 mg/L to a stable level of less than 10 mg/L. Bentonite rapidly forms a heavy compact sediment and is often employed in conjunction with final filtration to remove precipitated colloids.

The important clarifying agents that have a selective affinity for tannins, proanthocyanidins, and other polyphenols include proteins and certain synthetic resins, such as the polyamides and polyvinylpyrrolidone (PVP). Gelatin and isinglass (obtained from the swim-bladder of fish) are the proteins most commonly used to clarify beverages. It appears that the most important type of linkage between tannins and proteins, although probably not the only type, involves hydrogen bonding between phenolic hydroxyl groups and amide bonds in proteins. The addition of a small amount of gelatin (40–170 g/380 L) to apple juice causes aggregation and precipitation of a gelatin–tannin complex that on settling enmeshes and removes other suspended solids. The exact amount of gelatin for each use must be determined at the time of processing. Juices containing low levels of polyphenolics are supplemented with added tannin or tannic acid (0.005%–0.01%) to facilitate flocculation of the gelatin.

At low concentrations, gelatin and other soluble clarifying agents can act as protective colloids; at higher concentrations, they can cause precipitation and at still higher concentrations they can again fail to cause precipitation. Hydrogen bonding between the colloidal clarifying agents and water accounts for their solubilities. Molecules of the clarifying agent and polyphenol can combine in different proportions to either neutralize or enhance the hydration and solubility of a given colloidal particle. The most nearly complete disruption of H bonding between water and either the protein or the polyphenol gives the most complete precipitation. This would be expected to occur when the amount of dissolved clarifying agent roughly equals the weight of the tannin being removed.

The synthetic resins (polyamides and PVP) have been used to prevent browning in white wines [6] and to remove haze for beers [12]. These polymers are available in both soluble and insoluble forms, but requirements for little or no residual polymer in beverages have stimulated the use of the high-molecular-weight cross-linked forms that are insoluble. The synthetic resins have been particularly useful in the brewing industry where reversible refrigeration-induced haze (chill haze) and permanent haze (that which is associated with the development of oxidized flavors) are serious problems. These hazes are caused by formation of complexes between native proteins and proanthocyanidins from malted barley. Excessive removal of proteins leads to defective foam character, but the selective removal of polyphenols extends the stability of beer. Initial applications involved polyamides (Nylon 66), but greater efficiency has been achieved with cross-linked PVP (XXI). Treatment with 1.4–2.3 kg of insoluble PVP per 100 barrels of beer provides control of chill haze and improves storage stability [12].

PVP is added after fermentation and before filtration, and it rapidly adsorbs polyphenols. Just as bentonite removes some tannins along with preferentially adsorbed protein, selective tannin adsorbents remove some proteins along with the phenolics.

Polyvinylpyrrolidone (XXI)

In addition to the adsorbents already discussed, activated charcoal and some other materials have been employed. Activated charcoal is quite reactive, but it adsorbs appreciable amounts of smaller molecules (flavors, pigments) along with the larger compounds that contribute to haze formation. Tannic acid (tannin) is used to precipitate proteins, but its addition can potentially lead to the undesirable effects described previously. Other proteins with low solubility (keratin, casein, and zein) and soluble proteins (sodium caseinate, egg albumen, and serum albumin) also have selective adsorptive capacities for polyphenols, but they have not been extensively employed.

12.15 FLOUR BLEACHING AGENTS AND BREAD IMPROVERS

Freshly milled wheat flour has a pale yellow tint and yields a sticky dough that does not handle or bake well. When the flour is stored, it slowly becomes white and undergoes an aging or maturing process that improves its baking qualities. It is a usual practice to employ chemical treatments to accelerate these natural processes [54] and to use other additives to enhance yeast leavening activity and to retard the onset of staling.

Flour bleaching involves primarily the oxidation of carotenoid pigments. This results in disruption of the conjugated double bond system of carotenoids to a less conjugated colorless system. The dough improving action of oxidizing agents is believed to involve the oxidation of sulfhydryl groups in gluten proteins. Oxidizing agents employed may participate in bleaching only, in both bleaching and dough improvement or in dough improvement only. One commonly used flour bleaching agent, benzoyl peroxide [$(C_6H_5CO)_2O_2$], exhibits a bleaching or decolorizing action, but does not influence baking properties. Materials that act both as bleaching and improving agents include chlorine gas (Cl_2), chlorine dioxide (ClO_2), nitrosyl chloride (NOCl), and oxides of nitrogen (nitrogen dioxide, NO_2, and nitrogen tetroxide (N_2O_4) [43]. These oxidizing agents are gaseous and exert their action immediately upon contact with flour. Oxidizing agents that

serve primarily as dough improvers exert their action during the dough stages rather than in the flour. Included in this group are potassium bromate ($KBrO_3$), potassium iodate (KIO_3), calcium iodate [$Ca(IO_3)_2$], and calcium peroxide (CaO_2).

Benzoyl peroxide is usually added to flour (0.025%–0.075%) at the mill. It is a powder and is usually added along with diluting or stabilizing agents such as calcium sulfate, magnesium carbonate, dicalcium phosphate, calcium carbonate, and sodium aluminum phosphate. Benzoyl peroxide is a free radical initiator (see Chapter 4), and it requires several hours after addition to decompose into available free radicals for initiation of carotenoid oxidation.

The gaseous agents for oxidizing flour show variable bleaching efficiencies, but effectively improve baking qualities of suitable flours. Treatment with chlorine dioxide improves flour color only slightly, but yields flour with improved dough handling properties. Chlorine gas, often containing a small amount of nitrosyl chloride, is used extensively as a bleach and improver for soft wheat cake flour. Hydrochloric acid is formed from oxidation reactions of chlorine, and the resulting slightly lowered pH values lead to improved cake baking properties. Nitrogen tetroxide (N_2O_4) and other oxides of nitrogen, produced by passing air through an intense electric arc, are only moderately effective bleaching agents, but they produce good baking qualities in treated flours.

Oxidizing agents that function primarily as dough improvers can be added to flour (10–40 ppm) at the mill. They are, however, often incorporated into a dough conditioner mix containing several inorganic salts and then added at the bakery. Potassium bromate, an oxidizing agent used traditionally as a dough improver, remains unreactive until yeast fermentation lowers the pH of the dough sufficiently to activate it. As a result, it acts rather late in the process and causes increased loaf volume, improved loaf symmetry, and improved crumb and texture characteristics.

Early investigators proposed that the improved baking qualities resulting from treatment with oxidizing agents were attributable to inhibition of the proteolytic enzymes present in flour. However, a more recent belief is that dough improvers, at an appropriate time, oxidize sulfhydryl groups (−SH) in the gluten to yield an increased number of intermolecular disulfide bonds (−S−S−). This cross-linking would allow gluten proteins to form thin, tenacious networks of protein films that comprise the vesicles for leavening. The result is a tougher, drier, more extensible dough that gives rise to improved characteristics in the finished products. Excessive oxidation of the flour must be avoided since this leads to inferior products with gray crumb color, irregular grain, and reduced loaf volume.

The addition of a small amount of soybean flour to wheat flour intended for yeast-leavened doughs has become a common practice. The addition of soybean lipoxygenase (see Chapters 4 and 6) is an excellent way to initiate the free radical oxidation of carotenoids [15]. Addition of soybean lipoxygenase also greatly improves the rheological properties of the dough by a mechanism not yet elucidated. While it has been suggested that lipid hydroperoxides become involved in the oxidation of gluten−SH groups, evidence indicates that other protein–lipid interactions are also involved in dough improvement by oxidants [15].

Inorganic salts incorporated into dough conditioners include ammonium chloride (NH_4Cl), ammonium sulfate [$(NH_4)_2SO_4$], calcium sulfate ($CaSO_4$), ammonium phosphate [$(NH_4)_3PO_4$], and calcium phosphate ($CaHPO_4$). They are added to dough to facilitate growth of yeast and to aid in control of pH. The principal contribution of ammonium salts is to provide a ready source of nitrogen for yeast growth. The phosphate salts apparently improve dough by buffering the pH at a slightly lower than normal value. This is especially important when water supplies are alkaline.

Other types of materials are also used as dough improvers in the baking industry. Calcium stearoyl-2-lactylate (Figure 12.25) and similar emulsifying agents are used at low levels (up to 0.5%)

FIGURE 12.25 Reactions employed in the manufacture of stearoyl-2-lactylate emulsifiers.

to improve mixing qualities of dough and to promote increased loaf volume [56]. Hydrocolloid gums have been used in the baking industry to improve the water holding capacity of doughs and to modify other properties of doughs and baked products [34]. Carrageenan, carboxymethylcellulose, locust bean gum, and methylcellulose are among the more useful hydrocolloids in baking applications. Methylcellulose and carboxymethylcellulose have been found to retard retrogradation and staling in bread, and they also retard migration of moisture to the product surface during subsequent storage. Carrageenan (0.1%) softens the crumb texture of sweet dough products. Several hydrocolloids (e.g., carboxymethylcellulose at 0.25%) may be incorporated into doughnut mixes to significantly decrease the amount of fat absorbed during frying. This benefit apparently arises because of improvements in the dough and because a more effective hydrated barrier is established on the surface of the doughnuts.

12.16 ANTICAKING AGENTS

Several conditioning agents are used to maintain free-flowing characteristics of granular and powdered forms of foods that are hygroscopic in nature. In general, these materials function by readily absorbing excess moisture, by coating particles to impart a degree of water repellency, and/or by providing an insoluble particulate diluent. Calcium silicate ($Ca\ SiO_3 \cdot XH_2O$) is used to prevent caking in baking powder (up to 5%), table salts (up to 2%), and other foods and food ingredients. Finely divided calcium silicate absorbs liquids in amounts up to 2.5 times its weight and still remains free flowing. In addition to absorbing water, calcium silicate also effectively absorbs oils and other nonpolar organic compounds. This characteristic makes it useful in complex powdered mixes and in certain spices that contain free essential oils.

Food grade calcium and magnesium salts of long-chain fatty acids, derived from tallow, are used as conditioning agents in dehydrated vegetable products, salt, onion, and garlic salt and in a variety of other food ingredients and mixes that exist in powder form. Calcium stearate is often added to powdered foods to prevent agglomeration, to promote free flow during processing, and to insure freedom from caking during the shelf life of the finished product. Calcium stearate is essentially insoluble in water but adheres well to particles and provides a partial water-repellent coating for the particles. Commercial stearate powders have a high bulk density (about 27 kg/m^3) and possess large surface areas that make their use as conditioners (0.5%–2.5%) reasonably economical. Calcium stearate is also used as a release lubricant (1%) in the manufacture of pressed tablet-form candy.

Other anticaking agents employed in the food industry include sodium silicoaluminate, tricalcium phosphate, magnesium silicate, and magnesium carbonate. These materials are essentially insoluble in water and exhibit variable abilities to absorb moisture. Their use levels are similar to those for other anticaking agents (e.g., about 1% sodium silicoaluminate is used in powdered sugar). Microcrystalline cellulose powders are used to prevent grated or shredded cheese from clumping. Anticaking agents are either metabolized (starch, stearates) or exhibit no toxic actions at levels employed in food applications [19].

12.17 GASES AND PROPELLANTS

Gases, both reactive and inert, play important roles in the food industry. For example, hydrogen is used to hydrogenate unsaturated fats (Chapter 4), chlorine is used to bleach flour (see bleaching agents and dough improvers in this chapter) and sanitize equipment, SO_2 is used to inhibit enzymic browning in dried fruits (see sulfites and SO_2 in this chapter), ethylene gas is used to promote ripening of fruits (Chapter 16), ethylene oxide is used as a sterilant for spices (see epoxides in this chapter), and air is used to oxidize ripe olives for color development. However, the functions and properties of essentially inert gases used in food will be the topics of primary concern in the following sections.

12.17.1 Protection from Oxygen

Some processes for oxygen removal involve the use of inert gases, such as nitrogen or carbon dioxide, to flush a headspace, to strip or sparge a liquid, or to blanket a product during or after processing. Carbon dioxide is not totally without chemical influence because it is soluble in water and can lead to a tangy, carbonated taste in some foods. The ability of carbon dioxide to provide a dense, heavier-than-air, gaseous blanket over a product makes it attractive in many processing applications. Nitrogen blanketing requires thorough flushing followed by a slight positive pressure to prevent rapid diffusion of air into the system. A product that is thoroughly evacuated, flushed with nitrogen, and hermetically sealed will exhibit increased stability against oxidative deterioration [30].

12.17.2 Carbonation

The addition of carbon dioxide (carbonation) to liquid products, such as carbonated soft drinks, beer, some wines, and certain fruit juices, causes them to become effervescent, tangy, slightly tart, and somewhat tactual. The quantity of carbon dioxide used and the method of introduction vary widely with the type of product [30]. For example, beer becomes partially carbonated during the fermentation process, but is further carbonated before bottling. Beer usually contains 3–4 volumes of carbon dioxide (1 volume of beer at 16°C and 1 atmosphere pressure contains

3–4 volumes of carbon dioxide gas at the same temperature and pressure). Carbonation is often carried out at lowered temperatures (4°C) and elevated pressures to increase carbon dioxide solubility. Other carbonated beverages contain from 1.0 to 318 volumes of carbon dioxide depending upon the effect desired. The retention of large amounts of carbon dioxide in solutions at atmospheric pressure has been ascribed to surface adsorption by colloids and to chemical binding. It is well established that carbamino compounds are formed in some products by rapid, reversible reactions between carbon dioxide and free amino groups of amino acids and proteins. In addition, formation of carbonic acid (H_2CO_3) and bicarbonate ions (HCO_3^-) also aids in stabilizing the carbon dioxide system. Spontaneous release of carbon dioxide from beer, that is, gushing, has been associated with trace metallic impurities and with the presence of oxalate crystals that provide nuclei for nucleation of gas bubbles.

12.17.3 Propellants

Some fluid food products are dispensed as liquids, foams, or sprays from pressurized aerosol containers. Since the propellant usually comes into intimate contact with the food, it becomes an incidental food component or ingredient. The principal propellants for pressure dispensing of foods are nitrous oxide, nitrogen, and carbon dioxide [30]. Foam- and spray-type products are usually dispensed by nitrous oxide and carbon dioxide because these propellants are quite soluble in water and their expansion during dispensing assists in the formation of the spray or foam. Carbon dioxide is also employed for products such as cheese spreads where tanginess and tartness are acceptable characteristics. Nitrogen, because of its low solubility in water and fats, is used to dispense liquid streams in which foaming should be avoided (catsup, edible oils, syrups). The use of all of these gases in foods is regulated, and the pressure must not exceed 100 psig at 21°C or 135 psig at 54°C. At these conditions, none of the gases liquefy and a large portion of the container is occupied by the propellant. Thus, as the product is dispensed, the pressure drops and this can lead to difficulties with product uniformity and completeness of dispensing. The gaseous propellants are nontoxic, nonflammable, and economical and usually do not cause objectionable color or flavors. However, carbon dioxide, when used alone, imparts an undesirable taste to some foods.

Liquid propellants also have been developed and approved for food use, but environmental concerns regarding ozone depletion in the upper atmosphere have led to the banning of the chlorofluorocarbon substances. Those approved for foods were octafluorocyclobutane or Freon C-318 ($CF_2{-}CF_2{-}CF_2{-}CF_2$) and chloropentafluoroethane or Freon 115 ($CClF_2{-}CF_3$). Although potentially hazardous, the flammable hydrocarbons propane, butane, and isobutene are used as propellant gases for vegetable-oil-based aerosol and water-based emulsion cooking sprays. When used, a portion of the propellants exists in the container as a liquid layer situated on top of the food product, and an appropriate headspace containing vaporized propellant is also present. Use of a liquefied propellant enables dispensing to occur at a constant pressure, but the contents must be shaken first to provide an emulsion that will efficiently foam or spray upon discharge from the container. Constant pressure dispensing is essential for good performance of spray-type aerosols. These propellants are nontoxic at the levels encountered, and they do not impart off-flavors to foods. They give particularly good foams because they are highly soluble in any fat that may be present, and they can be effectively emulsified.

12.18 SUMMARY

Summarized in Table 12.5 [9,16,17,21,28,36] are various kinds of food additives and their functions in food.

TABLE 12.5
Selected Food Additives[a]

Class and General Function	Chemical Name	Additional or More Specific Function	Source of Discussion (Chapters)
I. Processing additives			
Aerating and foaming agents	Carbon dioxide	Carbonation, foaming	12
	Nitrogen	Foaming	12
	Sodium bicarbonate	Foaming	12
Antifoam agents	Aluminum stearate	Yeast processing	—
	Ammonium stearate	Beet sugar processing	—
	Butyl stearate	Beet sugar, yeast	—
	Decanoic acid	Beet sugar, yeast	—
	Dimethylpolysiloxane	General use	—
	Dimethylpolysilicone	General use	—
	Lauric acid	Beet sugar, yeast	—
	Mineral oil	Beet sugar, yeast	—
	Oleic acid	General use	—
	Oxystearin	Beet sugar, yeast	—
	Palmitic acid	Beet sugar, yeast	—
	Petroleum waxes	Beet sugar, yeast	—
	Silicone dioxide	General use	—
	Stearic acid	Beet sugar, yeast	—
Catalysts (including enzymes)	Nickel	Lipid hydrogenation	4
	Amylase	Starch conversion	3, 6
	Glucose oxidase	Oxygen scavenger	6
	Lipase	Dairy flavor developer	6
	Papain	Chill-proofing beer	6
	Pepsin	Meat tenderizer	6
	Rennin	Cheese production	6
Clarifying and flocculating agents	Bentonite	Absorbs proteins	12
	Gelatin	Complexes polyphenols	12
	Polyvinylpyrrolidone	Complexes polyphenols	12
	Tannic acid	Complexes proteins	12
Color control agents	Ferrous gluconate	Dark olives	—
	Magnesium chloride	Canned peas	10
	Nitrate, nitrite (potassium, sodium)	Cured meat	10, 12
	Sodium erythorbate	Cured meat color	10
Freezing and cooling agents	Carbon dioxide	—	12
	Liquid nitrogen	—	—
Malting and fermenting aids	Ammonium chloride	Yeast nutrients	12
	Calcium carbonate	—	—
	Calcium phosphate	—	—
	Calcium phosphate (dibasic)	—	—
	Calcium sulfate	—	—
	Potassium chloride	—	—
	Potassium phosphate	—	—
Material handling aids	Aluminum phosphate	Anticaking, free flow	12
	Calcium silicate	Anticaking, free flow	12
	Calcium stearate	Anticaking, free flow	12

(*Continued*)

TABLE 12.5 (*Continued*)
Selected Food Additives[a]

Class and General Function	Chemical Name	Additional or More Specific Function	Source of Discussion (Chapters)
	Dicalcium phosphate	Anticaking, free flow	12
	Dimagnesium phosphate	Anticaking, free flow	12
	Kaolin	Anticaking, free flow	12
	Magnesium silicate	Anticaking, free flow	
	Magnesium stearate	Anticaking, free flow	
	Sodium carboxymethylcellulose	Bodying, bulking	12
	Sodium silicoaluminate	Anticaking, free flow	12
	Starches	Anticaking, free flow	
	Tricalcium phosphate	Anticaking, free flow	12
	Tricalcium silicate	Anticaking, free flow	13
	Xanthan (other gums)	Bodying, bulking	3, 12
Oxidizing agents	Acetone peroxides	Free radical initiator	12
	Benzoyl peroxide	Free radical initiator	12
	Calcium peroxide	Free radical initiator	12
	Hydrogen peroxide	Free radical initiator	
	Sulfur dioxide	Dried fruit bleach	12
pH control and modification agents			
Acidulants (acids)	Acetic acid	Antimicrobial agent	12
	Citric acid	Chelating agent	4, 12
	Fumaric acid	Antimicrobial agent	12
	δ-Gluconolactone	Leavening agent	12
	Hydrochloric acid	—	12
	Lactic acid	—	12
	Malic acid	Chelating agent	12
	Phosphoric acid	—	12
	Potassium acid tartrate	Leavening agent	12
	Succinic acid	Chelating agent	12
	Tartaric acid	Chelating agent	12
Alkalines (bases)	Ammonium bicarbonate	Carbon dioxide source	12
	Ammonium hydroxide	—	—
	Calcium carbonate	—	—
	Magnesium carbonate	—	—
	Potassium carbonate	Carbon dioxide source	—
	Potassium hydroxide	—	—
	Sodium bicarbonate	Carbon dioxide source	12
	Sodium carbonate	—	—
	Sodium citrate	Emulsifier salt	12
	Trisodium citrate	Emulsifier salt	12
Buffering salts	Ammonium phosphate (mono-, dibasic)	—	12
	Calcium citrate	—	—
	Calcium gluconate	—	—
	Calcium phosphate (mono-, dibasic)	—	—
	Potassium acid tartrate	—	—
	Potassium citrate	—	—
	Potassium phosphate (mono-, dibasic)	—	—

(*Continued*)

TABLE 12.5 (*Continued*)
Selected Food Additives[a]

Class and General Function	Chemical Name	Additional or More Specific Function	Source of Discussion (Chapters)
	Sodium acetate	—	—
	Sodium acid pyrophosphate	—	—
	Sodium citrate	—	—
	Sodium phosphate (mono-, di-, tribasic)	—	—
	Sodium potassium tartrate	—	—
Release and antistick agents	Acylated monoacylglycerols	—	4, 12
	Beeswax	—	4, 12
	Calcium stearate	—	4, 12
	Magnesium silicate	—	4, 12
	Mineral oil	—	—
	Mono- and diacylglycerols	Emulsifiers	4
	Starches	—	3
	Stearic acid	—	4, 12
	Talc	—	—
Sanitizing and fumigating agents	Chlorine	Oxidant	—
	Methyl bromide	Insect fumigant	—
	Sodium hypochlorite	Oxidant	—
Separation and filtration aids	Diatomaceous earth	—	—
	Ion-exchange resins	—	—
	Magnesium silicate	—	—
Solvents, carriers, and encapsulating agents	Acetone	Solvent	—
	Agar-agar	Encapsulation	3
	Arabinogalactan	Encapsulation	3
	Cellulose	Carrier	3
	Glycerine	Solvent	4, 12
	Guar gum	Encapsulation	3
	Methylene chloride	Solvent	—
	Propylene glycol	Solvent	12
	Triethyl citrate	Solvent	—
Washing and surface removal agents	Sodium dodecylbenzenesulfonate	Detergent	—
	Sodium hydroxide	Lye peeling	—
II. Final product additives			
Antimicrobial agents	Acetic acid (and salts)	Bacteria, yeast	12
	Benzoic acid (and salts)	Bacteria, yeast	12
	Ethylene oxide	General sterilant	12
	p-Hydroxybenzoate alkyl esters	Molds, yeast	12
	Nitrates, nitrites (K, Na)	*C. botulinum*	10, 12
	Propionic acid (and salts)	Mold	12
	Propylene oxide	General sterilant	12
	Sorbic acid (and salts)	Mold, yeast, bacteria	12
	Sulfur dioxide and sulfites	General	12
Antioxidants	Ascorbic acid (and salts)	Reducing agent	4, 8
	Ascorbyl palmitate	Reducing agent	12
	BHA	Free radical terminator	4, 12
	BHT	Free radical terminator	4, 12

(*Continued*)

TABLE 12.5 (*Continued*)
Selected Food Additives[a]

Class and General Function	Chemical Name	Additional or More Specific Function	Source of Discussion (Chapters)
	Gum guaiac	Free radical terminator	4, 12
	Propyl gallate	Free radical terminator	4, 12
	Sulfite and metabisulfite salts	Reducing agents	4, 12
	Thiodipropionic acid (and esters)	Peracid decomposer	12
Appearance control agents			
Colors and color modifiers	Annatto	Cheese, butter, baked goods	10
	Beet powder	Frosting, soft drinks	10
	Caramel	Confectionary	10
	Carotene	Margarine	10
	Cochineal extract	Beverages	10
	FD&C No. 3	Mint jelly, beverages	10
	FD&C No. 3 (erythrosine)	Canned fruit cocktail	10
	Titanium dioxide	White candy, Italian cheeses	10
	Turmeric	Pickles, sauces	10
Other appearance agents	Beeswax	Gloss, polish	4
	Glycerine	Gloss, polish	4
	Oleic acid	Gloss, polish	4
	Sucrose	Crystalline glaze	3
	Wax, carnauba	Gloss, polish	—
Flavors and flavor modifiers			
Flavoring agents[b]	Essential oils	General	11
	Herbs and spices	General	11
	Plant extractives	General	11
	Synthetic flavor compounds	General	—
Flavor potentiators	Disodium guanylate	Meats and vegetables	11
	Disodium inosinate	Meats and vegetables	11
	Maltol	Bakery goods, sweets	11
	Monosodium glutamate	Meats and vegetables	11
	Sodium chloride	General	—
Moisture control agents	Glycerine	Plasticizer, humectant	3, 11
	Gum acacia	—	—
	Invert sugar	—	3
	Propylene glycol	—	11
	Mannitol	—	3, 11
	Sorbitol	—	3, 11
Nutrient, dietary supplements			
Amino acids	Alanine	—	5
	Arginine	Essential	5
	Aspartic acid	—	5
	Cysteine	—	5
	Cystine	—	5
	Glutamic acid	—	5
	Histidine	—	5
	Isoleucine	Essential	5
	Leucine	Essential	5

(*Continued*)

TABLE 12.5 (*Continued*)
Selected Food Additives[a]

Class and General Function	Chemical Name	Additional or More Specific Function	Source of Discussion (Chapters)
	Lysine	Essential	5
	Methionine	Essential	5
	Phenylalanine	Essential	5
	Proline	—	5
	Threonine	Essential	5
	Valine	Essential	5
Minerals	Boric acid	Boron source	9
	Calcium carbonate	Breakfast cereals	9
	Calcium citrate	Cornmeal	9
	Calcium phosphates	Enriched flour	9
	Calcium pyrophosphate	Enriched flour	9
	Calcium sulfate	Bread	9
	Cobalt carbonate	Cobalt source	9
	Cobalt chloride	Cobalt source	9
	Cupric chloride	Copper source	9
	Cupric gluconate	Copper source	9
	Cupric oxide	Copper source	9
	Calcium fluoride	Water fluoridations	—
	Ferric phosphate	Iron source	9
	Ferric pyrophosphate	Iron source	9
	Ferrous gluconate	Iron source	9
	Ferrous sulfate	Iron source	9
	Iodine	Iodine source	9
	Iodide, cuprous	Table salt	9
	Iodate, potassium	Iodine source	9
	Magnesium chloride	Magnesium source	9
	Magnesium oxide	Magnesium source	9
	Magnesium phosphates	Magnesium source	9
	Magnesium sulfate	Magnesium source	9
	Manganese citrate	Manganese source	9
	Manganese oxide	Manganese source	9
	Molybdate, ammonium	Molybdenum source	9
	Nickel sulfate	Nickel source	9
	Phosphates, calcium	Phosphorous source	9
	Phosphates, sodium	Phosphorous source	9
	Potassium chloride	NaCl substitute	—
	Zinc chloride	Zinc source	9
	Zinc stearate	Zinc source	9
Vitamins	*p*-Aminobenzoic acid	B complex factor	8
	Biotin	—	8
	Carotene	Provitamin A	8
	Folic acid	—	8
	Niacin	—	8
	Niacinamine	Enriched flour	8
	Pantothenate, calcium	B complex vitamin	8

(*Continued*)

TABLE 12.5 (*Continued*)
Selected Food Additives[a]

Class and General Function	Chemical Name	Additional or More Specific Function	Source of Discussion (Chapters)
	Pyridoxine hydrochloride	B complex vitamin	8
	Riboflavin	B complex vitamin	8
	Thiamine hydrochloride	Vitamin B_1	8
	Tocopherol acetate	Vitamin E	8
	Vitamin A acetate	—	—
	Vitamin B_{12}	—	8
	Vitamin D	—	8
Miscellaneous nutrients	Betaine hydrochloride	Dietary supplement	8
	Choline chloride	Dietary supplement	8
	Inositol	Dietary supplement	8
	Linoleic acid	Essential fatty acid	4
	Rutin	Dietary supplement	8
Sequestrants (chelating agents)	Calcium citrate	—	12
	Calcium disodium EDTA	—	12
	Calcium gluconate	—	—
	Calcium phosphate (monobasic)	—	—
	Citric acid	—	12
	Disodium EDTA	—	12
	Phosphoric acid	—	12
	Potassium citrate	—	—
	Potassium phosphate (mono, dibasic)	—	—
	Sodium acid pyrophosphate	—	9, 12
	Sodium citrate	—	12
	Sodium gluconate	—	—
	Sodium hexametaphosphate	—	—
	Sodium phosphate (mono, di, tri)	—	—
	Sodium potassium tartrate	—	—
	Sodium tartrate	—	—
	Sodium tripolyphosphate	—	9, 12
	Tartaric acid	—	—
Specific gravity control agent	Brominated vegetable oil	Increase density of oil droplets	12
Surface tension control agents	Dioctyl sodium sulfosuccinate	—	—
	Ox bile extract	—	—
	Sodium phosphate (dibasic)	—	—
Sweeteners			
Nonnutritive	Acesulfame K	—	11, 12
	Ammonium saccharin	—	11, 12
	Calcium saccharin	—	11, 12
	Saccharin	—	11, 12
	Sodium saccharin	—	11, 12
Nutritive	Aspartame	—	11, 12
	Glucose	—	3
	Sorbitol	—	3, 12

(*Continued*)

TABLE 12.5 (*Continued*)
Selected Food Additives[a]

Class and General Function	Chemical Name	Additional or More Specific Function	Source of Discussion (Chapters)
Texture and consistency control agents			
Emulsifiers and emulsifier salts	Calcium stearoyl-2-lactylate	Dried egg white, bakery	4, 12
	Cholic acid	Dried egg white	4
	Desoxycholic	Dried egg white	4
	Dioctyl sodium sulfosuccinate	General	—
	Fatty acids (C10–C18)	General	4
	Lactylic esters of fatty acids	Shortening	4, 12
	Lecithin	General	4
	Mono- and diacylglycerides	General	4
	Ox bile extract	General	—
	Polyglycerol esters	General	12
	Polyoxyethylene sorbitan esters	General	4, 12
	Propylene glycol, mono-, diesters	General	4
	Potassium phosphate, tribasic	Processed cheese	12
	Potassium polymetabisulfite		—
	Potassium pyrophosphate	Processed cheese	12
	Sodium aluminum sulfate	General	12
	Sodium citrate	Processed cheese	12
	Sodium metaphosphate	General	12
	Sodium phosphate, dibasic	Processed cheese	12
	Sodium phosphate, monobasic	Processed cheese	12
	Sodium phosphate, tribasic	Processed cheese	12
	Sodium pyrophosphate	General	12
	Sorbitan monooleate	Dietary products	4
	Sorbitan monopalmitate	Flavor dispersion	4
	Sorbitan monostearate	General	4
	Sorbitan tristearate	Confection coatings	4
	Stearoyl-2-lactylate	Bakery shortening	4
	Stearoyl monoglyceridyl citrate	Shortenings	4
	Taurocholic acid	Egg whites	4
Firming agents	Aluminum sulfates (alum)	Pickles	12
	Calcium carbonate	General	12
	Calcium chloride	Canned tomatoes	12
	Calcium citrate	Canned tomatoes	12
	Calcium gluconate	Apple slices	12
	Calcium hydroxide	Fruit slices	12
	Calcium lactate	Apple slices	12
	Calcium phosphate, monobasic	Canned tomatoes	12
	Calcium sulfate	Canned potatoes, tomatoes	12
	Magnesium chloride	Canned peas	
Leavening agents	Ammonium bicarbonate	Carbon dioxide source	12
	Ammonium phosphate (dibasic)	—	12
	Calcium phosphate	—	12
	5-Gluconolactone	—	12

(*Continued*)

TABLE 12.5 (*Continued*)
Selected Food Additives[a]

Class and General Function	Chemical Name	Additional or More Specific Function	Source of Discussion (Chapters)
	Sodium acid pyrophosphate	—	12
	Sodium aluminum phosphate	—	12
	Sodium aluminum sulfate	—	12
	Sodium bicarbonate	Carbon dioxide source	12
Masticatory substances	Paraffin (synthetic)	Chewing gum base	12
	Pentaerythritol ester of rosin	Chewing gum base	12
Propellants	Carbon dioxide	—	12
	Nitrous oxide	—	12
Stabilizers and thickeners	Acacia gum	Foam stabilizer	3, 12
	Agar	Ice cream	3, 12
	Alginic acid	Ice cream	3, 12
	Carrageenan	Chocolate drinks	3, 12
	Guar gum	Cheese foods	3, 12
	Hydroxypropyl methylcellulose	General	3, 12
	Locust bean gum	Salad dressing	3, 12
	Methylcellulose	General	3, 12
	Pectin	Jellies	3, 12
	Sodium carboxymethylcellulose	Ice cream	3, 12
	Tragacanth gum	Salad dressing	3, 12
Texturizers	Carrageenan	General	3, 12
	Mannitol	—	3, 12
	Pectin	—	3, 12
	Sodium caseinate	—	5
	Sodium citrate	—	12
Tracers	Titanium dioxide	Vegetable protein extenders	13

[a] For additional information, see [9,16,17,21,28,36]; also see Further Reading.
[b] Individual members comprising flavoring agents are too numerous to mention. See [21,28] for comprehensive listings; also see Further Reading.

REFERENCES

1. Akoh, C. C. and B. G. Swanson (eds.) (1994). *Carbohydrate Polyesters as Fat Substitutes*, Marcel Dekker, New York, pp. 1–9.
2. Artz, W. E. and S. L. Hansen (1994). Other fat substitutes, in *Carbohydrate Polyesters as Fat Substitutes* (C. C. Akoh and B. G. Swanson, eds.), Marcel Dekker, New York, pp. 197–236.
3. Bakal, G. and A. Diaz (2005). Antimicrobial resistance: The lowdown on lauric arginate. *Food Qual.* February–March:54–61.
4. Brusick, D., M. Cifone, R. Young, and S. Benson (1989). Assessment of the genotoxicity of calcium cyclamate and cyclohexylamine. *Environ. Mol. Mutagen.* 14:188–199.
5. Bryan, G. T. and E. Erturk (1970). Production of mouse urinary bladder carcinomas by sodium cyclamate. *Science* 167:996–998.
6. Caputi, A. and R. G. Peterson (1965). The browning problem in wines. *Am. J. Enol. Vitic.* 16(1):9–13.
7. Chanamai, R. and D. J. McClements (2000). Impact of weighting agents and sucrose on gravitational separation of beverage emulsions. *J. Agric. Food Chem.* 48:5561–5565.
8. Chichester, D. F. and F. W. Tanner (1972). Antimicrobial food additives, in *Handbook of Food Additives* (T. E. Furia, ed.), CRC Press, Cleveland, OH, pp. 115–184.

9. Committee on Food Protection, Food and Nutrition Board (1965). Chemicals used in food processing, Publ. 1274. National Academy of Sciences Press, Washington, DC, 294pp.
10. Considine, D. M. (ed.) (1982). Masticatory substances, in *Foods and Food Production Encyclopedia*, Van Nostrand Reinhold Co., New York, pp. 1154–1155.
11. Crowell, E. A. and J. F. Guyman (1975). Wine constituents arising from sorbic acid addition, and identification of 2-ethoxyhexa-3,5-diene as source of geranium-like off-odor. *Am. J. Enol. Vitic.* 26(2):97–102.
12. Dahlstrom, R. W. and M. R. Sfat (1972). The use of polyvinylpyrrolidone in brewing. *Brewer's Dig.* 47(5):75–80.
13. Ellinger, R. H. (1972). Phosphates in food processing, in *Handbook of Food Additives* (T. E. Furia, ed.), CRC Press, Cleveland, OH, pp. 617–780.
14. Etchells, J. L., T. A. Bell, and L. J. Turney (1972). Influence of alum on the firmness of fresh-pack dill pickles. *J. Food Sci.* 37:442–445.
15. Faubian, J. M. and R. C. Hoseny (1981). Lipoxygenase: Its biochemistry and role in breadmaking. *Cereal Chem.* 58:175–180.
16. Food and Nutrition Board (1981). *Food Chemicals Codex*, 3rd edn., National Research Council, National Academy Press, Washington, DC, 735pp.
17. Food Chemical News (1983). USDA petitioned to remove titanium dioxide marker requirements for soy protein. March 21,1982, Informa UK ltd., London, UK, pp. 37–38.
18. Freese, E. and B. E. Levin (1978). Action mechanisms of preservatives and antiseptics, in *Developments in Industrial Microbiology* (L. A. Underkofler, ed.), Society of Industrial Microbiology, Washington, DC, pp. 207–227.
19. Furia, T. E. (1972). Regulatory status of direct food additives, in *Handbook of Food Additives* (T. E. Furia, ed.), CRC Press, Cleveland, OH, pp. 903–966.
20. Furia, T. E. (1972). Sequestrants in food, in *Handbook of Food Additives* (T. E. Furia, ed.), CRC Press, Cleveland, OH, pp. 271–294.
21. Furia, T. E. and N. Bellanca (eds.) (1976). *Fenaroli's Handbook of Flavor Ingredients*, Chemical Rubber Co., Cleveland, OH, 2 vols.
22. Gardner, W. H. (1972). Acidulants in food processing, in *Handbook of Food Additives* (T. E. Furia, ed.), CRC Press, Cleveland, OH, pp. 225–270.
23. Goddard, S. J. and B. L. Wedzicha (1992). Kinetics of the reaction of sorbic acid with sulphite species. *Food Addit. Contam.* 9:485–492.
24. Griffin, W. C. and M. J. Lynch (1972). Polyhydric alcohols, in *Handbook of Food Additives* (T. E. Furia, ed.), 2nd edn., CRC Press, Cleveland, OH, pp. 431–455.
25. Griswold, R. M. (1962). Leavening agents, in *The Experimental Study of Foods* (R. M. Griswold, ed.), Houghton Mifflin Co., Boston, MA, pp. 330–352.
26. Haddon, W. F., M. L. Mancini, M. McLaren, A. Effio, L. A. Harden, R. L. Degre, and J. L. Bradford (1994). Occurrence of ethyl carbamate (Urethane) in U.S. and Canadian breads: Measurements by gas chromatography-mass spectrometry. *Cereal Chem.* 71:207–215.
27. Ham, R. (1971). Interactions between phosphates and meat proteins, in *Symposium: Phosphates in Food Processing* (J. M. DeMan and P. Melnychyn, eds.), AVI Publishing Co., Westport, CT, pp. 65–84.
28. Heath, H. B. (1981). *Source Book of Flavors*, AVI Publishing Co., Westport, CT, 861pp.
29. Ishii, H. and T. Koshimizu (1981). Toxicity of aspartame and its diketopiperazine for Wistar rats by dietary administration for 104 weeks. *Toxicology* 21:91–94.
30. Joslyn, M. A. (1964). Gassing and deaeration in food processing, in *Food Processing Operations: Their Management, Machines, Materials, and Methods* (M. A. Joslyn and J. L. Heid, eds.), Vol. 3, AVI Publishing Co., Westport, CT, pp. 335–368.
31. Kabara, J. J., R. Varable, and M. S. Jie (1977). Antimicrobial lipids: Natural and synthetic fatty acids and monoglycerides. *Lipids* 12:753–759.
32. Karahadian, C. and R. C. Lindsay (1988). Evaluation of the mechanism of dilauryl thiodipropionate antioxidant activity. *J. Am. Oil Chem. Soc.* 65:1159–1165.
33. Khandelwal, G. D., Y. L. Rimmer, and B. L. Wedzicha (1992). Reaction of sorbic acid in wheat flour doughs. *Food Addit. Contam.* 9:493–497.
34. Klose, R. E. and M. Glicksman (1972). Gums, in *Handbook of Food Additives* (T. E. Furia, ed.), CRC Press, Cleveland, OH, pp. 295–359.
35. Labuza, T. P., N. D. Heidelbaugh, M. Silver, and M. Karel (1971). Oxidation at intermediate moisture contents. *J. Am. Oil Chem. Soc.* 48:86–90.
36. Lewis, R. J., Sr. (1989). *Food Additives Handbook*, Van Nostrand Reinhold, New York.

37. Lucca, P. A. and Tepper, B. J. (1994). Fat replacers and the functionality of fat in foods. *Trends Food Sci. Technol.* 5:12–19.
38. Mahon, J. H., K. Schlamb, and E. Brotsky (1971). General concepts applicable to the use of polyphosphates in red meat, poultry, seafood processing, in *Symposium: Phosphates in Food Processing* (J. M. DeMan and P. Malnychyn, eds.), AVI Publishing Co., Westport, CT, pp. 158–181.
39. Miller, M. S. (1994). Proteins as fat substitutes, in *Protein Functionality in Food Systems* (N. S. Hettiarachchy and G. R. Ziegler, eds.), Marcel Dekker, New York, pp. 435–465.
40. Miller, W. T. (1987). The legacy of cyclamate. *Food Technol.* 41(1):116.
41. Minifie, B. W. (1970). *Chocolate, Cocoa and Confectionery: Science and Technology*, AVI Publishing Co., Westport, CT, pp. 38–41.
42. Peng, S. K., C. Taylor, J. C. Hill, and R. J. Marin (1985). Cholesterol oxidation derivatives and arterial endothelial damage. *Arteriosclerosis* 54:121–136.
43. Pomeranz, Y. and J. A. Shallenberger (1971). *Bread Science and Technology*, AVI Publishing Co., Westport, CT.
44. Pratter, P. J. (1980). Neohesperidin dihydrochalcone: An updated review on a naturally derived sweetener and flavor potentiator. *Perfumer Flavorist* 5(6):12–18.
45. Price, J. M., C. G. Biana, B. L. Oser, E. E. Vogin, J. Steinfeld, and H. L. Ley (1970). Bladder tumors in rats fed cyclohexylamine or high doses of a mixture of cyclamate and saccharin. *Science* 167:1131–1132.
46. Ramarathnam, N. and L. J. Rubin (1994). The flavour of cured meat, in *Flavor of Meat and Meat Products* (F. Shahidi, ed.), Blackie Academic & Professional, London, U.K., pp. 174–198.
47. Salant, A. (1972). Nonnutritive sweeteners, in *Handbook of Food Additives* (T. E. Furia, ed.), 2nd edn., CRC Press, Cleveland, OH, pp. 523–586.
48. Scharpf, L. G. (1971). The use of phosphates in cheese processing, in *Symposium: Phosphates in Food Processing* (J. M. deMan and P. Melnychyn, eds.), AVI Publishing Co., Westport, CT, pp. 120–157.
49. Scott, U. N. and S. L. Taylor (1981). Effect of nisin on the outgrowth of *Clostridium botulinum* spores. *J. Food Sci.* 46:117–126.
50. Sebranek, J. G. and R. G. Cassens (1973). Nitrosamines: A review. *J. Milk Food Technol.* 36:76–88.
51. Singleton, V. L. (1967). Adsorption of natural phenols from beer and wine. *MBAA Tech. Q.* 4(4):245–253.
52. Smith, R. E., J. W. Finley, and G. A. Leveille (1994). Overview of Salatrim, a family of low-calorie fats. *J. Agric. Food Chem.* 42:432–434.
53. Stahl, J. E. and R. H. Ellinger (1971). The use of phosphates in the baking industry, in *Symposium: Phosphates in Food Processing* (J. M. Deman and P. Melnychyn, eds.), AVI Publishing Co., Westport, CT, pp. 194–212.
54. Stauffer, C. E. (1983). Dough conditioners. *Cereal Foods World* 28:729–730.
55. Stukey, B. N. (1972). Antioxidants as food stabilizers, in *Handbook of Food Additives* (T. E. Furia, ed.), CRC Press, Cleveland, OH, pp.185–224.
56. Thompson, J. E. and B. D. Buddemeyer (1954). Improvement in flour mixing characteristics by a stearoyl lactylic acid salt. *Cereal Chem.* 31:296–302.
57. van Buren, J. P., J. C. Moyer, D. E. Wilson, W. B. Robinson, and D. B. Hand (1960). Influence of blanching conditions on sloughing, splitting, and firmness of canned snap beans. *Food Technol.* 14:233–236.
58. van der Wel, H. (1974). Miracle fruit, katemfe, and serendipity berry, in *Symposium: Sweeteners* (G. Inglett, ed.), AVI Publishing Co., Westport, CT, pp. 194–215.
59. Weast, R. C. (ed.) (1988). *Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL, pp. D161–D163.
60. Wedzicha, R. L. (1984). *Chemistry of Sulphur Dioxide in Foods*, Elsevier Applied Science Publishers, London, U.K.
61. Wedzicha, R. L. (1992). Chemistry of sulphiting agents in foods. *Food Additives Contam.* 9:449–459.
62. Wesley, F., F. Rourke, and O. Darbishire (1965). The formation of persistent toxic chlorohydrins in foodstuffs by fumigation with ethylene oxide and with propylene oxide. *J. Food Sci.* 30:1037–1042.

FURTHER READING

Ash, M. and I. Ash (2002). *Handbook of Food Additives*, 2nd edn., Synapse Information Resources, Endicott, New York.

Burdock, G. A. (1997). *Encyclopedia of Food and Color Additives*, CRC Press, Boca Raton, FL.

Burdock, G. A. (ed.) (2004). *Fenaroli's Handbook of Flavor Ingredients*, 5th edn., CRC Press, Boca Raton, FL.

Davidson, P. M., J. N. Sofos, and A. L. Branen (eds.) (2005). *Antimicrobials in Food*, 3rd edn., CRC Press, Boca Raton, FL.

Food and Nutrition Board (1981). *Food Chemicals Codex*, 3rd edn., National Research Council, National Academy Press, Washington, DC.

Gould, G. W. (1989). *Mechanisms of Action of Food Preservation Procedures*, Elsevier Applied Science, London, U.K.

Heath, H. B. (1981). *Source Book of Flavors*, AVI Publishing Co., Westport, CT.

Lewis, R. J., Sr. (1989). *Food Additives Handbook*, Van Nostrand Reinhold, New York.

Molins, R. A. (1991). *Phosphates in Food*, CRC Press, Boca Raton, FL.

Nabors, L. O. and R. C. Geraldi (eds.) (1991). *Alternative Sweeteners*, 2nd edn., Marcel Dekker, New York.

Phillips, G. O., D. J. Wedlock, and P. A. Williams (eds.) (1990). *Gums and Stabilizers for the Food Industry*, IRL Press, Oxford University Press, Oxford, U.K.

Roller, S. (2003). *Natural Antimicrobials for the Minimal Processing of Foods*, CRC Press, Boca Raton, FL.

Taylor, R. J. (1980). *Food Additives*, John Wiley & Sons, New York.

Wood, R., L. Foster, A. Damont, and P. Key (2004). *Analytical Methods for Food Additives,* CRC Press, Boca Raton, FL.

Yannai, S. (2004). *Dictionary of Food Compounds*, CRC Press, Boca Raton, FL.

13 Bioactive Food Components
Nutraceuticals and Toxicants

Hang Xiao and Chi-Tang Ho

CONTENTS

13.1 Examination of Health Effects of Food Components 865
13.2 Health-Promoting Nutraceuticals 866
  13.2.1 Carotenoids 866
  13.2.2 Flavonoids 869
  13.2.3 Proanthocyanidins 874
  13.2.4 Other Polyphenolic Compounds 875
  13.2.5 Organosulfur Compounds 878
  13.2.6 Isothiocyanates and Indoles 879
13.3 Generalized Health-Promoting Mechanisms of Nutraceuticals 882
13.4 Interactions among Nutraceuticals 884
13.5 Bioavailability of Nutraceuticals 885
13.6 Botanical Dietary Supplements 886
13.7 Extraction Technologies for Botanicals 888
13.8 Process-Induced Nutraceuticals in Foods 890
13.9 Food Components with Adverse Effects 891
13.10 Process-Induced Toxicants in Foods 893
References 898

Bioactive food components encompass a wide range of dietary substances that exhibit various biological effects on humans, and they are classified as nutraceuticals and toxicants. Nutraceuticals are naturally derived food components that have beneficial effects on humans such as health-promoting and disease-preventing activities that are beyond basic nutritional needs. In contrast, toxicants are naturally occurring or process-induced dietary substances that have adverse effects on human health. Fruits, vegetables, common beverages, grains, nuts, oils, marine products, medicinal plants, and herbal products all contain both nutraceuticals and toxicants. Depending on the food source, the types and abundance of the bioactive components vary. Generally, most fruits and vegetables contain more nutraceuticals than toxicants and therefore have the potential to provide health benefits. Nutraceuticals and toxicants are often consumed together as a part of one's diet where complex interactions may occur among them inside the human body. These interactions can have an impact on overall health outcomes (beneficial vs. adverse and decreased vs. increased risk of disease). Due to the enormous and growing public interest, bioactive food components is currently one of the most intensively studied areas in the field of food and nutrition. This chapter provides a brief overview on the major nutraceuticals and toxicants found in foods.

13.1 EXAMINATION OF HEALTH EFFECTS OF FOOD COMPONENTS

The initial evidence for the potential health benefits of consuming more fruits and vegetables was provided by observational epidemiological studies in humans. These studies compare the health outcomes among human populations at different levels of intake of foods or food components.

However, the observational epidemiological studies have been attributed to a number of limitations since they primarily examine the health outcomes in relation to a single nutraceutical, food, or category of foods. These epidemiological methods are not fully representative of the actual dietary intake since people do not eat individual nutraceuticals, foods, or well-defined mixtures of them. Instead, a variety of foods with many nutraceuticals are consumed. Complementary methods have been developed to analyze dietary patterns and to overcome these limitations [1]. While more refinements and solutions for these limitations are being sought, we should acknowledge that valuable information has been obtained using observational epidemiological methods; a successful example is that of vitamins in human nutrition.

An examination of observational epidemiological studies has shown that a higher intake of fruits and vegetables is associated with a lower risk of all-cause mortality, in particular cardiovascular disease (CVD) mortality [2]. Studies also suggest that certain dietary components present in fruits and vegetables can interfere with the development of multiple types of cancers, and the consumption of nutraceuticals such as resveratrol from grapes, sulforaphane from broccoli, and isoflavones from soy products may reduce both morbidity and mortality from cancer [3]. Some of the foods and botanicals that have demonstrated anticancer activities include garlic, ginger, licorice, onions, flax, turmeric, cruciferous vegetables, tomatoes, peppers, brown rice, wheat, and umbelliferous vegetables such as carrots, celery, and parsley [3].

A great deal of scientific evidence has been obtained from laboratory research to demonstrate the health effects of food components. Laboratory research makes use of well-characterized model systems or test organisms to determine the biological responses to prospective bioactive agents. *In vitro* assays are conducted outside the organism in an artificial environment that simulates conditions within the organism. These include studies using chemical cocktails, isolated enzymes, cell cultures, tissues, and organs. *In vivo* models are usually based on observing a relevant physiological response of a living organism to a treatment such as a nutraceutical or a toxicant. Specific models are developed for different disease states, and the required biological materials for these models can be obtained either from specialized vendors or from the laboratories where the models have been originally developed. Many techniques are used to generate these models; for example, models for cancers at different organ sites can be obtained by treating laboratory animals such as mice and rats with organ-specific carcinogens. Genetic engineering is a powerful tool to create many valuable mouse models called "knockout mice."* Although laboratory models all have limitations, well-designed laboratory studies do provide important information that can be translated to human physiology. As for the laboratory research on bioactive food components, one key factor is to make sure the model systems used are of physiological relevance to the oral consumption of the food. For example, to determine the efficacy of soy isoflavones in fighting breast cancer in a rodent model, the soy isoflavones should be orally administered to the rodents rather than being injected intraperitoneally or into the bloodstream.

13.2 HEALTH-PROMOTING NUTRACEUTICALS

Many nutraceuticals have been isolated from different foods, and their health-promoting effects have been studied. The most studied nutraceuticals can be categorized into several main classes, each of which is characterized in the following pages.

13.2.1 Carotenoids

Carotenoids are fat-soluble pigments that provide coloration to plants and animals. One defining characteristic of carotenoids is their backbone chemical structure, a 40-carbon polyene chain, derived from isoprene (Figure 13.1). The polyene backbone consists of conjugated double bonds,

* *Knockout mice*: Genetically engineered mice where one or more genes are rendered inoperable.

(a)

HO
(b)

HO
(c)

(d)

FIGURE 13.1 Structures of (a) β-carotene, (b) lutein, (c) zeaxanthin, and (d) lycopene.

which allows the carotenoids to absorb excess energy from other molecules [4]. This characteristic may be responsible for the antioxidant activity of carotenoids such as the scavenging effects on reactive oxygen species (ROS) and free radicals.

The characteristic conjugated double bond structure of carotenoids is also responsible for their chemical instability. These compounds are sensitive to light, air, heat, and acid exposure. This sensitivity leads to their vulnerabilities during food processing and storage, which may cause their degradation and production of rancidity. Care should be taken to minimize carotenoid losses in food products. For example, incorporation of carotenoids in emulsion systems could increase their stability by preventing them from contacting common prooxidants in food systems. Furthermore, multilayer emulsion systems could provide additional protection for carotenoids [5].

β-Carotene (Figure 13.1) is the most common carotenoid primarily found in red palm oil, palm fruits, leafy green vegetables, carrots, sweet potatoes, mature squashes, pumpkins, mangoes, and papayas. β-Carotene gives a strong red-orange color and is known for its various health effects, such as provitamin A activity and preventive effects against multiple diseases including CVDs, age-related macular degeneration, and cataract formation. β-Carotene is a precursor of vitamin A, and one molecule of β-carotene is enzymatically converted to two molecules of vitamin A in the small intestine. β-Carotene provides about 30% of dietary vitamin A in developed countries. It is absorbed and transported mainly through the lymphatic system to various locations in the body. The amount of β-carotene absorbed and converted to vitamin A is regulated according to the body's need for vitamin A. Therefore, high oral intake of β-carotene is less likely to cause vitamin A toxicity, but carotenosis, a condition where the skin turns orange, may result from excessive intake.

The cancer-preventive effects of β-carotene are controversial. Some studies showed no protective effects of β-carotene against cancers of the pancreas, breast, and skin, while other studies support the hypothesis that β-carotene can prevent cancer in humans [6]. It is noteworthy that a systematic review and meta-analysis of the results from several randomized controlled human

trials revealed that dietary supplementation of β-carotene may increase the risk of lung and stomach cancer in smokers and asbestos workers [7]. Mechanistic studies indicated that β-carotene itself may be anticarcinogenic, but its oxidized products may promote cancer development. This information provides a potential explanation to the increased cancer risks caused by β-carotene among certain populations. In other words, the degradation of β-carotene in a free radical–rich environment, for example, the lungs of cigarette smokers, may produce oxidized products that are actually carcinogenic [6].

The antioxidant activity of β-carotene may contribute to certain health effects. For example, high serum β-carotene content was associated with the lower incidence of metabolic syndrome caused by oxidative stress [8]. β-Carotene also exhibits free radical–trapping ability at partial pressures of oxygen substantially less than that in the atmosphere [9]. Such low oxygen partial pressures exist in most tissues under physiological conditions. In contrast, at higher oxygen pressure, β-carotene may lose the antioxidant activity and show a prooxidant effect [9]. These characteristics of β-carotene emphasize the importance of understanding the chemistry of specific nutraceuticals under physiological conditions, for example, in the lungs of smokers. If nutraceutical research is conducted without consideration of the physiological relevance, a conundrum of results such as those from the β-carotene cancer trials will be generated, adding more confusion to the field of bioactive food components.

Lutein and its isomer, zeaxanthin, are yellow pigments that belong to the classes of nonprovitamin A carotenoids (Figure 13.1). They are distinguished from other carotenoids by constitutive hydroxyl groups on the ring structures at the end of the conjugated double bond chains. Therefore, they are also called oxycarotenoids or xanthophylls. Lutein is found predominantly in dark green, leafy vegetables such as spinach and kale, while zeaxanthin gives corn its yellow color. Other food sources include squash, peas, cabbage, peppers, oranges, kiwis, and grapes. Lutein and zeaxanthin have been used as safe dyes in chromovitrectomy [10]. Although there is not sufficient evidence to support a direct causal relationship between lutein or zeaxanthin and protection from any chronic disease, there is a growing body of evidence (from *in vivo*, *in vitro*, and epidemiological studies) supporting the claim that lutein and zeaxanthin may lower the risk of cataract and age-related macular degeneration [11]. The evidence for the beneficial roles of lutein and zeaxanthin in eye health is compelling because of the exclusive presence of lutein and zeaxanthin in the ocular tissues of humans and the large numbers of epidemiological studies that have been conducted on this subject. With their high accumulation in the macula of the eye, the area of highest visual acuity, lutein and zeaxanthin were proposed to have the ability to absorb short-wavelength, high-energy light, filter out harmful blue light, while at the same time act as antioxidants to quench potentially damaging ROS [12]. Absorbed lutein and zeaxanthin mainly exist in the cell membrane due to their suitable molecular structures to align parallel to acyl chains of phospholipid molecules in the cell membrane. This localization facilitates the protection of cell membrane and phospholipids bilayer against prooxidants. Accumulating evidence supports the notion that lutein and zeaxanthin can protect the skin from light-induced damage, especially that caused by the ultraviolet wavelengths.

Lycopene (Figure 13.1) is a red-colored carotenoid found in tomatoes, watermelons, papayas, apricots, and grapefruits. About 80% of dietary lycopene is from tomatoes and tomato-related products, with watermelon being the major secondary dietary source. Most naturally occurring lycopene exists in the all-*trans* form. The bioavailability of lycopene in the raw tomatoes can be improved by thermal processing. This is because thermal processing, as well as light and certain chemical interactions, can induce the transformation of lycopene from all-*trans* form to *cis* isomers that are thermodynamically more stable. The *cis* isomers account for more than 50% of lycopene present in human tissues and plasma. After ingestion, all-*trans* lycopene is transformed to the *cis* form, which offers improved antioxidant activity. *Cis* lycopene isomers have higher bioavailability, since they can be incorporated more easily into bile acid micelles and chylomicrons. Linear all-*trans* lycopene forms crystals in some foods, which hinders its intestinal absorption [13,14].

Lycopene has strong antioxidant activities due to multiple conjugated double bonds in its chemical structure. Many studies have demonstrated the antioxidant effects of lycopene against ROS

that may cause damage to DNA, cell membranes, and proteins [6]. Lycopene is the most abundant carotenoid in human blood where it is generally bound to low-density lipoprotein (LDL) and transported to various tissue sites, such as the liver, testes, and adrenal and prostate glands. Although these beneficial effects of lycopene are thought to be partially due to its antioxidant properties, evidence is accumulating to suggest the roles of other mechanisms of action such as modulation of endocrine and immune systems. The higher consumption of tomato-based products has been associated with lower risks of a variety of cancers. These activities may be ascribed to the ability of lycopene to interfere with cancer-related signaling pathways, modulate cancer cell communication, or inhibit the formation of blood vessels (angiogenesis) that support cancer growth. The inhibitory effects of lycopene on prostate cancer have been the focus of many studies including several human trials. However, a meta-analysis on the available randomized controlled human trials failed to find evidence for the protective effects of lycopene against prostate cancer [15]. Another health benefit of lycopene is its activity against CVDs. The results from clinical research support consuming tomato-based foods as the source of lycopene as an approach to promote cardiovascular health [16].

13.2.2 Flavonoids

Flavonoids are ubiquitous in plants, and almost all plant tissues are able to produce flavonoids. Flavonoids are important in plant physiology. They are involved in plant growth and reproduction and can provide resistance to pathogens and predators [17]. A wide variety (more than 4000) of naturally occurring flavonoids have been identified. They are found in edible fruits, leafy vegetables, roots, tubers, bulbs, herbs, spices, legumes, tea, coffee, and red wine. They can be classified into seven groups: flavones, flavanones, flavonols, flavanonols, isoflavones, flavanols (catechins), and anthocyanidins. The structures of these flavonoids are given in Figure 13.2. Examples of common

Flavone

Flavanone

Flavonol

Flavanonol

Isoflavone

Flavanol

Anthocyanidin

FIGURE 13.2 Structures of flavonoids (ring classification identified in the flavone).

TABLE 13.1
Different Classes of Flavonoids, Their Substitution Patterns, and Dietary Sources

Class	Name	Substitution	Dietary Source
Flavone	Apigenin	5,7 –OH	Parsley, celery
	Rutin	5,7,3′,4′ –OH, 3-*O*-rutinose	Buckwheat, citrus
Flavanone	Naringin	5,4′ –OH	Citrus
	Naringenin	5,7,4′ –OH	Orange peel
Flavonol	Kaempferol	3,5,7,4′ –OH	Broccoli, tea
	Quercetin	3,5,7,3′,4′ –OH	Onion, broccoli, apples, berries
Flavanonol	Taxifolin	3,5,7,3′,4′ –OH	Fruits
Isoflavone	Genistein	5,7,4′ –OH	Soybean
	Daidzein	4′ –OH, 7-*O*-glucose	Soybean
	Puerarin	7,4′ –OH, 8-*C*-glucose	Kudzu
Flavanol (catechin)	(−)-Epicatechin	3,5,7, 3′,4′ –OH	Tea
	(−)-Epigallocatechin	3,5,7, 3′,4′,5′ –OH	Tea
	(−)-Epigallocatechin gallate	5,7, 3′,4′,5′ –OH, 3-gallate	Tea
Anthocyanidin	Cyanidin	3,5,7,3′,4′ –OH	Cherry, strawberry
	Delphinidin	3,5,7, 3′,4′,5′ –OH	Dark fruits

flavonoids in foods are listed in Table 13.1. Flavonoids occur as aglycones and glycosides in plants. In general, the leaves, flowers, and fruits of the plant itself contain flavonoid glycosides; woody tissues contain aglycones; and seeds may contain both.

As a result of their ubiquity in plants, flavonoids are an integral part of the human diet. It was estimated that the mean total flavonoid intake by U.S. adults was 345 mg/day, mainly from flavan-3-ols (55.7%), followed by proanthocyanidins (28.5%), flavanones (6.5%), flavonols (5.2%), anthocyanidins (2.7%), isoflavones (0.7%), and flavones (0.3%) [18]. The major daily intake of flavonoids was from the following foods: tea, citrus fruit juices, and citrus fruits.

Flavonoids possess several common chemical and biological properties: (1) antioxidant activity, (2) the ability to scavenge active oxygen species, (3) the ability to scavenge electrophiles, (4) the ability to inhibit nitrosation, (5) the ability to chelate metals (such as Fe and Cu), (6) the potential to produce hydrogen peroxide in the presence of certain metals, and (7) the capability to modulate certain cellular enzyme activities [2]. It appears that diets rich in flavonoids may provide protection against CVDs, neurodegenerative disorders, and some forms of cancers.

Among the most studied health-promoting flavonoids in recent years are green tea catechins. Tea is the second most consumed beverage in the world, well ahead of coffee, beer, wine, and carbonated soft drinks [19]; it has been used for medicinal purposes in China and Japan for thousands of years. More than 300 different kinds of tea are produced from the leaves of *Camellia sinensis* by different manufacturing processes. Generally, they are divided into three types: "nonfermented" green tea (produced by drying and steaming fresh tea leaves to inactivate polyphenol oxidase and thus, minimizing oxidation), "semifermented" oolong tea (produced when the fresh tea leaves are subjected to a partial fermentation stage before drying), and "fermented" black and red (Pu-Erh) teas (produced by a postharvest fermentation before drying and steaming; the fermentation of black tea is mainly due to oxidation reaction catalyzed by polyphenol oxidase, whereas Pu-Erh tea is fermented by microorganisms) [19]. Green tea and oolong tea are more popular in China, Japan, Korea, and some African countries, while black tea is preferred in India and Western countries. Laboratory and epidemiological studies have linked the consumption of tea to reduced risk of several chronic diseases, especially CVD and cancer. In addition, several studies suggest beneficial effects of green tea intake on bone density, cognitive function, dental caries, and kidney stones.

Common Name	Abbreviation	R_1	R_2
Epicatechin	EC	H	H
Epicatechin gallate	ECG	Gallate	H
Epigallocatechin	EGC	H	Gallate
Epigallocatechin gallate	EGCG	Gallate	Gallate

FIGURE 13.3 Structures of major green tea catechins.

These effects have been attributed to the polyphenolic compounds in tea [20]. Catechins are the most abundant polyphenols in green tea. A typical green tea contains, by dry weight, 30%–40% catechins including epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC) (Figure 13.3). EGCG is the most abundant catechin in green, oolong, and black teas. Green and oolong teas typically contain 30–130 mg of EGCG per cup (237 mL), whereas black teas may contain up to 70 mg of EGCG per cup [21]. The main pigments in black tea are theaflavins and thearubigins that are formed by the oxidation and polymerization of catechins during fermentation. The structures of four theaflavins are shown in Figure 13.4. The structures of thearubigins are not well characterized, but several studies have proposed structures and formation mechanism of some thearubigins [22]. Theaflavins have important contributions to the properties of black tea, such as color, taste, and mouth feel.

Green tea and its constituents have been extensively studied both *in vitro* and in animal models of carcinogenesis. Whereas these compounds have been shown to be efficacious in a number of animal models of carcinogenesis, the epidemiological evidence regarding the effects of tea consumption on

Common Name	Abbreviation	R_1	R_2
Theaflavin	TF	H	H
Theaflavin-3-gallate	TF3G	Gallate	H
Theaflavin-3′-gallate	TF3′G	H	Gallate
Theaflavin-3,3′-digallate	TFDG	Gallate	Gallate

FIGURE 13.4 Structures of theaflavins in black tea.

cancer risk in humans is conflicting [21]. Some studies have shown reduced cancer incidence and recurrence associated with green tea consumption, whereas others have failed to show an effect. Most of the epidemiological studies showing an inverse relationship between tea consumption and development of cancer were conducted on gastrointestinal (GI) cancer in Japan and China, where green tea is the main form of tea consumed. In Japan, women consuming more than 10 cups of tea daily have shown remarkable reduction of relative risk for lung, colon, liver cancer, and breast cancer metastasis* and recurrence [23]. A case–control study conducted in China showed that risk of prostate cancer declined with increasing frequency, duration, and quantity of green tea consumed [24]. Tea drinking was also shown to be associated with a lower risk of cancers in the digestive tract and urinary tract of women in a prospective cohort study in Iowa [25]. Numerous potential mechanisms have been proposed for the cancer-preventive activity of tea and tea constituents based on studies on cancer cell lines. *In vitro*, tea polyphenols, especially EGCG, have been shown to cause growth inhibition and induce apoptosis in a number of human tumor cell lines including melanoma, breast cancer, lung cancer, leukemia, and colon cancer [20,26]. The importance of any of these mechanisms remains to be determined in humans. In general, biologically important activities that can be modulated by low concentration of an agent are likely to be more relevant *in vivo*. One problem faced by most studies is the relatively high concentrations of tea compounds used in the *in vitro* studies. These concentrations often far exceed those found in plasma or tissue of animals and humans following tea consumption.

The potential health effects of green tea catechins depend not only on the total amount consumed but also on their bioavailability and biotransformation that appears to be variable under different conditions. Several studies of the systemic bioavailability of orally administered green tea and catechins in human volunteers have been conducted. It has been shown that oral administration of 20 mg green tea solids/kg body weight results in maximum plasma levels for EGC, EC, and EGCG of 729, 428, and 170 nM, respectively. Plasma EC and EGC are present mainly as the glucuronide or sulfate conjugates, whereas 77% of the EGCG was in the nonconjugated form [27]. Another study compared the bioavailability of equimolar doses of pure EGC, ECG, and EGCG in 10 healthy volunteers. Average peak plasma concentrations after a single dose of 1.5 mmol were 5.0 μM for EGC, 3.1 μM for ECG, and 1.3 μM for EGCG. After 24 h, plasma EGC and EGCG returned to the baseline level, but plasma ECG remained elevated [28]. This type of study provides an idea of the achievable levels of tea catechins in humans after oral consumption. This information can be used to guide studies using *in vitro* models to ensure the physiological relevance.

Another commonly occurring flavonoid is quercetin (Figure 13.5). Quercetin is found typically in grapes, wine, tea, onions, apples, and leafy green vegetables. Quercetin is associated with various health benefits, including protection against osteoporosis, certain types of cancers, and pulmonary diseases and CVDs. The antioxidative ability of quercetin to scavenge highly reactive species, such as peroxynitrite and hydroxyl radicals, was suggested to be involved in its possible beneficial health

HO O OH OH OH OH O (a)

OH O OH HO O (b)

FIGURE 13.5 Structures of (a) quercetin and (b) genistein.

* *Metastasis*: The spread of a cancer from one organ to another not directly connected with it.

effects [29]. However, human studies are needed to confirm the role of antioxidant activities of quercetin in its potential health benefits. In a cross-sectional human study, based on dietary record and biochemical analysis of blood samples, the dietary intake of quercetin was associated with lowered levels of total plasma cholesterol and plasma LDL cholesterol, which may lead to protection against CVDs [30]. Quercetin was also reported to inhibit chemical-induced carcinogenesis in the lung, colon, mammary gland, and liver of laboratory animals [31].

Health benefits of soybean and its products have been recognized in recent years. Genistein (Figure 13.5), an isoflavone, is the major nutraceutical found in soybean and its products such as soy flour, soy flakes, soybean oils, isolated soy protein, tofu, and soy drinks. One gram of powdered soybean chips contains over 500 μg of genistein, whereas 1 g of soy protein contains approximately 250 μg of genistein [32]. As a phytoestrogen, genistein lacks estrogenic activity in humans but exhibits antiestrogenic activity.* The structure of genistein is similar to that of human estrogens, which accounts for the antagonistic relationship between genistein and human estrogens. Competitive binding of genistein to estrogen receptors is believed to alter the production of specific proteins that translocate into the cell nucleus and bind to specific DNA regulatory sites. This binding can affect production of other important cellular proteins by increasing or decreasing their gene expression [33]. The reported health benefits of genistein include prevention of cancer, CVDs, obesity and osteoporosis, and attenuation of postmenopausal problems [34]. Evidence from animal studies demonstrated the anticancer effects of genistein in multiple organ sites, including chemically induced carcinogenesis in the mammary gland, prostate, and stomach, UV-induced skin carcinogenesis, mutation-induced endometrial carcinoma, and transplanted tumors of prostate, leukemia, and bladder cancer cells. Evidence from human studies also suggested the potential protective effects of genistein against cancers. The plasma isoflavone (including genistein) concentration due to dietary exposure was inversely associated with the risk of breast cancer among a cohort of women in China. Consumption of a soy-rich diet resulted in relatively high levels of soy isoflavones in the serum, urine, and prostatic fluid of Asian men, which was associated with lowered incidence of prostate cancer. Furthermore, American men with high consumption of soy milk were also found to have reduced risk of prostate cancer [34]. Randomized controlled clinical trials are needed to confirm the causal relationship between genistein (soy isoflavones) and lowered risk of cancer.

Polymethoxyflavones (PMFs) are a unique class of flavonoids found mainly in citrus fruits, especially in the peels of sweet oranges (*Citrus sinensis*) and mandarin oranges (*Citrus reticulate*) that have been used as herbal medicine for centuries in Asian countries. PMFs are flavones bearing two or more methoxy groups on their basic benzo-γ-pyrone skeleton with a carbonyl group at the C_4 position. Three examples of PMFs are shown in Figure 13.6. PMFs are of increasing research interests because accumulating evidence has demonstrated that PMFs have a broad spectrum of biological activities including anticarcinogenic, anti-inflammatory, antiatherogenic, antiviral, and antioxidative activities [35]. More than 20 PMFs have been isolated and identified, among which tangeretin, nobiletin, and 5-demethylnobiletin (Figure 13.6) are the most studied in terms of their potential health-promoting effects. Anti-inflammatory activity is one of the most significant biological effects of PMFs. Multiple mechanisms have been reported to be responsible for the anti-inflammatory effects of PMFs, such as inhibition of pro-inflammatory enzymes, for example, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), and downregulation of pro-inflammatory cytokines, for example, interleukin (IL)-1α, IL-1β, tumor necrosis factor-alpha (TNF-α), and IL-6. Multiple *in vitro* and *in vivo* studies have demonstrated protective effects of PMFs against different types of cancer by various mechanisms including blocking the metastasis cascade, inhibition of cancer cell mobility, apoptosis, selective cytotoxicity, and antiproliferation. 5-Hydroxylated PMFs are a subclass of PMFs, and they could be formed by autohydrolysis of their permethoxylated counterparts, for example, 5-demethylnobiletin (Figure 13.6) can be formed from

* *Estrogenic activity*: The biological activity elicited by an estrogen or analogs.

FIGURE 13.6 Structures of (a) tangeretin, (b) nobiletin, and (c) 5-demethylnobiletin.

nobiletin. It has been demonstrated that 5-hydroxylated PMFs have more potent inhibitory effects on different types of cancer cells in comparison with permethoxylated counterparts, such as nobiletin and tangeretin [36,37].

13.2.3 Proanthocyanidins

Proanthocyanidins are oligomers or polymers of flavan-3-ols and are found in fruits, berries, beans, nuts, cocoa, and wine. The abundance of proanthocyanidins in plants makes them an important part of the human diet [38]. In proanthocyanidin structures, flavan-3-ol units are linked mainly through C4 → C8 bonds. This linkage is called a B-type linkage. An additional ether bond between C2 → C7 resulting in double linkage of the flavan-3-ol units is called an A-type [38]. B-type proanthocyanidins are more abundant than A-type proanthocyanidins in natural plant sources. Figure 13.7 shows chemical structures of the most common dimers B1, B2, and A2 and the trimers C1 and C2. In different plants, the content of proanthocyanidins varies due to its high structural diversity and its wide range of degree of polymerization.

Proanthocyanidins are considered to have a wide range of biological activities, including antioxidant, anticarcinogenic, cardioprotective, antimicrobial, and neuroprotective activities, demonstrated by a number of *in vitro* and *in vivo* studies. However, the data on the health effects, bioavailability, and biotransformation of proanthocyanidins in humans are limited. In general, the bioavailability of proanthocyanidins is poor due to its high degree of polymerization and large molecular weight [39]. The available studies indicate that dietary proanthocyanidins are poorly absorbed, and the majority of polymeric anthocyanidins pass unaltered through the small intestine to the large intestine where they are degraded into small phenolic acids by the colonic microflora [38]. Accumulating evidence has suggested that it is the metabolites derived from the microbial catabolism of proanthocyanidins that potentially produce beneficial health effects in the human body [39].

B1: R_1 = OH; R_2 = H; R_3 = H; R_4 = OH
B2: R_1 = OH; R_2 = H; R_3 = OH; R_4 = H

Dimer A_2

C1: R_1 = OH; R_2 = H
C2: R_1 = H; R_2 = OH

FIGURE 13.7 Structures of common proanthocyanidin dimers and trimers.

13.2.4 Other Polyphenolic Compounds

Besides flavonoids, there exist many other polyphenolic compounds in food, particularly fruits, vegetables, and spices. Table 13.2 shows some of these compounds and their potential health benefits.

The dried rhizome of the plant *Curcuma longa* Linn has been used for centuries to treat inflammation and other diseases. The major pigment in the powdered rhizome, commonly known as turmeric spice, is identified as curcumin (Figure 13.8). Turmeric spice, consumed daily in India, has been used as a preservative and a coloring agent in food. Studies have shown that curcumin and/or turmeric have potent anti-inflammatory activity, which makes curcumin as a potential protective agent against various diseases that are promoted by inflammation. Both *in vitro* and *in vivo* laboratory studies have demonstrated that curcumin was effective in inhibiting cancer cell growth

TABLE 13.2
Other Polyphenols, Their Dietary Source, and Potential Health Benefits

Polyphenol	Dietary Source	Potential Health Benefits
Curcumin	Turmeric root	Anticarcinogenic, anti-inflammatory, cardioprotective, suppress diabetes
Gingerols, shogaols	Ginger root	Anticarcinogenic, antiemetic, nausea
Carnosic acid, carnosol	Rosemary, sage	Antioxidative, anticarcinogenic
Resveratrol	Grape, red wine	Anticarcinogenic, antioxidative, cardioprotective
Pterostilbene	Blueberry	Anticarcinogenic, antioxidative, cardioprotective

Curcumin

[6]-Gingerol

Resveratrol

Pterostilbene

FIGURE 13.8 Structures of curcumin, 6-gingerol, 6-shogaol resveratrol, and pterostilbene.

by inducing apoptosis. Several clinical trials have indicated that curcumin has preventive effects against certain types of cancers [40]. However, curcumin has low bioavailability because of its poor intestinal absorption, rapid first-pass metabolism, and rapid systemic elimination in the human body, which limits its potential as an effective nutraceutical for the prevention of chronic diseases.

Ginger has been used as a folk medicine for thousands of years. Gingerols and shogaols are believed to be the major bioactive components of ginger, and they are also the main pungent components of ginger. Gingerols are a series of homologues with varied alkyl chains, whereas shogaols are a series of dehydration (at C5 and C4) derivatives of gingerols (Figures 13.8 and 13.9). Gingerols are sensitive to heat. During drying or thermal processing, gingerols either undergo dehydration to form the corresponding shogaols or are degraded by a retroaldol reaction to zingerone and the corresponding aldehydes (Figure 13.9) [41]. Gingerols, especially 6-gingerol, are more abundant in fresh ginger, whereas the amount of shogaols increases in dried ginger. For example, the ratio of 6-gingerol to 6-shogaol is about 10:1 and 1:1 in fresh ginger and dried ginger, respectively. 6-Gingerol

FIGURE 13.9 Degradation reaction of gingerols.

and 6-shogaol (Figure 13.8) are the most studied among all bioactive components in ginger. They have been shown to have anti-inflammatory and anticancer effects in both *in vitro* and *in vivo* models. Some studies have demonstrated the stronger anticancer and antioxidant activities of 6-shogaol in comparison to 6-gingerol. After oral ingestion, both 6-gingerol and 6-shogaol are extensively metabolized to their glucuronide and sulfate conjugates and there is no detectable 6-gingerol in the blood while a small amount of 6-shogaol can be found in the blood [42].

Resveratrol (3,5,4′-trihydroxystilbene; Figure 13.8) is a compound found mainly in grapes, peanuts, mulberries, and red wines. It is synthesized in the plant tissue from *p*-coumaroyl CoA and malonyl CoA in response to stress, injury, infection, or UV irradiation. Resveratrol is classified as a phytoalexin, which confers disease resistance to plants. Both *in vitro* and *in vivo* studies have shown that resveratrol has many biological effects such as protection against atherosclerosis, antioxidant activity, inhibition of platelet aggregation, and antimutagenic and anticarcinogenic properties [43]. However, several clinical studies indicated that the low oral bioavailability and rapid first-pass metabolism of resveratrol might limit its beneficial health effects [44].

Pterostilbene (*trans*-3,5-dimethoxy-4-hydroxystilbene; Figure 13.8), a dimethylether analog of resveratrol, is found primarily in blueberries and *Pterocarpus marsupium* heartwood. Similar to resveratrol, pterostilbene is also a phytoalexin. Accumulating studies demonstrated that pterostilbene possesses various health benefits including protection against cancer, inflammation, and vascular disease. In animal studies, pterostilbene exhibits greater bioavailability and stronger bioactivity when compared to resveratrol. It is suggested that two methoxy groups in pterostilbene enhanced its lipophilicity and hence the oral bioavailability [44].

Rosemary and sage leaves are commonly used as spices and flavoring agents. The dried leaf of the rosemary plant is one of the most widely used spices in food processing because not only does it have a desirable flavor but also possesses potent antioxidant activity. Several phenolic diterpenes with antioxidant activity have been isolated from rosemary and sage leaves, and the

FIGURE 13.10 Oxidative transformation of carnosic acid.

most notable one is carnosic acid. Results from *in vitro* experiments indicated that carnosic acid could protect biological membranes and prevent lipid peroxidation through scavenging hydroxyl radicals and lipid peroxy radicals [45]. Besides antioxidant activity, both rosemary extract and carnosic acid have been shown to inhibit chemically induced tumor initiation in animal models. Carnosic acid is not stable during food processing and storage. It first oxidizes to form carnosol and then undergoes further oxidative transformation to form rosmanol. The mechanism of this transformation is shown in Figure 13.10 [46]. Both carnosol and rosmanol have antioxidant activity comparable to that of carnosic acid [46].

13.2.5 Organosulfur Compounds

A major source of organosulfur compounds are vegetables of the *Allium* family including onions, garlic, scallions, chives, and leeks. Examples of organosulfur compounds found in some commonly consumed *Allium* vegetables are shown in Figure 13.11. As shown in epidemiological studies, higher dietary intakes of *Allium* vegetables were associated with reduced risk of different types of cancers [47]. The protective effects of *Allium* vegetables against cancer have been attributed to the high levels of organosulfur compounds. Laboratory studies demonstrated the cancer-preventive effects of organosulfur compounds in the stomach, esophagus, mammary glands, breast, skin, and lungs of experimental animals. These effects were associated with a combination of different mechanisms

Alliin Allicin

(*E*)-Ajoene (dominant) (*Z*)-Ajoene

1,3-Vinyldithiin (dominant) 1,2-Vinyldithiin

Allyl methyl trisulfide

FIGURE 13.11 Structures of alliin, allicin, ajoenes, vinyldithiin, and allyl methyl trisulfide.

such as induction of carcinogen detoxification, inhibition of cancer cell proliferation, induction of cancer cell death, antimicrobial activity, and free radical scavenging activity, and inhibition of the formation of DNA adducts. Despite the potential anticancer effects of organosulfur compounds suggested by epidemiological and animal studies, the results from randomized controlled clinical trials were not conclusive. For example, two small clinical trials suggested anticancer effects of garlic against colon tumor, while another large trial showed no such effect [48].

Allicin (diallyl thiosulfinate) is the major organosulfur compound identified in fresh minced garlic (*Allium sativum* L.), and it contributes to the major health benefits of garlic. However, allicin does not occur in intact garlic tissue. Upon cutting or crushing, alliin (*S*-allylcysteine *S*-oxide, Figure 13.11), a major organosulfur compound in intact garlic cloves, is converted to allicin by the action of alliinase (illustrated in Chapters 6 and 11). The chemical transformation of allicin upon heating yields other organosulfur compounds such as ajoenes, vinyldithiin, and allyl methyl trisulfide (Figure 13.11), which may be responsible for increases in fibrinolytic activity and inhibition of platelet aggregation.

Garlic has been the subject of a number of clinical trials on its potential beneficial effects on common cold, hypercholesterolemia, hypertension, peripheral arterial disease, and preeclampsia. However, the overall results showed that there was no convincing evidence for the beneficial effects of garlic on the aforementioned conditions [48]. Several animal studies indicated anti-inflammatory and inhibition of coronary calcification effects of garlic-derived polysulfides. While garlic breath is the most well documented adverse effect associated with garlic use, other potential adverse effects include allergic reactions, alteration of platelet function and coagulation, and alteration of efficacy of prescription drugs [49].

13.2.6 Isothiocyanates and Indoles

The other major classes of bioactive organosulfur compounds are the isothiocyanates and indoles, generated from enzymatic hydrolysis of glucosinolates. These compounds are particularly abundant in cruciferous vegetables, such as broccoli, Brussels sprouts, cauliflower, collards, kale, mustard greens, and kohlrabi. The abundance of glucosinolates is largely responsible for the bitter, and sometimes tart, taste of Cruciferae. When cruciferous vegetable cells are damaged, for example, by microbial attack, insect predation, food processing, or mastication, cellular compartments are

FIGURE 13.12 Production of sulforaphane from myrosinase-mediated hydrolysis of glucoraphanin.

broken and an enzyme known as myrosinase (β-thioglucosidase glucohydrolase) gains access to glucosinolates, which leads to hydrolysis of glucosinolates and subsequent formation of isothiocyanates and indoles. For example, the most well-known bioactive compound in the isothiocyanate family, sulforaphane, is not present in broccoli prior to disruption of the plant tissues. The principal glucosinolate responsible for the production of sulforaphane in broccoli is glucoraphanin (Figure 13.12) [50]. Microbial myrosinase produced by the gut microbiome can also convert glucosinolates to isothiocyanates and indoles during digestion.

Isothiocyanates are thermally labile during food processing, and the major decomposition products are derivatives of thiourea. Figure 13.13 shows the decomposition pathway of sulforaphane. Sulforaphane is first hydrolyzed into an amine that subsequently reacts with sulforaphane to form *N*,*N′*-di(methylsulfinyl)butyl thiourea [51]. The consequence of this decomposition on bioactivity is not well understood. On the other hand, heat may inactivate myrosinase, which reduces the production of isothiocyanates from their precursor glucosinolates, thus reducing their abundance. Glucosinolates are water-soluble compounds that may be leached into the cooking water used to blanch the vegetables and become wasted. Therefore, cooking cruciferous vegetables may reduce their beneficial effects on health [50].

Many animal studies have demonstrated the anticancer effects of isothiocyanates including phenethyl isothiocyanate, sulforaphane, allyl isothiocyanate, and benzyl isothiocyanate (Figure 13.14) [52]. This effect has been shown in cancers of multiple sites, for example, liver, lung, colon, breast, prostate, and pancreas. Moreover, several epidemiological studies of humans support the anticancer effects of isothiocyanates observed in the animal models by showing an inverse association between intake of cruciferous vegetables and cancer risk [50]. Randomized controlled clinical trials are needed to confirm the efficacy of isothiocyanates as anticancer agents in humans. Isothiocyanates exert their anticancer effects by diversified mechanisms among which induction of phase II detoxification enzymes (e.g., glutathione *S*-transferases [GSTs], UDP-glucuronosyltransferases [UGTs]) has been widely demonstrated in many studies. Increased activities of these enzymes can potentially promote the elimination of carcinogens and genotoxic chemicals from the human body.

Indole-3-carbinol is produced from maceration-induced myrosinase hydrolysis of glucobrassicin in cruciferous vegetables (Figure 13.15). It is believed that indole-3-carbinol is partially responsible for the potential health benefits of cruciferous vegetables such as the anticancer effects. After oral consumption, indole-3-carbinol undergoes condensation under acidic conditions found in the stomach to form several oligomeric products, particularly 3,3′-diindolylmethane (Figure 13.15).

FIGURE 13.13 Pathway for the formation of *N*,*N*′-di(methylsulfinyl)butyl thiourea from sulforaphane.

(a)

(b)

(c)

(d)

FIGURE 13.14 Structures of (a) phenethyl isothiocyanate, (b) benzyl isothiocyanate, (c) sulforaphane, and (d) allyl isothiocyanate.

In fact, indole-3-carbinol was not detectable in the blood of human subjects who received oral doses of indole-3-carbinol, while a considerable amount of 3,3′-diindolylmethane was found in the blood of these subjects. It is noteworthy that 3,3′-diindolylmethane has been shown to possess anticancer effects. Therefore, it is valid to propose that 3,3′-diindolylmethane may be responsible for the *in vivo* anticancer effects of indole-3-carbinol.

Glucobrassicin → (Myrosinase) → Indole-3-carbinol → (Stomach acid) → Indolinium ion intermediate → 3,3′-diindolylmethane

FIGURE 13.15 Formation of indole-3-carbinol from 3,3′-diindolylmethane in the stomach.

13.3 GENERALIZED HEALTH-PROMOTING MECHANISMS OF NUTRACEUTICALS

Various nutraceuticals have been shown to provide a wide range of health-promoting benefits. Some important general mechanisms of these nutraceuticals include anti-inflammation; antioxidation; induction of detoxification enzymes, apoptosis, and cell cycle arrest; improved immune function; and hormone modulation [3,53–55].

The anti-inflammatory effects of many nutraceuticals have been well documented. There are strong links between chronic inflammation and various diseases, such as cancer, obesity, diabetes, and cardiovascular and neurological diseases. For example, obesity and insulin resistance in type 2 diabetes patients are often associated with a chronic low-grade systemic inflammation evidenced by increased expression level of a major pro-inflammatory cytokine,* TNF-α [56]. One of the most important mechanisms of inflammation involves the overproduction of nitric oxide (NO), superoxide, and other reactive oxygen and nitrogen species by macrophages, neutrophils, lymphocytes, and other immune cells during inflammatory responses. These immune cells can infiltrate inflamed tissue, induce DNA damage in proliferating cells, and contribute to malignant transformation [3]. The general mode of anti-inflammatory action of many nutraceuticals is the inhibition of two important pro-inflammatory proteins, namely, COX-2 and iNOS, by decreasing their expression levels and/or inhibiting their enzymatic activities [3]. Both COX-2 and iNOS have been shown to be involved in antigen-stimulated inflammatory responses. Other important mechanisms by which nutraceuticals elicit their anti-inflammatory effects include inhibition of pro-inflammatory cytokines, chemokines, and cell cycle regulatory molecules and modulation of key signaling pathways and transcription factors† associated with inflammation [57].

Antioxidant protection from damage caused by free radicals is vital for the integrity of cellular structures and macromolecules in the human body [58]. This defense system operates through a series of complex networks among vitamins C and E, carotenoids, zinc, copper, selenium, magnesium-dependent antioxidant enzymes, and other nutraceuticals, which together perform highly integrated recycling and regeneration reactions to balance and optimize oxidative status. Deficiencies in the aforementioned components could potentially lead to a compromised defense system [53,58].

* Cytokines are proteinaceous signaling substances critical to functioning of innate and adaptive immune responses.
† Lack of regulation of these transcription factors is often associated with inflammatory responses.

Oxidative stress is created when there is an imbalance between the generation of ROS and the antioxidative stress defense system in the body. These ROS include free radicals such as hydroxyl radical, peroxyl radical, superoxide anion radical, and other reactive species such as hydrogen peroxide and singlet oxygen generated as a result of naturally occurring processes (e.g., mitochondrial electron transport and exercise), environmental stimuli (e.g., ionizing radiation from the sun), inflammatory cytokines, environmental pollutants, changed atmospheric conditions (e.g., hypoxia), and lifestyle stressors (e.g., cigarette smoke and excess alcohol consumption). Defense mechanisms to remove the ROS include antioxidants and antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which can be produced endogenously in the body or obtained from the diet. Under oxidative stress, oxidative damage can occur to macromolecules such as protein, DNA, and lipid. DNA-based alterations, strand breakage, and mutations are problems that are usually associated with free radical attacks on DNA. It has been shown that this type of DNA damage can be stopped, reduced, and even reversed by antioxidant supplementation [3]. These protective effects of antioxidants were seen not only in populations with high oxidative stress (smokers) but also in populations where oxidative stress was not the highest risk factor [59].

Cancer is a major cause of death around the world. Anticancer effects of nutraceuticals have been intensively studied. The process of carcinogenesis includes three stages: initiation, promotion, and progression. The course of initiation is the transformation of a normal cell to an initiated cell, which requires carcinogen metabolism, gene mutations, and dysfunction of DNA repair [3]. During initiation, environmental carcinogens (e.g., certain toxic compounds, tobacco, and pollutants) cause one or more simple mutations such as transitions or small deletions in the key genes controlling the process of carcinogenesis. Two types of enzymes balance the initiation process. The phase I enzymes (e.g., cytochromes p450) can activate procarcinogens to produce ultimate carcinogens that can react with DNA and cause mutations. On the other hand, the phase II enzymes (e.g., glucuronidases and sulfotransferases) promote the removal of carcinogens from the body. Certain nutraceuticals activate various upstream signaling pathways and lead to activation of transcription factors such as nuclear factor-erythroid 2-related factor 2 (Nrf2), AhR, and pregnane X receptor (PXR), which results in increased expression levels of phase II and antioxidant enzymes, such as GST, UGT, heme oxygenase-1, and other cellular defensive enzymes [60]. These enzymes can lead to detoxification of carcinogens, reduction of free radicals and ROS, and removal or repair of oxidative-damaged proteins, which in turn decrease the risk of carcinogenesis.

The stages of promotion and progression during carcinogenesis are characterized by dysregulated cellular signaling pathways that lead to increased cell proliferation and decreased cell death, particularly the programmed cell death, also known as apoptosis. Dysregulated cell proliferation is one of the major characteristics of carcinogenesis. In cancer, genes that regulate cell cycle progression are often mutated and thereby lead to the loss of cell cycle regulation, which is one of the hallmarks of cancer. The cell cycle consists of four phases (G1, S, G2, and M phases). Each phase of the cell cycle is tightly controlled by many signaling proteins such as cyclin-dependent kinases (CDKs)/cyclin complexes and CDK inhibitors (e.g., p16, p21, and p27). As a consequence, mutations in these proteins can stimulate growth and result in high proliferation of transformed cells [61]. Apoptosis is the major type of programmed cell death that occurs when DNA damage is irreparable, and it plays a critical role in the death of tumor cells. In general, apoptosis is divided into two distinct pathways: intrinsic (mitochondrial-mediated) pathway and extrinsic (death receptor–mediated) pathway. In both intrinsic and extrinsic pathways, caspases, a family of cysteine proteases, play the central role in regulating apoptosis. Caspase-3 is a main executioner of apoptosis, and it can be activated by an initiator caspase such as caspase-9. The activated caspase-3 inactivates poly ADP-ribose polymerase (PARP) by enzymatically cleaving it. Inactivation of PARP results in the loss of DNA repair functions, which eventually leads to apoptosis [62]. An effective strategy to inhibit cancer cell growth is to induce cell cycle arrest and/or apoptosis. Many nutraceuticals have been shown to cause cell cycle arrest and apoptosis in cancer cells by modulating key signaling molecules associated with cell cycle progression and apoptosis [3].

The human immune system is vital for the protection against various disease-causing agents including malignant and mutant cells. The immune system, with its bioactive molecules produced by highly interactive immune cells, is susceptible to the effects that can be debilitating to the system such as stress, environmental exposure, deficiencies in nutrients, and aging. Studies have shown that the use of some nutraceuticals can improve immune function by helping increase the number and activity of certain immune cells, such as natural killer cells, T-helper cells, and transferring receptor IL-2-dependent cells [63].

Another health-promoting mechanism demonstrated by nutraceuticals is the modulation of hormones [64]. Phytoestrogens, a group of plant-derived compounds found in various plants, most notably soy, have been shown to have a series of health benefits associated with their ability to modulate hormone functions, such as inhibiting breast cancer development, increasing bone density, improving plasma lipid profile, lowering serum cholesterol, and ameliorating menopausal symptoms [65].

13.4 INTERACTIONS AMONG NUTRACEUTICALS

The human diet is highly complex and consists of many different nutraceuticals. These nutraceuticals in combination may produce health effects that are different from those produced by individual nutraceuticals. There is a growing body of evidence suggesting that the combination of bioactive agents with distinct mechanisms of action may produce a synergistic type of interaction [66]. The synergy among these agents can result in considerably stronger health benefits than those produced by each agent individually. The enhanced efficacy of the combination regimen can also lower the dose required for each agent in the combination, which lowers the cost of the regimen and offers a positive economic impact on society. Indeed, research showed that ingestion of certain nutraceuticals together could lead to enhanced health benefits [66]. Studies on the combinatory effects of nutraceuticals thus far have been primarily focused on examining their efficacy in altering the pathology of cancer.

The combined dietary treatment of curcumin and green tea polyphenols was shown to produce enhanced inhibitory effects on both the initiation and progression stages of colon carcinogenesis in rats in comparison with curcumin or green tea polyphenols alone [67]. A major green tea polyphenol has also been found to produce synergy with fish oil in inhibiting intestinal tumor formation in mice [68]. Studies on whole food have revealed enhanced anticarcinogenic effects that are attributed to the combination of multiple components present in the whole food or several foods combined. For example, whole tomato powder as part of the diet was shown to significantly decrease prostate tumor weight in rats, whereas lycopene, the major nutraceutical in tomato at a level twice as high as that found in the whole tomato powder diet, did not significantly decrease tumor weight [69]. Most interestingly, the combination of whole tomato powder and broccoli powder caused an even greater effect on diminishing tumor weight. Tomato has also been used in combination with garlic to produce a stronger effect on the inhibition of cancerous lesion in the rat colon than tomato or garlic alone [70]. In another study, the dietary combination treatment with fish oil plus butyrate resulted in a significant inhibition of the formation of cancerous lesions in the rat colon [71]. In contrast, the combination of corn oil plus butyrate promoted the formation of cancerous lesions in the rat colon. Thus, when combined with butyrate, two different dietary oils produced opposite effects on colon carcinogenesis. These findings highlight the important role of interactions between dietary components and their effects on human health.

Interactions between nutraceuticals and macronutrients in the GI tract have profound impacts on the bioavailability of nutraceuticals, which in turn affects their bioactivities [72]. For example, polyphenols can form complexes with proteins in the GI tract, which delays the absorption of polyphenols. Dietary fat can enhance the absorption of polyphenols by increasing the solubility of polyphenols in the GI tract. Carbohydrates can also enhance the absorption of polyphenols by increasing paracellular transport and suppressing the bacterial degradation of polyphenols.

These interactions illustrate the need to evaluate the nutraceutical behavior in relation to the food matrix and the diet as a whole to better predict and ensure the desired health effects.

13.5 BIOAVAILABILITY OF NUTRACEUTICALS

The oral bioavailability of a nutraceutical is defined as the fraction of the orally ingested nutraceutical that actually reaches the systemic (blood) circulation in an active form. The active nutraceuticals in the systemic circulation are available to be distributed to the tissues and organs where they can exert their beneficial health effects. For orally ingested nutraceuticals, there are a few barriers preventing them from reaching the systemic circulation in an active form, for example, entrapment in the food matrix, chemical instability during digestion, poor solubility in GI fluids, poor absorption by enterocytes, and first-pass metabolism. The oral bioavailability (F) of a nutraceutical can be estimated by the following equation [73]:

$$F = F_B \times F_A \times F_M$$

Here, F_B is the fraction of an ingested nutraceutical that survives passage through the upper GI tract and that is released from the food matrix into the GI fluids, thereby becoming bioaccessible for absorption by enterocytes. F_A is the fraction of the bioaccessible nutraceutical that is actually absorbed by the enterocytes and then transported to the portal blood or lymph (and into the systemic circulation). F_M is the fraction of the absorbed nutraceutical that is in an active form after first-pass metabolism in the GI tract and liver (and any other forms of metabolism).

As food components, nutraceuticals are usually entrapped in the food matrix, and they need to be released during digestion before they can be absorbed in the GI tract. To be bioaccessible to enterocyte absorption, the released nutraceuticals need to be solubilized in the GI fluids during digestion. Many nutraceuticals, such as carotenoids and curcuminoids, have low bioaccessibility due to their poor solubility in aqueous GI fluids. Nutraceuticals are exposed to various physicochemical environments during the passage through the GI tract, that is, mouth, stomach, small intestine, and large intestine. These varied conditions may cause changes in the physical state and chemistry of nutraceuticals, therefore decreasing or increasing their bioaccessibility. For example, EGCG, a green tea polyphenol, is unstable under the pH conditions found in small intestinal fluids. The small intestine is the place where most nutraceuticals are absorbed. To be available for absorption by enterocytes, nutraceuticals in the GI fluids need to be transported across the aqueous mucus layer, which may be challenging for highly hydrophobic nutraceuticals. The cell membrane of enterocytes is another barrier for the absorption of nutraceuticals. Generally, it is more difficult for hydrophilic nutraceuticals to pass through the lipid-based cell membrane. First-pass metabolism (also known as first-pass elimination) is a process during which nutraceuticals are metabolized by a wide array of enzymes present in the GI tract and liver. The result of first-pass metabolism is that only a fraction of the absorbed nutraceuticals reaches the systemic circulation in active forms, which leads to a decreased oral bioavailability. In order to improve bioavailability of nutraceuticals, new food technologies are under development to overcome the aforementioned barriers of bioavailability [3].

Extensive research has demonstrated the importance of bioavailability on the biological fate and health effects of nutraceuticals. For example, most polyphenols exist as glycosides that are poorly absorbed in the GI tract. This greatly limits the biological effects of these compounds. However, intestinal enzymes produced by human cells and gut microbiome can convert glycosides to aglycones that can be absorbed by enterocytes and transported into the liver through portal blood. The aglycones undergo extensive metabolism in the liver to be converted to conjugates (mainly glucuronides and sulfates). These conjugates are transported back either to the small intestine with the bile or to systemic circulation ultimately to be excreted through urine. Aglycone polyphenols and their metabolites (conjugates) that are not absorbed in the small intestine will reach the large intestine where they will be subject to metabolism by the gut microbiota. The microbial glucuronidases

and sulfatases convert the conjugates back to aglycones that can be reabsorbed by large intestinal mucosa. In the meantime, the gut microbiota can also metabolize aglycones to produce simple metabolites such as hydroxyphenylacetic acids, hydroxyphenylpropionic acids, and phenylvalerolactones. These metabolites can be absorbed in the large intestine and further metabolized in the liver and/or excreted through feces. During the journey of polyphenols passing through the GI tract, many metabolites are generated, which has profound impacts on health effects of polyphenols. Some metabolites can be easily absorbed while others cannot. Additionally, some metabolites have low biological activities as others have potent biological activities. For example, ingested PMFs, for example, nobiletin and 5-demethylnobiletin, were transformed to different metabolites in mice, and these metabolites showed much stronger anticancer effects than their parent compounds [74,75]. Another example worth mentioning is the case of daidzein, a soy isoflavone that can be converted by the gut microbiota to equol, a metabolite with much greater biological activities in comparison to its predecessor daidzein [76].

13.6 BOTANICAL DIETARY SUPPLEMENTS

With the increasing demand for health promotion and disease prevention, botanical products have become popular in the international market, not to mention in those countries with a long history of using botanical medicines such as China, India, Japan, and Germany. Currently, there are about 20,000 medicinal plants listed by the World Health Organization and about 400 medicinal plants widely traded in the world. Botanical products are frequently used in alternative medicine and dietary supplements for their pharmacological effects and health-promoting potential, even though their efficacy and safety have often not been scientifically confirmed. This is in part due to the passage of the Dietary Supplement Health and Education Act (DSHEA) by the U.S. Congress in 1994, which resulted in significantly reduced requirements for the labeling of dietary supplements as compared to the labeling requirements for drugs and food additives. The Act substantially weakened the authority of the U.S. Food and Drug Administration (FDA) to ensure regulation on safety, purity, and efficacy of dietary supplements; in fact, dietary supplements are not legally considered a food or a drug [77].

In the United States, botanical product–based dietary supplements are sold in different forms such as fresh plant products, dried botanical powders, liquid botanical extracts, soft extracts, dry extracts, tinctures, and purified natural compounds. Many kinds of botanical products can be sold in the United States with ginseng, ginkgo, garlic, saw palmetto, echinacea, soy, bilberry, grape seed, and green tea extracts among the top-selling ones. Table 13.3 lists some of the popular botanical dietary supplements and the presumptive active components and functions [78].

For different commercial purposes, there are different quality control and quality assurance standards for botanical dietary supplements. They usually follow some general rules [79]; the botanicals must be authenticated, safe to use, and be within a limit of foreign materials, heavy metals, aflatoxins, and pesticides. The pH value, ash contents, moisture contents, and particle size should be in a reasonable range. Also, the products must pass certain microbiological tests. However, there are some potential safety issues related to botanical dietary supplements. Some botanical products, when used in combination with various prescription and over-the-counter drugs, can cause adverse drug interactions that are sometimes life threatening [80]. For example, the combination of a botanical product that lowers blood pressure together with a prescription blood pressure–lowering drug may result in a dangerously low blood pressure. The quality of botanical products can be largely inconsistent because standardization is lacking for botanical products; chemical compositions of botanicals vary greatly due to differences in the area where they are grown, effects of drying, type of extraction used to enrich/remove specific components, storage conditions, different environmental factors, etc. [81]. Moreover, adulteration is not uncommon in the marketplace of botanical products, sometimes even with banned substances.

TABLE 13.3
Popular Botanical Dietary Supplements, Their Potential Active Components, and Functions

Dietary Supplements	Latin Name	Potential Active Components	Major Potential Health Benefit
Astragalus	*Astragalus membranaceus*	Polysaccharides, saponins (astragalosides)	Immunomodulatory, hepatoprotective
Black cohosh	*Cimicifuga racemosa*	Fukinolic acid 23-epi-26-deoxyactein	Relief of menopausal symptoms
Cranberry	*Vaccinium macrocarpon*	Proanthocyanidins	Prevention and treatment of urinary tract infections
Dang Gui	*Angelica sinensis*	Ligustilide	Treatment of gynecological conditions
Echinacea	*Echinacea purpurea, E. pallida, E. angustifolia*	Polysaccharides and glycoproteins, cichoric acid, alkamides	Treatment of common cold, cough, and upper respiratory infections
Feverfew	*Tanacetum parthenium*	Parthenolide and other sesquiterpene lactones	Alleviation of fever, headache, and women's ailments
Garlic	*Allium sativum*	Allyl sulfur compounds	Antibacterial, anticarcinogenic, antithrombotic, hypolipidemic
Ginger	*Zingiber officinale Roscoe*	Gingerols	Antiemetic, anti-inflammatory, digestive aid
Ginkgo biloba	*Ginkgo biloba*	Ginkgolids, flavonoids	Treatment of cerebral dysfunction and circulatory disorders
American ginseng	*Panax quinquefolius*	Ginsenosides	Therapeutic effects on immune function, cardiovascular diseases, cancer, sexual function
Asian ginseng	*Panax ginseng*	Ginsenosides	Combat psychophysical tiredness and asthenia
Goldenseal	*Hydrastis canadensis*	Alkaloid berberine and β-hydrastine	Soothing irritated skin and mucous membranes, easing dyspepsia
Grape seed extract	*Vitis vinifera*	Proanthocyanidins	Antioxidant, anti-inflammatory, immunostimulatory, antiviral, and anticancer
Green tea polyphenols	*Camellia sinensis*	Epigallocatechin gallate and catechins	Preventive effects on heart diseases, cancer, neurodegenerative disorders, and diabetes
Kava	*Piper methysticum*	Kava lactones	Effects on relaxing and mood calming
Licorice	*Glycyrrhiza glabra*	Triterpene saponins, flavonoids, and other phenolics	Possess soothing, anti-inflammatory, and antitussive properties
Maca	*Lepidium meyenii*	Aromatic isothiocyanates	Use for aphrodisiac purpose
Milk thistle	*Silybum marianum*	Silymarin	Treatment of liver disorders
Pycnogenol	*Pinus pinaster* ssp. *atlantica*	Procyanidins	Use for protection of the circulation and to store capillary healing
Red clover	*Trifolium pratense*	Isoflavones	Treatment for menopausal symptoms
Reishi mushroom	*Ganoderma lucidum*	Triterpenoids, polysaccharides	Antitumor and immunomodulating effects

(*Continued*)

TABLE 13.3 (*Continued*)
Popular Botanical Dietary Supplements, Their Potential Active Components, and Functions

Dietary Supplements	Latin Name	Potential Active Components	Major Potential Health Benefit
Saw palmetto	*Serenoa repens*	Unknown	Use for prostate health
Soy isoflavones	*Glycine max*	Genistein, daidzein	Prevention of menopausal symptoms, osteoporosis, coronary heart disease, and cancer
St. John's wort	*Hypericum perforatum*	Hyperforin, hypericin	Treatment of mild depression
Valerian	*Valeriana officinalis* L.	Valepotriates (iridoids)	Use for mild sedative and sleep disturbance
Yohimbe	*Pausinystalia johimbe*	Yohimbine	Use for aphrodisiac purpose

13.7 EXTRACTION TECHNOLOGIES FOR BOTANICALS

Nutraceuticals are widely used as important ingredients in different products such as functional foods and dietary supplements. To obtain nutraceuticals from botanical materials, effective extraction techniques are required to concentrate bioactive compounds and remove unwanted constituents. Traditional techniques, such as Soxhlet extraction, have been used for many decades, while novel techniques have been developed more recently to improve extraction efficiency and the cost-effectiveness of the process. Before the extraction process, the raw botanical materials often undergo pretreatment such as breaking, crushing, and milling, which can greatly facilitate the extraction efficacy. Extraction efficiency depends substantially on polarity of the nutraceuticals and the type of solvent used during the extraction. Extraction solvents can be selected based on solvent selectivity, capacity, reactivity, stability, solubility, regeneration capability, and toxicity. This section provides a brief overview on the novel extraction techniques suitable for botanical materials [82].

Accelerated solvent extraction (ASE) is also known as pressurized solvent extraction, subcritical solvent extraction, or pressurized liquid extraction [83]. Currently, ASE has been widely used in the extraction of nutraceuticals from botanicals. Because the extraction vessel is pressurized, temperatures higher than the boiling points of extraction solvents can be used during ASE [84]. The higher temperature and higher pressure can facilitate the extraction by increasing the solubility of target compound, increasing the rate of solvent diffusion, and decreasing the solvent viscosity and surface tension [84]. However, some thermally unstable components might degrade when the temperature is set too high, resulting in lower quality extracts. It is noteworthy that ASE often allows for the use of environmentally safe solvents, such as water and aqueous ethanol, to achieve more exhaustive extractions of botanical materials relative to use of these same solvents under normal extraction conditions. ASE is a relatively fast process, as prolonged extraction time was found not to significantly increase extraction efficacy. Moreover, the extraction process can be automated and therefore, less labor intensive [82].

Supercritical fluid extraction (SFE) utilizes supercritical fluid that has advantages in the extraction of various nutraceuticals. Supercritical state is a phase between gas and liquid, where temperature and pressure are above the critical point [83]. Supercritical fluid has a density similar to liquid and viscosity similar to gas, but its diffusion coefficient is much higher than that of liquid, which leads to much faster extraction rate in comparison to the conventional solvent extraction. Due to its high dissolving capacity compared to many nutraceuticals, supercritical fluid has been used as an

efficient extraction solvent. Carbon dioxide has been used as the major SFE solvent due to its relatively low critical conditions, low toxicity, nonflammability, and low cost [85,86]. By manipulating the pressure and temperature of supercritical CO_2, the solubility of nutraceuticals can be changed. Consequently, SFE conditions can be developed to increase the selectivity of the extraction and to obtain products with higher purity. The solubility of the nutraceuticals in supercritical CO_2 decreases when the density of supercritical CO_2 decreases. Therefore, the extracted phytochemicals can be easily recovered by decreasing the pressure of CO_2. Due to its low critical conditions, supercritical CO_2 offers relatively low extraction temperature, which makes it an ideal method for the extraction of thermally labile nutraceuticals [85,86]. Due to the nonpolarity of supercritical CO_2, it may not be an efficient extraction solvent for polar nutraceuticals such as phenolics. However, the efficiency of SFE for polar compounds is significantly increased when supercritical CO_2 is combined with organic solvent modifiers such as ethanol [87,88]. The high cost and sophisticated operation conditions required by SFE limit its broad utilization.

Ultrasound-assisted extraction (UAE) is a simple and cost-effective way to achieve efficient extraction of many plant materials. Ultrasound waves can change the physical and chemical properties of plant material and induce cavitations causing an increase in pressure and temperature close to the substrate surface, disrupting cell walls, and enhancing the release of intracellular compounds into the extraction solvent [89,90]. UAE can also cause swelling of the plant material that can facilitate the extraction of phytochemicals. UAE has been shown to improve the extraction of different phytochemicals such as polyphenols and carotenoids. One benefit of UAE is that it is suitable for thermally labile nutraceuticals because satisfactory extraction can be achieved at relatively low temperatures [82].

The principle of enzyme-assisted extraction (EAE) is to utilize enzymes such as cellulases, pectinases, and hemicellulase to break down the plant cell wall, thereby facilitating the extraction of targeted components from botanical materials [91]. EAE has been successfully used in the extraction of various components such as protein, phenolics, lycopene, and oils. Although EAE may offer better extraction efficiency in comparison with conventional extraction methods, it usually takes considerable amount of time, and it may not provide higher extraction efficacy in comparison with other novel techniques for preextraction treatment such as UAE. During EAE, specific enzymes are needed for different raw materials to effectively break down the cell wall to release target components. Additional factors that limit the application of EAE include relatively high cost of enzymes and environmental sensitivity regarding the use of these enzymes [82].

Pulsed electric field (PEF)-assisted extraction uses an electrical field to induce electroporation of cell membranes, which results in an increased permeability of extraction solvent into the plant matrix and extraction efficiency [83]. PEF treatment improves the extraction of compounds such as anthocyanins and tannins. In comparison with other pretreatment methods such as UAE, PEF-assisted extraction requires less time and lower energy costs. Moreover, PEF has been used as a pretreatment before pressing to enhance the yield and quality (turbidity and odor intensity) of juices from different fruits and vegetables [82].

Microwave-assisted extraction (MAE) utilizes microwaves to penetrate plant materials and interact with polar molecules such as water to generate heat. The heat generated internally in the plant materials can cause cell structure disruption, which facilitates the dissolution of nutraceuticals from the plant matrices [92]. Moreover, the homogeneously increased temperature of the extraction solvent and the plant materials by microwave treatment generally leads to enhanced extraction efficacy. MAE also reduces extraction time in comparison to conventional extraction methods. However, in certain cases, unwanted products can be generated during MAE, especially when excessively high temperatures are encountered. The major advantage of MAE is that it offers significant reduction in extraction time and solvent usage with similar or even better extraction efficacy in comparison to the conventional extraction methods [82,83].

13.8 PROCESS-INDUCED NUTRACEUTICALS IN FOODS

Almost all food items consumed are processed to some extent. Food processing plays a crucial role in food quality, safety, and characteristics of the specific food items. The chemical reactions involved in food processing are numerous and complicated. Although some bioactive compounds are destroyed in the process, many initially not present in the intact tissue or raw material are generated. Some of these compounds have potential health-promoting effects.

Hydroxycinnamic acids such as ferulic acid and caffeic acid are widely distributed in nature. They exist in a free form or as simple esters in fruits, vegetables, and cereals. The abundance of hydroxycinnamic acid–related compounds in coffee beans is high. Green coffee beans contain 6%–9% chlorogenic acid, which is the quinic acid ester of caffeic acid [93]. During the roasting process, the chlorogenic acid content decreases dramatically. Roasted coffee shows anticarcinogenic and antibacterial activities, suggesting that the degradation products of chlorogenic acid may contribute to the beneficial effects [94].

Caffeic acid is one of the major catecholic constituents in green coffee. Under thermal conditions, caffeic acid undergoes rapid decarboxylation to form simple catechol monomers and more complex condensed cyclic dimers and polymers. Two major compounds, 1,3-*cis*- and 1,3-*trans* tetraoxygenated phenylindans, were isolated as mild pyrolysis products of caffeic acid. *In vitro* studies indicate that these two compounds have potent antioxidant and antimutagenic activities. Figure 13.16 shows the mechanism for the formation of 1,3-*cis*- and 1,3-*trans* tetraoxygenated phenylindans. These two compounds were found to be present at levels ranging from 10 to 15 ppm in roasted coffee [95].

Acid treatment during food processing may cause the transformation of nutraceuticals. Formation of active antioxidant in sesame oil during the refining process is a good example. Sesamolin is the major lignin in sesame seeds. During the refining process, the abundance of sesaminol significantly increased as a result of a high yield conversion from sesamolin to sesaminol via intramolecular group transfer catalyzed by acid clay used for bleaching [96]. Sesaminol is responsible for the

FIGURE 13.16 Formation of 1,3-tetraoxygenated phenylindans from caffeic acid.

FIGURE 13.17 Formation of sesaminol from sesamolin.

antioxidant activity of unroasted sesame oil. Figure 13.17 shows the formation mechanism of sesaminol. Transformation of nutraceuticals can also be induced by fermentation. For example, genistein contents significantly increase during fermentation of soybean due to hydrolysis of genistin by microbial enzymes [97]. Genistein is reported to have more potent anticancer effects than genistin.

13.9 FOOD COMPONENTS WITH ADVERSE EFFECTS

Besides beneficial bioactive components, foods derived from plant and animal resources also contain components with adverse effects on humans known as toxins and antinutrients (Table 13.4; [98]). In plants, toxins are often used to protect the host plants against predation, but they can cause from mild to severe symptoms in humans depending on the type of toxins and the doses received [99]. Some of these toxins are considered carcinogens (Table 13.5). However, only a few such toxins consumed through normal diets have actually caused harm to humans.

Alkaloids are widely distributed in the plant kingdom and have been found toxic to other organisms. For example, pyrrolizidine alkaloids can cause liver damage, GI problems, and even death. Their basic ring structure is shown in Figure 13.18. There are many ways humans can become exposed to toxic alkaloids, from milk of a cow that has consumed alkaloid-containing feed to ingestion of processed grains that have been cultivated and/or processed with weeds containing the alkaloids. Two glycoalkaloids, α-solanine and α-chaconine (Figure 13.19), are present in potatoes. They are neurotoxic at high concentrations. FDA regulations limit the solanine content in potato to not be more than 20 mg/100 g [100].

Cyanogenic glycosides are another group of toxins of concern because they are metabolized to hydrocyanic acid (HCN) after consumption. Three cyanogenic glycosides have been identified in edible plants. They are amygdalin (benzaldehyde cyanohydrin β-glucosido-6-β-glucoside), dhurrin (*p*-hydroxybenzaldehyde cyanohydrin glucoside), and linamarin (acetone cyanohydrin glucoside) (Figure 13.20). Amygdalin can be found in bitter almonds, dhurrin in sorghum, and linamarin in pulse, linseed, and cassava [100]. Figure 13.21 shows the mechanism for the release of HCN from hydrolysis of linamarin.

TABLE 13.4
Examples of Inherent Toxins in Plants

Toxins	Chemical Nature	Main Food Source	Major Toxicity Symptoms
Protease inhibitor	Proteins (molecular weight 4,000–24,000)	Beans (soy, mung, kidney, navy, lima), chickpea, peas, potatoes (sweet, white), cereals	Impaired growth and food utilization, pancreatic hypertrophy
Amylase inhibitor	Proteins	Wheat flour	Food allergy in sensitive individual
Antithiamine compound	Proteins (enzyme)	Fish, crab, clam, blueberries, black currants, Brussel sprouts	Food allergy in sensitive individual
Hemagglutinin	Proteins (molecular weight 10,000–124,000)	Beans (castor, soy, kidney, black, yellow, jack), lentils, peas	Impaired growth and food utilization, agglutination of erythrocytes *in vitro*, mitogenic activity to cell cultures *in vitro*
Saponin	Steroid or triterpene glycosides	Soybeans, sugar beets, peanuts, spinach, asparagus	Hemolysis of erythrocytes *in vitro*
Glucosinolate (specific ones)	Thioglycosides	Cabbage and related species, turnips, rutabaga, radish, rapeseed, mustard	Hypothyroidism and thyroid enlargement
Cyanogen	Cyanogenic glycosides	Peas and beans, pulses, linseed, flax, fruit kernels, cassava	Hydrocyanic poisoning
Gossypol pigment	Gossypol	Cottonseeds	Liver damage, hemorrhage, edema
Lathyrogen	β-Aminopropionitrile and derivatives	Chickpea, vetch	Neurolathyrism (central nervous system damage)
Allergen	Proteins	Practically all foods (particularly grains, legumes, and nuts)	Allergic responses in sensitive individuals
Cycasin	Methylazoxymethanol	Nuts of *Cycas* genus	Cancer of liver and other organs
β-Thujone	Monoterpene ketone	Sage, clary, tansy, and wormwood	Tonic–clonic convulsion, absinthism
Erucic acid	Long-chain monounsaturated fatty acid (22:1)	Rapeseed oil	Myocardial lipidosis in a variety of animal species
Myristin	Mescaline-related compound	Black peppers, carrots, celeries, parsleys, and dills	Psychotropic effects
Favism	Vicine and convicine (pyrimidine β-glucosides)	Fava beans	Acute hemolytic anemia
Phytoalexin	Simple furans (ipomeamarone)	Sweet potatoes	Pulmonary edema, liver and kidney damage
	Benzofurans (psoralens)	Celery, parsnips	Skin photosensitivity
	Acetylenic furans (wyerone)	Broad beans	Cell lysis *in vitro*
	Isoflavonoids (pisain and phaseolin)	Peas, French beans	
Pyrrolizidine alkaloid	Dihydropyrroles	Families Compositae and Boraginaceae herbal teas	Liver and lung damage, carcinogens

(*Continued*)

TABLE 13.4 (*Continued*)
Examples of Inherent Toxins in Plants

Toxins	Chemical Nature	Main Food Source	Major Toxicity Symptoms
Solanine and chaconine	Glycoalkaloids	Potatoes	Acetylcholinesterase inhibitors, hyperesthesia, gastrointestinal symptoms
Safrole	Allyl-substituted benzene	Sassafras and black pepper	Carcinogens
α-Amanitin	Bicyclic octapeptides	Mushrooms (*Amanita phalloides*)	Salivation, vomiting, convulsions, death
Atractyloside	Steroidal glycoside	Thistle (*Atractylis gummifera*)	Depletion of glycogen

Source: Adapted from Hu, F.B., *Curr. Opin. Lipidol.*, 13(1), 3, 2002; Palozza, P. and Krinsky, N.I., *Methods Enzymol.*, 213, 403, 1992.

Goitrogen is a substance that interferes iodine uptake in the body. When *Brassica* vegetables are consumed, myrosinase catalyzes transformation from glucosinolates to isothiocyanates that are then cyclized to produce goitrin, one of the goitrogenic substances (Figure 13.22) [99]. Goitrin lowers iodine level that is important for thyroxine production in thyroid, potentially resulting in an enlargement of the thyroid gland.

Some seafoods contain potent toxins. Tetrodotoxin, found in most of the tissues of pufferfish and is a neurotoxin that causes paralysis of the central nervous system and peripheral nerves. It is stable at high temperature but not under alkaline conditions. Pyropheophorbide-A found in certain marine snails known as abalones is a derivative of chlorophyll in the seaweed that the abalones feed on. This toxin is photoactive and produces amine compounds from amino acids, leading to inflammation and other toxic reactions. Ciguatoxin occurs in more than 300 species of fish consuming plants and dinoflagellates that contain the toxin. Ciguatoxin inhibits cholinesterase, an important enzyme in neurotransmission, thus disrupting nerve functions [99].

Antinutrients are compounds that interact with nutrients and negatively affect the digestion, absorption, and beneficial activities of nutrients. Three main classes of antinutrients in food are antiproteins, antiminerals, and antivitamins. Chymotrypsin inhibitors are examples of antiproteins. They inhibit proteolytic activities of digestive enzymes that are necessary for breakdown of proteins prior to their absorption. Chymotrypsin inhibitors are present in egg whites, legumes, vegetables, milk, and potatoes. Since they are heat labile, boiling can effectively inactivate these enzyme inhibitors. Oxalates are antiminerals found in spinach, rhubarb, and tomatoes. They can reduce the solubility of zinc, iron, and calcium, causing lower absorption [100]. Certain coumarins found in tonka beans, melilot, and woodruff are antivitamins. In the presence of molds during food spoilage, these coumarins are metabolized to dicoumarols and have a similar structure as vitamin K. They act as competitive inhibitors to deplete active vitamin K in the blood [101].

13.10 PROCESS-INDUCED TOXICANTS IN FOODS

The chemical reactions initiated in food by heat can be beneficial, as in the case of the formation of desirable flavors, or harmful, as in the generation of toxic chemicals. Important examples of toxicants produced by heat-induced reactions in foods are heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs), and acrylamide.

HCAs and PAHs are generated when proteinaceous food products, such as beef, pork, fish, and poultry, are cooked at high temperatures, such as pan frying and grilling directly over an open flame [102]. Laboratory studies have shown that rodents fed a diet containing HCAs or PAHs developed

TABLE 13.5
Some Naturally Occurring Carcinogens Inherent in Food

Rodent Carcinogen	Plant Food	Concentration (ppm)
5-/8-Methoxypsoralen	Parsley	14
	Parsnip, cooked	32
	Celery	0.8
	Celery, new cultivar	6.2
	Celery, stressed	25
p-Hydrazinobenzoate	Mushrooms	11
Glutamyl *p*-hydrazinobenzoate	Mushrooms	42
Sinigrin (allyl isothiocyanate)	Cabbage	35–590
	Collard greens	250–788
	Cauliflower	12–66
	Brussels sprouts	110–1,560
	Mustard (brown)	16,000–72,000
	Horseradish	4,500
Estragole (1-Allyl-4-methoxybenzene)	Basil	3,800
	Fennel	3,000
Safrole (1-Allyl-3,4-methylene dioxybenzene)	Nutmeg	3,000
	Mace	10,000
	Pepper, black	100
Ethyl acrylate	Pineapple	0.07
Sesamol	Sesame seeds (heated oil)	75
α-Methylbenzyl alcohol	Cocoa	1.3
Benzyl acetate	Basil	82
	Jasmine tea	230
	Honey	15
	Coffee (roasted beans)	100
Caffeic acid	Apple, carrots, celery, cherry, eggplant, endive, grapes, lettuce, pear, plum, and potato	50
	Absinthe, anise, basil, caraway, dill, marjoram, rosemary, sage, savory, tarragon, and thyme	>1,000
	Coffee (roasted beans)	1,800
	Apricot, cherry, peach, and plum	50–500
Chlorogenic acid (caffeic acid)	Coffee (roasted beans)	21,600
Neochlorogenic acid (caffeic acid)	Apple, apricot, broccoli, Brussels sprout, cabbage, cherry, kale, peach, pear, and plum	50–500
	Coffee (roasted beans)	11,600
Coumarin (2*H*-1-Benzopyran-2 one)	Tonka beans, melilot, and woodruff	
Quercetin (2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4one)	Berries fruits, cereal grains, tea, and coffee	

Source: Adapted from Hu, F.B., *Curr. Opin. Lipidol.*, 13(1), 3, 2002; Boon, C.S. et al., *Crit. Rev. Food Sci. Nutr.*, 50(6), 515, 2010.

R—O
O—R
N

FIGURE 13.18 General structure of pyrrolizidine alkaloids.

FIGURE 13.19 Structures of (a) α-solanine and (b) α-chaconine.

FIGURE 13.20 Structures of (a) amygdalin, (b) dhurrin, and (c) linamarin.

FIGURE 13.21 Release of HCN from hydrolysis of linamarin.

FIGURE 13.22 Formation of goitrin.

tumors in multiple organs [103]. However, the doses of HCAs and PAHs used in these studies were extremely high (thousands of times higher than the dose a person would consume in a normal diet). Human studies have not confirmed a definitive link between HCA and PAH exposure from cooked meat and increased cancer risk. Nevertheless, epidemiological studies suggested an association between consumption of well-done, fried, or barbecued meats and increased risks of several cancers in humans [104].

Aminoimidazoazaarenes (AIAs) are a major type of HCAs. Figure 13.23 shows the structures of commonly occurring AIAs, including 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ),

Abbreviation	Z	R^1	R^2	R^3
IQ	C	H	H	H
MeIQ	C	Me	H	H
MeIQx	N	H	H	Me
4,8-DiMeIQx	N	Me	H	Me

FIGURE 13.23 Structures of commonly occurring aminoimidazoazaarenes.

2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline (MeIQx), and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx). All of these AIAs have been isolated from fried meat products [105]. IQ and MeIQ have also been isolated from boiled, sun-dried sardines [106].

Although the exact mechanism for the formation of HCAs in food has not been elucidated, it has been suggested that the 2-aminoimidazo part of the molecules originates from naturally occurring creatine in the muscle [105]. The quinoline and quinoxaline parts are believed to be formed from Maillard reaction products, especially precursors of pyrazines or pyridines and aldehydes [105]. Figure 13.24 shows a probable mechanism for the formation of 4,8-DiMeIQx. The first step of the mechanism is the Maillard reaction between the reducing sugar and amino acids to form reactive dicarbonyl compounds such as pyruvaldehyde. Then, the Strecker degradation between a dicarbonyl compound and amino acid generates a reactive dihydropyrazine molecule. Finally, the condensation among dihydropyrazine, creatinine, and acetaldehyde yields 4,8-DiMeIQx. Acetaldehyde is the Strecker degradation product of amino acids such as alanine and cysteine. On the other hand, acetaldehyde is also an important product of lipid oxidation. This may be the reason why the presence of triacylglycerols facilitates the formation of HCAs [107].

The Maillard reaction is the major reaction pathway for the formation of acrylamide, a well-studied neurotoxin [108,109]. Acrylamide also exhibits reproductive toxicity, genotoxicity, and carcinogenicity in animals. The mechanism for the formation of acrylamide from asparagine through the Maillard reaction is shown in Figure 13.25 [109]. There is a concern about the possible health risks to humans due to dietary acrylamide; however, a population-based study found no association between dietary acrylamide and cancer of the large bowel, kidney, and bladder [110].

Nitrosamines are formed by reaction of secondary or tertiary amines with a nitrosating agent during drying, curing, and preserving of foods. The nitrosating agent found in foods is usually nitrous anhydride, formed from nitrite under acidic and aqueous conditions [111]. Nitrosamines

FIGURE 13.24 Mechanism for the formation of 4,8-DiMeIQx.

FIGURE 13.25 Mechanism for the formation of acrylamide from asparagine through the early Maillard reaction.

are found in a variety of different foods such as cheese, soybean oil, canned fruit, cured or smoked meats, fish products, spices used for meat curing, and beer and other alcoholic beverages [101]. Among them, meat and fish products are the main sources of exposure. *N*-Nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosopiperidine (NPIP) are found most frequently in foods and are reasonably considered to be human carcinogens based on evidence of *in vivo* studies [101]. Evidence from case–control human studies also supported an association between nitrite and nitrosamine intake with gastric cancer in humans [112].

Many food products contain nanosized materials, especially metallic nanoparticles, which have been intentionally and unintentionally added to food products to improve product quality and for ease of use. For example, titanium dioxide (TiO_2, E171) and silica (SiO_2, E551) nanoparticles are used as food additives, while silver (Ag, E174) and zinc oxide (ZnO) nanoparticles are used in food packaging [113]. Although the fate and biological effects of these inorganic nanoparticles after entering the GI tract of the human body are not yet clearly understood, a growing body of evidence indicates that the oral exposure to metallic nanoparticles has a potential to cause health complications [114]. Studies have shown that TiO_2 and SiO_2 nanoparticles can cross the placenta barrier in pregnant mice and cause neurotoxicity in the offspring [115]. Hence, the utilization of these nanoparticles in foods should be approached with caution, and more research is warranted to ensure food safety.

REFERENCES

1. Hu, F.B., Dietary pattern analysis: A new direction in nutritional epidemiology. *Curr Opin Lipidol*, 2002. **13**(1): 3–9.
2. Wang, X., Y. Ouyang, J. Liu, M. Zhu, G. Zhao, W. Bao, and F.B. Hu, Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: Systematic review and dose-response meta-analysis of prospective cohort studies. *Br Med J*, 2014. **349**: g4490.

3. Pan, M.H. and C.T. Ho, Chemopreventive effects of natural dietary compounds on cancer development. *Chem Soc Rev*, 2008. **37**(11): 2558–2574.
4. Palozza, P. and N.I. Krinsky, Antioxidant effects of carotenoids in vivo and in vitro: An overview. *Methods Enzymol*, 1992. **213**: 403–420.
5. Boon, C.S., D.J. McClements, J. Weiss, and E.A. Decker, Factors influencing the chemical stability of carotenoids in foods. *Crit Rev Food Sci Nutr*, 2010. **50**(6): 515–532.
6. Álvarez, R., B. Vaz, H. Gronemeyer, and Á.R. de Lera, Functions, therapeutic applications, and synthesis of retinoids and carotenoids. *Chem Rev*, 2013. **114**(1): 1–125.
7. Druesne-Pecollo, N., P. Latino-Martel, T. Norat, E. Barrandon, S. Bertrais, P. Galan, and S. Hercberg, Beta-carotene supplementation and cancer risk: A systematic review and metaanalysis of randomized controlled trials. *Int J Cancer*, 2010. **127**(1): 172–184.
8. Liu, J., W.Q. Shi, Y. Cao, L.P. He, K. Guan, W.H. Ling, and Y.M. Chen, Higher serum carotenoid concentrations associated with a lower prevalence of the metabolic syndrome in middle-aged and elderly Chinese adults. *Br J Nutr*, 2014: 1–8.
9. Burton, G.W. and K.U. Ingold, beta-Carotene: An unusual type of lipid antioxidant. *Science*, 1984. **224**(4649): 569–573.
10. Maia, M., B.A. Furlani, A.A. Souza-Lima, D.S. Martins, R.M. Navarro, and R.J. Belfort, LUTEIN: A new dye for chromovitrectomy. *Retina*, 2014. **34**(2): 262–272. 10.1097/IAE.0b013e3182a0b7f4.
11. Carpentier, S., M. Knaus, and M.Y. Suh, Associations between lutein, zeaxanthin, and age-related macular degeneration: An overview. *Crit Rev Food Sci Nutr*, 2009. **49**(4): 313–326.
12. Roberts, R.L., J. Green, and B. Lewis, Lutein and zeaxanthin in eye and skin health. *Clin Dermatol*, 2009. **27**(2): 195–201.
13. Anese, M., G. Mirolo, A. Fabbro, and G. Lippe, Lycopene bioaccessibility and bioavailability from processed foods. *J Sci Ind Res*, 2013. **72**(9–10): 543–547.
14. Viuda-Martos, M., E. Sanchez-Zapata, E. Sayas-Barberá, E. Sendra, J.A. Pérez-Álvarez, and J. Fernández-López, Tomato and tomato byproducts. Human health benefits of lycopene and its application to meat products: A review. *Crit Rev Food Sci Nutr*, 2013. **54**(8): 1032–1049.
15. Ilic, D. and M. Misso, Lycopene for the prevention and treatment of benign prostatic hyperplasia and prostate cancer: A systematic review. *Maturitas*, 2012. **72**(4): 269–276.
16. Burton-Freeman, B. and H.D. Sesso, Whole food versus supplement: Comparing the clinical evidence of tomato intake and lycopene supplementation on cardiovascular risk factors. *Adv Nutr*, 2014. **5**(5): 457–485.
17. Ross, J.a. and C.M. Kasum, Dietary flavonoids: Bioavailability, metabolic effects, and safety. *Annu Rev Nutr*, 2002. **22**: 19–34.
18. Wei Bai, C.W. and C. Ren, Intakes of total and individual flavonoids by US adults. *Int J Food Sci Nutr*, 2014. **65**(1): 9–20.
19. Cabrera, C., Beneficial effects of green tea—A review. *J Am Coll Nutr*, 2006. **25**: 79–99.
20. Sang, S., J.D. Lambert, C.-T. Ho, and C.S. Yang, Green tea polyphenols, in *Encyclopedia of Dietary Supplements* (P. Coates, M.R. Blackman, G.M. Cragg, M. Levine, J. Moss, and J.D. White, eds.), Marcel Dekker, New York, 2005, pp. 327–336.
21. Ju, J., G. Lu., J.D. Lambert, and C.S. Yang, Inhibition of carcinogenesis by tea constituents. *Semin Cancer Biol*, 2007. **17**(5): 395–402.
22. Kuhnert, N., J.W. Drynan, J. Obuchowicz, M.N. Clifford, and M. Witt, Mass spectrometric characterization of black tea thearubigins leading to an oxidative cascade hypothesis for thearubigin formation. *Rapid Commun Mass Spectrom: RCM*, 2010. **24**: 3387–3404.
23. Khan, N. and H. Mukhtar, Tea polyphenols for health promotion. *Life Sci*, 2007. **81**: 519–533.
24. Jian, L., L.P. Xie, A.H. Lee, and C.W. Binns, Protective effect of green tea against prostate cancer: A case-control study in southeast China. *J Int Cancer* 2004. **108**: 130–135.
25. Lambert, J.D. and C.S. Yang, Cancer chemopreventive activity and bioavailability of tea and tea polyphenols. *Mutat Res*, 2003. **523–524**: 201–208.
26. Yang, C.S., P. Maliakal, and X. Meng, Inhibition of carcinogenesis by tea. *Annu Rev Pharmacol Toxicol*, 2002. **42**: 25–54.
27. Yang, C.S., L. Chen, M.J. Lee, D. Balentine, M.C. Kuo, and S.P. Schantz, Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol Biomarkers Prev*, 1998. **7**: 351–354.
28. Higdon, J.V. and B. Frei, Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. *Crit Rev Food Sci Nutr*, 2003. **43**: 89–143.

29. Boots, A.W., G.R.M.M. Haenen, and A. Bast, Health effects of quercetin: From antioxidant to nutraceutical. *Eur J Pharmacol*, 2008. **585**: 325–337.
30. Arai, Y., S. Watanabe, M. Kimira, K. Shimoi, R. Mochizuki, and N. Kinae, Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *J Nutr*, 2000. **130**: 2243–2250.
31. Gibellini, L., M. Pinti, M. Nasi, J.P. Montagna, S. De Biasi, E. Roat, L. Bertoncelli, E.L. Cooper, and A. Cossarizza, Quercetin and cancer chemoprevention. *Evid Based Complement Alternat Med*, 2011. **2011**: 591356.
32. Szkudelska, K. and L. Nogowski, Genistein—A dietary compound inducing hormonal and metabolic changes. *J Steroid Biochem Mol Biol*, 2007. **105**: 37–45.
33. De Naeyer, A., W. Vanden Berghe, D. De Keukeleire, and G. Haegeman, Characterization and beneficial effects of kurarinone, a new flavanone and a major phytoestrogen constituent of *Sophora flavescens* Ait., Chapter 25, in *Phytopharmaceuticals in Cancer Chemoprevention* (D. Bagchi and H. G. Preuss, eds.), CRC Press, Boca Raton, FL, 2005, pp. 427–448.
34. Banerjee, S., Y. Li, Z. Wang, and F.H. Sarkar, Multi-targeted therapy of cancer by genistein. *Cancer Lett*, 2008. **269**: 226–242.
35. Li, S., M.-H. Pan, C.-Y. Lo, D. Tan, Y. Wang, F. Shahidi, and C.-T. Ho, Chemistry and health effects of polymethoxyflavones and hydroxylated polymethoxyflavones. *J Funct Foods*, 2009. **1**: 2–12.
36. Qiu, P., P. Dong, H. Guan, S. Li, C.T. Ho, M.H. Pan, D.J. McClements, and H. Xiao, Inhibitory effects of 5-hydroxy polymethoxyflavones on colon cancer cells. *Mol Nutr Food Res*, 2010. **54**(Suppl. 2): S244–S252.
37. Charoensinphon, N., P. Qiu, P. Dong, J. Zheng, P. Ngauv, Y. Cao, S. Li, C.T. Ho, and H. Xiao, 5-Demethyltangeretin inhibits human nonsmall cell lung cancer cell growth by inducing G2/M cell cycle arrest and apoptosis. *Mol Nutr Food Res*, 2013. **57**(12): 2103–2111.
38. Rasmussen, S.E., H. Frederiksen, K.S. Krogholm, and L. Poulsen, Dietary proanthocyanidins: Occurrence, dietary intake, bioavailability, and protection against cardiovascular disease. *Mol Nutr Food Res* 2005. **49**(2): 159–174.
39. Monagas, M., M. Urpi-Sarda, F. Sánchez-Patán, R. Llorach, I. Garrido, C. Gómez-Cordovés, C. Andres-Lacueva, and B. Bartolomé, Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. *Food Funct*, 2010. **1**: 233–253.
40. Rahmani, A.H., M.A. Al Zohairy, S.M. Aly, and M.A. Khan, Curcumin: A potential candidate in prevention of cancer via modulation of molecular pathways. *Biomed Res Int*, 2014. **2014**: 761608.
41. Chen, C.-C., R.T. Rosen, and C.-T. Ho, Chromatographic analyses of isomeric shogaol compounds derived from isolated gingerol compounds of ginger (*Zingiber officinale* Roscoe). *J Chromatogr A*, 1986. **360**: 175–184.
42. Yu, Y., S. Zick, X. Li, P. Zou, B. Wright, and D. Sun, Examination of the pharmacokinetics of active ingredients of ginger in humans. *AAPS J*, 2011. **13**(3): 417–426.
43. Wu, J.M., X. Lu, J. Guo, and T.C. Hsieh, Vascular effects of resveratrol, in *Phytochemicals: Mechanisms of Action* (M.S. Meskin, W.R. Bidlack, A.J. Davies, D.S. Lewis, and R.K. Randolph, eds.), CRC Press, Boca Raton, FL, 2004, pp. 145–161.
44. Kapetanovic, I.M., M. Muzzio, Z. Huang, T.N. Thompson, and D.L. McCormick, Pharmacokinetics, oral bioavailability, and metabolic profile of resveratrol and its dimethylether analog, pterostilbene, in rats. *Cancer Chemother Pharmacol*, 2011. **68**: 593–601.
45. Manoharan, S., M. Vasanthaselvan, S. Silvan, N. Baskaran, A. Kumar Singh, and V. Vinoth Kumar, carnosic acid: A potent chemopreventive agent against oral carcinogenesis. *Chem-Biol Interact*, 2010. **188**: 616–622.
46. Ho, C.T., T. Ferraro, Q. Chen, R.T. Rosen, and M.T. Huang, Phytochemicals in teas and rosemary and their cancer-preventive properties. ACS Symposium series 547, Washington DC, pp. 2–19, 1994.
47. Moriarty, R.M., R. Naithani, and B. Surve, Organosulfur compounds in cancer chemoprevention. *Mini Rev Med Chem*, 2007. **7**(8): 827–838.
48. Pittler, M.H. and E. Ernst, Clinical effectiveness of garlic (*Allium sativum*). *Mol Nutr Food Res*, 2007. **51**(11): 1382–1385.
49. Borrelli, F., R. Capasso, and A.A. Izzo, Garlic (*Allium sativum* L.): Adverse effects and drug interactions in humans. *Mol Nutr Food Res*, 2007. **51**(11): 1386–1397.
50. Herr, I. and M.W. Buchler, Dietary constituents of broccoli and other cruciferous vegetables: Implications for prevention and therapy of cancer. *Cancer Treat Rev*, 2010. **36**(5): 377–383.
51. Jin, Y., M. Wang, R.T. Rosen, and C.T. Ho, Thermal degradation of sulforaphane in aqueous solution. *J Agric Food Chem*, 1999. **47**(8): 3121–3123.

52. Zhang, Y., Allyl isothiocyanate as a cancer chemopreventive phytochemical. *Mol Nutr Food Res*, 2010. **54**(1): 127–135.
53. Calder, P.C. and S. Kew, The immune system: A target for functional foods? *Br J Nutr*, 2002. **88**(Suppl. 2): S165–S177.
54. Fenech, M., C. Stockley, and C. Aitken, Moderate wine consumption protects against hydrogen peroxide-induced DNA damage. *Mutagenesis*, 1997. **12**(4): 289–296.
55. Somjen, D., E. Knoll, J. Vaya, N. Stern, and S. Tamir, Estrogen-like activity of licorice root constituents: Glabridin and glabrene, in vascular tissues in vitro and in vivo. *J Steroid Biochem Mol Biol*, 2004. **91**(3): 147–155.
56. Leiherer, A., A. Mundlein, and H. Drexel, Phytochemicals and their impact on adipose tissue inflammation and diabetes. *Vascul Pharmacol*, 2013. **58**(1–2): 3–20.
57. Reuter, S., S.C. Gupta, M.M. Chaturvedi, and B.B. Aggarwal, Oxidative stress, inflammation, and cancer: How are they linked? *Free Radic Biol Med*, 2010. **49**(11): 1603–1616.
58. De la Fuente, M., Effects of antioxidants on immune system ageing. *Eur J Clin Nutr*, 2002. **56**(Suppl. 3): S5–S8.
59. Zhang, J., S. Jiang, and R.R. Watson, Antioxidant supplementation prevents oxidation and inflammatory responses induced by sidestream cigarette smoke in old mice. *Environ Health Perspect*, 2001. **109**(10): 1007–1009.
60. Lee, J.H., T.O. Khor, L. Shu, Z.Y. Su, F. Fuentes, and A.N. Kong, Dietary phytochemicals and cancer prevention: Nrf2 signaling, epigenetics, and cell death mechanisms in blocking cancer initiation and progression. *Pharmacol Ther*, 2013. **137**(2): 153–171.
61. Gupta, S.C., J.H. Kim, S. Prasad, and B.B. Aggarwal, Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. *Cancer Metastasis Rev*, 2010. **29**(3): 405–434.
62. Ouyang, L., Z. Shi, S. Zhao, F.T. Wang, T.T. Zhou, B. Liu, and J.K. Bao, Programmed cell death pathways in cancer: A review of apoptosis, autophagy and programmed necrosis. *Cell Prolif*, 2012. **45**(6): 487–498.
63. Ganjali, S., A. Sahebkar, E. Mahdipour, K. Jamialahmadi, S. Torabi, S. Akhlaghi, G. Ferns, S.M. Parizadeh, and M. Ghayour-Mobarhan, Investigation of the effects of curcumin on serum cytokines in obese individuals: A randomized controlled trial. *Scientific World Journal*, 2014. **2014**: 898361.
64. Branca, F. and S. Lorenzetti, Health effects of phytoestrogens. *Forum Nutr*, 2005. **57**: 100–111.
65. Patisaul, H.B. and W. Jefferson, The pros and cons of phytoestrogens. *Front Neuroendocrinol*, 2010. **31**(4): 400–419.
66. DiMarco-Crook, C. and H. Xiao, Diet-based strategies for cancer chemoprevention: The role of combination regimens using dietary bioactive components. *Annu Rev Food Sci Technol*, 2015. **6**: 505–526.
67. Sengupta, A., S. Ghosh, and S. Das, Tomato and garlic can modulate azoxymethane-induced colon carcinogenesis in rats. *Eur J Cancer Prev*, 2003. **12**(3): 195–200.
68. Bose, M., X. Hao, J. Ju, A. Husain, S. Park, J.D. Lambert, and C.S. Yang, Inhibition of tumorigenesis in ApcMin/+ mice by a combination of (−)-epigallocatechin-3-gallate and fish oil. *J Agric Food Chem*, 2007. **55**(19): 7695–7700.
69. Canene-Adams, K., B.L. Lindshield, S. Wang, E.H. Jeffery, S.K. Clinton, and J.W. Erdman, Jr., Combinations of tomato and broccoli enhance antitumor activity in dunning r3327-h prostate adenocarcinomas. *Cancer Res*, 2007. **67**(2): 836–843.
70. Sungupta, A., S. Ghosh, and S. Das, Modulatory influence of garlic and tomato on cyclooxygenase-2 activity, cell proliferation and apoptosis during azoxymethane induced colon carcinogenesis in rat. *Cancer Lett*, 2004. **208**(2): 127–136.
71. Crim, K.C., L.M. Sanders, M.Y. Hong, S.S. Taddeo, N.D. Turner, R.S. Chapkin, and J.R. Lupton, Upregulation of p21Waf1/Cip1 expression in vivo by butyrate administration can be chemoprotective or chemopromotive depending on the lipid component of the diet. *Carcinogenesis*, 2008. **29**(7): 1415–1420.
72. Zhang, H., D. Yu, J. Sun, X. Liu, L. Jiang, H. Guo, and F. Ren, Interaction of plant phenols with food macronutrients: Characterisation and nutritional-physiological consequences. *Nutr Res Rev*, 2014. **27**(1): 1–15.
73. Yao, M.F., H. Xiao, and D.J. McClements, Delivery of lipophilic bioactives: Assembly, disassembly, and reassembly of lipid nanoparticles. *Annu Rev Food Sci Technol*, 2014. **5**: 53–81.
74. Zheng, J., M. Song, P. Dong, P. Qiu, S. Guo, Z. Zhong, S. Li, C.T. Ho, and H. Xiao, Identification of novel bioactive metabolites of 5-demethylnobiletin in mice. *Mol Nutr Food Res*, 2013. **57**(11): 1999–2007.
75. Wu, X., M. Song, M. Wang, J. Zheng, Z. Gao, F. Xu, G. Zhang, and H. Xiao, Chemopreventive effects of nobiletin and its colonic metabolites on colon carcinogenesis. *Mol Nutr Food Res*, 2015. **59**(12): 2383–2394.

76. Valdes, L., A. Cuervo, N. Salazar, P. Ruas-Madiedo, M. Gueimonde, and S. Gonzalez, The relationship between phenolic compounds from diet and microbiota: Impact on human health. *Food Funct*, 2015. **6**(8): 2424–2439.
77. Hathcock, J., Dietary supplements: How they are used and regulated. *J Nutr*, 2001. **131**(3s): 1114S–1117S.
78. Coates, P.M., J.M. Betz, M.R. Blackman, G.M. Cragg, M. Levine, J. Moss, and J.D. White, *Encyclopedia of Dietary Supplements*, Marcel Dekker, New York, 2005.
79. Yi, J., H. Chi-Tang, W. Mingfu, W. Qing-Li, and E.S. James, Chemistry, pharmacology, and quality control of selected popular Asian herbs in the U.S. market, in *Asian Functional Foods* (J. Shi, F. Shahidi, and C.-T. Ho, eds.), CRC Press, Boca Raton, FL, 2005, pp. 73–102.
80. Elvin-Lewis, M., Should we be concerned about herbal remedies. *J Ethnopharmacol*, 2001. **75**(2–3): 141–164.
81. Pawar, R., H. Tamta, J. Ma, A. Krynitsky, E. Grundel, W. Wamer, and J. Rader, Updates on chemical and biological research on botanical ingredients in dietary supplements. *Anal Bioanal Chem*, 2013. **405**(13): 4373–4384.
82. Blumberg, J.B., B.W. Bolling, C.Y.O. Chen, and H. Xiao, Review and perspective on the composition and safety of green tea extracts. *Eur J Nutr Food Safety*, 2015. **5**(1): 1–31.
83. Wijngaard, H., M.B. Hossain, D.K. Rai, and N. Brunton, Techniques to extract bioactive compounds from food by-products of plant origin. *Food Res Int*, 2012. **46**(2): 505–513.
84. Hossain, M.B., C. Barry-Ryan, A.B. Martin-Diana, and N.P. Brunton, Optimisation of accelerated solvent extraction of antioxidant compounds from rosemary (*Rosmarinus officinalis* L.), marjoram (*Origanum majorana* L.) and oregano (*Origanum vulgare* L.) using response surface methodology. *Food Chem*, 2011. **126**(1): 339–346.
85. Herrero, M., J.A. Mendiola, A. Cifuentes, and E. Ibanez, Supercritical fluid extraction: Recent advances and applications. *J Chromatogr A*, 2010. **1217**(16): 2495–2511.
86. Diaz-Reinoso, B., A. Moure, H. Dominguez, and J.C. Parajo, Supercritical CO_2 extraction and purification of compounds with antioxidant activity. *J Agric Food Chem*, 2006. **54**(7): 2441–2469.
87. Santos, S.A.O., J.J. Villaverde, C.M. Silva, C.P. Neto, and A.J.D. Silvestre, Supercritical fluid extraction of phenolic compounds from *Eucalyptus globulus* Labill bark. *J Supercrit Fluids*, 2012. **71**: 71–79.
88. Casas, L., C. Mantell, M. Rodríguez, E.J.M.D.L. Ossa, A. Roldán, I.D. Ory, I. Caro, and A. Blandino, Extraction of resveratrol from the pomace of *Palomino fino* grapes by supercritical carbon dioxide. *J Food Eng*, 2010. **96**: 304–308.
89. Chen, B.Y., C.H. Kuo, Y.C. Liu, L.Y. Ye, J.H. Chen, and C.J. Shieh, Ultrasonic-assisted extraction of the botanical dietary supplement resveratrol and other constituents of *Polygonum cuspidatum*. *J Nat Prod*, 2012. **75**(10): 1810–1813.
90. Chemat, F., Zill-e-Huma, and M.K. Khan, Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrason Sonochem*, 2011. **18**(4): 813–835.
91. Puri, M., D. Sharma, and C.J. Barrow, Enzyme-assisted extraction of bioactives from plants. *Trends Biotechnol*, 2012. **30**(1): 37–44.
92. Kaufmann, B., P. Christen, and J.L. Veuthey, Parameters affecting microwave-assisted extraction of withanolides. *Phytochem Anal*, 2001. **12**(5): 327–331.
93. Daglia, M., M.T. Cuzzoni, and C. Dacarro, Antibacterial activity of coffee. *J Agric Food Chem*, 1994. **42**(10): 2270–2272.
94. Daglia, M., M.T. Cuzzoni, and C. Dacarro, Antibacterial activity of coffee - Relationship between biological-activity and chemical markers. *J Agric Food Chem*, 1994. **42**(10): 2273–2277.
95. Stadler, R.H., D.H. Welti, A.A. Stampfli, and L.B. Fay, Thermal decomposition of caffeic acid in model systems: Identification of novel tetraoxygenated phenylindan isomers and their stability in aqueous solution. *J Agric Food Chem*, 1996. **44**(3): 898–905.
96. Jongen, W.M.F., Food phytochemicals for cancer prevention II: Teas, spices and herbs, (ACS Symposium Series 547). *Trends Food Sci Technol*, 1995. **6**: 216.
97. Chiou, R.Y.Y. and S.L. Cheng, Isoflavone transformation during soybean koji preparation and subsequent miso fermentation supplemented with ethanol and NaCl. *J Agric Food Chem*, 2001. **49**(8): 3656–3660.
98. Pariza, M., Toxic substances. *Food Chem*, 1996. **3**: 825–841.
99. Omaye, S.T., *Food and Nutritional Toxicology*, CRC Press, Boca Raton, FL, 2004.
100. Rietjens, I.M., M.J. Martena, M.G. Boersma, W. Spiegelenberg, and G.M. Alink, Molecular mechanisms of toxicity of important food-borne phytotoxins. *Mol Nutr Food Res*, 2005. **49**(2): 131–158.
101. Dolan, L.C., R.A. Matulka, and G.A. Burdock, Naturally occurring food toxins. *Toxins*, 2010. **2**(9): 2289–2332.

102. Cross, A.J. and R. Sinha, Meat-related mutagens/carcinogens in the etiology of colorectal cancer. *Environ Mol Mutagen*, 2004. **44**(1): 44–55.
103. Sugimura, T., K. Wakabayashi, H. Nakagama, and M. Nagao, Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Sci*, 2004. **95**(4): 290–299.
104. Cross, A.J., L.M. Ferrucci, A. Risch, B.I. Graubard, M.H. Ward, Y. Park, A.R. Hollenbeck, A. Schatzkin, and R. Sinha, A large prospective study of meat consumption and colorectal cancer risk: An investigation of potential mechanisms underlying this association. *Cancer Res*, 2010. **70**(6): 2406–2414.
105. Jagerstad, M., A.L. Reutersward, R. Oste, A. Dahlqvist, S. Grivas, K. Olsson, and T. Nyhammar, Creatinine and Maillard-reaction products as precursors of mutagenic compounds formed in fried beef. *ACS Symposium Series*, 1983. **215**: 507–519.
106. Kasai, H., Z. Yamaizumi, K. Wakabayashi, M. Nagao, T. Sugimura, S. Yokoyama, T. Miyazawa, and S. Nishimura, Structure and chemical synthesis of Me-Iq, a potent mutagen isolated from broiled fish. *Chem Lett*, 1980. **9**(11): 1391–1394.
107. Johansson, M., K. Skog, and M. Jagerstad, Effects of edible oils and fatty-acids on the formation of mutagenic heterocyclic amines in a model system. *Carcinogenesis*, 1993. **14**(1): 89–94.
108. Mottram, D.S., B.L. Wedzicha, and A.T. Dodson, Acrylamide is formed in the Maillard reaction. *Nature*, 2002. **419**(6906): 448–449.
109. Stadler, R.H., F. Robert, S. Riediker, N. Varga, T. Davidek, S. Devaud, T. Goldmann, J. Hau, and I. Blank, In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. *J Agric Food Chem*, 2004. **52**(17): 5550–5558.
110. Mucci, L.A., P.W. Dickman, G. Steineck, H.O. Adami, and K. Augustsson, Dietary acrylamide and cancer of the large bowel, kidney, and bladder: Absence of an association in a population-based study in Sweden. *Br J Cancer*, 2003. **88**(1): 84–89.
111. Scanlan, R.A., Formation and occurrence of nitrosamines in food. *Cancer Res*, 1983. **43**(5): 2435–2440.
112. Jakszyn, P. and C.A. Gonzalez, Nitrosamine and related food intake and gastric and oesophageal cancer risk: A systematic review of the epidemiological evidence. *World J Gastroenterol*, 2006. **12**(27): 4296–4303.
113. Wang, H.F., L.J. Du, Z.M. Song, and X.X. Chen, Progress in the characterization and safety evaluation of engineered inorganic nanomaterials in food. *Nanomedicine*, 2013. **8**(12): 2007–2025.
114. Martirosyan, A. and Y.J. Schneider, Engineered nanomaterials in food: Implications for food safety and consumer health. *Int J Environ Res Public Health*, 2014. **11**(6): 5720–5750.
115. Yamashita, K., Y. Yoshioka, K. Higashisaka, K. Mimura, Y. Morishita, M. Nozaki, T. Yoshida et al., Tsutsumi, Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. *Nat Nanotechnol*, 2011. **6**(5): 321–328.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Section III

Food Systems

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

14 Characteristics of Milk

David S. Horne

CONTENTS

14.1 Introduction 908
14.1.1 Some Facts and Figures 909
14.2 Lactation, Evolution, and Milk Synthesis 910
14.2.1 Milk Biosynthesis 912
14.3 Cow's Milk Composition 913
14.3.1 Milk Proteins 914
14.3.1.1 Caseins and Casein Micelles 915
14.3.1.2 Casein Primary Structure and Interactions 915
14.3.1.3 Casein Micelle Structure and Assembly 918
14.3.1.4 Whey Proteins 920
14.3.2 Milk Lipids and Milk Fat Globules 921
14.3.2.1 Milk Lipids 921
14.3.2.2 Milk Fat Globule Membrane 924
14.3.2.3 Composition of Milk Fat Globule Membrane 924
14.3.2.4 Structure of Milk Fat Globule Membrane 927
14.3.3 Milk Salts and Minerals, Lactose, and Minor Enzyme Compounds 927
14.3.3.1 Milk Salts and Minerals 927
14.3.3.2 Lactose 929
14.3.3.3 Enzymes 929
14.4 Commodity Milk Products 930
14.4.1 Introduction 930
14.4.2 Liquid Milks 930
14.4.2.1 Pasteurization 932
14.4.2.2 Effects of Heat Treatment 932
14.4.3 Cultured Milk Products 934
14.4.4 Cheese 934
14.4.5 Butter 936
14.4.6 Evaporated Milk, Condensed Milk, and Milk Powder 936
14.4.7 Membrane Separation Processing 937
14.5 Milk Proteins as Functional Ingredients 938
14.5.1 Bioactive Peptides Derived from Milk Protein 941
14.5.1.1 Derived from Caseins 941
14.5.1.2 Derived from Whey Proteins 942
14.6 Nutritive Value of Milk 943
14.6.1 Lactose Intolerance 947
References 948
Further Reading 953

14.1 INTRODUCTION

We are perhaps more familiar with milk as the white liquid we pour over our breakfast cereal or add to whiten our tea or coffee or as the precursor of the many dairy products found on grocery shelves or chill cabinets, but we should also be aware that milk is the first food that we consume, a characteristic shared by all mammalian species. For milk is that first perfect food designed by nature to supply, through the mother, all the requirements for nutrition, sustenance, and growth of the neonate, but only through the first few months of new life.

Milk has featured frequently in mythology and legend down through the ages. Behind many of these tales is the idea that the imbiber takes on the characteristics of the mother. Thus, the galaxy of stars, the Milky Way, arose because in Greek myth the child Heracles was put to the breast of the sleeping goddess, Hera, in the hope of acquiring godlike attributes by his father, the god Zeus. On awakening the goddess threw off the child and the droplets of milk spurting from her breast formed the stars of the Milky Way. Legend also has it that the founding fathers of Rome, Romulus and Remus, were suckled by a she-wolf. Not for them, the docility and meekness of a ewe, rather the strength and cunning of the wolf. Continuing on the Roman theme, Aurelia, the mother of Julius Caesar, breastfed all her children, considering it irresponsible to hand them over to wet-nurse slaves, as most Roman aristocrats were doing in a clear and shocking symptom of their degeneracy. At the same time, the Romans justified the invasion of Britannia as bringing civilization to a barbaric society that indulged in unspeakable habits such as cannibalism and drinking milk [46]!

It is not known when man first realized the benefits of consuming and preserving the milks of other species, certainly beyond any written history, probably predating any settled farming, and possibly in a transition period from hunter-gathering. Neolithic farmers began the domestication of cattle, goats, and sheep between 10,000 and 11,000 years BP (Before Present) in Anatolia (modern Turkey), western Iran, and northern Mesopotamia [78]. From there it spread north and west into the Balkans and the Caucasus and across Europe. When dairying became part of this is a matter of conjecture. Lipids extracted from broken unglazed ceramic vessels show the presence of milk fats in northwestern Anatolia around 7000–8000 years BP and in central and southeastern Anatolia more than a millennium later [28]. Indications of the widespread practice of dairying in Britain 5000–7000 years BP are evidenced by the detection of ruminant fats on pottery shards at several sites, this being prior to the arrival of farming (crop rearing) spreading from its Middle Eastern origins [14]. Such detection relies on the presence of the pottery container and dairying may predate this technology, since it is thought that the discovery of fermented milk products such as cheese and yogurt was due to the serendipital storage of milk in bags made from animal stomachs, allowing enzymatic digestion and the production of curds.

Not only did this discovery allow the storage and preservation of many of the nutritional components of milk for later consumption, it also removed the lactose whose presence rendered milk unsuitable as a food for most adults (see Section 14.6.1). We are all born with a lactase enzyme active in our stomachs, but this naturally is downregulated in childhood, rendering the adult lactose intolerant, unless we possess the genetic modification that preserves lactase activity. This genetic modification appeared in central northern Europe, in what is now Hungary, approximately 8000 years ago [66]. It spread from there, gaining high frequency in the populations of northern Europe through a culture/evolutionary feedback. The fledging dairy industry it spawned spread across the world in the train of the European diaspora. In modern times, other factors contributing to the growth of the dairy industry include technological innovation in the preservation of milk quality and in dairy product manufacture as well as improvements in breeding and rearing of dairy cattle for milk production.

14.1.1 Some Facts and Figures

Worldwide production of milk in 2011 was in the region of 734 × 10^6 tonnes [30]. Apart from an insignificant amount of mare's milk, this was obtained from ruminants, and by far the majority (approx. 84%) was bovine milk. Other contributors to the total were buffalo (13%), goat (1.8%), sheep (1.1%), and camel (0.3%). Reindeer milk is also consumed locally in Lapland. In terms of quantity, the aggregate production of milk ranked third in agricultural products behind sugar (1800 × 10^6 tonnes) and maize (885 × 10^6 tonnes). Bovine milk production is spread worldwide (Table 14.1), while buffalo and camel are more regionally specific: buffalo in India and Southeast Asia and camel in the Arabian peninsula and the Horn of Africa. As listed in Table 14.1, the United States ranked first in 2011 in fresh cow milk production, but the contribution of buffalo and goat milk to the figures listed for cow milk lifted India to the number 1 position for overall milk production, and almost all of that was consumed within the country.

Milk yield per cow depends on a number of factors including the production system and diet. What the cow eats has the greatest impact on yield obtained. Highest yields of around 12,500 kg milk per cow per year were obtained in Israel for cows kept in barns on an energy-rich mixed diet, while average production in New Zealand was approximately 4000 kg per cow per year with cows grazing outside all year round [41]. Milk yield per cow in the United States averages 10,000 kg per year, but in India, the average yield is only 1200 kg, testament to the more efficient production regime in the United States and the poor diet available in India. Herrero et al. [44] estimate, however, that in 2000, mixed-crop livestock produced 69% of the milk produced

TABLE 14.1
Milk Production Statistics for 2011 (Fresh Whole Milk, Cow), Top 20 Producing Countries

Rank	Area	Production (Metric Tonnes)
	World	614,578,723
1	USA	89,015,235
2	India	57,400,000 (unofficial figures)
3	China (mainland)	36,578,000
4	Brazil	32,096,214
5	Germany	30,301,359
6	Russian Federation	31,385,732
7	France	24,361,094
8	New Zealand	17,893,848
9	Turkey	13,802,428
10	United Kingdom	13,849,000
11	Pakistan	12,906,000
12	Poland	12,413,796
13	Netherlands	11,627,312
14	Argentina	11,206,000
15	Mexico	10,724,288
16	Italy	10,479,053
17	Ukraine	10,804,000
18	Australia	9,101,000
19	Canada	8,400,000
20	Japan	7,474,309

globally, contributing substantially to livelihoods of the poorer farmers in the developing world. They also calculate that feed efficiencies for the production of edible protein from milk are between 1.5 and 5 times higher than that of meat production from ruminants, whatever the region they considered. The cow is an efficient converter of grass and low digestibility fiber into a food acceptable to man.

14.2 LACTATION, EVOLUTION, AND MILK SYNTHESIS

Mammals are warm-blooded vertebrate animals that nourish their young with milk produced by mammary glands. They first appeared approximately 166 million years ago. The phylogenetic tree of Figure 14.1 shows the approximate divergence times of major classes and infraclasses with extant examples of their members. The reproductive strategy, developmental requirements of the young, and environment of the mother–infant pair drive variation in milk composition among species. Platypus and marsupial neonates are embryonic in appearance and dependent on milk for growth and immunological protection during the equivalent of the fetal period in placental mammals. In contrast, placental mammals have relatively longer gestation and shorter lactation periods. These reproductive strategies directly impact milk composition. The data in Table 14.2 illustrate that even the gross macronutrient composition of milk can be highly variable among species. Note also that these are representative compositions that can vary significantly depending on the stage of lactation (particularly) and the nutritional status of the mother.

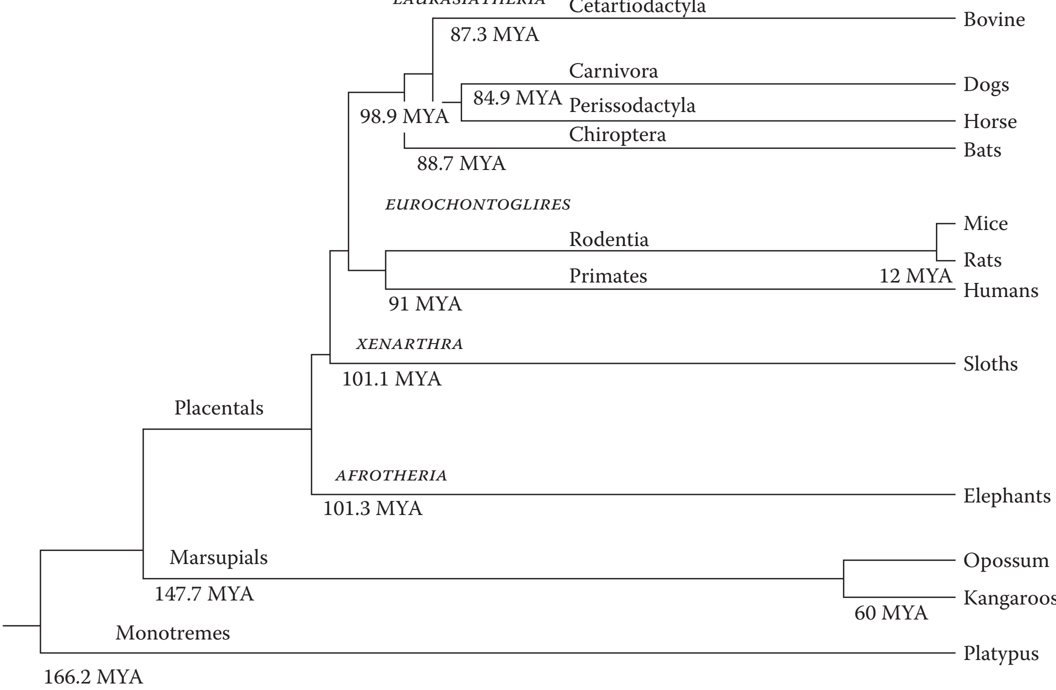


FIGURE 14.1 Simplified phylogenetic tree illustrating the relationship of representative extant mammalian species. Estimates of origin of major branch points are given in millions of years ago (MYA). The two earliest splits established monotremes (166.2 MYA) and marsupials and placentals (147.7 MYA). Approximately 50 million years pass before the origin of any extant groups, and then the four placental supergroups (italicized capitals) arose within 2.4 million years of each other. (Reproduced from LeMay, D.G. et al., *Genome Biol.*, 10, R43, 1, 2009. With permission.)

TABLE 14.2
Gross Macronutrient Composition of Mammalian Milk

Species	Fat (%)	Protein (%)	Lactose and Sugar (%)
Bovine	3.7	3.4	4.6
Dog	9.5	7.5	3.8
Horse	1.9	2.5	6.2
Bat	13.5	7.4	3.3
Mouse	27	12.5	2.6
Rat	8.8	8.1	3.8
Human	4	1	7
Sloth	6.9	6.1	?
Elephant	11.6	4.9	4.7
Opossum	7.4	10	10 (oligosaccharides)
Wallaby (~36 weeks)	20	13	12
Platypus	22.2	8.2	3.7 (difucosylactose)
Gray seal	53.1	11.2	0.7

Source: Adapted from Lemay, D.G. et al., *Genome Biol.*, 10, R43.1, 2009, with additions.

Milk, being the sole source of nourishment for the neonate, contains all the essential nutrients required for growth and development. The daily output of the well-fed cow might contain the equivalent of 2 kg of cheese, 1 kg of butter, and 1 kg of sugar. These do not come shrink or foil-wrapped or packaged for the supermarket shelf or chill cabinet. Nor is milk simply a soup of the individual nutrients. Rather it is the ensemble of biomolecules in complex higher-ordered structures. The most abundant milk proteins, the caseins, are delivered as micelles; fat is delivered as milk fat globules (MFG); and carbohydrate is delivered in solution as the disaccharide lactose.

Casein micelles and fat globules are considered in detail in later sections. Here, it is sufficient to mention that in an analysis of genomes from a range of animal species [77], milk fat globule membrane (MFGM) proteins were found to be very highly conserved, with 98%–100% of their amino acid sequences unchanged in the 160 million years since the common mammalian ancestor. The same analysis showed that the most divergent milk proteins were those with nutritional or immunological properties, such as the most abundant proteins, the caseins, but that on average milk and mammary genes were *per se* more highly conserved than other genes in the mammalian genome. Moreover, though there is significant divergence in the caseins, their organized assembled structure in milk, the casein micelle, is found in all milks examined to date, including those of marsupials. This suggests that the secretory process for milk production was established more than 150 million years ago and inherited from a premammalian ancestor.

A variety of theories have been put forward to explain the origin of the mammary gland, but the absence of direct fossil evidence of any stage of its development makes it difficult to validate or disprove any suggestions. Such hypotheses as have been proposed are summarized by Oftedal [100–102]. Ancestral sweat glands, sebaceous glands, hair follicles, and apocrine glands have all been proposed as candidates for the precursor of primordial mammary glands. Confounding the problem of tracing the evolutionary development is the recognition that the extent of glandular proliferation and output, the remarkable repeated cycles of proliferation and secretion followed by cell apoptosis and gland involution, and the multiplicity of secretory products formed in the mammary gland all represent evolutionary novelties [102]. Many of these processes involved the development of elaborate hormonal controls that require activation through other bodily changes, such as pregnancy and parturition.

14.2.1 Milk Biosynthesis

The mammary gland is an amazingly productive organ for biosynthesis. Most research on the physiology and functioning of the gland has been performed on human, ruminant, or rodent tissue, but all show such striking similarities in their results as to suggest the same mechanisms of secretion are common across all species.

Milk originating in the secretory tissue collects in ducts that increase in size as the teat region is approached. The smallest complete milk factory, which includes a storage region, is the alveolus (Figure 14.2). It is a roughly spherical organism consisting of a central storage volume (the lumen) surrounded by a single layer of secretory epithelial cells, which is connected directly to the duct system. These cells are directionally oriented such that the apical end with its unique membrane is positioned next to the lumen and the basal end is separated from blood and lymph by a basement membrane. Consequently, a directional flow of metabolites occurs through the cell, with the building blocks of milk entering through the basolateral membrane. The pathways followed through the cell by the main

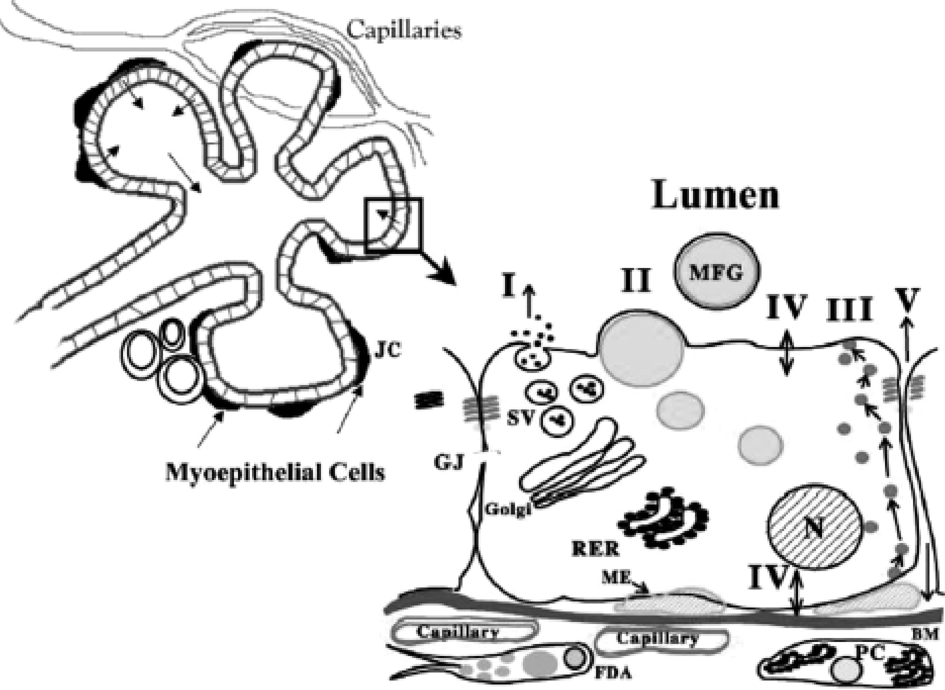


FIGURE 14.2 Diagram of mammary alveolus and alveolar epithelial cell showing pathways for milk secretion. Milk is secreted by alveolar epithelial cells into the lumen (arrows). It is then expressed through the ducts by contraction of myoepithelial cells that surround alveolar and ductal epithelial cells. The alveolus is surrounded by a well-developed vasculature and a stroma comprising extracellular matrix components, fibroblasts, and adipocytes. The region indicated by the box is expanded to show key structural and transport properties of alveolar cells. Pathway I depicts exocytotic secretion of milk proteins, lactose, calcium, and other components of the aqueous phase of milk. Pathway II depicts milk fat secretion with formation of cytoplasmic lipid droplets that move to the apical membrane to be secreted as membrane-bound milk fat globules. Pathway III depicts vesicular transcytosis of proteins such as immunoglobulins from the interstitial space. Pathway IV depicts transporters for the direct movement of monovalent ions, water and glucose across the apical and basal membranes of the cell. Pathway V depicts transport through the paracellular pathway for plasma components and leukocytes. Pathway V is open only during pregnancy, during involution, and in inflammatory states such as mastitis. Abbreviations: SV, secretory vesicle; RER, rough endoplasmic reticulum; BM, basement membrane; N, nucleus; PC, plasma cell; FDA, fat depleted adipocyte; JC, junctional complex containing the tight and adherens junctions; GJ, gap junctions; ME, myoepithelial cell. (Reproduced from McManaman, JL and Neville, MC. *Adv. Drug Delivery Rev*, 55, 451, 2003. With permission.)

milk components, proteins, fat, lactose, ions, etc., are depicted in the diagram in Figure 14.2. The basic components are synthesized on the production line of the endoplasmic reticulum. The components are then packaged in secretory vesicles in the Golgi apparatus or as lipid droplets in the cytoplasm.

These pathways have been known for a long time. What is not known with any certainty are the factors controlling the rates of synthesis at the molecular level. It is clear that the proteins being produced are milk specific, with very minor or no expression in tissues other than the lactating (or approaching lactation) mammary gland. Investigations so far have clearly shown that the expression of major milk proteins increases dramatically and in a concerted way during the onset of lactation and remains high until lactation declines (with some exceptions in marsupials) [3]. It is known that several hormones, the extracellular matrix, and cell-to-cell interaction play a pivotal role in inducing adaptation, with differences between species. Nutrition is known to influence the quantity of milk proteins produced, with energy content of the diet being particularly important. It has been demonstrated that the supply and transport of amino acids does not limit protein synthesis in the mammary gland. Indeed some studies [3] have found a decrease in the expression of ribosomal proteins, that is, a decrease in protein synthesis machinery within the mammary epithelial cell. But this may only be a mechanism for optimizing the translation and synthesis of milk-related proteins in order to increase the mammary gland's primary function, production of milk.

Fat is also synthesized within the mammary epithelial cell, appearing first as small lipid droplets originating from the endoplasmic reticulum. It appears that the droplet lipids accumulate between the outer and inner halves of the bilayer membrane and are then released from the endoplasmic reticulum into the cytosol as droplets coated with the outer half of the endoplasmic reticulum membrane [43]. Droplets appear to grow in volume by fusing with each other, giving rise to larger droplets termed cytoplasmic lipid droplets (CLDs), but as yet the mechanism controlling their growth, whether random or regulated, remains unknown. Lipid droplets must then transit from their sites of origin to the apical region of the cell (Pathway II in Figure 14.2). Again, what guides this apical migration is not known with certainty. At the apical membrane, the lipid droplets are progressively enveloped by that membrane up to the point where the lipid droplets become pinched off from the cell completely surrounded by plasma membrane. The outer coat on the MFGs appears as a classic bilayer membrane, termed *m*ilk *f*at *g*lobule *m*embrane (MFGM) derived mostly if not entirely from apical plasma membrane [43]. The mechanisms controlling milk fat secretion remain unknown.

14.3 COW'S MILK COMPOSITION

From now on, when we refer to milk, we will be concerned solely with bovine milk. As already noted, the majority of milk utilized by the dairy industries across the world comes from the cow. Its average composition with respect to major classes of compounds is given in Table 14.3 for some

TABLE 14.3
Composition (g/100 g) of Bovine Milk from Major Dairy Cattle Breeds

Breed	Fat	Protein	Lactose	Ash	Total Solids
Holstein	3.6	3.0	4.6	0.7	11.9
Brown Swiss	3.8	3.2	4.8	0.7	12.7
Ayrshire	4.0	3.3	4.6	0.7	12.7
Guernsey	4.6	3.5	4.6	0.8	13.7
Jersey	5.0	3.7	4.7	0.8	14.2

Source: After Huppertz, T. and Kelly, A.L., Properties and constituents of cow's milk, in *Milk Processing and Quality Management*, Tamime, A.Y., ed, Wiley-Blackwell Publishing, Oxford, U.K., 2009, pp. 23–47.

of the major breeds of dairy cattle. The greatest variability in composition is exhibited by the lipid fraction. Milk composition is influenced both by diet and breed, and the success of selection of cows for high fat production is readily evident.

Because of the major contribution of lactose and milk salts to osmolality, and the required matching of milk's osmotic pressure with that of blood, very little variability is observed in the sum of these constituents. It should be noted that ash does not truly represent milk salts because organic acids are destroyed by ashing; for example, various salts of citrate are principal components of the milk salt system [82].

14.3.1 Milk Proteins

Milk contains from 30 to 36 g/L of total protein. Table 14.4 lists the concentrations of major proteins in the skimmed milk fraction, that is, the subphase remaining when the lipid (fat) has been removed by centrifugation. There are six major gene products of the mammary gland. Four belong to the casein family, α_{S1}-casein, α_{S2}-casein, β-casein, and κ-casein, while two are termed whey proteins, β-lactoglobulin and α-lactalbumin. All of the caseins, in combination with calcium phosphate, exist in milk in a unique, highly hydrated spherical aggregated complex known as the casein micelle. Casein micelles have a broad range of sizes from 30 to 600 nm in diameter, with an average around 200 nm. Whey proteins, in contrast, are largely monomeric or dimeric and exist in the nonmicellar, aqueous phase of milk.

Milk proteins can be readily separated into casein and whey protein fractions. Curd may be formed by the agglomeration of casein micelles following enzyme treatment in cheese manufacture. The other proteins pass into the cheese whey, hence their designation as whey proteins. Separation of the caseins may also be achieved by their precipitation at their isoelectric point (around pH 4.6), yielding so-called acid casein, and a solution of whey proteins. High-speed centrifugation can also be employed to sediment the casein micelles, yielding a supernatant containing the whey proteins. Finally membrane filtration techniques using suitable pore-sized membranes have been developed for this purpose.

In addition to β-lactoglobulin and α-lactalbumin, which are gene products of the mammary gland, whey also contains serum albumin and immunoglobulins (Igs) that are derived from blood, as well as trace amounts of enzymes. Milk also contains several "protein" components that are actually large polypeptides; they are termed proteose-peptones. These arise from posttranslational proteolysis of milk proteins (mainly β-casein) by indigenous milk enzyme plasmin, which is derived

TABLE 14.4
Concentrations of Major Proteins in Milk

Protein		Concentration (g/L)	Percentage of Total Protein (Approx.)
Caseins		24–28	80
	α_{S1}-Caseins	12–15	34
	α_{S2}-Caseins	3–4	8
	β-Caseins	9–11	25
	κ-Caseins	3–4	9
	γ-Caseins	1–2	4
Whey proteins		5–7	20
	β-Lactoglobulin	2–4	9
	α-Lactalbumin	1–1.5	4
	Proteose-peptones	0.6–1.8	4
Blood proteins			
	Serum albumin	0.1–0.4	1
	Immunoglobulins	0.6–1.0	2
Total			100

from blood. Their larger fraction, so-called γ-caseins, remain attached to and precipitate with the casein micelle. Large or increasing amounts of γ-caseins are often indicative of mastitis.

14.3.1.1 Caseins and Casein Micelles

The caseins are a family of phosphoproteins, comprising approximately 80% of bovine milk protein. In the bovine family, the more highly phosphorylated members (α_{S1}-casein, α_{S2}-casein, and β-casein) are termed calcium-sensitive and individually are readily precipitated by Ca^{2+}. By contrast, κ-casein, with only one or at most two phosphoserine residues, is calcium-insensitive and in admixture with the calcium-sensitive members stabilizes their calcium-induced aggregates to nonprecipitating colloidal dimensions. Because the caseins utilize the same calcium-sequestering mechanisms to regulate the calcium phosphate concentration of their environment, they have been identified as members of a wider family of secretory calcium-binding phosphoproteins (SCPP) descended from a common ancestor gene [68,69]. All SCPP are descended from early primordial genes by duplication and divergence to serve their specialized adaptive functions. It is thought that primordial calcium-sensitive caseins diverged from matrix protein genes before the appearance of monotremes in the Jurassic era [68]. More controversially, Kawasaki et al. [70] have argued that all caseins, both calcium-sensitive and calcium-insensitive, that is, κ-type caseins, have a common ancestor gene, odontogenic ameloblast–associated gene, and evolved from it via two different pathways.

Casein allows milk to appear supersaturated with calcium phosphate. Essentially, in micellar form, it transports safely through the mammary gland, the mineral calcium phosphate that is essential for the developments of bones and teeth in the suckling infant.

14.3.1.2 Casein Primary Structure and Interactions

The amino acid compositions and primary sequences of the caseins are known from both chemical sequencing and inference from gene sequencing. For a more complete discussion of the characteristics of the individual caseins, the reader is referred to several excellent reviews [61,62,104]. For earlier work, the chapter by H.E. Swaisgood in the fourth edition of *Fennema's Food Chemistry* (2007) is an excellent source [127].

As a result of sequence analysis, the caseins were identified as members of the wider secretory calcium phosphate binding family by their possession of functional and sequence features common to that family [68,69]. Among the conserved motifs is the SXE peptide (Ser-X_{aa}-Glu) where X_{aa} may be any amino acid. In the caseins, this peptide provides a recognition template for posttranslational phosphorylation of the serine in the mammary gland by a casein kinase [93]. Moreover, in the caseins, the serine residues are often found clustered in groups of two, three, or four. Such clusters in α_{S1}-casein and β-casein are highly conserved [89], and their numbers attest to the significance of the calcium phosphate requirement for postnatal growth in mammals, even more so when it is noted that bovine α_{S1}- and α_{S2}-caseins possess two or more such clusters (Figure 14.3).

α_{S1}-casein (8P)
---- -SerP$_{46}$-Glu-SerP$_{48}$-Thr-Glu- ----
-SerP$_{64}$-Ile-SerP$_{66}$-SerP$_{67}$-SerP$_{68}$-Glu-Glu ------ -SerP$_{75}$- -----SerP$_{115}$- ------

α_{S1}-casein (11 or 12P)
---- SerP$_{8}$-SerP$_{9}$-SerP$_{10}$-Glu-Glu- --- -SerP$_{16}$- ------
-SerP$_{56}$-SerP$_{57}$-SerP$_{58}$-Glu-Glu-SerP$_{61}$-Ala-Glu- ---------

β-casein (5P)
-------SerP$_{15}$-Leu-SerP$_{17}$-SerP$_{18}$-SerP$_{19}$-Glu-Glu---------SerP$_{35}$-Glu-Glu---------

κ-casein (1P)
---------------- -SerP$_{149}$-Pro-Glu- -------

FIGURE 14.3 Anionic clusters in bovine caseins.

These clusters of phosphoserine residues and the necessary glutamic acid residues templating their existence would, in the individual molecule, carry significantly high densities of negative charge at normal milk pH. There is a charge density of −9*e* within the span of residues 65–72 of α_{S1}-casein and a further −6*e* along the sequence 48–53 of the same protein. A similarly high charge density of −9*e* is found between residues 16–23 of β-casein, encompassing the phosphoserine cluster there. Similar high densities are found around the phosphoserine clusters of α_{S2}-casein. The existence of the phosphoserine clusters and their accompanying high charge are at the root of the calcium sensitivity of the α_S- and β-caseins, since neutralizing these charges through calcium binding leads to the precipitation of the protein.

Away from the phosphoserine clusters, the casein molecules are distinctly hydrophobic. This segregation of hydrophilic and hydrophobic residues confers on the caseins a definite amphipathic nature, which contributes to their ability to function successfully as stabilizers in oil-in-water emulsions [54,57]. The sequence hydrophobicity plots obtained by computing a moving average of amino acid hydrophobicity with a five-residue window are plotted in Figure 14.4, for the four casein sequences. We can consider these in more detail by locating hydrophobic clusters along the caseins. These are recognized as discrete when separated by four or more nonhydrophobic residues. The hydrophobic acids are VILFMYW (see Chapter 5 for one-letter codes for amino acids), any other is taken as nonhydrophobic. The hydrophobic clusters so isolated are indicated as discrete bars above the hydrophobicity profiles (Figure 14.4). In hydrophobic bonding, we would envisage one of these clusters interacting with any other on its own molecule or on any other molecule. The possibilities are therefore legion. Though many, such bonds are ephemeral, labile, and weak.

The individual caseins do self-associate but apparently in such a way that the hydrophilic phosphoserine clusters protrude into the aqueous phase as spatially spread as possible. β-Casein forms detergent-like micelles, while α_{S1}-casein forms wormlike chains, the degree of polymerization promoted by decreasing solution pH or increasing ionic strength (see Ref. 54 for more extended discussion). Compared with hydrophobic interaction, electrostatic repulsion is a long-range force. The importance of charge in controlling the extent of aggregation of the caseins cannot be stressed too highly. It manifests itself in limiting the self-association of these proteins, in their isoelectric precipitation as pH is lowered, and in the calcium sensitivity of the α_S- and β-caseins. It also plays a significant part in casein micelle assembly, as discussed in the following section.

The calcium-insensitive κ-casein is also very amphipathic with distinct polar and hydrophobic domains, the latter with a number of discrete hydrophobic clusters (Figure 14.4). As we will see later, κ-casein plays an essential role in the formation of casein micelles. Experimental evidence points to a surface location for this protein and a role in limiting casein micelle size [17,25]. κ-Casein also has an important physiological function in that it possesses a unique recognition sequence that is specific for limited proteolysis by the enzyme, chymosin, in the stomach of the calf, allowing release of the polar domain and coagulation of the micelles. Provided by nature to aid the digestion of the milk by the calf, this proteolysis reaction is exploited by the dairy industry as the first stage leading to curd formation in cheesemaking.

Instead of anionic phosphoseryl clusters characteristic of the calcium-sensitive caseins, the seryl and threonyl residues located in the polar domain of κ-casein are often glycosylated [45]. This post-translational modification results in the attachment of tri- or tetrasaccharide moieties that include N-acetylneuraminic acid residues (AcNeu). There are no cationic residues in the C-terminal 53 residue polar domain, which in the nonglycosylated form has a net charge of −11 at pH 6.6. Potentially one or two seryl residues in the polar domain could be phosphorylated but these are remote from one another. Additional charges could accrue via attachment of the AcNeu chains. This high negative charge, many other polar residues, and the presence of eight evenly spaced proline residues create a highly hydrated, open, and flexible structure. This polar domain is attached sequentially to a very large hydrophobic domain with many hydrophobic cluster sites that are potentially able to interact with the other caseins. The κ-casein in a surface location on the casein micelle fulfils

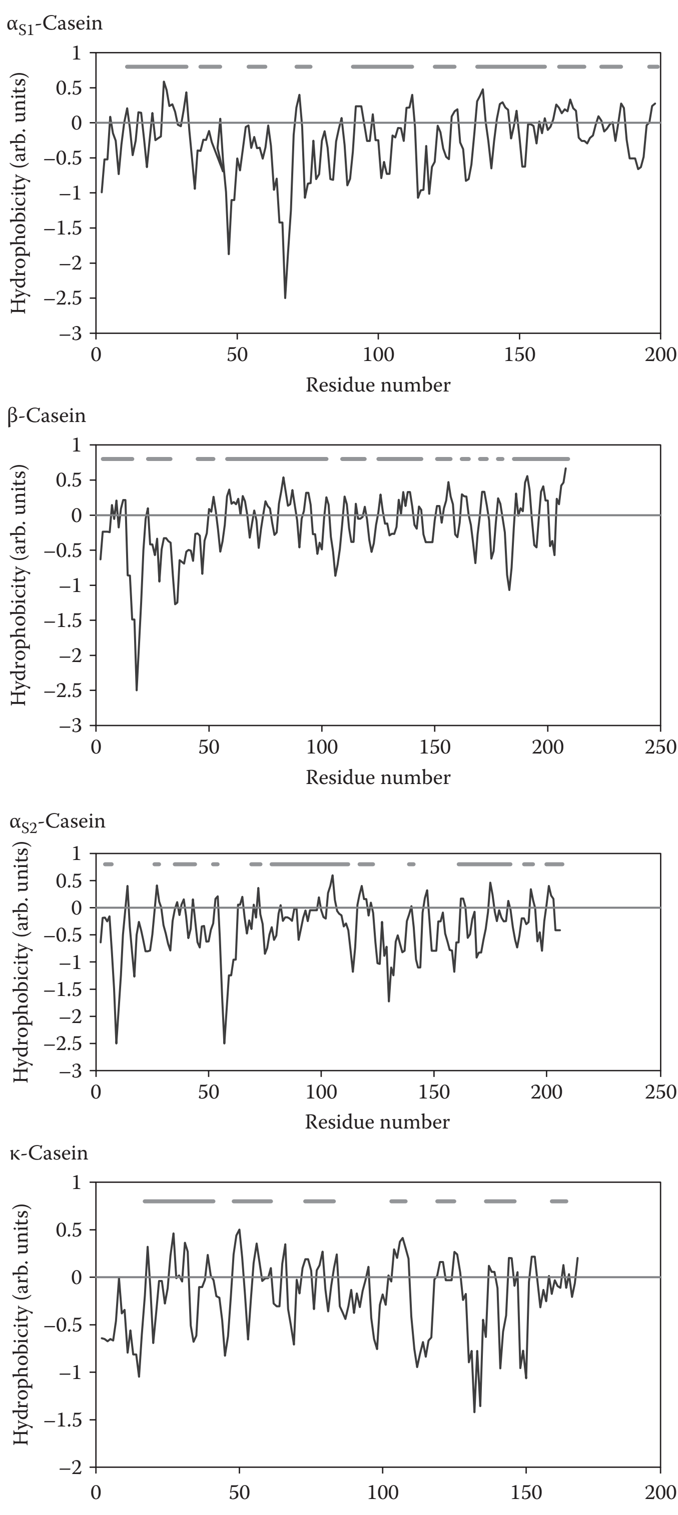


FIGURE 14.4 Hydrophobicity plots of the four caseins calculated as a moving average (window n = 3) of amino acid hydrophobicities taken from consensus scale used by Horne [51]. Bars above plots denote hydrophobic clusters, lengths denoting their size.

the role of a colloidal steric stabilizer. The chymosin proteolysis site of the Phe-Met bond at the N-terminus of the polar domain means that cleavage leads to the loss of the domain and its attendant steric stabilizing capability, followed by aggregation and curd formation in the milk.

14.3.1.3 Casein Micelle Structure and Assembly

As a consequence of their phosphorylation and amphiphilic structures, caseins interact with each other and calcium phosphate to form large spherical micelles with mean diameters of approximately 200 nm but with a wide size range. Light scattering by these complexes and the fat globules is responsible for the white appearance of milk. Bovine micelles are 92% by weight protein, composed of α_{S1}:α_{S2}:β:κ-caseins in approximate mole ratios 3:1:3:1 and 8% by weight milk salts, composed primarily of calcium phosphate but also significant amounts of Mg^{2+} and citrate. The characteristics of micelles determine the behavior of milk and milk products during industrial processing and storage; therefore, the properties of natural micelles and model systems, including so-called micellar phosphocaseinates (MPCs), have received considerable coverage (see reviews by DeKruif and Holt [20], Payens [108], Rollema [112], and Schmidt [114]).

Micelles have a porous, "spongy" structure with a large voluminosity, approximately 4 mL/g of casein, and exceptional hydration of 3.7 g H_2O/g casein. This hydration is an order of magnitude larger than that of typical globular proteins. Hence, large molecules, even proteins, have access to and can equilibrate with all parts of the micelle structure. All components of the micelle are apparently in slow equilibria with milk serum. Thus, under appropriate conditions, various caseins and milk salts can be reversibly dissociated from the micelle. Somewhat surprisingly such dissociation may occur to a limited extent without any apparent change in micelle (hydrodynamic) size. Lowering the temperature to near 0°C causes some β-casein, κ-casein, and colloidal calcium phosphate to reversibly dissociate. Lowering pH promotes loss of calcium phosphate. However, at physiological temperature and natural pH, the amount of individual caseins in the serum is extremely small.

Because the structure of the micelle has not been directly determined, there is no certainty as to the location of individual casein molecules. Nevertheless, as already mentioned, all evidence points to a predominant surface location for κ-casein, while α_S- and β-caseins dominate in the interior. The distribution may not be exclusive, however, because the calcium-sensitive caseins may also be accessible on the surface, a picture confounded by the openness of the micelle structure. That κ-casein should dominate the surface is based on the observation that the amount of κ-casein increases linearly with the surface/volume ratio. The same results show that the content of β-casein in the region of the surface decreases linearly. Note that the surface/volume ratio for a sphere increases as the reciprocal of size.

Early electron micrographs of the casein micelle showed a raspberry-like appearance (see, e.g., Reference 115). More recent electron microscopy studies [85,88,125] have suggested that these well-defined structures are likely to be artifacts of the fixation process, although micrographs obtained by field emission scanning electron microscopy show complex surface structure of cylindrical or tubular, but not spherical, protrusions between 10 and 20 nm in diameter extending from the surface of the micelle [18]. These samples were not metal-coated, although they were of necessity subjected to a fixation and dehydration process, which might have introduced some collapse of more loosely bound protein onto a denser skeleton. The cryo-TEM pictures of Marchin et al. [88] illuminate the finer detail of the micellar structure showing small regions of high electron density, approximately 2.5 nm in diameter, apparently uniformly distributed in a homogeneous web of protein. This gives the micelle a granular aspect that diminished as the pH was reduced from 6.7 to 5.2 [88], paralleling the known dissociation of calcium phosphate on acidification, but without loss of micellar integrity. This confirms the involvement of more than one type of bonding in maintaining that integrity. Though apparently homogeneous in the pictures resulting from the electron microscopy studies of McMahon and McManus [85] and Marchin et al. [88], the topographic images from the study of Trejo et al. [125], obtained by varying the angle of incidence of the electron beam, reveal a heterogeneous skeletal structure with water-filled channels and cavities, the skeletal structure converging on higher density nodes,

assumed to be calcium phosphate clusters. This openness of structure chimes with observations on the internal accessibility of the micelles to enzymes and the ready release of β-casein. It is also supported by the nonaffine response of casein micelle suspensions to osmotic compression [5].

Casein micelle structure is not fixed, but *dynamic*. In various ways, it responds to changes in micellar environment, temperature, and pressure. Cooling milk on release from the udder at 37°C to storage at refrigeration temperatures brings about significant solubilization of β-casein, some κ-casein and much lower amounts of α_{S1}- and α_{S2}-casein from the micelles. Raising the temperature back to 37°C reverses the process. None of this movement of β-casein does anything to disrupt the main internal structure of the micelle. Significantly, the colloidal calcium phosphate can also be solubilized by lowering the pH but, again, without substantial disruption of the micelle structure, though this response is temperature dependent in line with the observation regarding the flow of β- and κ-casein. Also, the remaining entity, following calcium phosphate loss, shows a softening in its response to AFM probes [106].

Casein micelle models have been extensively reviewed (see Reference 54 for a comprehensive list of references). Based on the biochemical and physical properties of the micelles and the casein proteins themselves, three main models have been proposed: the submicelle model [115,120], the nanocluster model [20,48], and the dual-binding model [52,53].

In the submicelle model, the casein micelles are composed of smaller proteinaceous subunits, the submicelles, which are preassembled and then linked together via colloidal calcium phosphate. In the Holt model, the nanoclusters of colloidal calcium phosphate are randomly distributed, cross-linking, through phosphoserine clusters, a three-dimensional web of casein molecules. Both of these models have been severely criticized [29,52] and the dual-binding model was created to overcome their deficiencies. It is rooted in the interactions and chemistry of both the caseins and calcium phosphate. Cross-linking of protein chains through the calcium phosphate nanocrystals contributes one pathway for network formation in the micelle, just as in the Holt model, but crucially, rather than simply adsorbing to a preformed crystal, the highly charged phosphoserine clusters of α_S- and β-caseins are envisaged in the dual-binding model to act as both a template for the initiation of mineral crystal growth and a terminating cap on that growth [60]. Network growth is achieved through the multiplicity of phosphoserine clusters on each α_S-casein molecule, but β-casein only has one such cluster (Figure 14.3) and would terminate such progression were it not for the fact that the neutralization of the high negative charge of the phosphoserine cluster allows sufficient diminution of electrostatic repulsion between the molecules to permit hydrophobic bonds to form. Similar interactions are permitted between hydrophobic regions of the α_S-caseins, up-sequence and down-sequence of the now neutralized phosphoserine clusters, forming two interlinked networks in the terminology of McMahon and Oommen [86] in the dual-binding structural model of the casein micelle.

This is essentially a polyfunctional condensation model with two types of functionalities, the phosphoserine clusters on the calcium-sensitive caseins and the hydrophobic clusters grouped in the hydrophobic domains of all of the caseins. Because κ-casein does not possess a phosphoserine cluster, it does not enter into the first reaction pathway, cross-linking of phosphoserine clusters through calcium phosphate nanocrystals. It does possess a hydrophobic domain and can enter into hydrophobic bonding with other caseins when favorable energy conditions ensue, that is, when local electrostatic repulsion has been neutralized. It cannot, however, further extend the network bridging between hydrophobic domains because the glycosylated C-terminal portion of the molecule is hydrophilic. Network growth is therefore terminated in this region on acquisition of a κ-casein molecule, and the micelle acquires a surface coating of κ-casein. Furthermore, the κ-casein content controls micelle size. Since the arrival of a κ-casein molecule at any point may be regarded as a random event, a range of micelle sizes is generated, with the mean dictated by overall κ-casein levels. The dual-binding model therefore fulfils a prime requirement set by empirical observations. Further examples of how the dual-binding model predicts the properties of the casein micelles and their response to the processing and destabilization treatments applied by the dairy industry are expanded in the texts of Horne [54] and Dalgleish and Corredig [16].

14.3.1.4 Whey Proteins

The whey proteins comprise approximately 20% by weight of the protein in bovine milk. β-Lactoglobulin and α-lactalbumin represent almost 80% by weight of whey protein, β-lactoglobulin alone approaching 55%. Their properties and behavior have been reviewed recently by Sawyer [113], Brew [6], and Edwards and Jameson [26]. Some methods of separation of whey proteins from milk have already been mentioned. Discussion on membrane separation techniques is covered in Section 14.4.7.

The structure of β-lactoglobulin and α-lactalbumin are typical of those of other globular proteins. Similar to the caseins, they carry a negative charge at the pH of milk; however, unlike the caseins, the sequence distribution of hydrophobic, polar, and charged residues is rather uniform. Consequently, these proteins fold intramolecularly, thereby burying most of their hydrophobic residues so that extensive self-association with other protein molecules does not occur. The three-dimensional structures have been determined by x-ray crystallography, and the aqueous solution structure of β-lactoglobulin by NMR spectroscopy [75,126].

14.3.1.4.1 β-Lactoglobulin

β-Lactoglobulin is a member of the lipocalin family of proteins, so called because of their ability to bind small hydrophobic molecules into a hydrophobic cuplike cavity. The quaternary structure of the protein ranges among monomers, dimers, or oligomers depending on the pH, temperature, and ionic strength, with the dimer being the prevalent form in bovine milk under physiological conditions. The dimer structure is shown in Figure 14.5 and shows bound 12-bromododecanoic acid in the hydrophobic cavities, one for each molecule of the dimer.

The thermal properties of β-lactoglobulin are of considerable industrial relevance because of their role in the fouling of processing equipment (heat exchangers and the like), as well as the functional properties that can be imparted to some dairy products by thermally induced β-lactoglobulin aggregation. At neutral pH, the midpoint of the thermal unfolding transition, as determined by

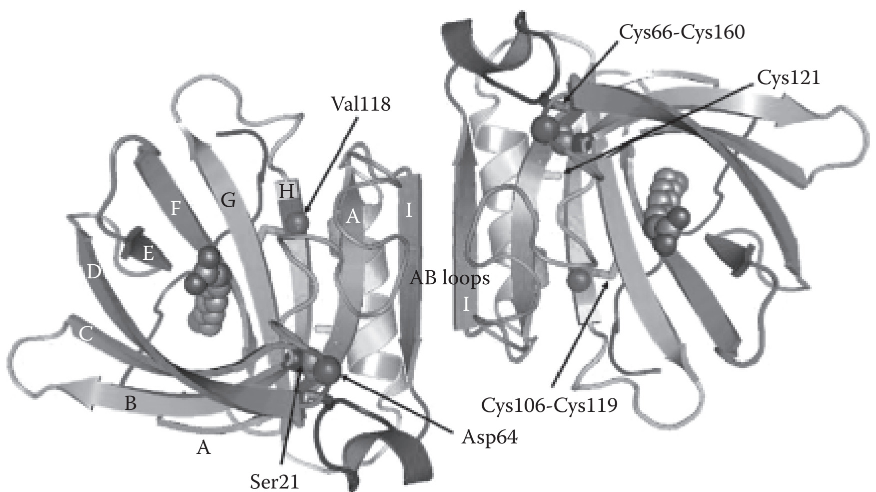


FIGURE 14.5 Diagram of the dimeric structure of bovine β-lactoglobulin A, looking down the twofold axis. The strands that form the β-barrel are labeled A to H. The I strand, together with part of the AB loop, forms the dimer interface at neutral pH. The location of the sites of difference between A and B variants are also shown. Ser_{21}, which shows conformational flexibility, and the bound 12-bromododecanoic anions are shown as spheres. (Reproduced from Edwards, P.J.B. and Jameson, G.B., Structure and stability of whey proteins, in *Milk Proteins: From Expression to Food*, 2nd edn., H. Singh, M. Boland, A. Thompson, eds, Elsevier/Academic Press, London, U.K., 2014, pp. 201–241. With permission.)

differential scanning calorimetry is ~70°C [21]. At this point, the dimer dissociates and the constituent molecules begin to unfold. This reveals the free thiol at Cys_{121} and a hydrophobic patch, opening up the possibility of both covalent and hydrophobic interactions [65]. The covalent disulfide interchanges can occur both intramolecularly and intermolecularly and, if in heated milk, with other whey proteins and importantly with κ-casein. The latter complexes impart favorable texture properties to acidified milk products such as yogurt, but impair the rennetability of milk. The reactions are complex and influenced by many factors. Summaries of current research can be found in the reviews of Anema [1] and Edwards and Jameson [26]. Denaturation and consequent reactions can also be achieved by high pressure treatments. Work in this area has been reviewed by Patel and Huppertz [107].

14.3.1.4.2 α-Lactalbumin

About 20% of the protein in bovine whey is α-lactalbumin. α-Lactalbumin is a compact, globular protein with striking primary sequence homology to hen's egg-white lysozyme. Fifty-four of the 123 residues are identical to those of lysozyme and the four disulfide bonds have similar locations. In the mammary secretory cell, the synthesis of lactose begins with the synthesis of α-lactalbumin in the rough endoplasmic reticulum. α-Lactalbumin is then transported to the Golgi apparatus. There it meets a transmembrane protein, β-1,4-galactosyl transferase, which, when bound to UDP-galactose, undergoes a conformational change that allows α-lactalbumin to be bound. With α-lactalbumin bound, the specificity of the β-1,4-galactosyl-transferase is altered to allow glucose to become the acceptor sugar for galactose transfer, resulting in the synthesis of lactose. Thus, α-lactalbumin acts as a regulator of β-1,4-galactosyl transferase, and without α-lactalbumin, the enzyme does not synthesize lactose under physiological conditions [6].

α-Lactalbumin is a metalloprotein, containing one Ca^{2+} per mole in a pocket containing four Asp residues. With the disulfide bonds intact, as the protein occurs in milk, the tertiary structure unfolds and refolds reversibly. Although α-lactalbumin denatures at a lower temperature than β-lactoglobulin, the transition is reversible except at very high temperatures. Thus, α-lactalbumin, unlike β-lactoglobulin, is not irreversibly thermally denatured under most milk processing conditions.

14.3.1.4.3 Serum Albumin, Immunoglobulins, and Lactoferrin

Normal milk contains 0.1–0.4 g/L of blood serum albumin, presumably as a result of leakage from blood. Its biological function in milk is unknown, but it may act as a promiscuous transporter of hydrophobic molecules (fatty acids) [26]. Its structure is notable for the high number of disulfide bridges.

Mature bovine milk contains from 0.6 to 1 g/L Igs, but colostrum contains 10% (w/v) Ig, the level of which declines rapidly postpartum. The young of the cow, sheep, and goat are born without Igs in their blood, but can absorb them from the intestine for several days after birth and thereby acquire passive immunity until they synthesize their own Ig within a few weeks after birth. In bovine milk the predominant species of Ig are members of the IgG subfamily. For a review of analysis and properties of bovine Ig, the reader may consult Gapper et al. [36].

Low levels of lactoferrin, less than 0.1% of total whey protein, are found in milk. Despite these low concentrations, commercial applications have emerged utilizing recovered bovine lactoferrin, and its partially digested peptides are appearing as nutraceuticals in infant formulas. Brock [7] has reviewed the properties and commercial applications of this protein.

14.3.2 Milk Lipids and Milk Fat Globules

14.3.2.1 Milk Lipids

Milk lipids or milk fat are an important source of dietary energy. For detailed characteristics of the various milk lipids and a discussion of their biosynthesis, the reader should consult one of the suggested reviews [43,71,83].

Milk contains about 3%–5% fat, secreted as globules, 2–6 μm in diameter, surrounded by a membrane of polar lipids and proteins termed the milk fat globule membrane (MFGM). Within the core of the fat globule, triacylglycerols are the main molecular form, comprising 96%–98% of the total weight (Table 14.5). Minor amounts of diacylglycerols, monoacylglycerols, free fatty acids, polar lipids and sterols, and trace amounts of fat-soluble vitamins and β-carotene are also present [83].

Triacylglycerols containing three different fatty acids have a chiral carbon at the sn-2 position of the glycerol skeleton. The fatty acid composition of milk fat is complex compared to other dietary fats (e.g., cocoa butter, sunflower oil, olive oil). More than 400 different fatty acids have been identified in bovine milk lipids. Consequently, if positional isomers are considered, 400^3 or 64 million triacylglycerol species are theoretically possible. However, only 13 fatty acids have been detected at concentrations exceeding 1% (w/w). The concentrations listed in Table 14.6 are representative of a fresh milk. Within a species, the fatty acid composition can vary as a function of genetics, stage of lactation, and diet. The fatty acids esterified into the triacylglycerol are derived either from plasma lipid or by *de novo* synthesis from small molecule precursors. In the bovine mammary gland, short- and

TABLE 14.5
Lipid Composition of Bovine Milk

Lipid	Weight Percent	g/L*
Triacylglycerols (triglycerides)	95.8	30.7
1,2-Diacylglycerols (diglycerides)	2.25	0.72
Monoacylglycerols (monoglycerides)	0.08	0.03
Free fatty acids	0.28	0.09
Phospholipids	1.11	0.36
Cholesterol	0.46	0.15
Cholesterol ester	0.02	0.006
Hydrocarbons	Trace	Trace

Source: Swaisgood, H.E., Characteristics of milk, in *Fennema's Food Chemistry*, 4th edn, Damodaran, S., Parkin, K.L., Fennema, O.R. eds., CRC Press, Boca Raton, FL, pp. 881–917, 2007.

* Based on butterfat percentage of a pasteurized whole milk, 3.2%.

TABLE 14.6
Major Fatty Acid Constituents of Bovine Milk Fat (Butter Fat Triglyceride Obtained after Cream Churning)

Fatty Acid	Weight Percent	Fatty Acid	Weight Percent
C4:0	3.81	C15:0	1.18
C6:0	2.51	C16:0	28.17
C8:0	1.60	C16:1	1.93
C10:0	3.68	C17:0	0.52
C12:0	4.26	C18:0	9.97
C14:0	11.74	C18:1	22.06
C14:1	0.94	C18:2	1.15
		C18:3	0.64

Source: Fong, B.Y. et al., *Int. Dairy J.*, 17, 275, 2007.

medium-chain length fatty acids, from C4:0 to C14:0 but also some C16:0, are the major products of *de novo* lipogenesis, whereas plasma lipids contribute longer chains and monounsaturated species.

Saturated fatty acids account for 65–70 wt% of bovine milk fatty acids, with C16:0 contributing 22–35 wt%. The unsaturated remainder is mainly C18:1 at about 20 wt%. The essential polyunsaturated fatty acids, C18:2 and C18:3, account for about 1.2–2 wt% and 0.5–0.7 wt%, respectively. In recent years, major research effort has been concentrated in attempting to shift the balance of saturated versus polyunsaturated fatty acids in milk fat. Various post- and on-farm strategies have been employed. Post-farm approaches include (1) dry fractionation of fat, (2) mixing milk fats with other fats richer in unsaturated fatty acids, for example, vegetable or fish oils, and (3) chemical and enzymatic interesterification. On-farm strategies employed include (1) animal selection and (2) feed manipulation with rapeseed or linseed oils. In the latter respect, the unsaturated fatty acid component must be fed in an encapsulated form to allow it to pass through the rumen unaltered.

With 13 fatty acids, the theoretical potential number of position isomers is reduced to 13^3 or 2197. Gresti et al. [42] isolated and identified 223 triacylglycerols with different composition, accounting for 80 wt% of the total milk fat. They determined that the distribution of fatty acids among the three sn-positions of the glycerol backbone is not random; this originates in the specific properties of acyl transferases in the ruminant mammary gland [95]. Briefly, the sn-glycerol-3-phosphate is the principal route for triacylglycerol biosynthesis, and long-chain acyl coenzyme A (especially palmitoyl coenzyme A) is the preferred substrate for acylation in position sn-1 and then sn-2. Conversely, the esterification of short-chain fatty acids in position sn-3, catalyzed by 1,2-diacyl-*sn*-glycerol acyl transferase, is more rapid than that of long-chain fatty acids. In consequence, it is found, by stereospecific analyses of total bovine triacylglycerols, that short-chain fatty acids are exclusively acylated on position sn-3, and long-chain saturated fatty acids are equally acylated in positions sn-1 and sn-2. Medium-chain fatty acids are esterified in the three positions, but proportions decrease at position sn-3 when the chain length of the fatty acid increases (Figure 14.6). Oleic acid is distributed nearly equally among the three positions. The overall pattern of fatty acid distribution in triacylglycerol molecules is not significantly affected by the diet of the animal. It has been suggested that this

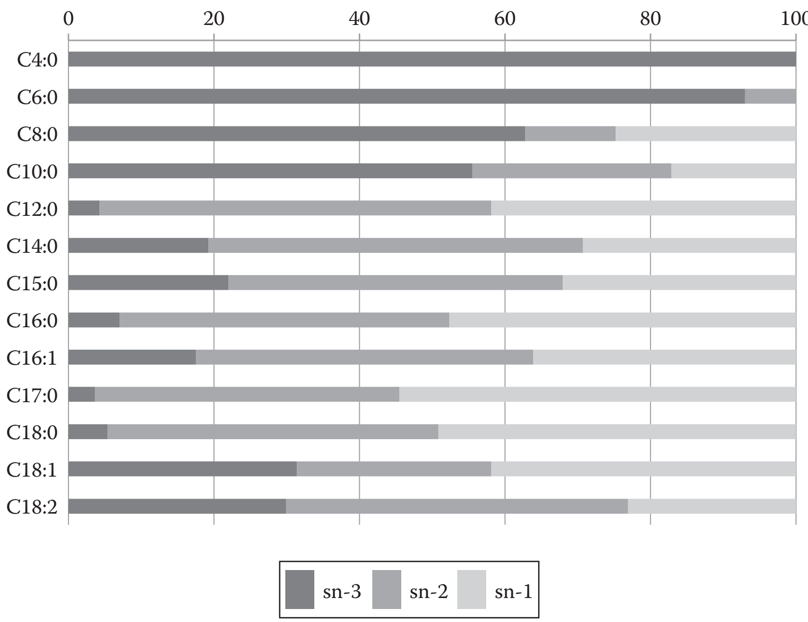


FIGURE 14.6 Percentage distributions of fatty acids among triacylglycerol positions, in bovine milk fat. (Adapted from Lopez, C., *Curr. Opin. Colloid Interface Sci.*, 16, 391, 2011.)

specific molecular bias results from a regulation during triacylglycerol synthesis to maintain fat in a liquid state at physiological temperatures.

Since the pioneering work of the late Professor Pieter Walstra in the 1960s, there have been few investigations of the crystallization properties of triacylglycerols within MFGs. The triacylglycerol lipids entrapped within the MFGs are liquid at the physiological temperature of their synthesis and secretion (36°C–39°C). Decreasing the temperature of the milk after collection and storage leads to a phase transition from the liquid to the solid state of triacylglycerol lipids and then to the formation of fat crystals. The crystallization and thermal properties of MFGs is a function of their fatty acid composition, the molecular structure of the triacylglycerols, and their polymorphism. The thermal history of the sample also has a significant influence on fat crystallization, as does the size of the fat globule. All of this means that bovine milk fat is a mixture of crystals and oil at temperatures of storage (4°C–7°C), of processing (7°C–30°C) and of consumption and digestion (37°C) of milk and dairy products.

As well as being of technological importance in the manufacture of dairy products, for example, in affecting the susceptibility of fat globules to churning or the structure and stability of ice cream, or influencing the rheological properties, consistency, and mouthfeel of high-fat products, the solid fat phase present within the MFG could have particular nutritional and health-related consequences. Triacylglycerol molecules that remain in the solid phase at the temperature of ingestion and digestion are composed of high melting point lipids that contain long-chain saturated fatty acids (C18:0 and C16:0). It has been reported that the presence of the solid phase limits the hydrolysis of triacylglycerols by digestive enzymes and their subsequent absorption [4], raising questions as to the bioavailability of fatty acids, especially long-chain saturated types.

14.3.2.2 Milk Fat Globule Membrane

The fat globules of milk are enveloped in a layer (or layers) of surface active material, which is called the MFG membrane. Essentially it is a tripartite structure (see Figure 14.7) thought to originate in the following fashion [43,92]. Within milk secretory cells, the triacylglycerides are synthesized in or on the surfaces of the rough endoplasmic reticulum membrane and accumulate in the form of microdroplets in the cytoplasm. These intracellular droplets are covered in a diffuse interfacial layer, which consists of phospholipids, glycosphingolipids, cholesterol, and proteins. The lipid microdroplets grow in volume by fusion to form CLDs of various sizes that are then translated to the apical pole of the cell through the cytoplasm by mechanisms yet unknown and are secreted from the epithelial cell. During secretion, the droplets are coated with the outer plasma membrane and are budded from the cell. The composition of the outer layer surrounding the fat globule is thus similar to that of the apical plasma membrane of the secretory cells.

Hence, the fat globules in milk are not a simple oil-in-water emulsion; the globules are surrounded by a complicated structured membrane, which cannot be considered as a simple monomolecular film of surface active material. Instead, the membrane has several distinct layers (Figure 14.7) that are laid down during its synthesis in the mammary secretory cell. As viewed from the lipid core outward, there is first an inner surface-active layer that surrounds the intracellular droplet, then a dense proteinaceous coat located on the inner surface of the bilayer membrane, and finally bilayer membrane.

14.3.2.3 Composition of Milk Fat Globule Membrane

The amount and composition of the membrane can vary considerably depending on both the fat content and the fat globule size, which are, in turn, affected by several factors, such as diet, breed, health, and stage of lactation of cows [92]. For example, in the fat globule size distribution, the smaller fat globules, because of their proportionately larger surface area, account for a larger proportion of the total MFGM material. It is estimated that the membrane material constitutes between 2 and 6 wt% of that of the total fat globules. Proteins and lipids together account for over 90% of the membrane dry weight, but the relative proportions of lipids and proteins may vary widely [118].

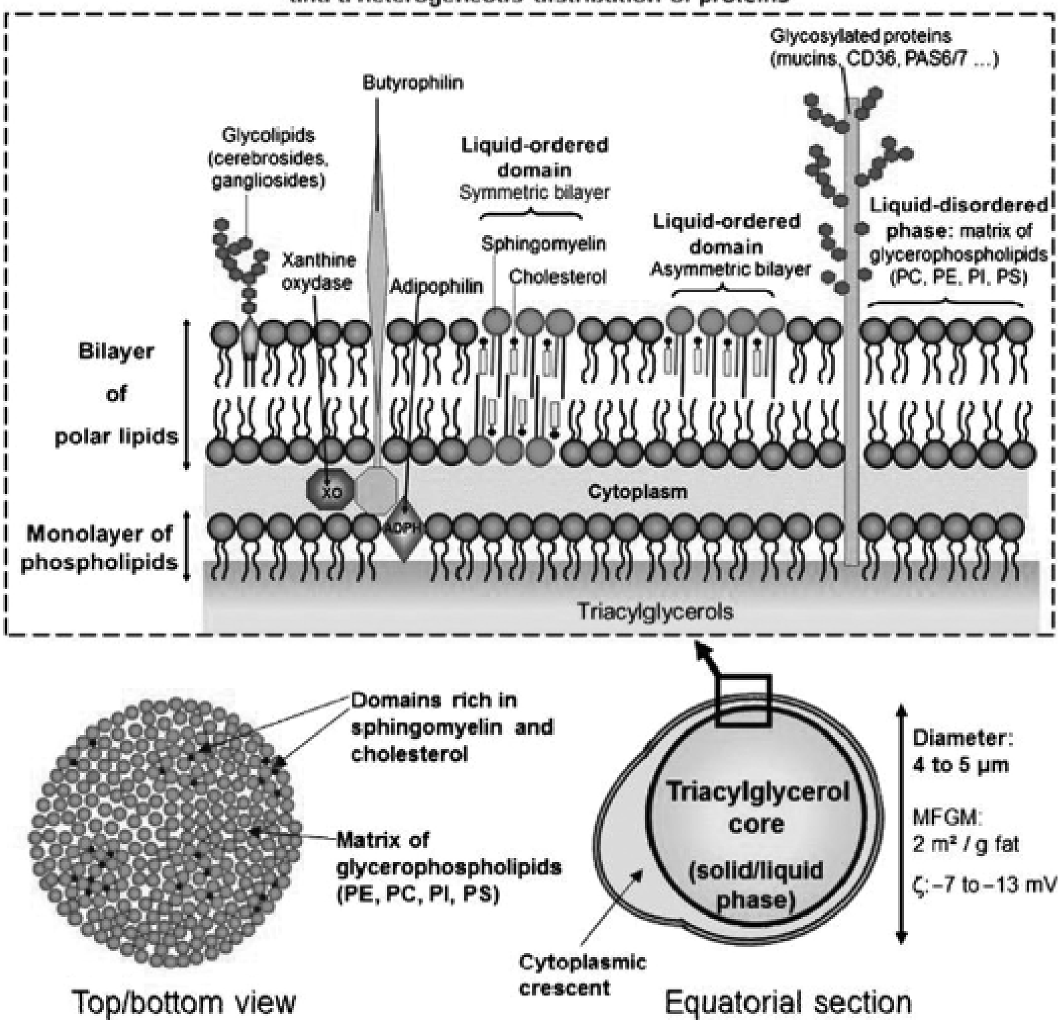


FIGURE 14.7 Milk fat globule membrane structure: a trilayer structure with a lateral organization of polar lipids and a heterogeneous distribution of protein. (Reproduced from Lopez, C., *Curr. Opin. Colloid Interface Sci.*, 16, 391, 2011. With permission.)

14.3.2.3.1 Lipid Composition

The lipid composition of MFGM is complex but has been detailed in several reviews [33,71,83]. Triacylglycerides are the major fraction of lipids in the MFGM fraction, >60%. However, the majority of these appear to originate from contamination by the core of the fat globule during isolation of the membrane. Hence, the method used for isolating membrane material has a large influence on the triacylglyceride content [33]. The MFGM-associated triacylglycerides contain higher proportions of longer-chain saturated fatty acids than the triacylglycerides of the globule core fat. Reported measured contents of sterols and sterol esters vary widely but range up to only 2 wt% of membrane total lipids, with cholesterol accounting for 90 wt% of the total sterols. Mono- and di-acylglycerides are also present and, together with free fatty acids and sterols, make up about 10 wt% of the total lipid

in MFGM. The remaining ~30 wt% is phospholipid, mainly the zwitterionic forms, phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin, at around 25%–30% each of total phospholipid, with smaller percentages of the anionic forms, phosphatidylinositol, phosphatidylserine, and trace amounts of lysophosphatidylcholine [71].

The major fatty acids associated with the glycerol are C16:0, C18:0, C18:1, and C18:2. Sphingomyelin was found with a high proportion of C20:0, C23:0, C24:1, and C24:0 fatty acids linked to the sphingoid base [33]. Short- and medium-chain fatty acids are present at very low levels. MFGM contains two major gangliosides, neutral glycol-sphingolipids, composed of ceramide and an oligosaccharide chain attached to one or more sialic acids and several sugars. The quantity of gangliosides is about 8 μg/mg membrane protein. In MFGM, the major forms found are glucosylceramide (35%) and lactosylceramide (65%). Their composition is identical to that found in apical plasma membranes of the secretory cells in the mammary gland.

The polar lipid composition of MFGM is specific to milk, particularly its high proportion of sphingomyelin, which makes it drastically different from other sources of phospholipids, for example, egg lecithin or soybean lecithin, which contain low amounts of sphingolipids.

14.3.2.3.2 Protein Content of MFGM

Proteins account for 25%–60% of the mass of MFGM depending on the method of isolation employed and the sample history. They also constitute about 1%–2% of total milk protein. About 40 different proteins can be resolved from MFGM protein fraction, using a combination of electrophoresis and isoelectric focusing. The structures, amino acid sequences, and properties of the major MFGM proteins are reviewed and discussed in detail by Mather [90]: many are glycoproteins. The main MFGM proteins are butyrophilin (glycoprotein that comprises over 40% of the mass of total protein associated with bovine MFGM), the redox enzyme xanthine oxidase (12% total bovine MFGM protein), and the heavily glycosylated mucin-like proteins (MUC1, MUC15, etc.), PAS 6/7 (lactadherin), PAS III, the heavily glycosylated CD36, and nonglycosylated proteins, adipophilin and fatty acid–binding protein.

Butyrophilin, a glycoprotein, makes up over 40% by wt of the total protein associated with MFGM in milks from Holstein cows and approximately 20% in MFGM from Jersey milk [91]. It has an apparent molecular weight of 67,000 Da and contains approximately 5% carbohydrate. Butyrophilin is synthesized as a peptide of 526 amino acids that has an N-terminal hydrophobic signal sequence of 26 amino acids. Butyrophilin is expressed specifically in the mammary gland and is concentrated in apical plasma membrane and the MFGM.

Xanthine oxidase, which accounts for approximately 12%–20% of total MFGM protein, belongs to the family of iron–sulfur–molybdenum flavin hydroxylases that convert xanthine to uric acid. Bovine xanthine oxidase is a dimer with two identical subunits of molecular weight approximately 150,000 Da. In milk secretory cells, this protein is concentrated along the inner face of the apical plasma membrane and the MFGM. There is evidence that butyrophilin and xanthine oxidase interact specifically with each other [43]. In cows, these two proteins are expressed in variable amounts but in constant molecular proportions (4:1) throughout lactation. This interaction appears to involve disulfide bonds between the two proteins, as xanthine oxidase can be released from MFGM by reduction of disulfide bonds. Disrupting the expression of either of these proteins has demonstrated that they play essential roles in normal MFG secretion.

MUC1 is heavily glycosylated (up to 50% of its weight). It is readily dissociated from the fat globules into skim milk when milk is cooled or agitated. PAS 6 and PAS 7 are abbreviations for periodic acid/Schiff 6 and 7, respectively. Their molecular weights range from 48,000 to 54,000 Da. The amino acid sequences of PAS 6 and PAS 7 are identical: they differ only in the level of glycosylation. PAS 6 and PAS 7 are also loosely bound to MFGM and can be removed from the membrane by washing with salt solutions.

Adipophilin is a major constituent of the insoluble fraction remaining after MFGM is extracted with salts and nonionic detergent. The role played by adipophilin, if any, in fat

globule secretion is uncertain. Unlike butyrophilin and xanthine oxidase, it does not appear to be localized in the apical regions of the plasma membrane. Instead, this protein appears to be concentrated on intracellular lipid droplets where it may be involved in lipid droplet–plasma membrane interactions that lead to progressive droplet envelopment and secretion, though this remains speculative [43].

14.3.2.4 Structure of Milk Fat Globule Membrane

Although there has been a major upsurge in research interest in the topic in recent years, the structure of the MFGM is still not known in detail. The organization of the MFGM depicted in Figure 14.7 as a trilayer structure (thickness 10–50 nm) is now well accepted, with the inner layer composed of proteins and polar lipids from the endoplasmic reticulum and the outer bilayer of polar lipids originating from regions of the apical plasma membrane of the mammary epithelial cell. Most frequently MFGs are enveloped compactly by apical plasma membrane. However, in some instances closure of the plasma membrane around the droplet can entrain some cytoplasm between the membrane and the fat droplet surface (Figure 14.7, inset). The results can vary from a sliver of cellular material to cases where the trapped cytoplasm volume exceeds the fat globule volume. Application of confocal scanning laser microscopy with lipophilic probes has revealed heterogeneities in both the composition and structure of the fat globule membrane within and among fat globules from the same species. The studies of Lopez et al. [80] revealed phase separation of polar lipids in the plane of the fat globule membrane, believed to be lateral segregation of sphingomyelins and cholesterol in rigid liquid-ordered phase microdomains surrounded by a fluid matrix of glycerol-phospholipids in a liquid-disordered phase. A lateral diffusion of the sphingomyelin-rich domains was also observed. Confocal scanning microscopy also revealed that the proteins and glycoproteins are also heterogeneously distributed in the fat globule membrane and are organized as patches or networks but are not found in the sphingomyelin-rich domains [79].

Despite this knowledge of composition and structure, virtually nothing is known about the molecular mechanisms involved in any step from the intracellular formation of the lipid droplets through their secretion as MFGs from the cell.

14.3.3 Milk Salts and Minerals, Lactose, and Minor Enzyme Compounds

14.3.3.1 Milk Salts and Minerals

The principal milk salts include the citrate, phosphate, carbonate, and chloride salts of H^+, K^+, Na^+, Mg^{2+}, and Ca^{2+}, whether as free ions or complexes in solution or as colloidal species complexed with the caseins. There have been a number of reviews on the topic of milk salts, the term including both inorganic and organic (citrate) species [38,47,50,82].

The approximate concentrations of milk salts and their distribution between colloidal and serum phases is shown in Table 14.7. This composition is relatively constant but some variation is seen through lactation with the most important changes occurring around parturition [38]. Changes in milk calcium concentrations appear to be regulated by the amount of citrate and casein in milk [98]. Indeed calcium concentration shows a linear correlation when plotted against the casein concentrations in the milks of many species [50]. Most or all of the calcium in milk is likely derived through exocytosis of secretory vesicles derived from the Golgi compartment. The calcium is pumped therein where it distributes among various calcium-binding molecules present, equilibrating with these molecules in an amount determined by their concentrations, the calcium-binding concentrations, and the ionized calcium concentration, $[Ca^{2+}]$. The difficulty comes in understanding how the large amount of calcium necessary can be transferred into the Golgi and secretory compartments while cytosolic calcium is maintained in the micromolar range. Research in this area is summarized in Neville [98]. Citrate is a major binding partner for calcium and its concentration varies widely through lactation. Citrate has an indirect role in

TABLE 14.7
Concentration and Distribution of the Principal Salts in Milk

	Concentration		Colloidal (Micellar) (%)	Serum (Soluble) (%)
Cationic	mg/L	mmol/kg		
Calcium	1040–1280	26–32	69	31
Magnesium	100–150	4–6	47	53
Potassium	1210–1680	31–43	6	94
Sodium	350–600	17–28	5	95
Anionic				
Carbonate (including CO_2)	~200	~2		
Chloride	780–1200	22–34	5	95
Citrate	1320–2080	7–11	14	86
Total phosphorus (PO_4) (all forms)	1800–2180	30–32		
Inorganic phosphorus (as PO_4)	930–1000	19–23	53	47
Sulfate	~100	~1		

Source: Adapted from Lucey, J.A. and Horne, D.S., Milk salts: Technological significance, in *Advanced Dairy Chemistry*, Vol. 3, Lactose, Water, Salts and Minor Constituents, 3rd edn., McSweeney, P.L.H. and Fox, P.F., eds., Springer Science, New York, pp. 351–389, 2009.

de novo fatty acid synthesis, and dietary manipulations that lead to a decrease in *de novo* synthesis of fatty acids has been found to increase milk citrate concentration [37]. The major pathway for phosphate secretion into milk is also believed to be the Golgi vesicle route by a Na^+-P_i cotransport mechanism [116].

The milk salts have a crucially important impact on many properties of milk, including the formation and stability of the casein micelle system (already discussed in Section 14.3.2.3), acid–base buffering and various colligative properties, and their key biological functions in bone growth and development in the neonate. In addition, these components have a powerful influence on protein stability during processing. Rennet coagulation, heat and alcohol stability, the texture of various types of milk protein gels, cheese texture and functionality, and emulsion stability are all influenced by mineral content.

The soluble species, which are in equilibrium with the colloidal forms, may be obtained in the permeate by dialysis or ultrafiltration (UF). The multivalent ions, Ca^{2+} and Mg^{2+}, exist in the serum mainly as complexes, including large amounts of Ca-citrate and Mg-citrate and lesser quantities of $Ca(H_2PO_4)_2$. Thus, only 20%–30% of the total Ca and Mg in the ultrafiltrate exists as free divalent cations. Conversely, >95% of the citrate is complexed with these cations. Univalent ions, such as K^+, Na^+, and Cl^-, are present almost entirely in the serum phase as free ions. Taking into account these different associations, the diffusible fraction of milk at pH 6.6–6.7 appears to be supersaturated in calcium phosphate and to have an ionic strength of about 80 mM.

Around 70% of the calcium, 50% of the phosphate, and 50% of the magnesium in milk is to be found in the colloidal phase. Strictly speaking, all of these minerals are associated with casein micelles. As we have seen, the phosphoserine residues of the caseins can bind calcium ions, but mostly the micellar calcium phosphate is bound up in the calcium phosphate nanoclusters (see previous discussion on casein micelle structure, Section 14.3.2.3). An important aspect of the partitioning of the milk minerals between micellar and diffusible compartments is control of the concentrations of the various species exerted by the complicated series of equilibria involved, both in complex formation and in complex solubility. The distribution depends on environmental conditions, including pH, temperature, and concentration, as summarized by Gaucheron [38], and has important implications for stability and functional properties.

14.3.3.2 Lactose

Because of the biosynthetic requirements of isosmolality with blood, one would expect a reciprocal concentration relationship between milk salts and lactose. Such an inverse relationship has been documented between sodium and lactose contents and between sodium and potassium contents [47,111]. Consequently, milk has an essentially constant freezing point (−0.53 to −0.57°C), and this colligative property is used to detect illegal dilution with water.

Lactose (4-*O*-β-D-galactopyranosyl-D-glucopyranosyl) is the predominant carbohydrate in skim milk. Its synthesis is associated with that of the major whey protein, α-lactalbumin, which acts as a modifier protein for UTP-galactosyl transferase, changing the specificity of this enzyme such that the galactosyl group is transferred to glucose rather than to the glycoprotein. Lactose occurs in both α- and β-forms, with an equilibrium ratio of β/α = 1.68 at 20°C [99]. The β-form is far more soluble than the α-form, and the rate of mutarotation is rapid at room temperature but very slow at 0°C. The α-hydrate crystal form, which crystallizes under ordinary conditions, occurs in a number of shapes, but the most familiar is the "tomahawk" shape that imparts a "sandy" mouthfeel to dairy products, as in "sandy" ice cream. Lactose, with a sweetness about one-fifth that of sucrose, contributes to the characteristic flavor of milk. A more extended discussion of the properties of lactose can be found in the review of Fox [34].

14.3.3.3 Enzymes

This discussion is limited to enzymes indigenous to milk, but it should be noted that enzymes are also introduced into milk as a result of microbial growth. For a more complete exposé of the indigenous enzymes, the reader should consult O'Mahony et al. [104,105]. Of the 60 or so indigenous enzymes reported in bovine milk, about 20 have been isolated and characterized in considerable detail. Most of these have some technical significance. Table 14.8 lists some of the more important.

TABLE 14.8
Some Enzymes Indigenous to Bovine Milk[a]

Oxidoreductases	Hydrolases
Xanthine oxidase (xanthine:O_2 oxidoreductase)	Proteinases (plasmin, thrombin, aminopeptidase, and peptidyl peptide hydrolase)
Sulfhydryl oxidase (protein:peptide-SH:O_2 oxidoreductase)	Lipase (glycerol ester hydrolase)
Lactoperoxidase (donor:H_2O_2 oxidoreductase)	Lysozyme (mucopeptide N-acetylneuraminyl hydrolase)
Superoxide dismutase (O_2^-:O_2^- oxidoreductase)	Alkaline phosphatase (orthophosphoric monoester phosphohydrolase)
Glutathione peroxidase (GSH:H_2O_2 oxidoreductase)	ATPase (ATP phosphohydrolase)
Catalase (H_2O_2:H_2O_2 oxidoreductase)	*N*-Acetyl-β-D-glucosaminidase
Diaphorase (NADH:lipoamide oxidoreductase)	Cholinesterase (acylcholine acylhydrolase)
Cytochrome c reductase (NADH:cytochrome c oxidoreductase)	β-Esterase (carboxylic ester hydrolase)
Lactate dehydrogenase (L-lactase:NAD oxidoreductase)	α-Amylase (α-1,4-glucan-4-glucanohydrolase)
Transferases	β-Amylase (β-1,4-glucan maltohydrolase)
UDP-galactosyl transferase (UDP galactosyl:D-glucose-1-galactosyl transferase)	5′-Nucleotidase (5′-ribonucleotide phosphohydrolase
Ribonuclease (polyribonuclease 2-oligonucleotide transferase)	**Lyases**
γ-Glutamyl transferase	Aldolase (fructo-1,6-diphosphate D-glyceraldehyde-3-phosphate lyase)
	Carbonic anhydrase (carbonate hydrolase)

Source: Swaisgood, H.E., Characteristics of milk, in *Fennema's Food Chemistry*, 4th edn., Damodaran, S., Parkin, K.L., Fennema, O.R. eds., CRC Press, Boca Raton, FL, pp. 881–917, 2007. Table 15.12.

[a] The systematic name is given in brackets.

It is likely that these enzymes have no specific function in milk but enter milk owing to the peculiarities of the mechanisms involved in the secretion and excretion of milk constituents. Lipoprotein lipase and the plasmin system enter from the blood through leaky junctions between secretory cells. These enzymes tend to be associated with the casein micelles. Somatic cells transferred to the mammary gland from blood to fight bacterial infection in the gland give rise to cathepsins B and D. MFGM is a major location for the enzymes found in milk, most entering entrained in this membrane, for example, xanthine oxidase.

The physical compartmentalization of the enzymes facilitates their separation by techniques such as centrifugation. However, the storage and handling of milk cause redistribution of many of the indigenous enzymes, leading to consequential damage. Thus, cold-induced lipolysis of milk fat may be caused by transfer of lipase from micelles to fat globules, and cold storage may induce dissociation of proteinase from casein micelles. These enzymes can have significant effects on flavor and protein stability in dairy foods.

Among other important effects, some beneficial, some useful, some detrimental, are the following. Levels of plasmin or catalase are indices of animal health, especially mastitis. Alkaline phosphatase is a heat-resistant enzyme, whose inactivation is employed as an indicator of effective pasteurization. Some enzymes produce desirable changes in dairy products, for example, lipoprotein lipase, acid phosphatase, and xanthine oxidase in cheese ripening. Equally, the same enzymes can lead to a deterioration in product quality, especially lipoprotein lipase (hydrolytic rancidity), plasmin (proteolysis, off-flavors), and xanthine oxidase (oxidative rancidity).

Lactoperoxidase is found in high concentrations in bovine milk. When combined with hydrogen peroxide or thiocyanate, it has antibacterial properties, but neither partner is present in milk. A major function of this enzyme may be to limit the accumulation of toxic levels of peroxide in the udder.

14.4 COMMODITY MILK PRODUCTS

14.4.1 Introduction

Intended by nature as food for direct transmission to, and immediate consumption by the suckling, milk is a highly perishable commodity, readily open to bacterial contamination leading to spoilage of the product or possible disease in consumers. Cognizant of the nutritional benefits, man has developed a range of processes intended for the preservation of milk or its components, particularly, in the case of the historically earliest products, as a means of conservation in times of surplus production.

Among the wide range of products currently to be found are liquid milks, fermented milks, cheeses, butter and ghee, condensed and evaporated milks, ice cream, milk powders, cream, whey products, and casein. This listing is roughly in terms of the usable storage life of the products, though for each individual grouping there can be a considerable range and consequently a great deal of overlap. Table 14.9 indicates that consumption of a selection of the major products varies widely within and between product groupings across the world, from highs in North America and Europe to lows in Asia. Even within regions, such as Europe, fluid milk consumption is high in the Scandinavian countries, compared to France and Italy where historically cheeses have tended to dominate milk consumption. This may simply be reflective of the climate experienced in these regions, evidence of a culture of producing a more stable product (cheese) in hotter climates as a means of preservation before the advent of more modern technology relying on refrigeration, heat treatment, and/or aseptic packaging, all of which are important contributors to the availability of fluid milk supplies.

14.4.2 Liquid Milks

Milk leaves the udder at a temperature of about 37°C. Fresh milk from a healthy cow is practically free from bacteria but it is then exposed to contamination at all stages of handling. Bacteria thrive most vigorously at around 37°C; hence, the milk must be chilled immediately to around 4°C to

TABLE 14.9
Per Capita (kg) Consumption of Selected Commodity Dairy Products across the World

Country	Milk	Butter	Cheese	Skimmed Milk Powder	Whole Milk Powder
1. Ukraine	122.73	2.11 (8)	2.09 (10)	0.64 (14)	0.27 (13)
2. Australia	108.94	3.56 (4)	10.41 (5)	3.30 (1)	1.74 (5)
3. USA	90.61	2.50 (6)	15.14 (3)	1.64 (5)	0.08 (15)
4. Canada	87.26	2.78 (5)	15.41 (2)	2.02 (4)	—
5. Russia	77.11	2.34 (7)	5.53 (7)	1.07 (8)	0.65 (9)
6. New Zealand	61.66	4.71 (1)	7.17 (6)	0.67 (13)	0.45 (12)
7. EU-27	66.45	3.99 (2)	16.60 (1)	1.57 (6)	0.54 (10)
8. Brazil	58.96	0.40 (12)	3.64 (8)	0.88 (11)	3.03 (3)
9. Argentina	51.91	0.90 (9)	12.44 (4)	0.44 (15)	2.19 (4)
10. India	42.05	3.66 (3)	—	0.34 (16)	—
11. Mexico	34.49	1.87 (8)	2.89 (9)	2.08 (3)	1.27 (7)
12. Japan	31.79	0.61 (11)	2.22 (11)	—	—
13. Taiwan	14.61	0.77 (10)	0.92 (12)	0.90 (10)	1.33 (6)
14. China	9.82	—	—	0.19 (17)	1.12 (8)
15. Philippines	0.54	—	0.22 (13)	0.99 (9)	0.13 (14)

Countries are ranked for milk consumption per head. Bracketed figures in each row give ranking for commodity in that column. FAO 2012 data.

minimize their growth rate, and this temperature must be maintained during transportation and storage until further processing can be carried out. Even this simple change in temperature alters equilibria within the milk system; the possibility of such alterations must always be borne in mind when considering the impact of processing on product quality and functionality.

The fat and protein may undergo changes during storage. The fat may be oxidized at the double bonds of unsaturated fatty acids, those of lecithin being most susceptible to attack. Iron or copper salts accelerate the onset of autoxidation, as does the presence of dissolved oxygen or exposure to light, especially direct sunlight. In the presence of light and/or transition metal ions (such as Cu^{+} and Fe^{2+}), fatty acids are further broken down, ultimately into aldehydes and ketones, which give rise to off-flavors and rancidity. The fat may also undergo lipolysis, releasing fatty acids that make the milk taste rancid, but the naturally occurring lipase can only act on exposed triglyceride following damage to the MFGM. This can occur during pumping, splashing, or other agitation. Undue agitation should be avoided but a certain amount is necessary in silo storage to reduce creaming of the lower density fat globules.

Exposure to light can also bring about oxidation of protein-related components but the complexity of these reactions is beyond this simplified and limited discussion. Further details of the changes during milk storage and many other aspects of milk processing can be found in Walstra et al. [128].

Liquid milk is marketed with a range of fat contents, whole milk (as secreted), standardized (to normally 3.5% fat), semi-skimmed (half fat), and skimmed (no fat). The fat-containing varieties are generally homogenized. Homogenization prevents the lower density fat globules from forming a cream layer. The globules are reduced in size from 3 to 10 μm to less than 2 μm by forcing the globules through restricted passages under high pressure (~25 MPa). Homogenization is most efficient when the fat phase is liquid and temperatures normally applied are 60°C–70°C. The decrease in droplet diameter is accompanied by a 5- to 10-fold increase in surface area. There is insufficient membrane material to coat this newly created surface and the deficit is made up by rapid adsorption of protein. This can be micellar casein, micellar fragments, or whey proteins and this interfacial layer prevents the coalescence of the fat globules.

The major benefit of homogenization is the prevention of creaming. A secondary benefit is increased whiteness that occurs because the increased number and smaller size of the fat globules scatters light more effectively. Homogenization also has several disadvantages. The milk has reduced heat stability [119]. In addition, any product manufacture that relies on aggregation and gelation of the caseins, as in rennet curd formation or acid gelation in fermented milk, results in a product with different rheological and textural properties to comparable food made from unhomogenized milks. The fat globules are also more susceptible to light-induced oxidation, readily prevented by opaque packaging, and to lipolysis when active lipase is present.

14.4.2.1 Pasteurization

Pasteurization is a heat treatment process that extends the usable life of milk and reduces the number of possible pathogenic microorganisms to levels at which they do not present a health hazard. Pasteurization conditions are designed to effectively destroy the organisms *Mycobacterium tuberculosis*, recognized as the most resistant common organism, and *Coxiella burnetii*. Apart from pathogenic microorganisms, milk also contains other substances and microorganisms that lead to spoilage and reduce shelf life. Hence, a secondary purpose of heat treatment is to destroy many of these other organisms and enzyme systems (e.g., lipases). The desired level of lethality can be achieved in a wide range of temperature/time combinations. Those commonly employed by the dairy industry are listed in Table 14.10, some of them enforced by statute in various jurisdictions. The most common procedures used in modern plant are high temperature/short time (HTST) and ultrahigh temperature (UHT) flow sterilization. The actual time/temperature combinations employed in each process depend on the quality of the raw milk, the type of product treated, and the desired product shelf life. HTST pasteurized milks should be kept chilled and the cold chain maintained at home by the purchaser. In an unopened container, HTST pasteurized milk should have a shelf life of 8–10 days at 5°C–7°C (for a full description of time/temperature combinations used in thermal process, the reader should consult Walstra et al. [128]).

UHT treatment is a continuous flow process, which takes place in a closed system that prevents product from being contaminated by airborne microorganisms. The product passes through heating and cooling stages in quick succession. Aseptic filling, to avoid reinfection of the milk, is an integral part of the process. The resulting UHT milk can be stored sealed for months, rather than weeks, at ambient temperature.

14.4.2.2 Effects of Heat Treatment

Changes in temperature alter equilibria within the salt system, within the protein system, and between salts and protein. Often these changes are not completely reversible, even if the original conditions are restored, so that the final characteristics and properties of the dairy product depend on the processing conditions. For a review of this topic, see Huppertz and Kelly [62]. The milk serum is saturated or supersaturated with respect to various calcium phosphates and calcium citrate;

TABLE 14.10
Main Categories of Heat Treatment Employed in the Dairy Industry

Process	Temperature (°C)	Time
Thermization	63–65	15 s
LTLT pasteurization of milk (low temperature, long time)	63	30 min
HTST pasteurization of milk (high temperature, short time)	72–75	15–20 s
HTST pasteurization of cream	>80	1–5 s
Ultra-pasteurization	125–138	2–4 s
UHT (ultrahigh temperature) (flow sterilization)	Normally 135–140	A few seconds
Sterilization in-container	115–120	20–30 min

consequently small changes in environmental conditions cause significant shifts in these equilibria [47,50]. The solubility of calcium phosphate is strongly temperature dependent and, unlike most compounds, decreases with temperature.

Pasteurization or UHT processing irreversibly increases the amount of colloidal calcium phosphate at the expense of both soluble and ionized calcium and soluble phosphate. Heat-induced reductions (85°C) in levels of calcium and phosphate are rapidly and almost completely reversible on subsequent cooling [109,110]. More severe heat treatment (>90°C) may result in irreversible changes in the mineral balance [49]. Consequently, the pH also decreases due to the release of protons from primary and secondary phosphates. The calcium transformed to tertiary calcium phosphate does not come entirely from the serum phase because heating also draws calcium bound to protein. Thus, pasteurization and especially sterilization affects micellar stability properties.

Individual milks have been classified according to their pH-dependent heat stability, defined as the duration of heating at 140°C required for protein coagulation. For reviews, the reader is referred to Singh and Creamer [119], O'Connell and Fox [103], and Huppertz [61]. Type A milk exhibits a maximum, followed by a minimum, in the stability curve between pH 6.6 and 6.9, while Type B milk usually increases in stability as the pH is raised above pH 6.6. This phenomenon appears to be related to the ratio of β-lactoglobulin to κ-casein and the heat-induced interaction between these two proteins. Addition of κ-casein will convert a Type A milk to a Type B, and the reverse can be achieved by the addition of β-lactoglobulin to a Type B. Competitive heat-induced interactions of protein with heat-created products of urea transformation have also been implicated in the phenomena, but controversy still exists over the reaction mechanisms involved [55,59].

A brief overview of heat-induced changes in caseins and casein micelles is given in the following text. For extensive review, the reader is referred to O'Connell and Fox [103] and Huppertz and Kelly [62]. Heat treatments of increasing severity are accompanied by increased production of dehydroalanyl residues due to β-elimination of disulfide bonds and phosphoseryl residues, increased deamidation of asparaginyl and glutaminyl residues, and increased Maillard browning. Cross-linking of protein during heating can result from the reaction of dehydroalanyl residues with ε-amino groups of lysine residues to form lysinoalanine or reaction with sulfhydryl groups of cysteinyl residues to form lanthionine (see Chapter 5). Continued heating (e.g., 20–40 min at 140°C) destabilizes micelles, leading to gel formation. The prolonged heating also induces a drop in pH through degradation of lactose and hydrolyses phosphoserine over time, on top of the more rapid shift in calcium phosphate equilibria from primary and secondary phosphate to hydroxyapatite. The coagulation might possibly be seen as an isoelectric precipitation process, with some of the protein changes (e.g., reaction of acyl groups) contributing a stabilizing effect through increasing protein negative charge and consequently electrostatic repulsion.

Because denaturation of whey proteins occurs rapidly at temperatures above 70°C, normal commercial heat treatment denatures a portion of these proteins. Major whey proteins exhibit thermostability to structural unfolding in the order of α-lactalbumin < bovine serum albumin < Ig < β-lactoglobulin. However, thermal unfolding of α-lactalbumin is reversible, so that denaturation as measured by irreversible changes indicates an order of increasing thermostability of Ig < bovine serum albumin < β-lactoglobulin < α-lactalbumin. The exposure of the sulfhydryl group on denaturation initiates the aggregation of, say, β-lactoglobulin with other proteins through sulfhydryl–disulfide interchange. Potential partners in milk, alongside β-lactoglobulin itself, include other whey proteins, α_{S2}-casein, κ-casein, and some of the proteins of the MFGM. These interactions have significant effects on many processing characteristics of milk, positively in fermented milk but negatively in cheesemaking [62]. Intense heat treatment also has a deleterious effect on the appearance, taste, and nutritive value of milk.

Milk exposed to heat treatment must be of good quality and capable of withstanding the application of the temperature/time combination without showing instability. This is particularly important in UHT processing. In South and Latin America, milk quality for these purposes is assessed by an

alcohol stability test. Samples of milk are mixed with a solution of ethanol (usually 72% v/v). If no immediate flocculation is observed, the milk is adjudged suitable for further processing. The alcohol stability test was originally adopted as an indicator of souring in milk due to fermentative degradation, but some milks rejected for UHT processing show no significant pH drop. The factors affecting the alcohol stability test and its application in this context are discussed in more detail by Horne [55], who previously derived a mechanism for the alcoholic destabilization, involving dielectric effects on ionization and mineral equilibria.

14.4.3 Cultured Milk Products

Cultured or fermented milk products are prepared by lactic acid fermentation (e.g., yogurt) or a combination of this and yeast fermentation (e.g., kefir). Cultured milk originates from the Near East and subsequently became popular in eastern and central Europe. The first examples were presumably prepared accidentally, possibly by nomadic tribes. This milk turned sour and coagulated under the influence of certain microorganisms. Luckily, the bacteria were of a harmless, acidifying type and were not toxin-producing organisms. Kefir originates from the same region. The active organisms used in its production also include yeast capable of forming alcohol to a maximum content of about 0.8% v/v.

Yogurt is now produced on an industrial scale and its popularity is growing worldwide (Table 14.9). Consumption in the United States, where it is associated with a healthy lifestyle, has doubled in the last 10 years and now stands at 4.5×10^9 lb/annum [127].

Yogurt is produced by lowering the pH of milk proteins to their isoelectric points (about pH 4.6) by the fermentation of lactic acid, using starter bacteria such as a combination of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (also termed *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*). Before inoculation the milk must be heat-treated to destroy any competing microorganisms, normally at 85°C–90°C for 5–20 min. This heat treatment also denatures the whey proteins and allows disulfide exchange reactions with the cysteine-containing caseins. These reactions modify the micelle surface and contribute to favorable textural properties in the acidified coagulum. After heat treatment and inoculation, the milk is held at the optimum temperature for starter growth and lactic acid production. When ~pH 4.6 is reached, the product is cooled quickly to stop the fermentation process. This incubation and cooling can be carried out in the final packaging pot, in which case the product is referred to as set-style, or in tanks, where following gelation, the gel is stirred and pumped into retail pots as a stirred yogurt. In addition to flavor and aroma, correct appearance and consistency are important features. These are determined by the choice of preprocessing parameters. These include adequate heat treatment and homogenization, sometimes combined with methods to increase solids content. Stabilizers may be added to modify the textural properties or inhibit whey separation, where necessary. Commonly used stabilizers include starch, gelatin, and pectin.

Natural yogurts are retailed with a range of fat contents, made from fat-adjusted milks. Yogurts also come in fruit-flavored varieties, the fruit being either layered into the pot before milk addition and incubation, stirred into the set gel, or packed separately in a "twin-pot" integrated with the basic pot. There is also growing consumption of yogurts supplemented with lactobacillus acidophilus and bifidobacteria. These microorganisms are important members of human intestinal flora. Consumption of these in milk products is promoted as an ideal way of restoring the balance or maintain their levels, but some associated claims need to be substantiated.

14.4.4 Cheese

Cheese is another of the preservative milk products that date from prehistoric times. Like yogurt, cheese was probably first discovered by accident when milk stored in animal intestines was found to curdle and coagulate with further compression expressing the liquid whey [35].

Approximately 50% of the world's cheese is produced in Europe, although the biggest single producer country is the United States (~30% of world total in 2009 [30]). Given the large variety (~1400 named variants [35]) and the considerable body of literature available, it is beyond the scope of this chapter to discuss cheese in detail. A short summary follows.

Cheesemaking involves a number of main stages that are common to most types of cheese. The most important step, the coagulation of the casein component of the milk, is achieved by using one of the following methods: (1) limited proteolysis using enzymes, (2) acidification by adding acids or a starter culture, and (3) acidification combined with heating to about 90°C [35]. The majority of cheeses are produced by enzymatic coagulation; rennet from the stomachs of young calves, lambs, and buffalo was traditionally used. The active enzyme, chymosin, has been cloned and is widely available.

Despite much research summarized in Dalgleish [15], Hyslop [64], Lucey [81], and Horne and Banks [56], the mechanism of curd formation is not yet fully understood. The current view is that the casein micelle is an electrosterically stabilized colloidal system; the steric component and much of the electrostatic charge is provided by the κ-casein located on the surface of the micelle. The chymosin splits the κ-casein at a specific bond, Phe_{104}-Met_{105}. With the glycosylated macropeptide released to the serum phase of the milk, the steric component is removed and, as a result, the micelle is destabilized and aggregation and gelation follow. The whole process is governed by temperature, pH, and calcium concentration. The coagulation reaction is extremely temperature sensitive. For many years this was thought due to a decrease in hydrophobic attraction as temperature was lowered [15,64], but a recent reappraisal of the aggregation kinetics of fully renneted casein micelles has strongly suggested that the decrease in aggregation rate is due to a decrease in the level of calcium ion binding with temperature [58]. This highlighting of the importance of electrostatic repulsion energy in casein micelle interactions is in line with a universal stability behavior for the micelle and moves the role of ionic calcium and its binding to center stage.

Treatment of the coagulum follows a specific protocol for each type of cheese, a procedure developed in some cases over years of experimentation, and often with a local touch. In general terms, the curd is separated from the whey, the methodology dictated by the desired moisture content of the final cheese. Softer cheese of high moisture content, for example, Camembert, is simply ladled into molds and kept overnight, as the whey is allowed to drain off. In others, the curd is cut into cubes; the finer the cut, the lower the moisture content. When the whey is drained off, the curd may be subjected to heating, cooking, stirring, pressing, salting, and other operations to promote gel syneresis.

After this, the curd is pressed into molds or hoops and may undergo further compression. The majority of rennet-coagulated cheeses are then subjected to ripening. During this period, the cheese goes through a whole series of processes of a microbiological, biochemical, and physical nature. These changes affect the lactose, protein, and fat, especially the protein in the case of hard cheeses.

The techniques that have been devised for making different kinds of cheese are always directed toward controlling and regulating the growth and activity of lactic acid bacteria. In Cheddar cheese, lactose has already been fermented before the curd is hooped. For other cheeses, lactose fermentation ought to be controlled in such a way that most of the decomposition takes place during the pressing and, at the latest, during the first week or two of storage. The lactates provide a suitable substrate for the propionic acid bacteria that are an important component of the microbiological flora of Emmental, Gruyere, and similar types of cheese. Besides propionic acid, considerable amounts of carbon dioxide are formed, the direct cause of the formation of the round eyes characteristic of these cheeses.

The degree of protein decomposition brought about by bacterial enzymes strongly influences the cheese quality, particularly in respect of its taste and texture. The external storage conditions are adjusted to optimally control the ripening process with a specific temperature and relative humidity for every type of cheese. In medium-soft cheeses, such as Tilsiter where protein decomposition is assisted by a surface smear, initial conditions promote the smear growth, before transfer to another

regime advantageous to other processes and removal of the smear. In all cheeses, the casein is progressively broken down during ripening producing a myriad range of peptides and free amino acids.

In the blue-veined semi-soft cheeses, such as Roquefort, lipolysis is an important flavor generator. Roquefort cheese is produced from sheep milk that has a high fat content. Other blue-veined cheeses can be produced from bovine milk where fat is augmented by addition of cream. The milk is partially homogenized before renneting. This exposure makes the fat more sensitive to the lipolytic enzymes emanating from the inoculated *Penicillium roqueforti* mold, the characteristic of these cheeses. The cheeses need to be pierced with needles after about 5 days of ripening, to facilitate admission of the oxygen needed for the growth of the mold.

The ripening period depends on the final product. Hard cheeses, such as Parmesan, Manchego, or Cheddar, are stored for months, even years. Fresh cheeses, such as cottage cheese or quarg, undergo virtually no ripening whatsoever and are kept chilled for retail sale.

14.4.5 Butter

Butter is the third dairy product that has been produced since Neolithic times. Butter fat has been detected on shards of pottery at sites from crannogs in Scotland to archaeological digs in the Middle East. Until well into the nineteenth century, butter was still made from cream that had been allowed to sour naturally. The cream was then skimmed from the top of the milk and poured into a wooden tub. Butter was then made by hand churning. As the availability of cooling became commonplace, it became possible to skim the cream before milk had gone sour. The invention of the cream separator improved skimming efficiency and gave birth to large-scale butter-making.

Variations in the composition of butter are due to differences in production. Butter contains 80% fat and 16%–18% moisture, basically depending on whether it is salted or not. The color of butter varies with the content of carotenoids that make up 11%–50% of the total vitamin A activity of milk. As the carotenoid content normally fluctuates between winter and summer, butter produced in the winter period has a brighter color.

14.4.6 Evaporated Milk, Condensed Milk, and Milk Powder

These preserved milk products were introduced in the nineteenth and twentieth centuries. Preserving an evaporated milk by adding sugar was perfected around 1850. The high sugar content in sweetened condensed milk increases the osmotic pressure to such a level that most microorganisms are destroyed. The sugar content in the aqueous phase cannot be less than 62.5% or more than 64.5%. At the latter level, some sugar may crystallize and sediment. This product is used industrially in ice cream and chocolate manufacture, but it is also offered in cans for retail sale. An extensively heat-treated and therefore caramelized sweetened condensed milk is widely marketed in South American countries as dulce de leche.

The product referred to as evaporated milk is a sterilized product, light in color and with the appearance of cream. It is used extensively in situations where fresh milk is not available and widely in Europe as a coffee creamer. It is prepared by evaporating milk to 2× concentration, homogenizing, sealing into cans, and autoclaving at 110°C–120°C for 15–20 min. It is important that the milk should be able to tolerate the intensive heat treatment without the product coagulating in the can on storage. The heat stability must therefore be assessed before processing and can be improved by the addition of a stabilizer, usually disodium or trisodium phosphate. The quantity to be added is determined during the preprocessing tests. The ability to come through the sterilization treatment is essential; condensed milk can be stored for practically any length of time at a temperature of 0°C–15°C. Failures do occur, and to avoid such, each production batch should be tested by incubating at ambient, 30°C and 38°C for 10–14 days, after which quality tests (viscosity, bacteria and spore counts, color, odor, taste) are carried out.

Carrying water removal from milk to the extreme by drying leads to the production of milk powder. Drying extends the shelf life of milk, simultaneously reducing its weight and volume and drastically cutting the costs of transporting and storing the product. Today, milk powder is produced on a large scale in modern plants, allowing production excess in one country to be exported for the benefit of another. Skim milk powder has a maximum shelf life of about 3 years; whole milk powder has a maximum shelf life of about 6 months. The latter is because the fat in the powder oxidizes during storage, with a consequent gradual deterioration in taste.

Two principal methods of drying are employed by the dairy industry: roller drying and spray drying. In spray drying the milk is first concentrated by vacuum evaporation to a dry matter content of about 45%–55%, after which it is then dried in a spray tower. In the production of roller dried powder, the milk is admitted to the roller drier and the whole drying process takes place in one stage, but with a much more severe heating regime compared to spray drying.

Dried milk powder finds many applications. Examples (not exhaustive) include recombination of milk; in the baking industry where benefits are found in several products, such as bread and pastry goods; in producing milk chocolate; and in production of ice cream. Each field of application makes its own specific demands of the milk powder. If the milk powder is used for a recombined milk for consumption, it must be readily soluble and have the correct taste. Some degree of caramelization of the lactose is beneficial in chocolate production. In the first case, gentle drying of the product in a spray tower is essential, whereas in the second case, the powder must be subjected to intense heat treatment in a roller drier. Heat treatment denatures the whey proteins, with percentage denaturation increasing with the intensity of heat treatment. The degree of denaturation is normally expressed by the whey protein nitrogen index (WPN), as milligrams of undenatured whey protein per gram of powder. The higher this index, the less heat damage suffered by the milk powder. High heat powders have WPN < 1.5, whereas for a low heat powder, WPN > 6.0.

14.4.7 Membrane Separation Processing

Membrane technology, a proven separation technique based on molecular size, has seen significant growth in application in the dairy industry in the last 50 years. The range of size of milk components lends itself to physical separation using the whole gamut of membrane operations (Figure 14.8).

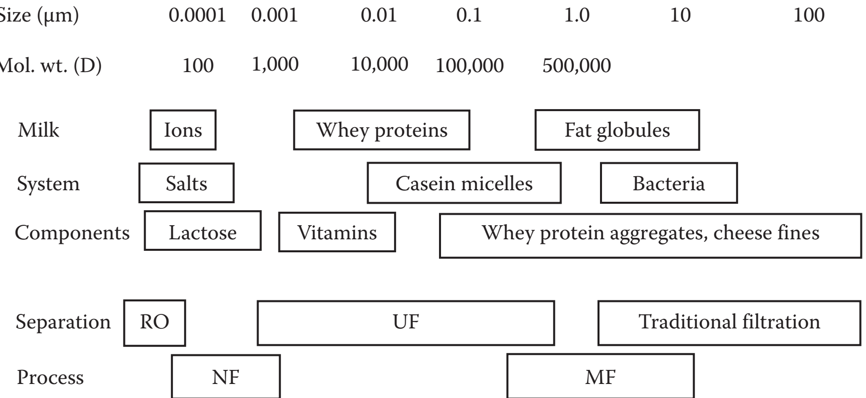


FIGURE 14.8 Milk components, their size, and membrane separation techniques applied in the dairy industry.

In the dairy industry, membrane technology is principally associated with

Reverse osmosis (RO)—Concentration of solution by removal of water
Nanofiltration (NF)—Concentration of organic components by partial removal of monovalent ions, like Na^+ and Cl^-
Ultrafiltration (UF)—Concentration of large particles and macromolecules
Microfiltration (MF)—Removal of bacteria, separation of macromolecules

All of these techniques feature cross-flow membrane filtration, in which the feed solution is forced through the membrane under pressure. The solution flows over the material; the larger component (retentate) is retained, while the permeate is removed. They only differ in pore size of membrane.

The separation techniques employed by the dairy industry serve different purposes. RO is used for the dehydration of whey, UF permeate, and condensate. NF is used when partial desalination of whey, UF permeate, or retentate is required. UF is typically employed for concentration of milk proteins in milk and whey and for protein standardization of milk intended for cheese, yogurt, and some other products. MF is basically used for reduction of bacteria in skim milk, whey, and brine, but is also used for defatting whey intended for whey protein concentrate (WPC) production and for protein fractionation, including casein micelle separation, giving MPC. Often these elements are operated in series, allowing separation and production of particular desirable milk components, for example, casein micelles or whey proteins.

14.5 MILK PROTEINS AS FUNCTIONAL INGREDIENTS

Proteins from traditional sources are finding increasing usage as functional ingredients in a growing number of formulated foods [19]. Milk proteins are especially useful in this respect because they can be separated from milk and its other components by a range of processes to release proteins, either alone or in mixtures with a wide spectrum of properties. A review of the methods of preparation and isolation has been provided by Mulvihill and Ennis [97]. Section 14.4.7 has already mentioned the techniques for membrane separation of milk proteins, but traditional commercial techniques predate this technology and are widely used.

Four types of casein products are available, namely, acid casein, rennet casein, caseinates, and coprecipitates. Acid (hydrochloric, lactic, or sulfuric) caseins are simply isoelectric precipitates (pH 4.6) and they are not very soluble. Likewise rennet casein that is prepared by treatment with chymosin (rennet) is not very soluble, especially in the presence of Ca^{2+}, because the polar domain of κ-casein has been removed. Rennet casein also has a high mineral content because colloidal calcium phosphate is included in the clotted (precipitated) micelles, whereas acid casein has a lower mineral content because the colloidal calcium phosphate is solubilized and passes into the whey. Coprecipitates of casein and whey protein are prepared by heat treatment of a milk sufficiently to fully denature the whey proteins, which then coprecipitate with the caseins on acidification to pH 4.6. Coprecipitates are more soluble than acid or rennet caseins but are not as soluble as the caseinates. Solubilization of coprecipitates can be improved by adjustment of aqueous suspensions to alkaline pH and addition of polyphosphates. The caseinates (sodium, potassium, and calcium) are prepared by neutralizing acid caseins with the appropriate alkali, prior to drying. These isolates, especially sodium and potassium caseinates, are very soluble and extremely heat stable over a wide range of conditions.

WPCs or whey protein isolates (WPIs) can be prepared from acid or cheese wheys. WPCs are produced by a combination of UF/diafiltration using a suitable membrane. Diafiltration involves dilution of the retentate with water and repeated UF, resulting in a more complete removal of low-molecular-weight solutes. WPIs are manufactured using ion-exchange technology. Below their isoelectric point, whey proteins carry a net positive charge and behave as cations that can be adsorbed

onto cation exchangers. Above the isoelectric point, these proteins carry a negative charge and can be adsorbed onto anion exchangers.

Two major processes are operated commercially. The steps involved in ion-exchange fractionation are as follows: (1) whey protein is acidified to pH < 4.6, pumped into a reactor and stirred to allow protein adsorption onto the cation exchanger; (2) lactose and other unadsorbed compounds are filtered off; (3) the resin is resuspended in water and pH is adjusted to >5.5 to release proteins from the ion exchanger; (4) the aqueous solution of protein is filtered off, and the protein-rich eluate is concentrated by UF and evaporation and finally spray-dried to yield WPI, containing ~95% protein. In the second process, adsorption is at high pH onto an anion exchanger, and release is by lowering the pH with acid. Control of the level of protein denaturation by limiting the extent of heat treatment is a key factor in determining the usefulness of these proteins as functional ingredients. It is also important to reduce mineral and lipid content, as these can have deleterious effects on functional performance.

Some applications of milk proteins in formulated foods and their functional roles are listed in Table 14.11. It is apparent that many of the food products listed predate the concept of functionality or the availability of separated milk protein components. In these instances, it is probable that milk or concentrated milks featured first in the recipes and our awareness of the role of milk components came later as ideas of functional performance developed in the 1980s.

The functional properties of proteins are manifestly related to their structure, primary, secondary, and tertiary. Structure governs the interaction properties, the solubility, and the surface activity. Solubility is important because the ability to function in other roles depends on complete dispersion into solution. Surface activity is a complex function of the protein's surface hydrophobicity and

TABLE 14.11
Some Applications of Milk Proteins and Products in Formulated Foods

Food category	Specific foods in which casein/caseinate/coprecipitate are useful	Specific foods in which whey proteins are useful
Bakery products	Bread, biscuits/cookies, breakfast cereals, cake mixes, pastries, frozen cakes	Breads, cakes, muffins, croissants
Functional role	*Emulsifier, dough consistency, texture, volume/yield, nutritional*	*Emulsifier, egg replacer, nutritional*
Dairy-type foods	Imitation cheese, coffee creamer, cultured milk products, milk beverages	Yogurts, cheeses, and cheese spreads
Functional role	*Texture, fat and water binding, emulsifier*	*Texture, consistency, emulsifier*
Confectionery	Toffee, caramel, fudges, marshmallow, nougat	Aerated candy mixes, meringues, sponge cakes
Functional role	*Texture, water binding, emulsifier, color*	*Whipping properties, emulsifier*
Desserts	Ice cream, frozen desserts, mousses, instant pudding, whipped topping	Ice cream, frozen juice bars, frozen dessert coatings
Functional role	*Whipping properties, texture, emulsifier*	*Whipping properties, texture, emulsifier*
Beverages	Drinking chocolate, fizzy drinks and fruit beverages, cream liqueurs, wine aperitifs, wine and beer	Soft drinks, fruit drinks, powdered or frozen orange beverages, milk-based flavored beverages
Functional role	*Stabilizer, whipping and foaming properties, emulsifier, clarification*	*Nutritional, viscosity, colloidal stability*
Meat products	Comminuted products	Frankfurters, luncheon rolls, and injection brine for fortification
Functional role	*Emulsifier, water binding, gel formation*	*Pre-emulsion, gelation*

Source: Compiled from Mulvihill, D.M. and Ennis, M.P., Functional milk proteins: Production and utilization, in *Advanced Dairy Chemistry*, Vol. 1B, Proteins, 3rd edn., Fox, P.F. and McSweeney, P.L.H., eds., Kluwer Academic/Plenum Publishers, New York, pp. 1175–1228, 2003.

its flexibility, which allows it to unfold and spread at an interface, either air/water (as in foams) or oil/water (as in emulsions). The order of surface activity for various milk protein components is β-casein > monodispersed casein micelles> serum albumin > α-lactalbumin > α_s-casein/κ-casein > β-lactoglobulin. The intermolecular interactions that occur between partially unfolded protein structures are extremely important to functionality but are a complex interplay of protein stability and structure and the type of surface or surface structure exposed in partially unfolded protein. Caseins are unique because their flexible structures allow interaction with many partially unfolded structures of other proteins by hydrophobic interactions and/or extensions of secondary structure. Control of protein/protein interactions can lead to aggregates and gels, contributing texture, elasticity, and extrudability to the food product.

Table 14.10 shows that casein products and whey protein products can function similarly in many food products with the possibility that one can replace the other depending on the desired effect. One particular application of particulate whey protein aggregates is their development as fat mimetics, conferring the illusion of creaminess without the fat. In this, they may be unique but this example illustrates the importance of the extent and type of protein denaturation to achieve a desired functionality in the final state.

Ultimately, the bulk properties of colloidal systems, including food colloids, are determined by the nature and strength of the interactions among the various kinds of constituent particles and polymers [22]. The particles in food systems are entities such as emulsion droplets, gas bubbles, fat crystals, and protein aggregates, whose interactions are sensitively influenced by the structure and composition of their surfaces. The polymers are proteins and polysaccharides, whose interactions with each other, and with the surfaces of the particles, are sensitively moderated by the presence of much smaller molecular species, such as salts, sugars, lipids, and surfactants. Full coverage of this complex topic is beyond this chapter, and the interested reader is referred to reviews by Dickinson [22,23]. Undoubtedly, as knowledge of these interactions increases, greater control can be exerted on the properties of the manufactured food product.

While studies of the physical aspects of protein functionality described earlier still constitute an active field of food science research, greater recognition is being given to biological consequences [32]. Biological function can be beneficial, as in supplying amino acids for protein synthesis or providing bioactive peptides (see Section 14.5.1), or it can be detrimental, as in causing a food allergic response. An example of nutritional benefit is seen in partial replacement of cereal proteins with casein in bread and breakfast cereals [97]. The limiting amino acid in most cereals is lysine, in which caseins are particularly rich. Only about 4% casein in a wheat flour/casein mixture is required to boost the lysine content by ~60%, without detrimentally affecting the bread produced. WPCs or WPIs also deliver desirable benefits because of their high content of sulfur-containing amino acids when compared to plant proteins, such as soy proteins.

Work to understand the allergenic properties of food proteins is an active field of research. Studies of the allergenicity of milk proteins have been reviewed by Kaminogawa and Totsuka [67]. Excellent reviews are available describing current knowledge of the mechanisms responsible for the pathogenesis of food allergy, including potential routes for sensitization in affected individuals [9,117]. It appears that most allergenic food proteins bind IgE and can cause cross-linking on the surface of mast cells, which results in downstream cascades leading to the allergic response. Portions of a protein that bind IgE are referred to as epitopes. Epitopes are attributed either to a given linear sequence of amino acids within the protein or to a portion of the three-dimensional structure of the protein and are designated as either linear or conformational, respectively. The majority of epitopes for allergic proteins are thought to be at least eight amino acids long.

Allergic proteins are typically consumed as a component within a complex food matrix. Processing and food matrix greatly influence the potential of food allergens to react with sensitized individuals [94]. Sugars, polysaccharides, and lipids are common examples of molecules that can react with proteins, typically during a processing operation, and affect the allergenic potential of the protein. For example, phospholipids were shown to have a protective effect on the allergenicity

of α-lactalbumin during simulated *in vitro* digestion [96]. Similarly, Maillard modifications to β-lactoglobulin have been shown to reduce allergenic potential of this major whey protein, with glycation essentially being hypothesized to mask epitopes along the protein [124]. Reduction in the allergenicity of milk proteins has also been observed when the proteins are hydrolyzed [67].

14.5.1 Bioactive Peptides Derived from Milk Protein

Milk proteins contain peptide sequences that may affect the major body systems, namely, the cardiovascular, digestive, immune, and nervous systems [11,31,72] (Figure 14.9). Some also influence microbial growth [12]. These peptides are inactive within the sequence of the parent protein and can be liberated by (1) gastrointestinal digestion of milk (or milk product), (2) fermentation of milk with proteolytic starter cultures, or (3) hydrolysis by proteolytic enzymes. A comprehensive review of milk-derived bioactive agents has been published [72,74]. In the following paragraphs, we list some of the bioactive peptides derived from casein and whey proteins. The list is by no means exhaustive, as research in this area is active and ongoing [73].

14.5.1.1 Derived from Caseins (Table 14.12)

A number of peptides (casokinins) derived from α_{S1}- and β-casein exhibit antihypertensive activity because they inhibit angiotensin-converting enzyme (ACE). Because ACE converts angiotensin I to angiotensin II, ACE inhibitors lower blood pressure and aldosterone and activate the depressor

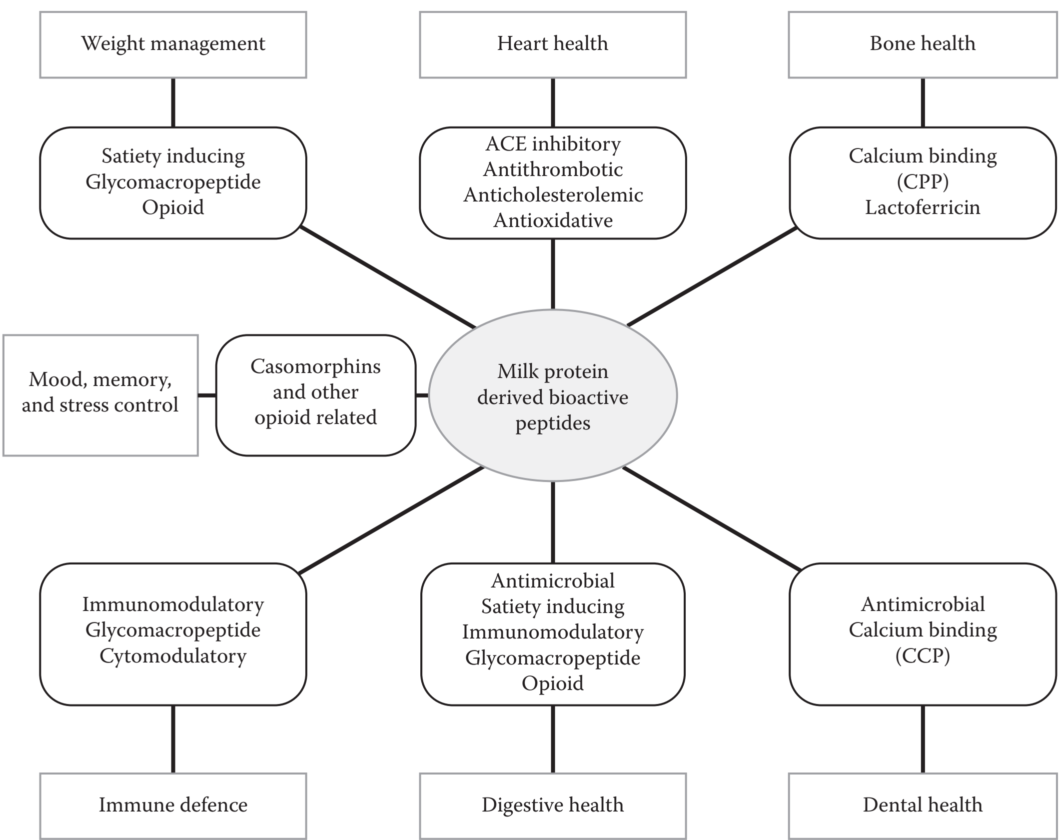


FIGURE 14.9 Milk protein bioactive peptides and areas of potential activity. (Reproduced from Korhonen, H., *J. Funct. Foods*, 1, 177, 2009. With permission.)

TABLE 14.12
Bioactive Peptides Derived from the Caseins of Milk

Protein	Common Name	Peptide	Biological Activity
α_{S1}-Casein	Isracidin	α_{S1}-Casein (f1–23)	Antimicrobial
	α_{S1}-Casokinin-5	α_{S1}-Casein (f23–27)	Antimicrobial and ACE inhibitor
	Caseinophosphopeptide	α_{S1}-Casein (f59–79)	Calcium binding and transport
	α-Casein exorphin	α_{S1}-Casein (f90–96)	Opioid agonist
	Casoxin D	α_{S1}-Casein (f158–164)	Opioid antagonist
α_{S2}-Casein	Casocidin-I	α_{S2}-Casein (f165–203)	Antimicrobial
β-Casein	β-Casokinin-7	β-Casein (f177–183)	ACE inhibitor
	β-Casokinin-10	β-Casein (f193–202)	ACE inhibitor and immunomodulatory
	β-Casomorphin-5	β-Casein (f60–64)	Opioid agonist
	Morphiceptin	β-Casein (f60–63 amide)	Opioid agonist
κ-Casein	Casoplatelin	κ-Casein (f106–116)	Antithrombotic
	Thrombin inhibitory peptide	κ-Casein (f112–116)	Antithrombotic
	Glycomacropeptide	κ-Casein (f106–169)	Inhibits platelet aggregation, anticariogenic, and immunomodulatory
	Casoxin C	κ-Casein (f25–34)	Opioid antagonist

action of bradykinin. Two peptides with antimicrobial activity, isracidin and casocidin-I, are derived from α_{S1}- and α_{S2}-caseins, respectively. These peptides are active against both Gram (+) and Gram (−) strains. The glycomacropeptide derived from κ-casein during cheesemaking exhibits anticariogenic activity due to its inhibition of the growth of oral streptococci and formation of dental plaque. Thus, it is used in some oral health-care products. The phosphopeptide domains of the calcium-sensitive caseins have excellent calcium-binding properties and are resistant to proteolysis. Hence, these caseinophosphopeptides, derived from α_{S1}-, α_{S2}-, and β-caseins, are present in the small intestine where they increase calcium solubility and enhance calcium transport across the intestinal wall, thus increasing calcium absorption and bone calcification.

A number of peptides that have opioid activity have been identified. Several derived from β-casein are known as β-casomorphins because of their morphine-like activity. Although their primary structure differs slightly from typical opioid peptides known as endorphins, these atypical peptides are opioid agonists because their structures fit well in the binding pocket of the opioid receptor. As a result they can modulate physiological activities such as gastrointestinal transient time, antidiarrheal action, amino acid transport, and endocrine activity of the pancreas causing an increase in insulin output. Two peptides, casoxin D from α_{S1}-casein and casoxin C from κ-casein, act as opioid antagonists.

Several peptides, principally from κ-casein, display antithrombotic and/or immunomodulating activities. For example, the glycomacropeptide promotes synthesis of IgA and induces proliferation of B-lymphocytes. It also inhibits platelet aggregation thus decreasing thrombosis. Several other peptides derived from the glycomacropeptide region of κ-casein also have antithrombotic activity.

14.5.1.2 Derived from Whey Proteins

A number of antimicrobial peptides derived from β-lactoglobulin and α-lactalbumin have been identified (Table 14.13). They appear to be active only against Gram (+) bacteria. The β-lactorphins derived from β-lactoglobulin exhibit ACE inhibitor activity and one is also an opioid agonist.

The best-known and well-characterized antimicrobial peptide is lactoferricin B derived from lactoferrin. Studies have shown that consumption of infant formulas or adult drinks supplemented with lactoferrin will produce relatively high levels of the peptide in the gastrointestinal tract that could have beneficial effects. Lactoferricin B displays bacteriocidal activity against pathogens such

TABLE 14.13
Bioactive Peptides Derived from Whey Proteins in Milk

Protein	Common Name	Peptide	Biological Activity
β-Lactoglobulin	None	β-Lactoglobulin (f15–20)	Antimicrobial
	None	β-Lactoglobulin (f25–40)	Antimicrobial
	None	β-Lactoglobulin (f78–83)	Antimicrobial
	None	β-Lactoglobulin (f92–100)	Antimicrobial
	β-Lactorphin	β-Lactoglobulin (f142–148)	ACE inhibitor
	β-Lactorphin (amide)	β-Lactoglobulin (f102–105)	Opioid agonist, ACE inhibitor
α-Lactalbumin	None	α-Lactalbumin (f17–31S-S109–114)	Antimicrobial
	None	α-Lactalbumin (f61-68S–S75-80)	Antimicrobial
Lactoferrin	Lactoferrin B	Lactoferrin (f17–41)	Antimicrobial

as *Staphylococcus aureus* and *Escherichia coli* as effectively as antibiotics. Additional biological activities are also associated with lactoferrin B. The peptide-induced apoptosis in a leukemic cell line that was mediated by intracellular reactive oxygen species and activated by endonucleases. Antiviral, immunoregulatory, and anti-inflammatory properties have also been ascribed to the peptide.

The potential health benefits of milk-protein-derived peptides have been the subject of growing commercial interest in the context of health-promoting functional foods. Bioactive peptides can be incorporated in the form of ingredients in functional and novel foods, dietary supplements, and even pharmaceuticals with the aim of delivering specific health benefits. Many scientific and technological issues must, however, be resolved before many of these can be realized. In particular, molecular studies are needed to assess the mechanisms by which bioactive peptides exert their activities. The majority of known bioactive peptides are not absorbed from the gastrointestinal tract into the bloodstream, and their effect is, therefore, probably mediated directly in the gut lumen or through receptors on the intestinal cell wall. In this respect, the target function of the peptide concerned is of the utmost importance.

14.6 NUTRITIVE VALUE OF MILK

Dairy foods make a significant contribution to the total nutrient diet of many populations, especially in northern Europe and North America. Table 14.14 lists the energy equivalent, fat, carbohydrate, protein, and important micronutrients in some dairy products. Epidemiology has pointed to a positive association between cow's milk and human health, the underpinning mechanisms of which are not well understood [27], for milk is a complex food. Most of the constituents in milk do not work in isolation but rather interact with other constituents. Often, they are involved in more than one biological process, sometimes with conflicting health effects, depending on the process in question.

Evidence, reviewed by McGregor and Poppitt [84], has been growing that milk proteins may improve or prevent a range of age-related chronic health problems, particularly those associated with metabolic health, including metabolic syndrome, Type 2 diabetes, atherosclerosis, and hypertension. Milk proteins may also play a role in the control of body weight and maintenance of lean body and/or skeletal muscle mass during aging and weight loss.

Another example is milk fat. The traditional health paradigm, developed in the 1960s and 1970s, held that consumption of fat, and saturated fat, in particular, raised total cholesterol and low-density lipoprotein (LDL) cholesterol levels, leading to coronary heart disease. More recent studies and analyses are now suggesting that not all saturated fats are the same and that consumption of milk fat may actually reduce the risks of heart disease [63,122].

It is also important to remember that dietary fats, in addition to being a concentrated energy source, serve as an important delivery mechanism for fat-soluble vitamins and contain various

TABLE 14.14
Contents of Selected Nutrients (per 100 g) of Whole Milk, Skim Milk, and Some Other Dairy Foods

USDA Food Name and Code	Energy (kcal)	Protein (g)	Total Fat (g)	Carbohydrate (g)	Calcium (mg)	Na (mg)	SFA (g)	MUFA (g)	PUFA (g)	Cholesterol (mg)
Milk, producer, fluid, 3.7% milk fat (01078)	64	3.3	3.7	4.7	119	49	2.3	1.1	0.1	14
Milk, nonfat, fluid, fat-free, or skim (01151)	34	3.4	0.1	5.0	122	42	0.1	0.0	0.0	2
Butter, without salt (01145)	717	0.9	81.1	0.1	24	11	51.4	21.0	3.0	215
Butter, salted (01001)	717	0.9	81.1	0.1	24	714	51.4	21.0	3.0	215
Yogurt, plain, low fat, 12 g protein per 8 oz. (01117)	63	5.3	1.6	7.0	183	70	1.0	0.4	0.0	6
Cheese, cheddar (01009)	403	24.9	33.1	1.3	721	621	21.1	9.4	0.9	105
Recommended daily amounts	2000	56	<65	300		<2400	<20			<300

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

fatty acids (e.g., conjugated linoleic acid) and bioactive factors beneficial to health (e.g., phospholipids) [40]. Similarly, to consider even saturated fatty acids as one uniform group of fats may be an oversimplification, since individual fatty acids have specific functions depending on their chain length. A further aspect for consideration and study is the influence of food structure on digestion and downstream biological processing. Nowadays, as opposed to the 1960s and 1970s, many fats are presented in foods in a highly stabilized emulsified form, often with a protein-based emulsifier. The influence of this on lipase accessibility and any consequent changes in fat digestion patterns has not been fully quantified.

Digestibility is also an important aspect in protein bioavailability, bioavailability being defined as getting the amino acids from the food structure in the gastrointestinal tract to the cells that need them throughout the body. Thus, it is necessary for the protein in foods to be accessible to the digestive enzymes of the stomach and small intestine. In casein-stabilized emulsions, it has been demonstrated that the conformation adopted by the adsorbed casein limits the accessibility of proteolytic enzymes, compared to the casein free in solution [76]. In general, however, animal proteins, including milk proteins, have good bioavailability compared to most plant and cereal proteins. Indeed, whey protein has an exceptional biological value that exceeds that of egg protein, the former benchmark, by about 15% [121].

The recommended daily intake of protein for adults is 800 mg protein/kg bodyweight [129]. This equates to 56 g/day for the reference adult male, weighing 154 lb, considered when drawing up *Dietary Guidelines for Americans, 2010*. In particular, this protein should ensure the consumption

TABLE 14.15
WHO-Recommended Daily Intake of Protein and Essential Amino Acids, Together with Amounts of These Provided by Sodium Caseinate and WPC80

Dietary Component	Rec. Daily Intake (mg/kg Body Weight)	Sodium Caseinate Contribution	WPC 80 Contribution
Protein	800	800	800
Histidine	10	19	*6*
Isoleucine	20	37	40
Leucine	39	71	85
Lysine	30	62	70
Methionine + cystine	15	25	64
Phenylalanine + tyrosine	25	81	46
Threonine	15	32	55
Tryptophan	4	8	14
Valine	26	45	*15*

Source: WHO, Protein and amino acid requirements in human nutrition: Report of a Joint FAO/WHO/UNU Expert Consultation, World Health Organization, Geneva, Switzerland, 2007.

Latter quantities based on 800 mg protein intake. Amounts are italicized for deficient DEAs in WPC80.

of a sufficient quantity of the dietary essential amino acids, whose WHO [24,129]-recommended intake is listed in Table 14.15. Also listed therein are the calculated levels of these amino acids in commercial samples of sodium caseinate and a WPC (WPC80), based on an 800 mg protein intake. Casein (and also cheese) is an excellent source of essential amino acids, exceeding the requirements for all. Whey protein is deficient in histidine and valine only, a shortfall easily made up if consumed in combination with the caseins of liquid milk.

Casein and whey protein are particularly abundant sources of lysine, in contrast to many staple plant proteins that are markedly deficient in this amino acid [10]. Having an adequate lysine content is made doubly important when it is noted that lysine is chemically unstable and undergoes a range of reactions when food is heated. The most important of these reactions is the Maillard reaction, in which the side chains of the lysine residues in the protein react with sugars to produce glycosyl derivatives. This reaction can occur under mild heating conditions but under more extreme conditions is responsible for much of the browning of food that occurs during cooking. At advanced stages of the Maillard reaction, lysine is no longer bioavailable, and losses of lysine by this route would be anticipated to be proportional to their content; hence, dairy protein should still be better. Another reaction, however, is of importance to dairy products. This is the reaction of lysine with phosphoserine that leads to the formation of lysinoalanine, which is not bioavailable. This problem is specific to casein-containing products (mainly milk powders and caseinate) because of their high phosphoserine content.

Labeling of foods to list macro- and micronutrients, and their energy equivalents, where appropriate, is becoming mandatory in many countries, parallel to increasing guidance on recommended intake to maintain and sustain a healthy weight. The typical nutrition facts label displayed on foods retailed in the United States is shown in Figure 14.10 for several dairy products. As well as the listing of nutrients, and their amount per serving, each label has a panel showing recommended daily values (DVs), based on a reference intake that should be consumed or should not be exceeded. Two sets of figures for caloric needs are given: the first level of 2000 kcal/day is expected to satisfy the requirements of a moderately active, adult female and the second higher level of 2500 kcal/day is applicable to the moderately active, adult male [24]. More intense activity burns more energy. A professional tennis player in a Grand Slam final might burn

Whole milk Nutrition facts	
Serving size 1 cup (240 mL)	
Amount per serving	
Calories 150	Calories from fat 70
	% Daily value[a]
Total fat 8 g	13%
Saturated fat 5 g	26%
Cholesterol 35 mg	11%
Sodium 120 mg	5%
Total carbohydrate 12 g	4%
Dietary fiber 0 g	0%
Sugars 12 g	
Protein 8 g	
Vitamin A 6%	• Vitamin C 4%
Calcium 30%	• Iron 0%

[a] Percent daily values are based on a 2000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2000	2500
Total fat	Less than	65 g	80 g
Sat. fat	Less than	20 g	25 g
Cholesterol	Less than	300 mg	300 mg
Sodium	Less than	2400 mg	2400 mg
Total carbohydrate		300 g	375 g
Dietary fiber		25 g	30 g

Low-fat milk (1%) Nutrition facts	
Serving size 1 cup (240 mL)	
Amount per serving	
Calories 110	Calories from fat 20
	% Daily value[a]
Total fat 2.5 g	4%
Saturated fat 1.5 g	7%
Cholesterol 10 mg	3%
Sodium 130 mg	5%
Total carbohydrate 12 g	4%
Dietary fiber 0 g	0%
Sugars 12 g	
Protein 9 g	
Vitamin A 10%	• Vitamin C 4%
Calcium 30%	• Iron 0%

[a] Percent daily values are based on a 2000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2000	2500
Total fat	Less than	65 g	80 g
Sat. fat	Less than	20 g	25 g
Cholesterol	Less than	300 mg	300 mg
Sodium	Less than	2400 mg	2400 mg
Total carbohydrate		300 g	375 g
Dietary fiber		25 g	30 g

Cheese (cheddar) Nutrition facts	
Serving size 1 oz. (28 g)	
Servings per container 16	
Amount per serving	
Calories 110	Calories from fat 80
	% Daily value[a]
Total fat 9 g	14%
Saturated fat 6 g	30%
Cholesterol 30 mg	10%
Sodium 170 mg	7%
Total carbohydrate 0 g	0%
Dietary fiber 0 g	0%
Sugars 0 g	
Protein 7 g	
Vitamin A 6%	• Vitamin C 0%
Calcium 20%	• Iron 0%

[a] Percent daily values are based on a 2000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2000	2500
Total fat	Less than	65 g	80 g
Sat. fat	Less than	20 g	25 g
Cholesterol	Less than	300 mg	300 mg
Sodium	Less than	2400 mg	2400 mg
Total carbohydrate		300 g	375 g
Dietary fiber		25 g	30 g

Yogurt Nutrition facts	
Serving size 1 cup (225 g)	
Amount per serving	
Calories 230	Calories from fat 20
	% Daily value[a]
Total fat 2.5 g	4%
Saturated fat 1.5 g	8%
Cholesterol 10 mg	3%
Sodium 130 mg	5%
Total carbohydrate 43 g	4%
Dietary fiber 0 g	0%
Sugars 34 g	
Protein 10 g	
Vitamin A 2%	• Vitamin C 2%
Calcium 35%	• Iron 0%

[a] Percent daily values are based on a 2000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2000	2500
Total fat	Less than	65 g	80 g
Sat. fat	Less than	20 g	25 g
Cholesterol	Less than	300 mg	300 mg
Sodium	Less than	2400 mg	2400 mg
Total carbohydrate		300 g	375 g
Dietary fiber		25 g	30 g

FIGURE 14.10 Exemplary food labels designated in the United States for a number of dairy products.

5000–6000 kcal in the match. A cyclist in a 150 km road race might require similar inputs from his diet. Sedentary consumption at these levels would lead to obesity.

The higher the percent DV, the more that serving of food contributes to an individual's intake of a specific nutrient. Foods that are "low" in a nutrient generally contain less than 5% of DV; foods that are a "good" source of a nutrient generally contain 10%–19% of DV per serving. Foods that are "high" or "rich in," or are an "excellent," source, generally contain 20% or more of DV per serving. On this basis, dairy products are "low" in carbohydrate and fiber, whole milk and cheese are "good" sources of fat, and all are "excellent" sources of calcium. DV for protein is not listed on these labels, but elsewhere in *Dietary Guidelines for Americans, 2010*, this is given as 56 g for the reference male, which would mean a single serving of any of these dairy products would provide at least 12% DV, ranking all of them as "good" sources of protein.

14.6.1 Lactose Intolerance

It is the norm that mammals lose most of their lactase intestinal activity and the ability to digest lactose shortly after weaning. In humans, this occurs in early childhood. Consumption of milk by such individuals results in lactose digestion by colonic bacteria, leading to the production of fatty acids and various gases, particularly hydrogen. In addition, the presence of lactose in the colon has an osmotic effect, drawing in water from the blood. The outcomes can include diarrhea, abdominal cramps, bloating, and chronic flatulence. Symptoms like this are referred to as lactose intolerance, but it would be more correct to label the condition as lactose maldigestion, or low intestinal lactase activity.

It is estimated that approximately 75 million Americans and 75% of the world population are lactose maldigesters [8]. This 75% is not uniformly spread. In Hispanic individuals, prevalence varies from 50% to 80%, with 60% to 80% affected in black populations or Ashkenazi Jews, and almost 100% of Asians or Native Americans. In contrast, lactase persistence is high, almost 98%, among northern Europeans, and it also occurs in pockets in Africa and the Middle East. In European populations, the occurrence of a single genetic mutation appears to explain the absence of the down-regulation of the lactase enzyme and its persistence into adulthood. Estimates for the age of lactase persistence–associated alleles bracket those for the age of animal domestication and the culturally transmitted practice of dairying. Depending on methodology, dates of origin range between 2,000 and 20,000 years ago by one method [2] and between 7,500 and 12,300 by another [13]. Such dates are still remarkably recent for alleles that are found in such high frequencies in multiple widely separated populations [39]. It is easy to envisage recent alleles being rare, since they change in frequency slowly and, in a directionless way, by genetic drift. However, a recent allele that has reached such high populations requires more than genetic drift alone: there has to be a selective advantage, an extragenetic inheritance, playing a key role in the survivor of the carrier. Since the trait has been mainly identified in dairy-practicing or pastoralist populations, and since fresh milk and some milk products are the only naturally occurring sources of lactose, it is unlikely that lactase persistence would be selected without a supply of fresh milk. It has therefore been suggested that lactase persistence coevolved with the cultural adaptation of dairying in a gene-culture coevolution process, and various hypotheses have been put forward as to how the selective pressures operated. The reader is directed to Gerbault et al. [39] for further details on this topic and its association with the spread and growth of dairying from the Middle East and through Europe. Computer simulation studies on the spread of the allele place its origin somewhere in the region of Hungary, in central Europe [66]. Farming and dairying may have originated in the Middle East, but so also did the manufacture of fermented milks and cheese, virtually lactose-free products. In the more temperate climes of central and northern Europe, milk may remain fresh for longer periods, despite the probable absence of good hygiene and the definite lack of refrigeration, aseptic packaging, and knowledge of pasteurization. Diarrhea and flatulence are not life-threatening conditions in modern societies, but in conditions of starvation childhood diarrhea can be fatal. Hence in primitive Neolithic times, the

ability to consume milk beyond weaning may have reduced child mortality and increased survival rates. Another scenario highlights the role of the vitamin D of milk in the absorption of its calcium content and the prevention of rickets in higher-latitude regions with low sunlight.

Nowadays, for those who suffer from lactose maldigestion, the option is available of drinking milk where lactose has been hydrolyzed by pretreatment with immobilized microbial lactase is available, allowing them to benefit fully from the major nutritional qualities of milk.

REFERENCES

1. Anema, S.G. (2014). The whey proteins in milk: Thermal denaturation, physical interactions and effects on the functional properties of milk. In *Milk Proteins: From Expression to Food*, 2nd edn. (H. Singh, M. Boland, A. Thompson, eds.). Elsevier/Academic Press, London, U.K., pp. 270–318.
2. Bersaglieri, T., Sabati, P.C., Patterson, N., Vanderploeg, T., Schaffner, S.F., Drake, J.A., Rhodes, M., Reich, D.E., Hirschhorn, J.N. (2004). Genetic signatures of strong recent positive selection at the lactase gene. *American Journal of Human Genetics*, **74**, 1111–1120.
3. Bionaz, M., Perlasamy, K., Rodriguez-Zas, S.L., Everts, R.E., Lewin, H.A., Hurley, W.L., Loor, J.J. (2012). Old and new stories: Revelations from functional analysis of the bovine mammary transcriptome during the lactation cycle. *PLoS ONE*, **7**, e33268, 1–14.
4. Bonnaire, L., Sandra, L., Helgason, T., Decker, E.A., Weiss, J., McClements, D.J. (2008). Influence of lipid physical state on the in vitro digestibility of emulsified lipids. *Journal of Agricultural and Food Chemistry*, **56**, 3791–3797.
5. Bouchoux, A., Gesan-Guiziou, G., Perez, J., Cabane, B. (2010). How to squeeze a sponge. Casein micelles under osmotic stress, a SAXS study. *Biophysical Journal*, **99**, 3754–3762.
6. Brew, K. (2013). α-Lactalbumin. In *Advanced Dairy Chemistry*, Vol. 1A, Proteins: Basic Aspects (P.L.H. McSweeney, P.F. Fox, eds.). Springer Science+Business Media, New York, pp. 261–273.
7. Brock, J.H. (2002). The physiology of lactoferrin. *Biochemistry and Cell Biology*, **80**, 1–6.
8. Brown-Esters, O., McNamara, P., Savaiano, D. (2012). Dietary and biological factors influencing lactose intolerance. *International Dairy Journal*, **22**, 98–103.
9. Burks, A.W. (2008). Peanut allergy. *Lancet*, **371**, 1538–2546.
10. Chatterjee, S., Sarkar, A., Boland, M.J. (2014). The world supply of food and the role of dairy protein. In *Milk Proteins: From Expression to Food*, 2nd edn. (H. Singh, M. Boland, A. Thompson, eds.). Elsevier/Academic Press, London, U.K., pp. 2–18.
11. Clare, D.A., Swaisgood, H.E. (2000). Bioactive milk peptides: A prospectus. *Journal of Dairy Science*, **83**, 1187–1195.
12. Clare, D.A., Castignani, G.L., Swaisgood, H.E. (2003). Biodefense properties of milk; the role of antimicrobial proteins and peptides. *Current Pharmaceutical Design*, **9**, 1239–1255.
13. Coelho, M., Luiselli, D., Bertorelle, G., Lopes, A.I., Seixas, S., Destro-Bisol, G., Rocha, J. (2005). Microsatellite variation and evolution of human lactase persistence. *Human Genetics*, **117**, 329–339.
14. Copley, M.S., Berstan, R., Dudd, S.N., Docherty, G., Mukherjee, A.J., Straker, V. et al. (2003). Direct chemical evidence for widespread dairying in prehistoric Britain. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 1524–1529.
15. Dalgleish, D.G. (1992). The enzymatic coagulation of milk. In *Advanced Dairy Chemistry*, Vol. I, Proteins (P.F. Fox, ed). Elsevier Applied Science, London, U.K., pp. 579–619.
16. Dalgleish, D.G., Corredig, M. (2012). The structure of the casein micelle of milk and its changes during processing. *Annual Review of Food Science and Technology*, **3**, 449–467.
17. Dalgleish, D.G., Horne, D.S., Law, A.J.R. (1989). Size-related differences in bovine casein micelles. *Biochimica et Biophysica Acta*, **991**, 383–387.
18. Dalgleish, D.G., Spagnuolo, P.A., Goff, H.D. (2004). A possible structure of the casein micelle based on field-emission scanning electron microscopy. *International Dairy Journal*, **14**, 1025–1031.
19. Damodaran, S., Paraf, A. (1997). *Food Proteins and Their Applications*, Marcel Decker Inc., New York.
20. DeKruif, C.G., Holt, C. (2003). Casein micelle structure, functions and interactions. In *Advanced Dairy Chemistry*, Vol. I, Proteins, 3rd edn., Part A (P.F. Fox, P.L.H. McSweeney, eds.). Kluwer Academic/Plenum Publishers, New York, pp. 233–276.
21. deWit, J.N., Swinkels, G.A.M. (1980). A differential scanning calorimetry study of the thermal denaturation of bovine β-lactoglobulin. Thermal behaviour at temperatures up to 100°C. *Biochimica Biophysica Acta Protein Structure*, **624**, 40–50.
22. Dickinson, E. (2006). Colloid science of mixed ingredients. *Soft Matter*, **2**, 642–652.

23. Dickinson, E. (2011). Food colloids research: Historical perspective and outlook. *Advances in Colloid and Interface Science*, **165**, 7–13.
24. U.S. Department of Agriculture and U.S. Department of Health and Human Services. (2010). Dietary guidelines for Americans, 2010, 7th edn. U.S. Government Printing Office, Washington, DC.
25. Donnelly, W.J., McNeill, G.P., Bucheim, W., McGann, T.C.A. (1984). A comprehensive study of the relationship between size and protein composition in natural casein micelles. *Biochimica et Biophysica Acta*, **789**, 136–143.
26. Edwards, P.J.B., Jameson, G.B. (2014). Structure and stability of whey proteins. In *Milk Proteins: From Expression to Food*, 2nd edn. (H. Singh, M. Boland, A. Thompson, eds.). Elsevier/Academic Press, London, U.K., pp. 201–241.
27. Elwood, P.C., Givens, D.J., Beswick, A.D., Fehily, A.M., Pickering, J.E., Gallagher, J. (2008). The survival advantage of milk and dairy consumption. An overview of cohort studies of vascular diseases, diabetes and cancer. *Journal of American College of Nutrition*, **27**, S723–S734.
28. Evershed, R.P., Payne, S., Sherrat, A.G., Copley, M.S., Coolidge, J., Uram-Kotsu, D. et al. (2008). Earliest date for milk use in the Near East and south-eastern Europe linked to cattle herding. *Nature*, **455**, 528–531.
29. Farrell, Jr., H.M., Malin, E.L., Brown, E.M., Qi, P.X. (2006). Casein micelle structure: What can be learned from milk synthesis and structural biology? *Current Opinion in Colloid and Interface Science*, **11**, 135–147.
30. FAOSTAT. (2011). Statistical database. Food and Agricultural Organization of the United Nations, Rome, Italy.
31. Fitzgerald, R.J., Meisel, H. (2003). Milk protein hydrolysates and bioactive peptides. In *Advanced Dairy Chemistry*, Vol. 1, Proteins, 3rd edn. (P.F. Fox, P.L.H. McSweeney, eds.). Kluwer Academic/Plenum Publishers, New York, pp. 675–698.
32. Foegeding, E.A., Davis, J.P. (2011). Food protein functionality: A comprehensive approach. *Food Hydrocolloids*, **25**, 1853–1864.
33. Fong, B.Y., Norris, C.S., MacGibbon, A.K.H. (2007). Protein and lipid composition of bovine milk fat globule membrane. *International Dairy Journal*, **17**, 275–288.
34. Fox, P.F. (2009). Lactose: Chemistry and properties. In *Advanced Dairy Chemistry*, Vol. 3, Lactose, Water, Salts and Minor Constituents, 3rd edn. (P.L.H. McSweeney, P.F. Fox, eds.). Springer Science, New York, pp. 1–15.
35. Fox, P.F., McSweeney, P.L.H. (2004). Cheese: An overview. In *Cheese Chemistry, Physics and Microbiology*, Vol. 1 (P.F. Fox, P.L.H. McSweeney, T.M. Cogan, T.P. Guinee, eds). Academic Press, London, U.K., pp. 1–18.
36. Gapper, L., Copestake, D., Otter, D., Indyk, H. (2007). Analysis of bovine immunoglobulin G in milk, colostrums and dietary supplements: A review. *Analytical and Bioanalytical Chemistry*, **389**, 93–109.
37. Garnsworthy, P.C., Masson, L.L., Lock, A.L., Mottram, T.T. (2006). Variation of milk citrate with stage of lactation and *de novo* fatty acid synthesis in dairy cows. *Journal of Dairy Science*, **89**, 1604–1612.
38. Gaucheron, F. (2005). The minerals of milk. *Reproduction, Nutrition, Development*, **45**, 473–483.
39. Gerbault, P., Liebert, A., Itan, Y., Powell, A., Currat, M., Burger, J., Swallow, D.M., Thomas, M.G. (2011). Evolution of lactase persistence: An example of human niche construction. *Philosophical Transactions of the Royal Society B*, **366**, 863–877.
40. German, J.B., Dillard, C.J. (2006). Composition, structure and adsorption of milk lipids: A source of energy, fat-soluble nutrients and bioactive molecules. *Critical Reviews in Food Science and Nutrition*, **46**, 57–92.
41. Gerosa, S., Skoet, J. (2013). Milk availability: Current production and demand and medium-term outlook. In *Milk and Dairy Products in Human Nutrition* (E. Muehlhoff, A. Bennett, D. McMahon, eds). Food and Agricultural Organization of the United Nations, Rome, Italy, pp. 11–40.
42. Gresti, J.M., Bugant, M., Maniongui, C., Bezard, J. (1993). Composition of molecular species of triacylglycerols in bovine milk fat. *Journal of Dairy Science*, **76**, 1850–1869.
43. Heid, H.W., Keenan, T.W. (2005). Intracellular origin and secretion of milk fat globules. *European Journal of Cell Biology*, **84**, 245–258.
44. Herrero, M., Havlik, P., Valin, H., Notenbaert, A., Rufino, M.C., Thornton, P.K. et al. (2013). Biomass use, production, feed efficiencies, and greenhouse gas emissions from global livestock systems. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 20888–20893.
45. Holland, J.W., Boland, M. (2014). Post-translational modification of the caseins. In *Milk Proteins: From Expression to Food*, 2nd edn. (H. Singh, M. Boland, A. Thompson, eds.). Elsevier/Academic Press, London, U.K., pp. 141–168.

46. Holland, T. (2003). *Rubicon, the Triumph and Tragedy of the Roman Republic*. Little, Brown and Co., New York.
47. Holt, C. (1985). The milk salts: Their secretion, concentration and physical chemistry. In *Developments in Dairy Chemistry*, Vol. 3, Lactose and Minor Constituents (P.F. Fox, ed.). Applied Science Publishers, London, U.K., pp. 143–181.
48. Holt, C. (1992). Structure and stability of casein micelles. *Advances in Protein Chemistry*, **43**, 63–151.
49. Holt, C. (1995). Effect of heating and cooling on the milk salts and their interaction with casein. In *Heat-Induced Changes in Milk* (P.F. Fox, ed.) Special Issue 9501, International Dairy Federation, Brussels, Belgium, pp. 105–133.
50. Holt, C. (1997). The milk salts and their interaction with casein. In *Advanced Dairy Chemistry*, Vol. 3, Lactose, Water, Salts and Vitamins, 2nd edn. (P.F. Fox, ed.). Chapman and Hall, London, U.K., pp. 233–256.
51. Horne, D.S. (1988). Prediction of protein helix content from an autocorrelation analysis of sequence hydrophobicities. *Biopolymers*, **27**, 451–477.
52. Horne, D.S. (2006). Casein micelle structure: Models and muddles. *Current Opinion in Colloid and Interface Science*, **11**, 148–153.
53. Horne, D.S. (1998). Casein interactions: Casting light on the Black Boxes, the structure in dairy products. *International Dairy Journal*, **8**, 171–177.
54. Horne, D.S. (2014). Casein micelle structure and stability. In *Milk Proteins: From Expression to Food*, 2nd edn. (H. Singh, M. Boland, A. Thompson, eds.). Elsevier/Academic Press, London, U.K., pp. 169–200.
55. Horne, D.S. (2016). Ethanol stability and milk composition. In *Advanced Dairy Chemistry*, Vol. 1B, Proteins (P.L.H. Mac Sweeney, J.A. O'Mahony, P.F. Fox, eds.). Springer Science+Business Media, New York, pp. 225–246.
56. Horne, D.S., Banks, J. (2004). Rennet-induced coagulation of milk. In *Cheese: Chemistry, Physics and Microbiology*, Vol. 1, General Aspects, 3rd edn. (P.F. Fox, P.L.H. McSweeney, T.M. Cogan, T.P. Guinee, eds.). Academic Press, London, U.K., pp. 47–70.
57. Horne, D.S., Leaver, J. (1995). Milk proteins on surfaces. *Food Hydrocolloids*, **9**, 91–95.
58. Horne, D.S., Lucey, J.A. (2014). Revisiting the temperature dependence of the coagulation of renneted bovine casein micelles. *Food Hydrocolloids*, 42, 75–80.
59. Horne, D.S., Muir, D.D. (1990). Alcohol and heat stability of milk protein. *Journal of Dairy Science*, **73**, 3613–3626.
60. Horne, D.S., Lucey, J.A., Choi, J. (2007). Casein interactions: Does the chemistry really matter? In *Food Colloids: Self Assembly and Materials Science* (E. Dickinson, M. Leser, eds.). Royal Society of Chemistry, Cambridge, U.K., pp. 155–166.
61. Huppertz, T. (2013). Chemistry of the caseins. In *Advanced Dairy Chemistry*, Vol. 1A, Proteins: Basic Aspects, 4th edn. (P.L.H. McSweeney, P.F. Fox, eds.). Springer, New York, pp. 135–160.
62. Huppertz, T., Kelly, A.L. (2009). Properties and constituents of cow's milk. In *Milk Processing and Quality Management* (A.Y. Tamime, ed). Wiley-Blackwell Publishing, Oxford, U.K., pp. 23–47.
63. Huth, P.J., Park, K.M. (2012). Influence of dairy product and milk fat consumption on cardiovascular disease risk: A review of the evidence. *Advances in Nutrition*, **3**, 266–285.
64. Hyslop, D.B. (2003). Enzymatic coagulation of milk. In *Advanced Dairy Chemistry*, Vol. I, Proteins, 2nd edn. (P.F. Fox, P.L.H. McSweeney, eds.). Kluwer Academic/Plenum Publishers, New York, pp. 839–878.
65. Iametti, S., De Gregori, B., Vecchio, G., Bonomi, F. (1996). Modifications occur at different structural levels during the heat denaturation of β-lactoglobulin. *European Journal of Biochemistry*, **237**, 106–112.
66. Itan, Y., Powell, A., Beaumont, M.A., Burger, J., Thomas, M.G. (2009). The origins of lactase persistence in Europe. *PLoS Computational Biology*, **5**(8), e1000491.
67. Kaminogawa, S., Totsuka, M. (2003). Allergenicity of milk proteins. In *Advanced Dairy Chemistry*, Vol. 1, Proteins, 3rd edn. (P.F. Fox, P.L.H. McSweeney, eds). Kluwer Academic/Plenum Publishers, New York, pp. 647–674.
68. Kawasaki, K., Weiss, K.M. (2003). Mineralized tissue and vertebrate evolution: The secretory calcium-binding phosphoprotein gene cluster. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 4060–4065.
69. Kawasaki, K., Weiss, K.M. (2006). Evolutionary genetics of vertebrate tissue mineralization: The origin and evolution of the secretory calcium-binding phosphoprotein family. *Journal of Experimental Zoology (Molecular Development and Evolution)*, **306B**, 295–316.

70. Kawasaki, K., Lafont, A.-G., Sire, J.-Y. (2012). The evolution of milk casein genes from tooth gene before the origin of mammals. *Molecular Biology and Evolution*, **28**, 2053–2061.
71. Keenan, T.W., Mather, I.H. (2006). Intracellular origin of milk fat globules. In *Advanced Dairy Chemistry, Lipids*, 3rd edn. (P.F. Fox, P.L.H. McSweeney, eds.). Springer, New York, pp. 137–171.
72. Korhonen, H. (2006). Technological and health aspects of bioactive components of milk. *International Dairy Journal*, **16**, 1227–1426.
73. Korhonen, H. (2009). Milk-derived bioactive peptides: From science to application. *Journal of Functional Foods*, **1**, 177–187.
74. Korhonen, H., Pihlanto, A. (2006). Bioactive peptides: Production and functionality. *International Dairy Journal*, **16**, 945–960.
75. Kuwata, K., Hoshino, M., Forge, V., Batt, C.A., Goto, Y. (1999). Solution structure and dynamics of bovine β-lactoglobulin. *Protein Science*, **8**, 2541–2545.
76. Leaver, J., Dalgleish, D.G. (1990). The topography of bovine β-casein at an oil/water interface as determined from the kinetics of trypsin-catalysed hydrolysis. *Biochimica et Biophysica Acta*, **1041**, 217–222.
77. Lemay, D.G., Lynn, D.J., Martin, W.F., Neville, M.C., Casey, T.M., Rincon, G. et al. (2009). The bovine lactation genome: Insights into the evolution of mammalian milk. *Genome Biology*, **10**, R43.1–18.
78. Leonardi, M., Gerbault, P., Thomas, M.G., Burger, J. (2012). The evolution of lactase persistence in Europe. A synthesis of archaeological and genetic evidence. *International Dairy Journal*, **22**, 88–97.
79. Lopez, C. (2011). Milk fat globules enveloped by their biological membrane: Unique colloidal assemblies with a specific composition and structure. *Current Opinion in Colloid and Interface Science*, **16**, 391–404.
80. Lopez, C., Madec, M.-N., Jimenez-Flores, R. (2010). Lipid rafts in the bovine milk fat globule membrane revealed by the lateral segregation of phospholipids and heterogeneous distribution of glycoproteins. *Food Chemistry*, **120**, 22–33.
81. Lucey, J.A. (2014). Milk protein gels. In *Milk Proteins: From Expression to Food*, 2nd edn. (H. Singh, M. Boland, A. Thompson, eds.). Elsevier/Academic Press, London, U.K., pp. 494–318.
82. Lucey, J.A., Horne, D.S. (2009). Milk salts: Technological significance. In *Advanced Dairy Chemistry*, Vol. 3, Lactose, Water, Salts and Minor Constituents, 3rd edn. (P.L.H. McSweeney, P.F. Fox, eds.). Springer Science, New York, pp. 351–389.
83. MacGibbon, A.K.H., Taylor, M.W. (2006). Composition and structure of bovine milk lipids. In *Advanced Dairy Chemistry, Lipids*, 3rd edn. (P.F. Fox, P.L.H. McSweeney, eds.). Springer, New York, pp. 1–35.
84. McGregor, R.A., Poppitt, S.D. (2014). Milk proteins and human health. In *Milk Proteins: From Expression to Food*, 2nd edn. (H. Singh, M. Boland, A. Thompson, eds.). Elsevier/Academic Press, London, U.K., pp. 541–555.
85. McMahon, D.J., McManus, W.R. (1998). Rethinking casein micelle structure using electron microscopy. *Journal of Dairy Science*, **81**, 2985–2993.
86. McMahon, D.J., Oommen, B.S. (2008). Supramolecular structure of casein micelles. *Journal of Dairy Science*, **91**, 1709–1721.
87. McManaman, J.L., Neville, M.C. (2003). Mammary physiology and milk secretion. *Advanced Drug Delivery Reviews*, **55**, 629–641.
88. Marchin, S., Putaux, J.-L., Pignon, F., Leonil, J. (2007). Effects of the environmental factors on the casein micelle structure studied by cryo-transmission electron microscopy and small-angle X-ray scattering/ultra-small-angle X-ray scattering. *Journal of Chemical Physics*, **126**, 045101.
89. Martin, P., Ferranti, P., Leroux, C., Addeo, F. (2003). Non-bovine caseins: Quantitative variability and molecular diversity. In *Advanced Dairy Chemistry*, Vol. 1, Proteins, 3rd edn. (P.F. Fox, P.L.H. McSweeney, eds.). Kluwer Academic/Plenum Publishers, New York, pp. 277–317.
90. Mather, I.H. (2000). A review and proposed nomenclature for major proteins of the milk fat globule membrane. *Journal of Dairy Science*, **83**, 203–247.
91. Mather, I.H., Jack, I.J.W. (1996). A review of the molecular and cellular biology of butyrophilin, the major protein of milk fat globule membrane. *Journal of Dairy Science*, **76**, 3832–3850.
92. Mather, I.H., Keenan, T.W. (1998). Origin and secretion of milk lipids. *Journal of Mammary Gland Biology and Neoplasia*, **3**, 259–273.
93. Mercier, J.-C. (1981). Phosphorylation of casein. Present evidence for an amino acid triplet code post-translationally recognized by specific kinases. *Biochimie*, **68**, 1–17.
94. Mills, E.N.C., Mackie, A.R. (2008). The impact of processing on the allergenicity of food. *Current Opinion in Allergy and Clinical Immunology*, **8**, 249–253.

95. Moore, J.H., Christie, W.W. (1979). Lipid metabolism in the mammary gland of ruminants. *Progress in Lipid Research*, **17**, 347–395.
96. Moreno, F.J., Mackie, A.R., Mills, E.N.C. (2005). Phospholipid interactions protect the milk allergen alpha-lactalbumin from proteolysis during in vitro digestion. *Journal of Agricultural and Food Chemistry*, **53**, 9810–9816.
97. Mulvihill, D.M., Ennis, M.P. (2003). Functional milk proteins: Production and utilization. In *Advanced Dairy Chemistry*, Vol. 1B, Proteins, 3rd edn. (P.F. Fox, P.L.H. McSweeney, eds.). Kluwer Academic/Plenum Publishers, New York, pp. 1175–1228.
98. Neville, M.C. (2005). Calcium secretion into milk. *Journal of Mammary Gland Biology and Neoplasia*, **10**, 119–128.
99. Nickerson, T.A. (1965). Lactose. In *Fundamentals of Dairy Chemistry* (B.H. Webb, A.H. Johnson, eds.). AVI Publishing, Westport, CT, pp. 224–260.
100. Oftedal, O.T. (2002). The mammary gland and its origin during synapsid evolution. *Journal of Mammary Gland Biology*, **7**, 225–252.
101. Oftedal, O.T. (2012). The evolution of milk secretion and its ancient origin. *Animal*, **6**, 355–368.
102. Oftedal, O.T. (2013). Origin and evolution of the constituents of milk. In *Advanced Dairy Chemistry*, Vol. 1A, Proteins: Basic Aspects (P.L.H. McSweeney, P.F. Fox, eds.). Springer Science+Business Media, New York, pp. 1–42.
103. O'Connell, J.E., Fox, P.F. (2003). Heat-induced coagulation in milk. In *Advanced Dairy Chemistry*, Vol. 1, Proteins (P.F. Fox, P.L.H. McSweeney, eds.). Kluwer Academic/Plenum Publishers, New York, pp. 879–945.
104. O'Mahony, J.A., Fox, P.F. (2014). Milk: An overview. In *Milk Proteins: From Expression to Food*, 2nd edn. (H. Singh, M. Boland, A. Thompson, eds.). Elsevier/Academic Press, London, U.K., pp. 19–73.
105. O'Mahony, J.A., Fox, P.F., Kelly, A.L. (2013). Indigenous enzymes of milk. In *Advanced Dairy Chemistry*, Vol. 1A, Proteins: Basic Aspects (P.L.H. McSweeney, P.F. Fox, eds.). Springer Science+Business Media, New York, pp. 337–386.
106. Ouanezar, M., Guyomarc'h, F., Bouchoux, A. (2012). AFM imaging of casein micelles: Evidence for structural rearrangement upon acidification. *Langmuir*, **28**, 4915–4919.
107. Patel, H.A., Huppertz, T. (2014). Effects of high-pressure processing on structure and interactions of milk proteins. In *Milk Proteins: From Expression to Food*, 2nd edn. (H. Singh, M. Boland, A. Thompson, eds.). Elsevier/Academic Press, London, U.K., pp. 243–267.
108. Payens, T.A.J. (1979). Casein micelles: The colloid chemical approach. *Journal of Dairy Research*, **46**, 291–306.
109. Pouliot, Y., Boulet, M., Paquin, P. (1989a). Observations on the heat-induced salt balance changes in milk. I. Effect of heating time between 4 and 90°C. *Journal of Dairy Research*, **56**, 185–192.
110. Pouliot, Y., Boulet, M., Paquin, P. (1989b). Observations on the heat-induced salt balance changes in milk. II. Reversibility on cooling. *Journal of Dairy Research*, **56**, 193–199.
111. Pyne, G.T. (1962). Some aspects of the physical chemistry of salts of milk. *Journal of Dairy Research*, **29**, 101–130.
112. Rollema, H.S. (1992). Casein association and micelle formation. In *Advanced Dairy Chemistry*, Vol. 1, Proteins (P.F. Fox, ed). Elsevier Applied Science, London, U.K., pp. 111–140.
113. Sawyer, L. (2013). β-Lactoglobulin. In *Advanced Dairy Chemistry*, Vol. 1A, Proteins: Basic Aspects (P.L.H. McSweeney, P.F. Fox, eds). Springer Science+Business Media, New York, pp. 211–259.
114. Schmidt, D.G. (1980). Colloidal aspects of the caseins. *Netherlands Milk and Dairy Journal*, **34**, 42–64.
115. Schmidt, D.G. (1982). Association of caseins and casein micelle structure. In *Developments in Dairy Chemistry*, Vol. 1 (P.F. Fox, ed). Applied Science Publishers, London, U.K., pp. 61–86.
116. Shennan, D., Peaker, M. (2000). Transport of milk constituents to the mammary gland. *Physiological Reviews*, **80**, 925–951.
117. Sicherer, S.H., Sampson, H.A. (2010). Food allergy. *Journal of Allergy and Clinical Immunology*, **125**, S116–S125.
118. Singh, H. (2006). The milk fat globule membrane. A biophysical system for food applications. *Current Opinion in Colloid and Interface Science*, **11**, 154–163.
119. Singh, H., Creamer, L.K. (1992). Heat stability of milk. In *Advanced Dairy Chemistry*, Vol. 1, Proteins (P.F. Fox, ed). Elsevier Applied Science, London, U.K., pp. 621–656.
120. Slattery, C.W., Evard, R. (1973). A model for the formation of casein micelles from sub-units of variable composition. *Biochimica et Biophysica Acta*, **317**, 529–538.

121. Smithers, G.W. (2008). Whey proteins—From "gutter to gold." *International Dairy Journal*, **18**, 695–704.
122. Soedamah-Muthu, S.S., Ding, E.L., Al-Delaimy, W.K., Hu, F.b., Engberink, M.F., Willett, W.C., Galeijnse, J.M. (2011). Milk and dairy consumption and incidence of cardiovascular diseases and all-cause mortality: Dose-response meta-analysis of prospective cohort studies. *American Journal of Clinical Nutrition*, **93**, 158–171.
123. Swaisgood, H.E. (2007). Characteristics of milk. In *Fennema's Food Chemistry*, 4th edn. (S. Damodaran, K.L. Parkin, O.R. Fennema, eds.). CRC Press, Boca Raton, FL., pp. 881–897.
124. Taheri-Kafrani, A., Gaudin, J.C., Rabesona, H., Nioi, C., Agarwal, D., Drouet, M. et al. (2009). Effects of heating and glycation of beta-lactoglobulin on its recognition by IgE of sera from cow milk allergy patients. *Journal of Agricultural and Food Chemistry*, **57**, 4974–4982.
125. Trejo, R., Dokland, T., Jurat-Fuentes, J., Harte, F. (2011). Cryo-transmission electron tomography of native casein micelles from bovine milk. *Journal of Dairy Science*, **94**, 5770–5775.
126. Uhrinova, S., Smith, M.H., Jameson, G.B., Uhrin, D., Sawyer, L., Barlow, P.N. (2000). Structural changes accompanying pH-induced dissociation of the β-lactoglobulin dimer. *Biochemistry*, **39**, 3565–3574.
127. Dairy Products 2012 Summary. (April, 2013). USDA National Agricultural Statistics Service, Washington, D.C.
128. Walstra, P., Wouters, J.T.M., Guerts, T.J. (2005). *Dairy Science and Technology*, 2nd edn. CRC Press, Boca Raton, FL.
129. WHO (2007). Protein and amino acid requirements in human nutrition: Report of a Joint FAO/WHO/UNU Expert Consultation. World Health Organization, Geneva, Switzerland.

FURTHER READING

Fox, P.F., McSweeney, P.L.H. (eds.) (2006). *Advanced Dairy Chemistry, Lipids*, 3rd edn. Springer, New York.
Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (eds). (2004). *Cheese Chemistry, Physics and Microbiology*, Vol. 1. Academic Press, London, U.K.
McSweeney, P.L.H., Fox, P.F. (eds.) (2009). *Advanced Dairy Chemistry*, Vol. 3, Lactose, Water, Salts and Minor Constituents, 3rd edn. Springer Science, New York.
McSweeney, P.L.H., Fox, P.F. (eds.) (2013). *Advanced Dairy Chemistry*, Vol. 1A, Proteins: Basic Aspects. Springer Science+Business Media, New York.
McSweeney, P.L.H., O'Mahony, J.A. (eds.) (2016). *Advanced Dairy Chemistry*, Vol. 1B, Proteins: Applied Aspects. Springer Science+Business Media, New York.
Muehlhoff, E., Bennett, A., McMahon, D. (eds.) (2013). *Milk and Dairy Products in Human Nutrition*, Food and Agricultural Organization of the United Nations, Rome, Italy.
Singh, H., Boland, M., Thompson, A. (eds.) (2014). *Milk Proteins: From Expression to Food*, 2nd edn. Elsevier/Academic Press, London, U.K.
Tamime, A.Y. (ed.) *Milk Processing and Quality Management*. Wiley-Blackwell Publishing, Oxford, U.K.
Walstra, P., Wouters, J.T.M., Guerts, T.J. (eds.) (2009). *Dairy Science and Technology*, 2nd edn. CRC Press, Boca Raton, FL.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

15 Physiology and Chemistry of Edible Muscle Tissues

Gale M. Strasburg and Youling L. Xiong

CONTENTS

15.1 Introduction 956
15.2 Nutritive Value 956
15.2.1 Summary 960
15.3 Structure and Function of Muscle 961
15.3.1 Structure of Skeletal Muscle 961
15.3.2 Structure of Cardiac Muscle 965
15.3.3 Structure of Smooth Muscle 965
15.3.4 Proteins of the Muscle Tissue 966
15.3.4.1 Connective Tissue or Extracellular Matrix Proteins 966
15.3.4.2 Sarcoplasmic Proteins 970
15.3.4.3 Contractile Proteins 973
15.3.4.4 Regulatory Proteins 976
15.3.4.5 Structural Proteins of the Myofibril 978
15.3.4.6 Proteins of the Sarcoplasmic Reticulum and Sarcolemma 982
15.3.5 Excitation–Contraction Coupling 983
15.3.6 Muscle Fiber Types 985
15.3.7 Summary 986
15.4 Conversion of Muscle to Meat 987
15.4.1 Postmortem Degradation of Muscle Proteins 988
15.4.2 Summary 989
15.5 Natural and Induced Postmortem Biochemical Changes Affecting Meat Quality 991
15.5.1 Pale, Soft, Exudative Meat 992
15.5.2 Dark, Firm, and Dry Meat 993
15.5.3 Cold Shortening 994
15.5.4 Thaw Rigor 996
15.5.5 Electrical Stimulation 996
15.5.6 Summary 997
15.6 Chemical Changes in Meat during Preservation 997
15.6.1 Chilling and Refrigeration 997
15.6.2 Freezing 998
15.6.3 Pressurization 999
15.6.4 Irradiation 1000
15.6.5 Summary 1001
15.7 Chemistry of Processed Meats 1001
15.7.1 Curing 1001
15.7.2 Hydration and Water Retention 1002
15.7.3 Formation of Protein Gel Matrix 1004

15.7.4 Fat Immobilization and Stabilization ... 1006
15.7.5 Meat Restructuring ... 1008
15.7.6 Chemistry of Surimi ... 1009
15.7.7 Summary ... 1009
Questions ... 1010
References ... 1010

15.1 INTRODUCTION

Archaeological evidence indicates that humans have consumed animal products, including meat, as sources of food for thousands of years. This fact was dramatically illustrated following the discovery by mountain hikers in 1991 of Eis Mann (iceman), the frozen remains of a man found in a glacier high in the Italian Alps [1]. Ötzi, as he affectionately came to be known by the area residents, had apparently died approximately 5100–5300 years ago. It appears that he was a hunter who may have died of arrow wounds as a result of a rivalry with another hunter or hunting group. His body was so well preserved by the glacial ice that it was possible for scientists to use recombinant DNA techniques to analyze the contents of his gastrointestinal tract in order to determine what he had eaten in his last two meals. The penultimate meal prior to his death consisted of meat from an ibex (a type of wild goat once found in the Alps) as well as cereal grains and other types of plant food. His final meal included red deer meat and possibly cereal grains.

Over the course of history, some cultures have eaten meat as a matter of choice, while others have done so as a matter of necessity. Likewise, religious and cultural factors have had considerable influence in defining meat that is considered acceptable for consumption versus meat that is considered unacceptable. For example, some religious groups prescribe avoidance of pork, whereas others mandate avoidance of beef. In the United States, eating horse meat is culturally considered taboo, while it is acceptable in other countries. Still other individuals avoid meat completely for ethical reasons or presumptive health concerns. Nevertheless, it is also evident that as economies of nations grow, particularly in developing countries, there is a parallel growth in demand for meat products.

The term "meat" in colloquial use sometimes connotes red muscle food (beef, pork, lamb), whereas poultry and fish muscle foods are in classes by themselves. In this chapter, we utilize the term "meat" more broadly to refer to skeletal muscle tissue from a mammal, bird, reptile, amphibian, or fish that has undergone a specific series of transformative biochemical reactions following the death of the animal. This term specifically excludes other organ foods, such as the liver, thymus, and kidney, but does include the heart and tongue as unique muscle tissues.

As with any food product, foundational understanding of the tissue(s) from which the product is derived is critical to managing and optimizing the functional characteristics of the product components in processed food. Accordingly, after describing the nutritive value of muscle from various species as food, this chapter will focus on the structure and function of muscle in the live animal, the characteristics of which are important determinants of the functional properties and quality indices of meat and meat products. Subsequently, this chapter will describe the conversion of muscle to meat, that is, the chemical and physiological changes occurring in the muscle upon the death of the animal. The chapter concludes with a description of chemical changes occurring in meat as a result of preservation and processing.

15.2 NUTRITIVE VALUE

The positive sensory appeal of fresh and processed meat products and the feeling of satiety following consumption of a meal that includes meat have combined to make muscle food products staples of human diets around the world. The variety, bioavailability, and density of nutrients (i.e., the concentrations of nutrients per kilocalorie) in meat combine to make it a substantial contributor of nutrients to the diets of many consumers.

The composition of meat is quite variable. Species, breed, sex, age, nutritional status, and activity level of the animal are major factors affecting the gross composition of meat [2]. Moreover, even for a given animal, the anatomical location of the retail cut, post-slaughter processing, storage, and, of course, cooking contribute significantly to the variability of meat composition. It is beyond the scope of this chapter to examine these factors beyond a few generalizations discussed later. Detailed information on the composition of meat products by species, retail cut, degree of trim, raw versus cooked product, etc., is available from the USDA and is periodically updated [3]. However, even these values must be regarded as approximations, given the many sources of variation.

The proximate composition of separable lean tissue (skeletal muscle trimmed of external fat) is somewhat variable, but in general, water accounts for about 70% of the weight of fresh, lean muscle (Table 15.1). Most of the water is trapped within or between the muscle cells, with lesser amounts bound in varying degrees to proteins. The variation in water content is generally offset by changes in lipid composition, while protein composition ranges from 18% to 23%, and ash or mineral content is approximately 1%–1.2% (Table 15.1).

The lipid content and composition are the most variable of the four primary components of meat. Because the lipid fractions associated with muscle tissue and adipose tissue vary in quantity and composition, the amount of adipose tissue present in a meat product profoundly affects the proximate composition of the product [4]. Moreover, as the adipose tissue content of meat decreases, the percentage of phospholipid contribution to the total increases (Table 15.2). Most of the lipid in meat consists of neutral triacylglycerols, lesser amounts of phospholipids that comprise cellular membranes, and a small amount of cholesterol found primarily in the muscle plasma membrane and nervous tissue. The neutral fat fraction consists of a higher percentage of saturated fatty acids than that of the phospholipid fraction, which is not surprising from a functional standpoint. The necessity of maintaining a fluid cell membrane at physiological temperatures requires that phospholipids have a higher percentage of unsaturated fatty acids, while the more solid nature of adipose tissue results from the large fraction of saturated fatty acids with their higher melting points. Despite the smaller contribution of phospholipid fraction to the total lipid composition, the polyunsaturated nature of the phospholipids together with the high surface-to-volume ratio makes this lipid fraction highly susceptible to oxidative reactions that contribute to deterioration of flavor and color of meat [5].

TABLE 15.1
Proximate Composition of Meat from Various Sources[a]

	Red Meat			Poultry		Fish	
	Beef[b]	Pork[c]	Lamb[d]	Chicken[e]	Turkey[f]	Cod[g]	Tuna[h]
Water	70.29	73.17	73.42	75.46	75.37	81.22	68.09
Protein	20.72	21.20	20.29	21.39	22.64	17.81	23.33
Lipid	7.37	4.86	5.25	3.08	1.93	0.67	4.90
Ash	1.02	1.00	1.06	0.96	1.04	1.16	1.18

Source: Compiled from U.S. Department of Agriculture, Agricultural Research Service, USDA National Nutrient Database for Standard Reference, Release 26, Nutrient Data Laboratory Home Page, https://ndb.nal.usda.gov/ndb/, 2013.

[a] Percent by weight of edible portion.

[b] Beef, brisket, whole, separable lean only, all grades, raw.

[c] Pork, fresh, composite of trimmed retail cuts (leg, loin, shoulder), separable lean only, raw.

[d] Lamb, domestic, composite of trimmed retail cuts, separable lean only, trimmed to 1/4″ fat, choice, raw.

[e] Chicken, broilers or fryers, meat only, raw.

[f] Turkey, whole, meat only, raw.

[g] Fish, cod, Atlantic, raw.

[h] Fish, tuna, fresh, bluefin, raw.

TABLE 15.2
Lipid Content of Various Meats

		Content[a]		
Species	**Muscle or Type**	**Lipid (%)**	**Neutral Lipids (%)**	**Phospholipids (%)**
Chicken	White	1.0	52	48
	Red	2.5	79	21
Turkey	White	1.0	29	71
	Red	3.5	74	26
Fish (sucker)	White	1.5	76	2
	Red	6.2	93	7
Beef	Lattisimus dorsi	2.6	78	22
		7.7	92	8
		12.7	95	5
Pork	Lattisimus dorsi	4.6	79	21
	Psoas major	3.1	63	37
Lamb	Lattisimus dorsi	5.7	83	10
	Semitendinosus	3.8	79	17

Source: Allen, C.E. and Foegeding, E.A., *Food Technol*., 35, 253, 1981.
[a] Percentage of gross muscle composition.

Lipid composition varies by species; the highest levels of polyunsaturated fatty acids are found in fish, whereas the lowest amounts are in beef and mutton (Table 15.3). Various fish, especially fatty sea species such as salmon, are uniquely rich sources of n-3 polyunsaturated fatty acids such as docosahexaenoic acid (C22:6) and eicosapentaenoic acid (C20:5). Fatty acid composition can be altered to some degree by diet. For example, maintaining beef cattle on a grass-based diet instead of finishing cattle on grain increases the percentage of polyunsaturated fats, including n-3 fatty acids [6]. However, despite the increase attributable to diet, the quantity of n-3 fatty acids in both grass-fed and grain-finished beef is quite small compared to that of most fatty fish [3]. Lipid composition also varies from muscle to muscle within a species, particularly when comparing muscles in which most of the fibers rely on oxidative metabolism (red muscles) with muscles that generally rely on glycolytic metabolism (white muscles).

The protein content of meat is typically derived from analysis of total nitrogen content of the product multiplied by 6.25, a factor based on the average nitrogen content of meat protein. However, this approach overestimates the amount of protein, because as much of 10% of muscle nitrogen comes from nonprotein sources including amino acids, peptides, creatine, nucleic acids, and other nitrogen-containing molecules.

Meat is an excellent source of dietary protein because the amino acid composition closely parallels human dietary amino acid requirements. The high quality together with the relative abundance of protein in meat means that a single 85 g serving of meat may provide 50%–100% of the daily protein intake recommended for maintenance of growth and health [7]. Moreover, the complete amino acid composition of meat enables complementation of other dietary sources of protein. For example, even a small amount of meat added to a cereal- or legume-based diet, which would be deficient in lysine and sulfur amino acids, respectively, dramatically improves the nutritional value of the plant-derived protein.

Meat protein is also recognized as a source for bioactive peptides that exert functions beyond normal nutritional roles. For example, many peptides produced during the gastric (pepsin) and intestinal (trypsin, chymotrypsin, and aminopeptidase) digestion of meat (mammalian, poultry, and fish) exhibit antioxidative, angiotensin-1-converting enzyme inhibitory, and metal ion–binding activities. Individual peptides with such bioactivities have been isolated from *in vitro* digests of myosin, sarcoplasmic proteins, collagen, and many other muscle proteins [8].

TABLE 15.3
Fatty Acid Composition of Meat from Various Sources[a]

	Red Meat			Poultry		Fish	
	Beef[b]	Pork[c]	Lamb[d]	Chicken[e]	Turkey[f]	Cod[g]	Tuna[h]
Total saturated	35.14	33.44	35.81	25.65	23.78	19.55	25.65
14:0	2.99	1.19	2.67	0.65	0.67	1.34	2.84
16:0	22.12	21.13	19.43	17.21	15.03	13.58	16.53
18:0	9.91	10.80	11.81	7.14	7.00	4.48	6.27
Total monounsaturated	46.95	42.45	40.19	29.22	24.72	14.03	32.65
16:1	4.75	2.61	3.05	3.90	2.23	2.39	3.31
18:1	42.06	39.18	36.38	24.68	21.87	9.10	18.86
Total polyunsaturated	3.12	10.68	9.14	24.35	21.30	34.48	29.24
18:2	2.44	9.05	6.86	17.86	20.67	0.75	1.08
18:3	0.27	0.41	1.33	0.65	0.88	0.15	0.00
20:4	0.41	1.01	0.95	2.60	1.61	3.28	0.88
20:5	0.00	0.00	0.00	0.32	0.00	9.55	5.77
22:5	0.00	0.00	0.00	0.65	0.16	1.49	2.55
22:6	0.00	0.00	0.00	0.97	0.10	17.91	18.20

[a] Percent of total fat in lean muscle tissue for selected red meats, poultry, and fish. Calculated based on information compiled from U.S. Department of Agriculture, Agricultural Research Service (2013). USDA National Nutrient Database for Standard Reference, Release 26. Nutrient Data Laboratory Home Page, https://ndb.nal.usda.gov/ndb/.
[b] Beef, brisket, whole, separable lean only, all grades, raw.
[c] Pork, fresh, composite of trimmed retail cuts (leg, loin, shoulder), separable lean only, raw.
[d] Lamb, domestic, composite of trimmed retail cuts, separable lean only, trimmed to 1/4″ fat, choice, raw.
[e] Chicken, broilers or fryers, meat only, raw.
[f] Turkey, whole, meat only, raw.
[g] Fish, cod, Atlantic, raw.
[h] Fish, tuna, fresh, bluefin, raw.

Muscle tissue is an excellent source of many water-soluble vitamins including thiamin, riboflavin, niacin, B_6, and B_{12} (Table 15.4). However, as with other nutrients, the vitamin content is strongly influenced by species, age, sex, and nutritional status of the animal. Most noteworthy are the very high levels of thiamin and the low levels of B_{12} in pork compared to beef and lamb. Vitamins C, D, E, and K tend to be low in all muscle foods. However, studies have indicated that levels of vitamin E in meat can be substantially elevated through increased dietary supplementation of animal feed. Because vitamin E functions as an antioxidant, its presence at elevated levels has beneficial effects in stabilizing meat color, reducing lipid oxidation, and enhancing human health [9].

Red meats are particularly good sources of iron because of their high myoglobin content; however, even white muscle of poultry and fish can be important sources of iron (Table 15.4). Moreover, the heme form of the iron imparts a high degree of bioavailability compared to most inorganic sources of iron. Potassium, phosphorus, and magnesium are relatively abundant in meat. Calcium, despite its importance in regulating muscle contraction, is present in muscle at very low levels relative to dietary requirements. In mechanically separated meats, calcium may be present at higher levels because of the presence of small amounts of microscopic bone fragments present in the final product [10].

Carbohydrates make only a small contribution to the composition of fresh meat (<1%). The major source of carbohydrate in muscle is glycogen, with minor amounts of monosaccharides and glycolytic metabolites. During conversion of muscle to meat, glycogen is largely converted to lactate by anaerobic glycolysis, thus making lactate the primary carbohydrate in meat [11].

TABLE 15.4
Mineral and Vitamin Composition of Meat from Various Sources[a]

	Red Meat			Poultry		Fish		DRI[b]
	Beef[c]	Pork[d]	Lamb[e]	Chicken[f]	Turkey[g]	Cod[h]	Tuna[i]	RDA or AI*
Minerals								
Potassium	330	363	280	229	235	413	252	4,700/4,700*
Phosphorous	201	216	189	173	190	203	254	700/700
Sodium	79	59	66	77	118	54	39	1,500/1,500*
Magnesium	23	24	26	25	27	32	50	420/320[j]
Calcium	5	13	10	12	11	16	8	1,000/1,000*
Zinc	4.31	2.21	4.06	1.54	1.84	0.45	0.6	11/8
Iron	1.92	0.82	1.77	0.89	0.86	0.38	1.02	8/18
Vitamins								
Thiamin	100	642	130	73	50	76	241	1,200/1,100
Riboflavin	170	254	230	142	192	65	251	1,300/1,100
Niacin	3,940	5,573	6,000	8,239	8,100	2,063	8,654	16,000/14,000
Pantothenic acid	350	936	700	1058	844	153	1054	5,000/5,000*
B6	420	644	160	430	652	245	455	1,300/1,300
Folate	7	2	23	7	7	7	2	400/400
B12	2.43	0.64	2.62	0.37	1.24	0.91	9.43	2.4/2.4

[a] Values are expressed as mg/100 g and μg/100 g for minerals and vitamins, respectively. U.S. Department of Agriculture, Agricultural Research Service (2013). USDA National Nutrient Database for Standard Reference, Release 26. Nutrient Data Laboratory Home Page, https://ndb.nal.usda.gov/ndb/.

[b] DRI (Dietary Reference Intake) values are expressed as RDA (recommended dietary allowances) or AI* (adequate intake) for male/female adults (age 19–50). Compiled from Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. DRI for calcium, phosphorus, magnesium, vitamin D, and fluoride; Food and Nutrition Board, Institute of Medicine, National Academy of Sciences (1998). DRI for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin, and choline; Food and Nutrition Board, Institute of Medicine, National Academy of Sciences (2000). DRI for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc; and Food and Nutrition Board, Institute of Medicine, National Academy of Sciences (2004) DRI for water, potassium, sodium, chloride, and sulfate.

[c] Beef, brisket, whole, separable lean only, all grades, raw.

[d] Pork, fresh, composite of trimmed retail cuts (leg, loin, shoulder), separable lean only, raw.

[e] Lamb, domestic, composite of trimmed retail cuts, separable lean only, trimmed to 1/4 in. fat, choice, raw.

[f] Chicken, broilers or fryers, meat only, raw.

[g] Turkey, whole, meat only, raw.

[h] Fish, cod, Atlantic, raw.

[i] Fish, tuna, fresh, bluefin, raw.

[j] Age 31–50.

15.2.1 Summary

- Meat consists of approximately 70% water, 21% protein, 7% fat, 1% carbohydrate, and 1% mineral (ash) content. The water and ash content are relatively constant, whereas the percentages of protein and fat are much more variable.
- Meat is a nutrient-dense food. It is an excellent source of complete protein, B vitamins, iron, potassium, magnesium, and phosphorus. It is notably deficient in vitamins C, D, and K and calcium.
- Lipid content and composition varies dramatically from species to species, but can be altered to some degree by management practices.

15.3 STRUCTURE AND FUNCTION OF MUSCLE

15.3.1 Structure of Skeletal Muscle

Individual skeletal muscles vary greatly in size and morphology. In general, muscles consist of a parallel arrangement of elongated, multinucleated cells called myofibers or muscle fibers. Individual myofibers range from 10 to 100 μM in width and from a few millimeters to several centimeters long, sometimes spanning the entire length of a muscle. Myofibers are arranged in hierarchical fashion with associated circulatory, nerve, and blood tissues forming the whole muscle organ (Figure 15.1). The extracellular matrix (ECM), a term synonymous with intramuscular connective tissue, serves as the scaffolding system in which the myofibers are assembled. Within the ECM, each myofiber is encased in a layer of connective tissue called the endomysium. Groups of myofibers are organized into primary and secondary bundles (fascicles) that are encased by

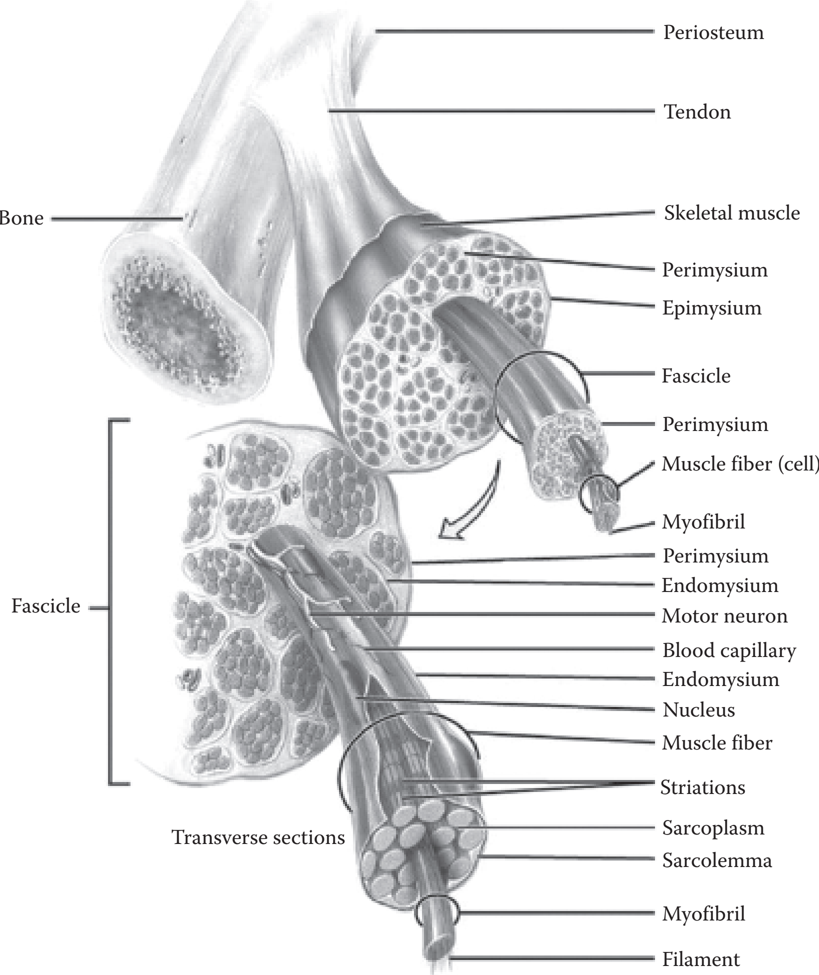


FIGURE 15.1 Diagrammatic representation of the structural organization of muscle from subcellular myofibrils to whole organ. Individual muscle cells (fibers) are surrounded by a layer of connective tissue (endomysium), which, in turn, are organized into bundles (fascicles), separated by another layer of connective tissue called the perimysium. Blood vessels and nerves penetrate the perimysium and serve as tissues supporting muscle function. (Reprinted from Tortora, G.J. and Derrickson, B., *Principles of Anatomy and Physiology*, 14th edn., John Wiley & Sons, Inc., Hoboken, NJ, 2014. With permission.)

another layer of connective tissue called the perimysium. A final sheath of heavy connective tissue, the epimysium, surrounds the whole muscle. The epimysium merges with the tendons to link the muscle to the bones. The molecular and structural properties of connective tissue are described in Section 15.3.4.1.

Muscle is infiltrated by a complex system of nerves involved in the regulation of muscle contraction and maintenance of muscle tone as well as a vascular system through which blood provides oxygen and nutrients while removing metabolic end products such as carbon dioxide and lactate (Figure 15.1). The perimysium and endomysium combine to provide the necessary framework for maintaining the structural integrity of these tissues within the muscle at rest and more importantly during the mechanical stress of contraction. Adipose tissue may also be found embedded in the perimysial layer and is visible in red meats as white flecks of fat (marbling) in contrast to the red background of myofibers. The abundance of marbling is often used as an indicator in visual appraisal of meat quality [12].

The unique structure of muscle cells enables translation of electrochemical impulses, triggered by neural stimulation, into increased intracellular Ca^{2+} concentrations that in turn trigger muscle contraction. Like all cells, a myofiber is bounded by a plasma membrane, referred to as the sarcolemma (SL) in muscle. However, the skeletal muscle SL is distinguished structurally from the plasmalemma of other cells by periodic perpendicular invaginations of the membrane into the interior of the muscle cell, much like fingers poking into the skin of a balloon. These inward extensions of the SL, referred to as transverse tubules or T-tubules, transmit the action potential or depolarization signal for contraction from the neuromuscular junction to the interior of the myofiber. The T-tubules are in contact with an extensive, highly developed intracellular membrane network called the sarcoplasmic reticulum (SR), which is the muscle equivalent of the endoplasmic reticulum (Figure 15.2). The SR network encircles the contractile organelles (myofibrils) and functions as a reservoir of Ca^{2+} that serves as the trigger for muscle contraction. Numerous proteins embedded in the SR are responsible for specific functions related to intracellular Ca^{2+} regulation. Some proteins in the

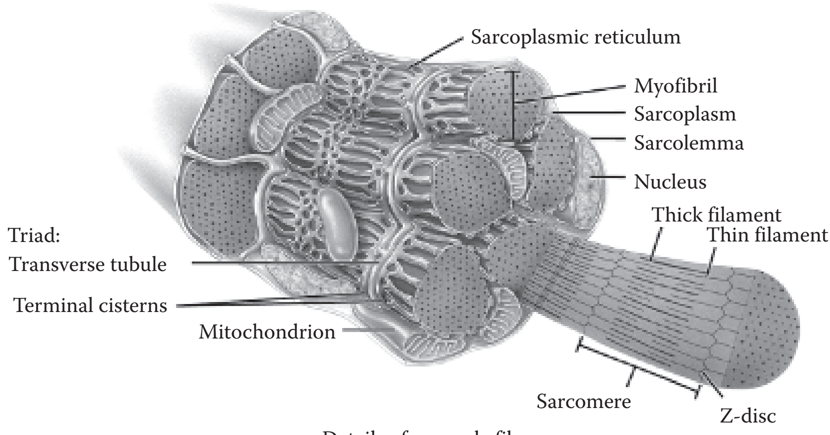


FIGURE 15.2 Schematic representation of the structural organization of the muscle fiber. Multinucleated muscle fibers are encased by the sarcolemma (muscle cell membrane). Invaginations of the sarcolemma into the center of the muscle fiber form structures called transverse tubules (T-tubules). Each T-tubule is sandwiched between and linked to terminal cisternae of the SR, forming a structure called a triad. The SR (endoplasmic reticulum of muscle) envelops the myofibrils, stores Ca^{2+} ions when muscle is at rest, and releases Ca^{2+} to the sarcoplasm during muscle contraction. (Reprinted from Tortora, G.J. and Derrickson, B., *Principles of Anatomy and Physiology*, 14th edn., John Wiley & Sons, Inc., Hoboken, NJ, 2014. With permission.)

interior (lumen) of the SR bind Ca^{2+} while the muscle is at rest [13]. Other proteins form channels that open in response to the depolarization signal, enabling diffusion of Ca^{2+} from within the SR to the sarcoplasm (cytoplasm); this process initiates muscle contraction [14]. Another SR protein pumps Ca^{2+} back into the lumen of SR thereby enabling relaxation. These proteins are discussed in greater detail in Section 15.3.4.6.

Muscle cells possess other organelles typical of all cells. Because of the developmental path by which skeletal muscle cells develop, myofibers are typically multinucleated cells (Figure 15.2). The nuclei are usually dispersed to the periphery of the cell and are typically found immediately beneath the SL. Mitochondria serve as energy transducers for the myofiber and are found throughout the cell in close association with myofibrils. Lysosomes serve as a major reservoir for a family of proteolytic enzymes known as cathepsins, which play a catabolic function in protein turnover.

The sarcoplasm may contain glycogen particles and lipid droplets, the quantity of which depends on the type of muscle fiber (oxidative or nonoxidative) and the nutritional and exercise/resting state of the organism. The oxygen-storage protein myoglobin is found in varying degrees within the sarcoplasm, as are various enzymes, metabolites, amino acids, nucleotides, and so forth.

Muscle contraction is effected through the action of specialized proteins that are organized into parallel, interdigitating thin and thick filaments (myofilaments) that comprise 80%–90% of the volume of the myofiber. Myofilaments are grouped into myofibrils that function in coordinated fashion as the contractile organelles of a muscle cell. The high degree of structural organization of the myofibrils is evident when thin longitudinal sections of skeletal muscle are viewed with a microscope. One sees a pattern of alternating light and dark bands that result from longitudinal repetition of the fundamental structural unit of muscle contraction known as the sarcomere (Figure 15.3a). When viewed with polarized light, the dark bands are anisotropic and are thus referred to as “A-bands.” The lighter bands, “I-bands,” are isotropic in polarized light. The boundaries of the sarcomere are defined by structures known as Z-discs (also called Z-lines), which are narrow, dark, electron-dense bands of proteins in the center of the I-band. The term Z-disc is derived from the German *zwischen* meaning “between,” indicating its position at the center of the I-band. The matrix of proteins that constitute the Z-disc serves as the anchoring structure for the proteins of the thin filaments that emanate from both sides of the Z-disc (Figures 15.3b and 15.4).

The sarcomere consists of alternately placed thin and thick filaments. The I-band consists of thin filaments, whereas the A-band consists of overlapping thin and thick filaments. The center of the A-band is slightly less dense than the distal regions and therefore appears brighter because this zone consists only of thick filaments with no overlapping thin filaments (Figures 15.3b and 15.4). This band is called the H-zone, derived from the German *helle* meaning bright. At the center of the H-zone is a dark zone analogous to the Z-disc. This structure, called the M-line, consists of several proteins that maintain the structural arrangement of the thick filament proteins and serve as an anchoring point for the protein titin that spans the entire distance from the M-line to the Z-disc (see Section 15.3.4.5).

In 1954, Huxley and Hanson proposed a theory of muscle contraction, called the sliding filament theory, which has survived largely intact to the present era [15]. The theory is based on the observation that lengths of both thin and thick filaments remain constant, independent of whether the muscle is stretched, contracting, or in the resting state. In contrast, sarcomere lengths, defined as the distance between the centers of adjacent Z-discs, vary depending on degree of contraction or stretch force applied to the fiber. In addition, electron micrographs of muscle cross sections indicated that the thin and thick filaments interdigitate in such a way that one thick filament is surrounded by a hexagonal array of six thin filaments. Huxley and Hanson proposed that when contraction takes place, the thin and thick filaments slide past each other such that the thin filaments at opposite ends of a sarcomere move toward each other. This results in shortening of the sarcomere length (Figure 15.4). Conversely, stretching results from an increase in Z-disc separation, again accomplished by sliding of opposing thin filaments of a sarcomere away from each other as they

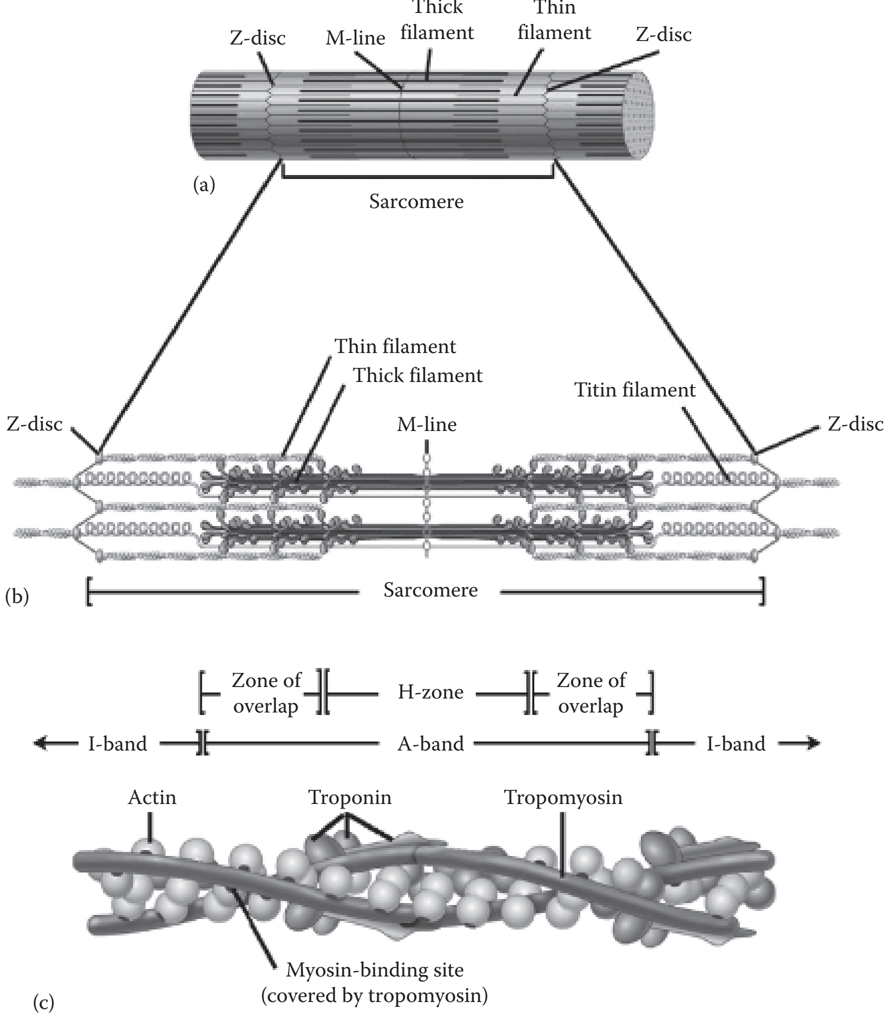


FIGURE 15.3 Structural arrangement of the sarcomere. (a) The sarcomeric unit of the myofibril begins at one Z-disc and extends to the next Z-disc. At the center of each sarcomere is an arrangement of proteins that form the M-line. (b) The major components of the sarcomere are thin filaments anchored at the Z-disc, thick filaments in the central region of the sarcomere that partially overlap with thin filaments, and titin filaments that span from the Z-disc to the M-line. (c) Actin monomers polymerize to form a double-stranded coiled coil that constitutes the backbone of the filament. Tropomyosin polymerizes in "head-to-tail" fashion and lies near the groove of the actin double helix, covering the myosin-binding sites on the actin backbone. One tropomyosin molecule spans seven actin monomers. One troponin molecule (consisting of subunits T, I, and C) binds to one tropomyosin molecule via the asymmetrically shaped TnT subunit. (Reprinted from Tortora, G.J. and Derrickson, B., *Principles of Anatomy and Physiology*, 14th edn., John Wiley & Sons, Inc., Hoboken, NJ, 2014. With permission.)

move along the A-band. The extent to which thin and thick filaments overlap has great practical significance with respect to meat tenderness. As we shall see later (Section 15.5.3), there is generally a negative correlation between sarcomere length and meat toughness. When muscle is maximally contracted, the sarcomeres are at their shortest, and the high degree of overlap between filaments, together with the large number of rigor bonds or crossbridges formed between the two types of filaments, results in increased toughness. The molecular details of how the sliding of filaments results in muscle contraction are discussed in Section 15.3.5.

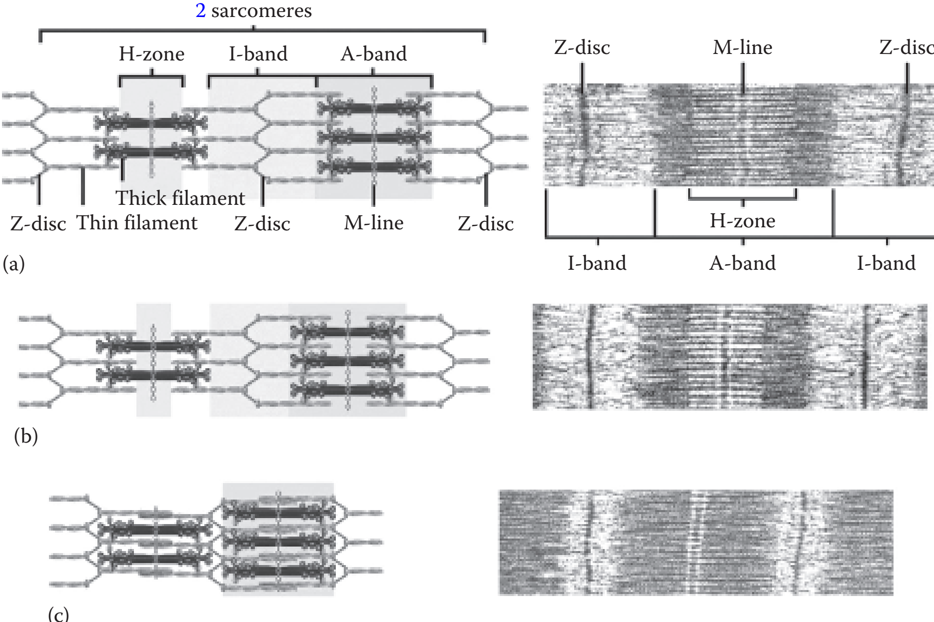


FIGURE 15.4 Illustration of the sliding filament theory: (a) relaxed, (b) partially contracted, (c) maximally contracted. Thin filaments and thick filaments interdigitate, allowing for sliding of filaments past each other. Note the shortening of the distance between Z-discs and the increasing overlap of thin and thick filaments as the muscle shortens. (Reprinted from Tortora, G.J. and Derrickson, B., *Principles of Anatomy and Physiology*, 14th edn., John Wiley & Sons, Inc., Hoboken, NJ, 2014. With permission.)

15.3.2 Structure of Cardiac Muscle

Heart muscle may be used as a food directly or more commonly may be minced and incorporated into processed meat products such as sausages. Like skeletal muscle, cardiac muscle is striated, which suggests an arrangement of the contractile proteins of cardiac muscle that is similar to that of skeletal muscle. From an anatomical point of view, the fiber arrangement is somewhat less regular than skeletal muscle fibers, and in contrast to the multinucleated skeletal muscle fibers, cardiac muscle typically has only 1–2 nuclei that are centrally located. Although the proteins comprising the cardiac contractile machinery are the same as skeletal, the isoforms are often specific to cardiac muscle. The differences in amino acid sequence between the skeletal and cardiac isoforms of the contractile proteins impart differences in functionality between proteins in foods from these tissues. In addition, the signaling mechanism for excitation of cardiac muscle contraction and the mechanism of Ca^{2+} release differ from that of skeletal muscle. All of these factors have important implications with respect to postmortem metabolism of cardiac muscle and utilization of cardiac muscle as a food. A detailed comparison is beyond the scope of this chapter. The reader is advised to consult other references for more details on cardiac muscle biology [16,17].

15.3.3 Structure of Smooth Muscle

Some smooth muscles (e.g., gizzard, stomach, and intestine) are consumed as specialized organ foods. Unlike skeletal and cardiac muscles, smooth muscles do not exhibit the pattern of striation when longitudinal sections are viewed under the microscope; hence, the term "smooth muscle." This appearance results from a relatively unstructured arrangement of contractile proteins within smooth muscle. Many of the same proteins are involved in smooth muscle contraction

as in skeletal muscle, but some (e.g., troponin) are notably absent, and there exist multiple mechanisms of regulation of muscle contraction among different smooth muscles as well as among species. As with cardiac muscle, even when the same proteins are present (e.g., myosin, actin, tropomyosin), the isoforms expressed are tissue specific and differ sufficiently from the skeletal isoforms such that their functional properties in processed meat products may differ substantially from that of skeletal muscle. The reader is referred to other references for more details on the physiology and biochemistry of smooth muscle [17,18].

15.3.4 Proteins of the Muscle Tissue

Proteins in skeletal muscle have been categorized according to solubility or biological function. Categories related to biological function generally refer to a protein's contribution to muscle structure, contraction, and metabolism, among others. The solubility category is classically based on differential solubilization of muscle proteins at varying salt concentrations, yielding three primary classes of proteins. These classes generally correlate with cellular localization and are identified as (1) sarcoplasmic proteins, (2) myofibrillar proteins, and (3) stromal proteins.

As the name implies, sarcoplasmic proteins comprise proteins found in the sarcoplasm of the myofiber including the glycolytic enzymes, myoglobin, and other enzymes involved in metabolism. These proteins are sometimes termed "water-soluble" proteins because they can be dissolved at low ionic strength (~0.3 mM). This fraction constitutes about 30% of the total muscle protein content [19].

The myofibrillar class of proteins constitutes the largest fraction (50%–60%) of muscle protein. These proteins require high salt concentrations (e.g., >0.3 M NaCl) for solubilization; thus, they are sometimes referred to as the "salt-soluble fraction" of muscle proteins. In muscle tissue, the physiological salt concentration is approximately 0.15 M. This concentration is sufficiently low to prevent these proteins from dissolving in the sarcoplasm, thereby maintaining the complex quaternary structure of the myofilaments.

Myosin and actin, the primary constituents of the thick and thin filaments (Figure 15.3b), respectively, comprise about 65% of the total myofibrillar protein content and about 40% of the total muscle protein content [20]. On the basis of abundance, the chemical behavior of these two proteins in salt solutions accounts for the solubility properties of this group as well as the development of processed meat products. It must be noted that although the myofibrillar proteins are generally equated with high salt solubility, this is a broad generalization. For example, in some cases complete solubilization of cod myofibrillar proteins has been observed at very low ionic strength (<0.0002) [21]. Other myofibrillar proteins such as the troponin complex, when purified, are also soluble at very low ionic strength.

The quality characteristics of fresh meat products are also highly dependent on the abundance and composition of the stromal proteins or connective tissue proteins, which constitute 10%–20% of total muscle protein content. The content of stromal proteins varies with species, age, and muscle [22]. These proteins are generally insoluble under the usual conditions of extraction: near-neutral pH, low-salt or high-salt concentrations, and cold temperatures. Collagen, the most abundant protein in the body, is the dominant protein in the stromal fraction. The stromal proteins form the connective tissue layers described previously that strengthen and protect muscles; thus, there may be some correlation of collagen quantity and quality with meat toughness. In addition, collagen molecules are covalently cross-linked (see the following text), and the number of cross-links increases with age of the animal [23]. The significance of collagen to meat quality is highlighted by the fact that various processing and cookery methods for meat are designed to disrupt and partially solubilize collagen fibers, thereby enhancing meat tenderness [24].

15.3.4.1 Connective Tissue or Extracellular Matrix Proteins

As noted earlier, the ECM serves as the organizing principle of the muscle in the form of a scaffolding network necessary to support muscle function while maintaining a degree of elasticity [23].

In addition, the ECM is permeated by a complex network of macromolecules including members of the collagen family, elastin, proteoglycans, and glycosaminoglycans as well as a variety of cell types, including fibroblasts that synthesize collagen, macrophages, lymphoid cells, mast cells, and eosinophils. Until rather recently, the primary role of ECM was thought to be static maintenance of the structural integrity of the muscle. It is now apparent that the ECM plays a dynamic role in regulating the behavior of surrounding cells [25,26]. The ECM communicates information to cells via the integrin complex, proteins embedded in the SL that link the intracellular cytoskeletal network to the ECM (Figure 15.5) [27]. Through the integrins, the ECM regulates and modulates various cell functions including gene expression, migration, adhesion, proliferation, and differentiation. Differentiation of skeletal muscle is absolutely dependent on proteoglycan synthesis [28], and the types of proteoglycans expressed changes as development proceeds [29]. Moreover, it has been suggested that reduced expression level of ECM proteins [30] as well as postmortem degradation of integrins [31] may contribute to the excessive drip loss associated with poor meat quality.

The dominant proteins comprising the ECM are members of the collagen family and thus will be the focus of most of the discussion of ECM proteins. The term "collagen" refers to a group of at least 29 different protein types that are products of more than 30 genes found in connective tissues throughout the body including bone, tendon, cartilage, blood vessels, skin, teeth, and muscle [32]. Collagen contributes to varying degrees to the toughness of some muscle foods, whereas in semipure form, it is an important functional ingredient as gelatin. The contribution of collagen to meat toughness is somewhat correlated with animal size and function. Skeletal muscles from small animals and fish, for example, have less requirement for weight-bearing strength and tend to be composed of lower levels of collagen and of lower cross-linking relative to collagens in larger land animals.

Collagens are grouped according to the supramolecular structures that they form. These groups include (1) striated or fibrillar, (2) nonfibrous or network forming, (3) microfibrillar or filamentous, and

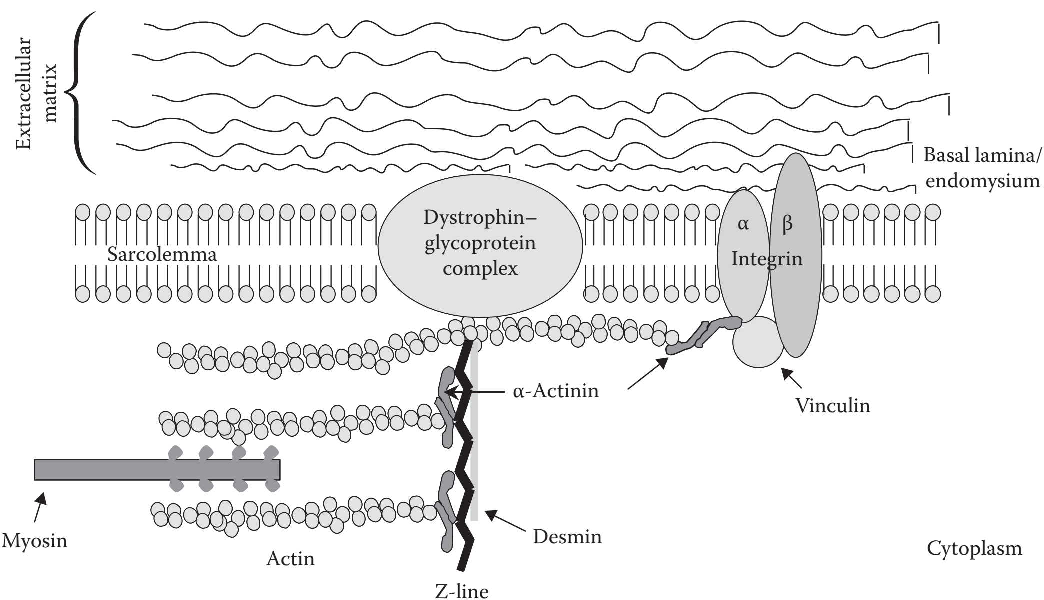


FIGURE 15.5 Diagrammatic representation of the localization and the interaction of extracellular matrix proteins with the intracellular cytoskeletal protein network. Extracellular matrix proteins connect to the intracellular cytoskeleton via the integrin complex embedded in the sarcolemma. (Modified from Lewis, M.P. et al., *Eur. J. Oral Sci.*, 109, 209, 2001. With permission.)

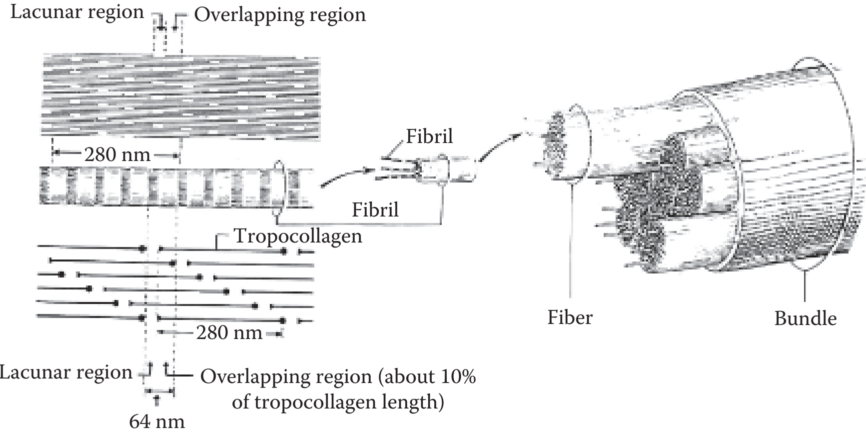


FIGURE 15.6 Formation of collagen fibrils. Tropocollagen units assemble in staggered, side-by-side arrays with head–tail overlap. Gaps and overlaps created in the staggered arrangement of tropocollagen units give rise to the appearance of light zones (lacunar regions) and dark zones (overlapping regions). Collagen fibrils encase muscle fibers, fiber bundles, and whole muscle. (Reprinted from Junqueira, L.C. et al., *Basic Histology*, Appleton & Lange, Norwalk, CT, 1989. With permission.)

(4) fibril-associated collagens with interrupted triple helices (FACITs). By convention, each collagen protein gene product is identified by a Roman numeral, for example, Type I, Type II, … to Type XXIX based on chronological order of discovery. Of the 29 types of collagen, Types I, III, IV, V, VI, XII, and XIV are found in muscle [33]. A few examples of types of collagen associated with muscle follow.

Type I, III, and V collagens are members of the fibrillar collagen family typically found in the ECM. Examination of these collagens by electron microscopy reveals repeating bands every 64–70 nm, resulting from staggered side-by-side arrays of collagen molecules (Figure 15.6). These three collagen types are associated with epimysium, which consists of only Type I collagen, and perimysium, which includes both Types I and III, with some Type V collagen [23]. Type III collagen is thought to be an embryonic form of collagen as its expression is highest in the embryo and neonate and decreases in concert with an increase in Type I collagen. Some of the older muscle literature refers to a heat-resistant, stromal protein fraction called reticulin that forms small fibers within the perimysium. It is now evident that these fibers are primarily Type III collagen with minor amounts of other collagens, glycoproteins, and proteoglycans [34]. There have been some studies suggesting a correlation between the Type I to Type III collagen ratio and meat toughness, but these studies have proven inconclusive [23].

Type IV collagen belongs to the network-forming collagen group [23], and unlike the fibrous collagens, Type IV collagen forms a sheetlike appearance resembling the structure of a chain-link fence. This structure arises from differences in amino acid sequence that prevent the side-by-side association observed in the fibrous collagen family. Type IV collagen is one of the predominant components of the basement membrane, a sheetlike structure sandwiched between and linking the SL to the endomysial layer of the ECM. Type IV collagen is also found in association with collagen Types I, III, and V in the endomysium [23].

The basic unit of collagen is called tropocollagen; it consists of three polypeptide chains entwined around each other in a coiled-coil, superhelical fashion forming a linear molecule about 280 nm long and 1.4–1.5 nm wide (Figure 15.7). The chains may be identical or they may differ in amino acid sequence depending on the type of collagen. For example, Type I collagen consists of two identical polypeptide chains termed α1(I) and one chain with a different amino acid sequence termed α2(I). Type III collagen consists of three identical chains: α1(III). By convention, the Arabic numbers are used to identify different collagen chains within a given type, whereas the Roman

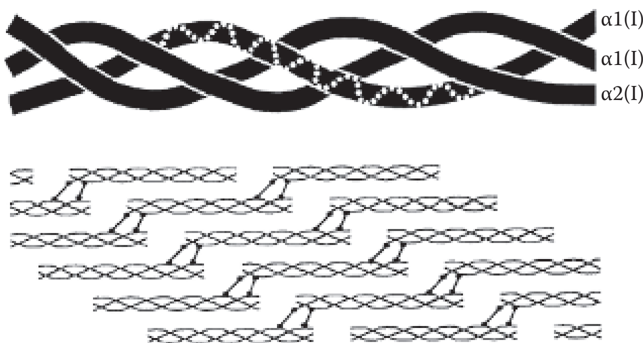


FIGURE 15.7 Schematic representation of the tropocollagen triple helix and cross-links between tropocollagen molecules in a collagen fibril. (Reprinted from Chiang, W. et al., in *Food Chemistry: Principles and Applications*, 2nd edn., Y.H. Hui, ed., Science Technology System, West Sacramento, CA, 2007. With permission.)

numerals refer to the collagen type. Accordingly, α1 chains from Type I collagen differ from α1 chains from Type III collagen.

In Type I collagen, an average polypeptide chain consists of approximately 1000 amino acid residues, with a characteristic repeating sequence throughout most of the chain of $(Gly\text{-}X\text{-}Y)_n$. Residue X in this sequence is often proline, and Y is often hydroxyproline or hydroxylysine. The latter amino acids are formed by posttranslational hydroxylation of proline and lysine by prolyl hydroxylase and lysyl hydroxylase, respectively. Overall, collagens generally contain approximately 33% glycine, 12% proline, 11% alanine, 10% hydroxyproline, 1% hydroxylysine, and small amounts of polar and charged amino acids. Tryptophan is notably absent from collagen; in fact, the absence of tryptophan in collagen preparations is sometimes used as a criterion of purity of collagen preparations. The dominance of the amino acids identified earlier and the notable absence of most essential amino acids make collagen (particularly when consumed as gelatin or as a collagen hydrolysate) a poor protein source in the human diet.

As with all proteins, the primary structure of collagen dictates the folding and assembly of the collagen family of proteins. The presence of glycine at the beginning of each amino acid triplet followed frequently by a proline residue gives rise to a highly extended polypeptide α-chain that forms a unique, shallow, left-handed helix. In Type I collagen, three α-chains form a right-handed triple-helical coiled coil that constitutes the tropocollagen molecule (Figure 15.7). Structural studies indicate that the side chain of each glycine residue, that is, a hydrogen atom, is directed toward the center of the coiled-coil helix. Because of the small size of the hydrogen atom compared to other amino acid side chains, glycine is the only amino acid whose side chain could be accommodated in such a structure. Moreover, each chain is slightly staggered with respect to the other two. This enables hydrogen bonding between the polypeptide amide hydrogen of a glycine residue with the carbonyl oxygen of the adjacent X residue on another chain. The presence of proline and hydroxyproline at frequent intervals along the sequence prevents the chain from adopting a classical α-helix because of constraints of allowable φ, ψ angles of these residues. Moreover, they lack amide hydrogen atoms that are characteristically involved in stabilization of α-helices. The hydroxyl groups of hydroxyproline and hydroxylysine are also thought to be stabilized by interchain hydrogen bonds. Thus, the secondary structure of collagen is an unusually extended, relatively rigid, distinctive helix among the proteins [34].

Collagen polypeptides are synthesized as precursors, termed pro-α chains. These precursor polypeptides include an amino-terminal signal sequence that directs the polypeptide to the lumen of the endoplasmic reticulum of the fibroblast. Following the signal sequence at the N-terminal end as well as at the C-terminus is a series of additional residues collectively termed propeptides. Upon

entry of the polypeptide into the lumen of the endoplasmic reticulum, selected proline and lysine residues are hydroxylated, and a few hydroxylysine residues are glycosylated. The pro-α chains then combine to form the triple-stranded procollagen molecule. The propeptide sequences are believed to initiate formation of the procollagen molecule, and they prevent formation of large fibrils within the cell, which could not easily be secreted [23].

Procollagen is secreted to the ECM where proteinases cleave the propeptides from both ends of the molecule, forming tropocollagen that then begins self-assembly with other tropocollagen molecules to form fibrils. Tropocollagen molecules assemble in a staggered array through side-by-side associations stabilized primarily through hydrophobic and electrostatic interactions. The N-terminal 14 and C-terminal 10 residues of tropocollagen do not display the characteristic Gly-X-Y sequence found throughout most of the tropocollagen molecule, and hence, these "telopeptide" regions do not form the characteristic collagen helix.

The telopeptide regions are involved in formation of covalent intermolecular covalent cross-links between the individual chains (Figure 15.7). These cross-links provide critically needed stability and tensile strength to the supramolecular structure. There are four key residues involved in the initial cross-linking of tropocollagen chains: two lysine or hydroxylysine residues of the N-terminal telopeptides and two lysine or hydroxylysine residues of the C-terminal telopeptides. The head-to-tail staggered arrangement of the tropocollagen molecules enables interactions of the N-terminal telopeptides with the adjacent C-terminal telopeptides.

The prerequisite step preceding cross-linking is oxidative deamination of the lysine or hydroxylysine residues to form allysine or hydroxyallysine, respectively, through the action of the enzyme lysyl oxidase. The subsequent cross-linking reactions occur spontaneously through aldol condensation or through formation of Schiff base intermediates resulting from condensation of an amino group from lysine or hydroxylysine with an aldehyde from allysine or hydroxyallysine. Several examples of these cross-linking reactions are shown in Figure 15.8. The divalent cross-links formed via these pathways are reducible by borohydride and are thus termed "reducible cross-links."

As an animal matures, these divalent, reducible cross-links are converted to more stable, nonreducible, trivalent cross-links. Two types of mature cross-links have been characterized: hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) (Figure 15.9), the latter being present in only negligible amounts in muscle. HP is probably formed via condensation of two ketoamine cross-links (Figure 15.8), as evidenced by concurrent stoichiometric disappearance of reducible cross-links with appearance of the nonreducible forms. In contrast to the reducible cross-links, nonreducible cross-links are very heat stable, which has important implications for the tenderness of meat. Moreover, additional cross-links that spontaneously form during maturation of the animal contribute to the increase in meat toughness, which is often found in older animals [23,33].

The cross-link content varies not only with age but also with muscle function (e.g., postural vs. locomotor), species, exercise, and treatment with growth promoters. Moreover, while both collagen content and degree of cross-linking are casually correlated with toughness of meat, it is evident that there is an additive effect of the two components. Less tender muscles such as bovine *biceps femoris* show high collagen concentrations as well as high concentrations of HP. In contrast, a relatively tender muscle such as *longissimus dorsi* possesses only half to two-thirds of the total amount of collagen and HP as the former muscle. Moreover, *gluteus medius* is high in collagen and low in HP content, whereas *pectoralis major* has low abundance of collagen but has high levels of HP. Both muscles are relatively tender. Thus, it appears that toughness is more strongly correlated with an additive effect of both collagen parameters [35].

15.3.4.2 Sarcoplasmic Proteins

Sarcoplasmic proteins are present in high abundance (25%–30% of total muscle protein), and as the name implies, they are located in the sarcoplasmic (cytoplasmic) fraction of the muscle cell [19]. Many, but not all, of the proteins are enzymes involved in glycolysis, glycogen synthesis, and

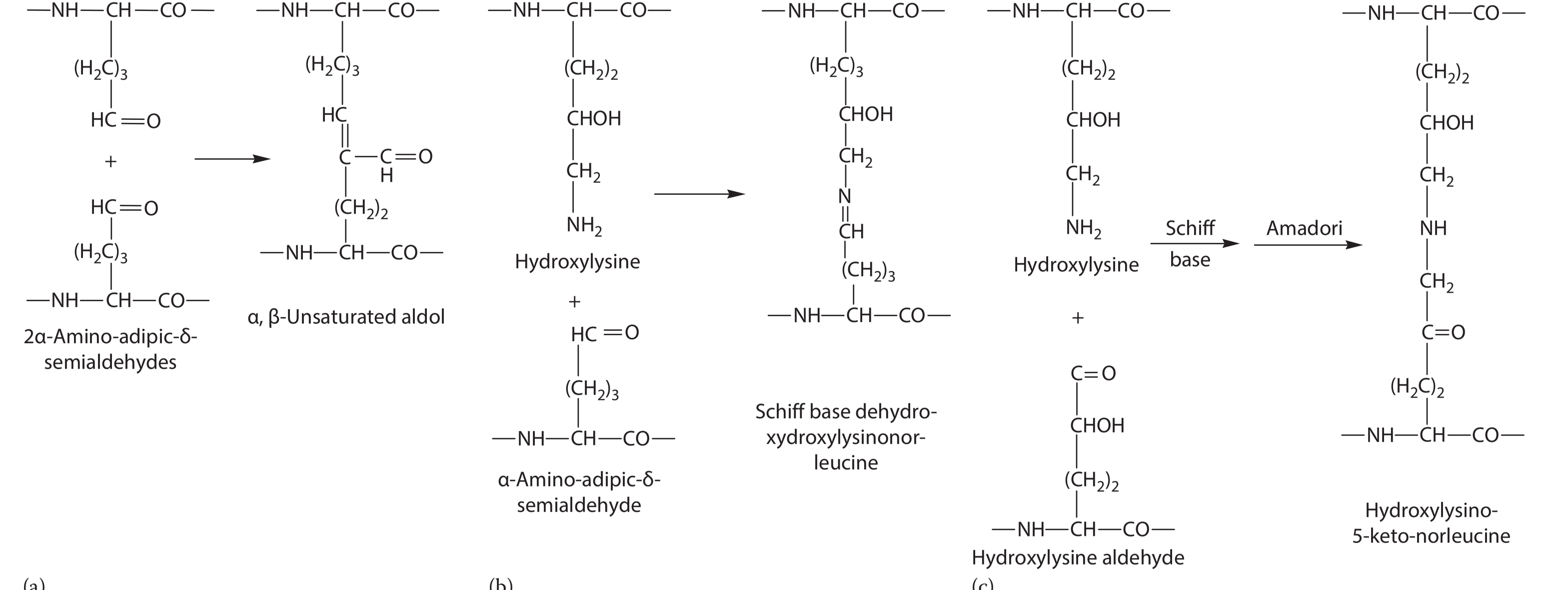


FIGURE 15.8 Mechanisms by which collagen side chains may form cross-links. (a) Aldol condensation of two allysine residues followed by loss of a water molecule, (b) Schiff base formation following condensation of hydroxylysine with allysine, and (c) Schiff base formation following condensation of hydroxylysine and hydroxyallysine. The Schiff base intermediate undergoes Amadori rearrangement to form hydroxylysino-5-keto-norleucine.

FIGURE 15.9 Structures of (a) HP and (b) LP collagen cross-links.

TABLE 15.5
Abundance of Sarcoplasmic Proteins in Muscle

Protein	mg/g
Phosphorylase B	2.5
Phosphoglucomutase	1.5
Phosphoglucose isomerase	1.0
Phosphofructokinase	1.0
Aldolase	6.0
Triose phosphate isomerase	2.0
α–Glycerophosphate dehydrogenase	0.5
Glyceraldehyde phosphate dehydrogenase	12
Phosphoglycerate kinase	1.2
Phosphoglycerate mutase	1.0
Enolase	5
Pyruvate kinase	3
Lactate dehydrogenase	4
Creatine kinase	5
Adenylate kinase	0.5
AMP deaminase	0.2
Myoglobin	0.5–2.0

Source: Compiled from Scopes, R.K. (1970). Characterization and study of sarcoplasmic proteins (Ch. 22). In *The Physiology and Biochemistry of Muscle as a Food*, 2nd edn., (E.J. Briskey, R.G. Cassens, and B.B. Marsh, eds.), The University of Wisconsin Press, Madison, WI, pp. 471–492.

glycogenolysis (Table 15.5). One enzyme, glyceraldehyde phosphate dehydrogenase, constitutes as much as 20% of the sarcoplasmic fraction. Together, the next four or five most abundant glycolytic enzymes comprise over half of the total sarcoplasmic protein content. Other proteins include enzymes of the pentose shunt and auxiliary enzymes such as creatine kinase (the soluble fraction), AMP deaminase, and the oxygen storage protein, myoglobin.

The abundance of some proteins found in the sarcoplasm may vary substantially depending on species, breed, muscle fiber type, age of the animal, and individual genetics. For example, myoglobin tends to be lower in abundance in young animals, thus the pale color of veal compared to adult beef.

Poultry breast muscles and many fish muscles have little redness because of the low levels of myoglobin, whereas poultry leg and thigh muscles are redder than breast muscles because of higher levels of myoglobin. At the other extreme, whale muscle contains the highest known levels of myoglobin; as much as 70% of the sarcoplasmic protein content of some whale muscles is myoglobin [19].

Another enzyme of great significance both in live muscle as well as in postmortem conversion of muscle to meat is creatine kinase. This enzyme is found both in the soluble sarcoplasmic protein fraction and as a component of the M-line protein matrix in the myofibril. Creatine kinase maintains stable levels of adenosine tripolyphosphate (ATP) for use by the muscle when subjected to intensive energy demands such as sprinting or lifting a heavy object [36]. ATP would quickly be depleted before glycolysis and oxidative metabolism could replenish the loss. Creatine phosphate (CrP) serves as a high-energy reservoir compound that can donate its phosphate to ADP in a reaction catalyzed by creatine kinase:

$$\text{CrP} + \text{MgADP} \rightarrow \text{MgATP} + \text{Cr}$$

In resting muscle, as glycolysis and oxidative metabolism restore ATP levels, this process is reversed and some of the excess metabolic energy is converted from ATP to the reservoir form of CrP.

Two other proteins of note in the sarcoplasmic fraction are adenylate kinase and AMP deaminase. As ATP is utilized to meet energy demands, ADP is converted back to ATP by glycolysis, oxidative phosphorylation, and creatine kinase. Adenylate kinase is another enzyme that supports energy demands by synthesis of ATP through the following reaction:

$$2\text{ADP} \rightarrow \text{ATP} + \text{AMP}$$

During periods of intense energy demands on muscle such as sprinting, as well as during the early phase of postmortem conversion of muscle to meat, ATP levels rapidly decline. This reaction becomes especially important when other sources of ATP generation become depleted. The other product, AMP, is deaminated by the action of AMP deaminase to inosine monophosphate by the following reaction:

$$\text{AMP} \rightarrow \text{IMP} + \text{NH}_3$$

IMP is further degraded to hypoxanthine, which has a bitter off-flavor. Hypoxanthine has long been regarded as a biochemical indicator of the postmortem age of fish muscle.

Finally, there are several sarcoplasmic proteinases that are likely involved in muscle growth, maintenance, and postmortem muscle protein degradation. The role of these enzymes in ageing and tenderization of meat will be described in Section 15.4.1. Another group of sarcoplasmic proteins is not soluble, but may be readily solubilized using various extraction methodologies. These proteins include members of the intermediate and microfilaments comprising the cytoskeletal protein network in the muscle.

15.3.4.3 Contractile Proteins

15.3.4.3.1 Myosin

Like collagen, myosin is a superfamily consisting of many closely related proteins that are found in nearly all cells and play important functions in cell motility. Myosins are organized into an ever-growing number of phylogenetic classes based largely on function and sequence similarity. Currently, there are 24 classes within the myosin superfamily; the skeletal, cardiac, and smooth muscle isoforms comprise the Class II myosins [37]. Even within the Type II myosin class, there are many different protein isoforms that are products of different genes or that undergo alternative splicing. These isoforms are differentially expressed depending on factors such as stage of development (embryo, neonate, adult), muscle tissue type (skeletal, cardiac, smooth), and fiber type (fast, intermediate, slow).

Myosin serves as the molecular motor for muscle contraction. It is the dominant protein of the A-band, and at 45% of the myofibrillar protein content, it is the most abundant skeletal muscle protein [20]. A myosin molecule consists of six subunits: two "heavy chains" of $M_r \sim 220,000$ and four "light chains" that vary in mass from about 16,000 to 20,000 [38]. These six subunits combine

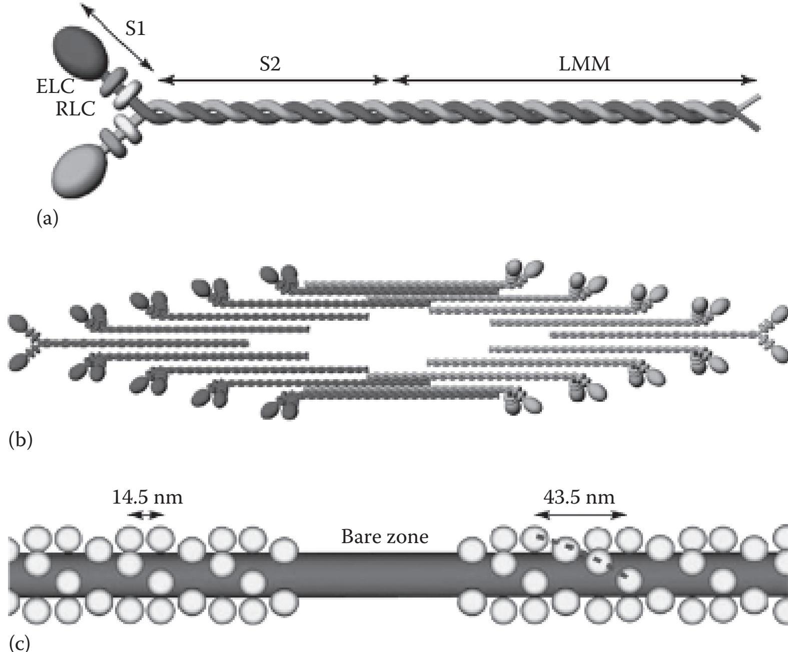


FIGURE 15.10 Schematic representation of the structural features of the myosin molecule and its assembly into thick filaments. (a) A functional myosin molecule consists of two heavy chains, each of which forms a globular head domain and a rodlike α-helix. The helices of the two heavy chains form a rodlike coiled coil. Two light chains bind to the neck region of each heavy chain: either of the two essential light chains (ELC) also known as light chains 1 and 3, and the regulatory light chain (RLC) also known as light chain 2. Limited of proteolysis of myosin yields HMM and LMM. Limited proteolysis yields two subfragments of HMM: subfragment 1 (S1), which consists of the head and neck domain of HMM, and subfragment 2 (S2), which serves as a linker between the myosin head and the portion of the rod that is involved in filament formation (LMM). (b) Myosin molecules assemble in bipolar, staggered arrays to form thick filaments. (c) Myosin heads radiate from the filament shaft in a spiraling fashion. Parallel, adjacent myosin molecules are staggered by a distance of 14.5 nm; myosin heads are separated by a translational distance of 43.5 nm. (Reprinted from Craig, R., and Woodhead, J.L., *Curr. Opin. Struct. Biol.*, 16, 204, 2006. With permission.)

to form a quaternary structure with a molecular mass of ~520,000 and the general appearance of two globular heads projecting from the end of a stick (Figure 15.10a). The N-terminal end of each heavy chain folds into the structure referred to as the myosin head, while the C-terminal portion (60% of the residues) consists of a sequence motif that gives rise to a long alpha-helix. The helices of two heavy chains intertwine to form a rodlike coiled coil. Two myosin light chains wrap each myosin head. The light chains of myosin consist of two alkali-extractable polypeptides, referred to as light chain 1 (LC1) (M_r ~ 20,900) and light chain 3 (LC3) (M_r ~ 16,600), respectively. Myosin light chain 2 (M_r ~ 18,000) is sometimes referred to as the DTNB light chain because treatment of myosin with (5,5′-dithiobis)-2-nitrobenzoic acid (DTNB) removes this protein from the myosin head. Each myosin head contains one of the alkali-extractable light chains and one DTNB light chain. The DTNB light chain is sometimes called the regulatory light chain, because it is required for myosin-based regulation of muscle contraction in molluscan muscles and smooth muscles of higher organisms. Likewise, LC1 and LC3 are sometimes referred to as essential light chains. Together, the light chains play important roles in binding of actin by the myosin heavy chains and of fine-tuning the kinetics of ATP hydrolysis and consequent contractile function.

The head and rod portions of the myosin molecule have distinctly different, important functions. Under physiological conditions, myosin molecules coalesce through staggered, side-by-side associations

of the rod portions of each molecule (Figure 15.10b). Thus, the myosin rod domain provides the structural basis for the formation of the thick filament. The thick filaments are arranged in bipolar fashion such that the origin of the filament is the M-line located at the center of the thick filament. Myosin molecules proceed in opposite directions away from the M-line (Figure 15.3c). The myosin heads project radially from each thick filament shaft and are directed toward the thin filaments (Figure 15.10c). The top of the myosin head binds to actin, the protein forming the backbone of the thin filament. The myosin head also contains an ATP-binding site and serves as the molecular motor that drives muscle contraction.

The size and solubility characteristics of myosin made it difficult to study the contractile and enzymatic properties of the native protein. However, researchers discovered in the early 1950s that brief proteolysis of myosin with trypsin or papain resulted in fragments of the protein that were more suitable for study under physiological conditions. Two primary products of this limited proteolysis were obtained, and each fragment retained specific structural and functional characteristics found in the intact protein [39]. One fragment, called heavy meromyosin (HMM), consisted of the two myosin heads plus a short portion of the myosin rod emanating from each head. HMM maintained the actin-binding and ATP-hydrolytic activities of the parent myosin molecule. The remaining rod portion was called light meromyosin (LMM); it retained the molecular assembly determinants that enable the organization of individual myosin molecules into thick filaments. Subsequently, it was shown that further proteolytic treatment of HMM resulted in cleavage at the head–tail junction, thus yielding two products. One product consisting of the myosin head was called subfragment 1 (S1), and the short, tail portion was called subfragment 2 (S2) (Figure 15.10a). Subfragment 2 possessed neither the self-assembly property nor the molecular motor activity of the parent molecule. Thus, it appeared to serve as a linker segment that separates the myosin head from the thick filament shaft [40].

Myosin subfragment 1 has been crystallized and its molecular structure has been determined at high resolution [41]. Among the many features observable in the structure of S1 are two prominent clefts. One pocket serves as the ATP-binding site, and the other cleft is the actin-binding site (Figure 15.11). These two functions constitute the essential features of the molecular motor activity of the myosin molecule. In addition, the structure shows a long alpha-helical segment that connects the myosin head (S1) to the myosin rod (S2). This helix serves as a lever arm at which the conformational change associated with myosin head movement takes place during muscle contraction.

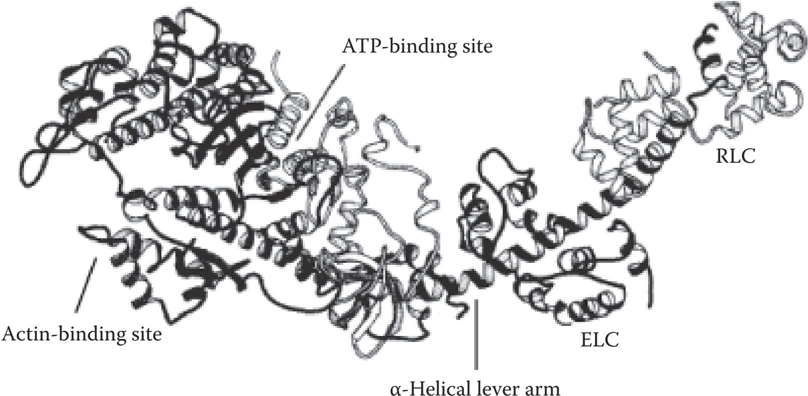


FIGURE 15.11 Representation of the three-dimensional structure of myosin subfragment 1. Two clefts are prominent in the myosin head: an actin-binding site that serves as the domain for crossbridge formation and a nucleotide-binding site where ATP undergoes hydrolysis to yield mechanical energy to support muscle contraction. The structure also indicates a long α-helix that serves as a binding domain for the light chains and as a lever arm on which myosin conformational changes occur during contraction and relaxation. Abbreviations: RLC, regulatory light chain; ELC, essential light chain. (Reprinted from Rayment, I. et al., *Annu. Rev. Physiol.*, 58, 671, 1996. With permission.)

15.3.4.3.2 Actin

Like myosin, actin is a nearly ubiquitous protein found in nearly every eukaryotic and prokaryotic cell. However, unlike myosin, actin is one of the most highly conserved families of proteins found in nature. At approximately 20% of the myofibrillar protein content, actin is the second most abundant protein in muscle [20]. The actin monomer, called globular actin or G-actin, is a single polypeptide chain with a molecular mass of 42,000 and contains a single adenine nucleotide-binding site. The term G-actin is rooted in the historical view that the monomer was approximately spherical. When the high-resolution crystal structure of G-actin was determined, it became clear that molecule is actually shaped rather like a peanut shell with two large domains subdivided into two additional subdomains [42]. Nevertheless, the term G-actin persists to describe the actin monomer.

G-actin remains monomeric at very low ionic strength. However, under conditions approaching physiological ionic strength coupled with the presence of MgATP, actin polymerizes in "head-to-tail" fashion to form double-stranded, coiled-coil, thin filaments called filamentous actin or F-actin [43]. One end of each filament is anchored in the Z-disc, and the filaments project toward the M-line located at the center of the sarcomere (Figure 15.3b). Because of the directional nature of the thin filaments, actin filaments on opposite sides of the M-line are directed toward each other like arrows within the sarcomere. The lengths of the thin filaments are remarkably precise at approximately 1 μm. It is believed that other thin filament proteins including nebulin, tropomodulin, and CapZ protein play key roles in controlling the size of individual thin filaments [38].

Actin plays a dual role in the myofibril. It binds myosin during muscle contraction, forming actomyosin crossbridges between the two filaments. Binding of actin to myosin activates the ATPase activity of myosin, causing myosin to act as a molecular motor that pulls thin filaments past the thick filaments to shorten the sarcomere. Secondly, actin forms the backbone for binding tropomyosin and troponin, two proteins that act in concert to regulate actin–myosin interaction in response to changes in sarcoplasmic Ca^{2+} levels.

15.3.4.4 Regulatory Proteins

15.3.4.4.1 Tropomyosin

Tropomyosin together with troponin constitutes the regulatory switch that turns muscle contraction on or off within the sarcomere [44]. Tropomyosin consists of two alpha-helical subunits (M_r ~ 37,000 Da) that intertwine to form a long, rodlike, coiled-coil protein with a molecular mass of ~74,000 Da. Two different tropomyosin isoform subunits, termed α- and β-tropomyosin, are found in skeletal muscle. These subunits may combine in various ways to form homodimers (αα or ββ) or heterodimers (αβ) [45]. The actual amounts of each isoform expressed in myofibers depend on muscle fiber type (fast vs. slow), muscle type (i.e., skeletal, cardiac, or smooth), developmental stage (embryonic, neonatal, or adult), and species. Moreover, posttranslational alternative splicing generates several additional tropomyosin isoforms that presumably provide an additional level of modulation of contractile properties of different types of myofibers.

Like actin, tropomyosin polymerizes at physiological ionic strength in head-to-tail fashion, with a small overlap of about 8–11 amino acids at each end of a tropomyosin molecule. An individual tropomyosin molecule is approximately 42 nm long and spans seven actin monomeric units (Figure 15.3c). The filamentous strand of tropomyosin binds to the actin backbone at specific sites along each actin monomer, near the grooves of the thin filament double helix. In this position, tropomyosin blocks the myosin-binding sites on the outer domain of the actin filament when muscle is at rest (Figure 15.12). During muscle contraction, tropomyosin is shifted to another set of binding sites located deeper within the groove formed by the actin coiled-coil filament. When the contraction signal ceases, tropomyosin reverts back to its resting position in which the myosin-binding sites are sterically blocked [45].

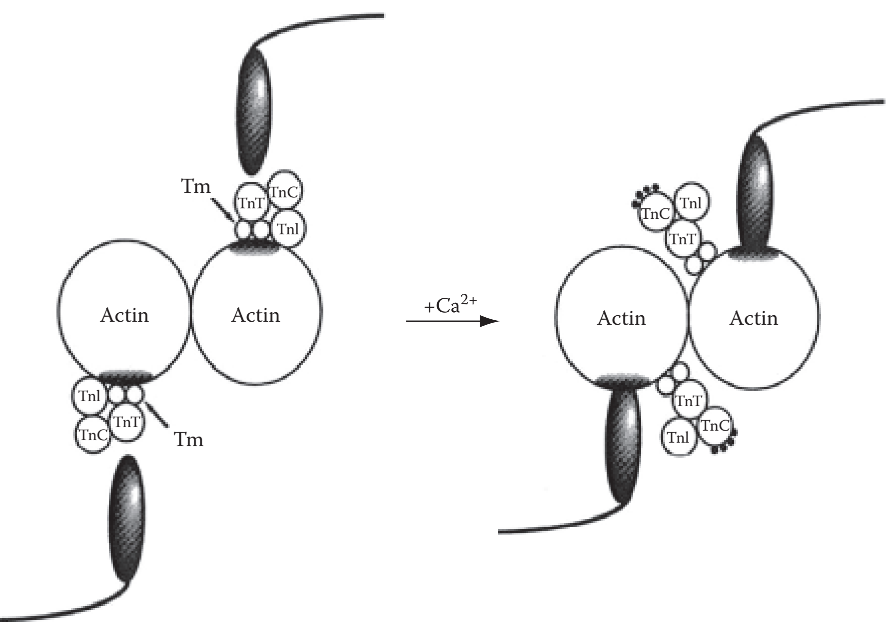


FIGURE 15.12 Regulation of contraction and relaxation by the tropomyosin–troponin complex. When muscle is in the resting state, tropomyosin sterically blocks myosin heads from binding to the myosin-binding site (shaded region) on the actin filament. When Ca^{2+} binds to troponin-C, the troponin complex undergoes a conformational change that causes tropomyosin to shift deeper into the actin groove, exposing myosin-binding sites and enabling myosin crossbridge formation with actin. (Reprinted from Chiang, W. et al., in *Food Chemistry: Principles and Applications*, 2nd edn., Y.H. Hui, ed., Science Technology System, West Sacramento, CA, 2007. With permission.)

15.3.4.4.2 Troponin

The troponin complex, which constitutes 5% of the myofibrillar protein, is a heterotrimer that binds to tropomyosin (Figure 15.12). Working in concert with tropomyosin, troponin responds to changes in sarcoplasmic Ca^{2+} concentration to control actin–myosin interaction and, thus, triggers contraction or relaxation of muscle [44].

Troponin-C (TnC) has a M_r of ~18,000 and is the Ca^{2+}-binding subunit of the troponin complex [46]. The TnC molecule consists of two ion-binding domains. In the C-terminal half of the molecule, there are two high-affinity Ca^{2+}-binding sites ($K_d \sim 10^{-9}$ M) that can also bind Mg^{2+}. In the N-terminal half of the molecule, there is either one or two lower affinity calcium-binding sites ($K_d \sim 10^{-6}$ M). The fast skeletal muscle TnC isoform contains two low-affinity sites, whereas the slow skeletal and cardiac muscle isoforms have only one low-affinity site. For all TnC isoforms, the low-affinity sites are specific for binding Ca^{2+}. In resting muscle, the low-affinity binding sites are believed to be empty because of the low Ca^{2+} concentration ($<10^{-7}$ M), whereas the high-affinity sites are likely to be occupied by Mg^{2+} because of its high intracellular concentration (~1–5 mM) relative to Ca^{2+} [47]. When a motor neuron initiates muscle contraction, sarcoplasmic Ca^{2+} concentrations increase more than 100-fold to $>10^{-5}$ M; some of the Ca^{2+} binds to the low-affinity sites, triggering a conformational change in the protein that is transmitted through the other troponin subunits to tropomyosin and actin. When the contraction signal ceases, the sarcoplasmic Ca^{2+} concentration decreases below 10^{-7} M, and Ca^{2+} is removed from the low-affinity TnC sites, thereby causing a reversion of the structure of the troponin complex to its resting state.

The troponin complex binds to tropomyosin primarily via the troponin T (TnT) subunit, a mallet-shaped protein of $M_r \sim 30,500$. The third subunit, troponin I (TnI), is so named

because it inhibits actomyosin ATPase activity. It interacts with the other two troponin subunits as well as with actin under resting conditions. The troponin complex is anchored near the head–tail junction of a tropomyosin filament at a ratio of one troponin molecule per tropomyosin molecule [44].

15.3.4.5 Structural Proteins of the Myofibril

15.3.4.5.1 Titin

Titin, sometimes called connectin, is the third most abundant protein of muscle myofibrils. With over 38,000 amino acid residues and a M_r of 3–4,000,000, titin is the largest single polypeptide chain known. Yet despite titin's size and abundance in muscle, it was discovered relatively late by K. Wang and coworkers in 1979 [48]. Ironically, this protein escaped notice because it is so large: titin's extremely high molecular mass prevented its migration into conventional SDS polyacrylamide gel systems used to characterize muscle proteins. Use of low acrylamide concentrations in sodium dodecyl sulfate polyacrylamide gels or SDS agarose gels created much larger pore sizes in gels that enabled titin to migrate into the gels, thus leading to the discovery of this protein as well as another large protein, nebulin (Box 15.1).

Titin is a flexible, elastic, filamentous protein of about 1 μm in length and consists of several structural and functional domains that contribute to the protein's various roles in muscle. The amino terminus of one titin molecule is attached to one Z-disc of a sarcomere, and the carboxyl terminus is anchored in the M-line. A corresponding titin molecule of opposite polarity spans the opposite half of the sarcomere. Given this structural arrangement, the titin molecule must be able to accommodate both stretching and contraction of muscle sarcomeres. The extensible region of titin is located in the I-band and is composed of tandemly arranged immunoglobulin-like domains, and a PEVK segment, so named because it is abundant in proline (P), glutamate (E), valine (V), and lysine (K) [49]. This region of titin is also believed to be intrinsically disordered, thus making it a target of protease activity in the postmortem conversion of muscle to meat. In addition to these segments, the extensible region of cardiac titin also contains a unique 572-residue sequence that is part of the cardiac-specific N2B element [50]. These segments have distinct bending rigidities, and as a result, stretching of resting-length sarcomeres initially gives rise to extension (unfolding) of tandem Ig segments followed by extension of PEVK segments. When muscle relaxes, titin undergoes structural rearrangement that restores muscle to its resting sarcomere length.

In addition to its role in maintaining muscle elasticity, titin serves numerous additional functions. As one of the earliest genes expressed in muscle protein synthesis in embryogenesis, titin serves as a template upon which myosin molecules form thick filaments [51]. The A-band portion of titin may serve as a molecular ruler, maintaining uniform length of the thick filaments. Moreover, titin binds a multitude of other muscle proteins leading to speculation that it serves as an adhesion template for assembly of the entire muscle sarcomere.

15.3.4.5.2 Nebulin

Like titin, nebulin was discovered rather recently because of its unusually large size (M_r ~ 800,000). It is present in amounts comparable to that of tropomyosin and troponin. Nebulin is located along both sides of the actin thin filaments of skeletal muscle, with the C-terminal end partially inserted into the Z-line and the N-terminal end extending to the opposite end of the thin filament. There is an amino acid sequence super-repeat in the nebulin sequence, analogous to one of the roles of titin. Nebulin may act as a molecular ruler for specifying the precise lengths of the thin filaments [52]. However, unlike titin, nebulin is inextensible and does not play a role in maintaining muscle elasticity. Nebulin may also be involved in signal transduction, contractile regulation, and myofibril force generation [53], but as its name suggests, the precise mechanisms by which nebulin fulfills these functions remain nebulous.

15.3.4.5.3 α-Actinin

A major component of the Z-line is α-actinin (M_r ~ 97,000) that functions as an actin-binding protein. α-Actinin contains three major domains: a globular N-terminal actin-binding domain, a

BOX 15.1 ELECTROPHORESIS IN THE STUDY OF MUSCLE PROTEINS

As early as the 1930s, electrophoresis was being used in studies on proteins. The original gels were made from starch as the separating matrix and were later followed by agarose and polyacrylamide. Interpretation of the early electrophoresis experiments was limited, however, by the fact that proteins migrated through the gel as a function of protein size (molecular weight), protein charge, and protein shape. Thus, if protein 1 and protein 2 have the same molecular weight and spherical shape but opposite net charges, one protein will migrate into the gel and the other will not. Likewise, two proteins might have the same molecular weight and similar overall charges; if one is approximately spherical while the other is rodlike, the spherical protein will migrate much more quickly through the gel than the rod-shaped protein.

The field of protein science was dramatically transformed by the seminal publication of K. Weber and M. Osborne [143] in 1969. These researchers observed that inclusion of the detergent sodium dodecyl sulfate (SDS) in a mixture of proteins resulted in complete unfolding of most proteins with an approximately constant amount of SDS bound per unit weight of protein. The SDS molecule is negatively charged, and the total number of SDS molecules bound per unit weight of protein generally overwhelms the native charge (positive or negative) on any protein. Thus, in the presence of SDS, all proteins adopt a rod shape and carry a net negative charge. The mobility of an SDS-denatured protein is inversely proportional to the logarithm of the molecular weight. By measuring the mobilities of several proteins with known molecular weights as standards, one can develop a standard curve to calculate the molecular weight of unknown proteins. Some proteins migrate with somewhat anomalous apparent molecular weights, but in general, the technique predicts molecular weight with an accuracy of ±10%.

Shortly after the publication by Weber and Osborne, SDS polyacrylamide gel electrophoresis (PAGE) was widely adopted for study of muscle proteins. For example, Greaser and Gergely used SDS-PAGE to prove that troponin, originally discovered in 1965 as a monomer, actually consisted of three subunits [143]. Moreover, SDS-PAGE could easily be used to determine the degree of purification of a protein, as contaminating proteins would be seen in gels with staining intensity proportional to their abundance.

The original SDS-PAGE protocol could be easily modified to address specific questions. For example, by adjusting the acrylamide monomer concentration and bis-acrylamide cross-linker concentration, it was possible to use SDS-PAGE to display and analyze what was—in the 1970s—thought to be the full spectrum of myofibrillar proteins from the smallest protein subunit of myosin (light chain 3, MW = 15,000) to the myosin heavy chain (MW = 200,000) (Figure B15.1.1a).

SDS gels of preparations of myofibrils often showed variable amounts of high-molecular-weight protein at or near the top of gel lanes (see Figure B15.1.1). These bands (or often smears) of protein were generally ignored by researchers; most believed this high-molecular-weight material was some sort of artifact, for example, undissociated/nondenatured protein. Suspecting that these bands might in fact be distinct and unique proteins of high molecular weight, Dr. K. Wang conducted experiments in the late 1970s with polyacrylamide concentrations of 3%–4% instead of the typical 10%–20% concentrations. The lower polyacrylamide concentration resulted in migration of all proteins further into the gel. Proteins smaller than molecular weight of about 35,000 were not retained on the gel; however, three high-molecular-weight proteins migrated further into the gel. These experiments led to the discovery of titin (bands 1 and 2 in figure) and nebulin (band 3), proteins so large that they were not previously “seen.”

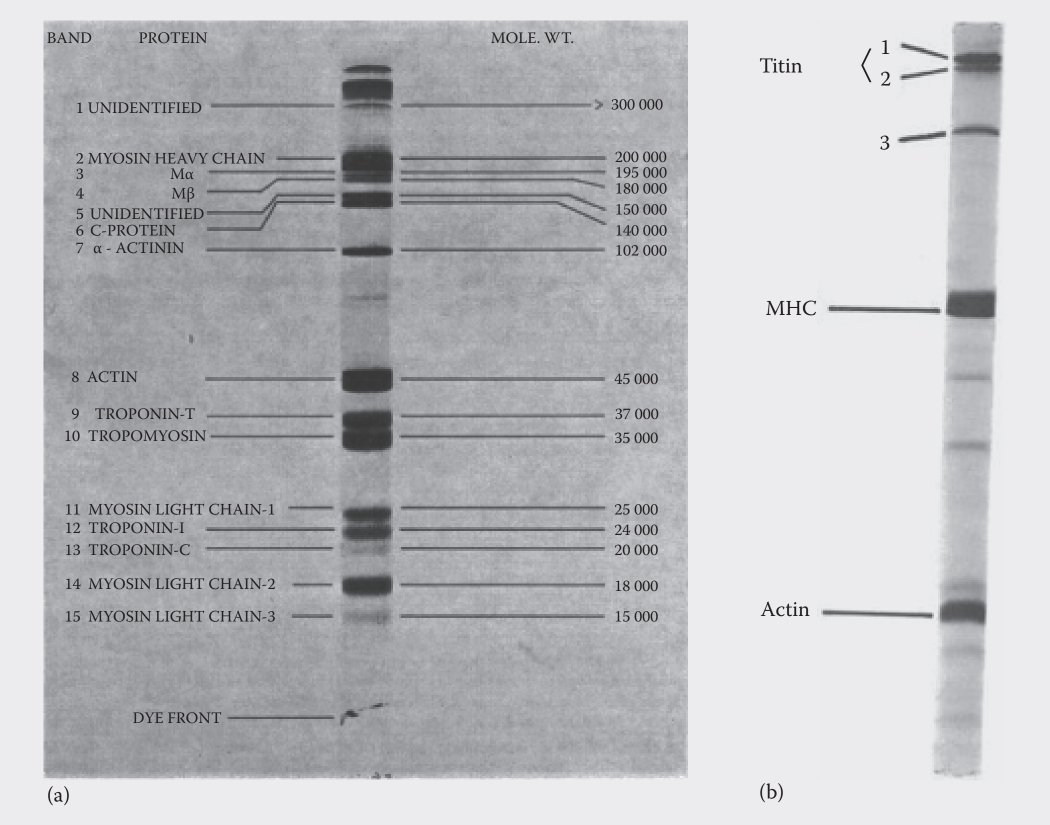


FIGURE B15.1.1 Use of electrophoresis in analysis of muscle proteins. (a) SDS-PAGE of a muscle myofibril preparation using 10% acrylamide monomer and 0.1% bisacrylamide cross-linker. The bands of protein are visualized by staining with Coomassie blue dye, and proteins are identified based on their apparent molecular weight. (Reprinted from *Biochim. Biophys. Acta—Protein Struct.*, 490, Porzio, A.M. and Pearson, A.M., 30, Copyright 1977, with permission from Elsevier.) (b) SDS-PAGE of a myofibril preparation using 3.2% acrylamide monomer and 0.1% bisacrylamide cross-linker. Titin bands 1 and 2 have molecular weights >3×10^6 and correspond to the intact protein and a proteolytic fragment of titin. Band 3 was identified as nebulin. (Reprinted from Wang, K. and Williamson, C.L., *Proc. Natl. Acad. Sci. USA*, 77, 3255, 1980. With permission.)

central rod domain, and a C-terminal domain that bears similarity to the calcium-binding protein calmodulin [54]. The rod domains of α-actinin monomers interact to establish antiparallel dimers that are capable of cross-linking actin and titin filaments from neighboring sarcomeres. In fact, α-actinin has the capacity to associate with numerous protein partners at the Z-line. These protein interactions provide tensile integrity to the Z-line and may also serve as an additional docking site for other Z-line-associated proteins (Figure 15.5).

15.3.4.5.4 CapZ and Tropomodulin

Actin filament formation is a dynamic process, and regulation of this process during myofilament assembly is a key factor in maintaining their uniform filament lengths [43]. CapZ, also known as β-actinin, and tropomodulin bind to and cap the thin filaments to block filament elongation and shortening [55,56]. CapZ is a heterodimer consisting of one α (M_r ~ 36,000) and one β (M_r ~ 32,000) subunit, both of which are required for its function in nucleation and stabilization of actin filaments. In striated muscle, CapZ is localized to Z-lines where it binds α-actinin and likely forms an anchoring complex

for the thin filaments. Tropomodulin (M_r ~ 40,000) has two domains, one of which binds tropomyosin, whereas the other binds actin, thus capping the pointed ends (opposite end from the Z-line) of the thin filaments and preventing the addition or loss of actin monomers from the thin filament [38]. Based on the specific protein–protein interactions of these molecules, one would predict a stoichiometry of 1 CapZ at the Z-line origin of a thin filament and two tropomodulin molecules at the opposite end.

15.3.4.5.5 Desmin

Desmin (M_r ~ 55,000) is the dominant protein of the intermediate filaments (10 nm diameter), key components that maintain the structural integrity of most cells. In muscle, desmin filaments are found at the periphery of Z-lines, and it appears that these filaments serve to cross-link adjacent myofibrils and to link the myofibrillar Z-lines to proteins of the SL (Figure 15.5) [57].

15.3.4.5.6 Filamin

Three filamins (α, β, and γ) (M_r ~ 300,000) have been identified in muscle. They share a similar molecular structure: an N-terminal actin-binding head followed by 24 Ig-like domains. γ-Filamin (also known as ABP-280) is the muscle-specific filamin isoform. Filamin is localized at the periphery of the Z-line and provides a critical link between the SL and sarcomeric cytoskeleton. Since γ-filamin exists at the very early stages of developing Z-lines, it is suggested that this filamin isoform might be involved in the formation of sarcomeric Z-lines [58].

15.3.4.5.7 Myosin-Binding Proteins C and H

Myosin-binding protein C (MyBP-C; M_r ~ 140,000) and myosin-binding protein H (MyBP-H, M_r ~ 58,000) are found at discrete intervals within thick filaments. They are both distributed in the C-zone (middle third of each half of A-band) of the thick filament, forming a series of transverse stripes spaced 43 nm apart, although H-protein also is sometimes found outside this zone [59]. MyBP-C (formerly known as C-protein) is found in approximately a 1:8 molar ratio with myosin heavy chains and interacts with both the myosin and titin filaments. MyBP-C and H-protein may function to link and/or align the thick filaments in the A-band [60].

15.3.4.5.8 Myomesin and Other M-Line Proteins

The main protein component of the M-line is myomesin, a single polypeptide of M_r = 162,000–185,000, depending on the isoform [61]. There are three different genes that code for myomesin: myomesin 1 is found in all striated muscles, whereas myosin 2 and myomesin 3 are found in fast-twitch and slow-twitch muscle fibers, respectively. In addition to serving as scaffolding for binding of titin and myosin, myomesin may be involved in maintaining structural integrity of thick filaments. Additional components of the M-line include creatine kinase and adenylate kinase, enzymes involved in regeneration of ATP in muscle as described in Section 15.3.4.2. Localization of these enzymes near the point of ATP utilization by myosin in muscle contraction is critical for the muscle function during periods of high ATP demand such as in sprinting.

15.3.4.5.9 Dystrophin and the Dystroglycan Complex

Dystrophin and the associated proteins comprising the dystroglycan complex play critical roles in muscle structure and function. Dystrophin (M_r = 427,000) is so named because mutations in the gene result in abnormal proteins associated with several types of muscular dystrophy [62]. The dystroglycan complex consists of a large multiprotein complex embedded in the SL that serves as the transmembrane structural link between specific proteins in the ECM and dystrophin on the intracellular side of the membrane (Figure 15.5) [63].

15.3.4.5.10 Integrins

Analogous to the dystroglycan complex, the integrins are intrinsic sarcolemmal proteins that structurally link proteins of the ECM to intracellular structural proteins [27]. The integrins also play

a key role in signal transduction from the ECM to the interior of the cell. The functional integrin protein is a heterodimer consisting of one α and one β subunit, and there are at least 18 α and 8 β gene products. Both subunits traverse the width of the SL and form linkages to proteins such as fibronectin, laminin, and collagen in the ECM. On the intracellular side, the integrins link to a variety of proteins of the cytoskeleton including α-actinin, vinculin, and talin. The specific linkages are determined in part by the identity of the individual subunits [64].

15.3.4.6 Proteins of the Sarcoplasmic Reticulum and Sarcolemma

The membranes of the various organelles of muscle—nuclei, mitochondria, SL, etc.—contain proteins that function to meet the specific needs of the organelles and of the cells. The proteins of the SR merit special attention because of their roles in storage, transport, and release of Ca^{2+} in the myofiber. Because Ca^{2+} triggers muscle contraction and regulates key glycolytic steps, these proteins play key roles in muscle activity of the live organism. The proteins of the SR may also serve as key determinants of meat quality, depending on the extent to which they control sarcoplasmic Ca^{2+} concentrations in the initial time period postmortem. While there are many proteins that are found in the SR and SL, our focus here is on three proteins. Two of these proteins, the ryanodine receptor (RyR) and the dihydropyridine receptor (DHPR), comprise the key elements of the Ca^{2+}-release mechanism responsible for initiating muscle contraction. The third protein, the Ca^{2+} pump, is primarily responsible for relaxation of muscle.

The DHPR is an intrinsic membrane protein complex embedded in the T-tubules that serves as a sensor of the change in voltage across the membrane when muscle contraction is initiated by neural stimulus [65]. It must be noted that although the term DHPR is still commonly used to describe this protein complex, this term is being displaced by recently adopted nomenclature that categorizes all voltage-gated channels Ca^{2+} channels within 1 of 10 groups within this family of proteins based on similarity of physiological, pharmacological, and genetic characteristics [66]. The skeletal muscle complex is defined as $Ca_V1.1$; the cardiac muscle complex is defined as $Ca_V1.2$. For the purposes of this chapter, however, the term DHPR will be retained.

The DHPR consists of four different subunits, referred to as α_1, β, γ, and $\alpha_2\delta$ subunits. The α_1 subunit forms a calcium channel, senses changes in voltage across the T-tubule, and contains the binding site for a class of pharmaceutical compounds called dihydropyridines that are used therapeutically to modulate the protein's function. The roles of the other protein subunits are less clear, but there is evidence supporting their role in modulating the process of excitation–contraction coupling [67]. The function of DHPRs in various tissues depends in large part on the isoform expressed in the tissues. For example, the cardiac-muscle-specific α_1 isoform allows inward diffusion of Ca^{2+} from the extracellular milieu, whereas the skeletal muscle isoform is essentially inactive as a channel but plays a critical role as a sensor of the membrane voltage.

A portion of the SR called the terminal cisternae is closely apposed to the T-tubule (Figure 15.2). This portion of the SR is enriched in a protein called the SR Ca^{2+}-release channel or ryanodine receptor (RyR), so named because it binds a toxic plant alkaloid (ryanodine) very specifically and with high affinity. The RyR family consists of a series of homologous proteins sometimes referred to as RyR1, RyR2, and RyR3. In mammals, RyR1 is the skeletal muscle isoform, while RyR2 is the dominant form of cardiac muscle. RyR2 is also found in other tissues including the brain; RyR3 is found in various mammalian nonmuscle tissues and in trace amounts in skeletal muscle. In avian, piscine, and amphibian species, RyR3 is found in most skeletal muscles in approximately equal abundance with that of RyR1 [68]. RyRs function as channels or transmembrane conduits for diffusion of Ca^{2+} from within the lumen of the SR, where the concentration is several millimolar, to the sarcoplasm where the resting muscle Ca^{2+} concentration is $<10^{-7}$ M.

The RyR is an extremely large protein consisting of four identical subunits, each with a relative molecular mass of approximately 550,000, resulting in a functional ion channel with a molecular mass of ~2,200,000. Several other proteins are tightly associated with the RyR and modulate Ca^{2+} conductance through the channel [13]. More than 80% of the RyR mass is on the cytoplasmic

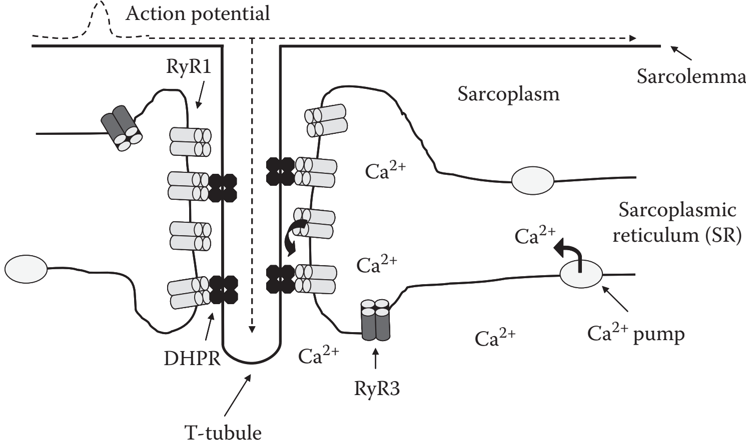


FIGURE 15.13 Schematic representation of the Ca^{2+} release and uptake mechanisms in skeletal muscle. DHPRs embedded in the T-tubule membrane serve as sensors of the action potential initiated by a motor neuron. Ryanodine receptors (RyR1 and RyR3) located in the SR serve as Ca^{2+} channel proteins that open to allow Ca^{2+} to diffuse from the SR to the sarcoplasm during contraction. When neuronal stimulation ceases, RyR channels close, and Ca^{2+} pump proteins lower the sarcoplasmic Ca^{2+} concentration by transporting Ca^{2+} into the SR. In mammalian skeletal muscle, every other RyR1 is coupled to a DHPR tetrad. RyR3 is localized in the peripheral region of the terminal cisternae distal to the T-tubule and does not associate with the DHPR. RyR3 channels are activated when Ca^{2+} released by RyR1 binds to the cytoplasmic side of RyR3 resulting in channel opening. RyR3 is present in very low abundance in mammalian skeletal muscle but is approximately equal in abundance to that of RyR1 in most avian, piscine, and amphibian muscle.

side of the SR membrane, forming a structure sometimes called a junctional foot that spans the gap between the terminal cisternae of the SR and the T-tubule. Skeletal muscle DHPRs and RyRs appear to be in physical association [14]. Electron micrographs show that DHPRs assemble into groups of four units called a "tetrad." Overlays of DHPR tetrads in the T-tubule with SR junctional foot proteins (RyRs) indicate that every other RyR interacts with a DHPR tetrad; conversely, every other RyR is not coupled with a DHPR (Figure 15.13) [69]. Within the SR of birds, fish, and amphibians, RyR3, which is generally found in equal abundance with RyR1, is confined to the periphery of the SR/T-tubule junction and thus does not interact with the T-tubule or with the DHPR tetrad [70].

The DHPR and RyR work in concert with their associated regulatory proteins to affect Ca^{2+}-release into the sarcoplasm that triggers muscle contraction. When the neuromuscular contraction signal ceases, these proteins return to their "resting" state, and the sarcoplasmic Ca^{2+} concentration is restored by the action of the Ca^{2+} pump protein. This protein is located primarily in the longitudinal SR, that is, the portion of the SR distal to the terminal cisternae. Using ATP as an energy source, the calcium pump protein transports two Ca^{2+} ions against the concentration gradient across the SR membrane and into the lumen of the SR for every ATP molecule hydrolyzed [71].

15.3.5 Excitation–Contraction Coupling

A detailed understanding of the mechanism of muscle contraction may, at first glance, seem irrelevant to meat quality. The difference between live muscle tissue and meat is quite literally the difference between life and death. However, at the point of slaughter, muscle tissue immediately begins a series of transitional reactions (described in Section 15.4) known as conversion of muscle to meat. The biochemical events associated with this transition involve exactly the same mechanisms of muscle contraction and the supporting physiological and biochemical reactions of live muscle, all of which are significant contributing determinants of meat quality.

When the muscle cell is at rest in the live animal, there is a voltage difference across the SL of about −90 mV and the intracellular Ca^{2+} concentration is very low (<10^{-7} M). In the myofibrils, tropomyosin is situated on the actin filament in a position so as to sterically prevent myosin–actin crossbridge formation. Upon stimulation of a myofiber by a motor neuron, the muscle cell is depolarized at the neuromuscular junction. This voltage change travels along the SL, entering the interior of the muscle via the T-tubule. The DHPR responds to the electrochemical transient across the membrane by undergoing a conformational change that is transmitted across the T-tubule/SR membrane junction to RyR1 located in the SR terminal cisternae. RyR1, in turn, responds by opening its channel pore. Ca^{2+} diffuses from the lumen of the SR, where the concentration is relatively high (>10^{-3} M), to the sarcoplasm, thus raising the sarcoplasmic concentration of Ca^{2+} over 100-fold to >10^{-5} M. Ca^{2+} binds to troponin-C, resulting in a conformational change in the troponin complex that is transmitted to tropomyosin. This causes a shift of the position of tropomyosin along the actin filament. Tropomyosin shifts deeper into the thin filament groove, thereby exposing myosin-binding sites on the actin monomers and enabling actin–myosin crossbridge formation (Figure 15.12) [17].

At this point in the muscle contraction cycle (Figure 15.14), the nucleotide-binding pocket of the myosin head contains ADP and inorganic phosphate (P_i), which are products of hydrolysis of ATP. Exposure of the myosin-binding sites on the actin filament enables weak binding of the myosin head to the thin filament, with the myosin heads binding in an approximately perpendicular orientation relative to the thick filament axis. Upon binding to actin, the myosin head releases P_i, causing a small conformational change in myosin and strengthening the binding of myosin to actin. This is immediately followed by a large conformational change in the myosin head called the power stroke in which the myosin heads pull the actin filament along the thick filament in a rowing-like motion toward the M-line. ADP is then released from the myosin head, and ATP then binds to the empty nucleotide-binding site that triggers release of the myosin head from actin. Hydrolysis of ATP within the myosin head results in a conformational change that "cocks" the myosin head so that it is again approximately perpendicular to the thick filament, and the reaction series undergoes another cycle of contraction [72]. Because the actin and myosin filaments within a sarcomere are oriented toward the M-line, each

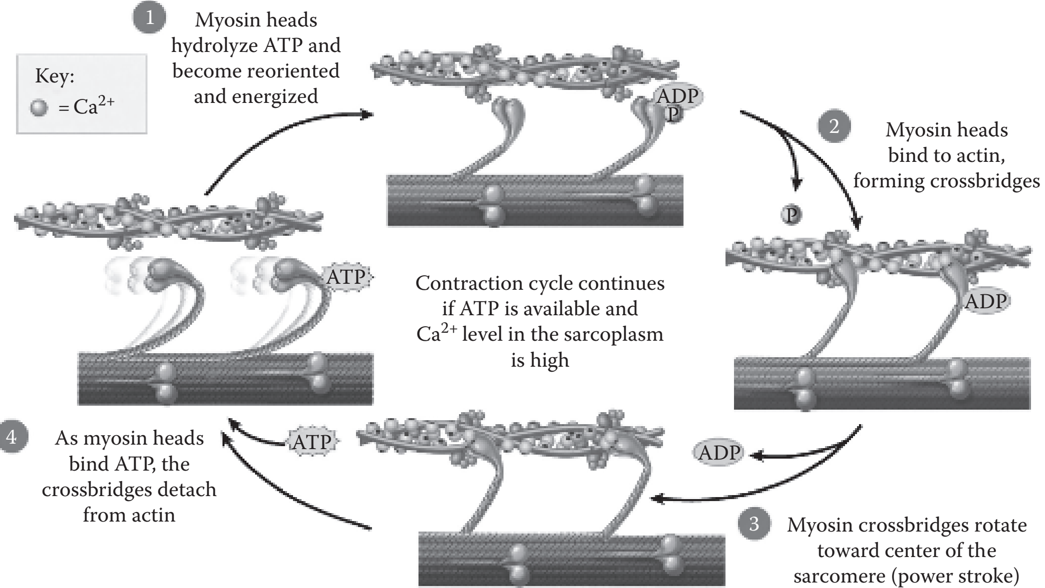


FIGURE 15.14 Crossbridge cycling during muscle contraction. (Reprinted from Tortora, G.J. and Derrickson, B., *Principles of Anatomy and Physiology*, 14th edn., John Wiley & Sons, Inc., Hoboken, NJ, 2014. With permission.)

contraction cycle results in shortening of the distance between the Z-discs, thereby affecting the contraction of muscle (Figures 15.4 and 15.14) [73].

The myosin crossbridge cycle of attachment, power stroke, and detachment continues to maintain tension or contraction as long as neuronal stimulation of the myofiber continues. Upon cessation of the stimulus, sarcoplasmic Ca^{2+} is reduced to resting muscle concentrations by the action of the Ca^{2+} pump protein. As the sarcoplasmic Ca^{2+} concentration is lowered, TnC is depleted of Ca^{2+} from the low-affinity Ca^{2+}-binding sites, resulting in a conformational change in TnC reverting to the structure associated with the relaxed state of muscle. This conformational change causes tropomyosin to move back to its resting-state position on the actin filament, thereby blocking myosin–actin crossbridge formation, resulting in muscle relaxation.

It is important to recognize the multiple roles of ATP in muscle contraction and to recognize its critical role in conversion of muscle to meat. As the dominant energy source for the cell, hydrolysis of the ATP phosphodiester bond is converted to mechanical energy by myosin resulting in muscle contraction. In addition to the other myriad chemical reactions requiring ATP, the role of ATP in support of the function of the Ca^{2+} pump is pivotal to the conversion of muscle to meat. As ATP levels decline, the ability of the Ca^{2+} pump to sequester sarcoplasmic Ca^{2+} is compromised. Finally, it should be evident that after death of the animal, ATP levels will eventually drop to the point at which there is no longer sufficient ATP to bind to myosin heads, which would keep myosin dissociated from actin. The position at the end of the power stroke in which the myosin head is bound to actin is sometimes referred to as the "rigor complex." A summary of the steps of excitation–contraction coupling is provided in Box 15.2.

15.3.6 Muscle Fiber Types

Skeletal muscle comprises several different types of muscle fibers that display differences in function, including speed of contraction (fast vs. slow) and supporting metabolism (oxidative vs. anaerobic). The muscle fiber types have great adaptive potential, and their phenotypic profiles are affected by the type of neuron innervating the muscle fiber, degree of neuromuscular activity, exercise/training, mechanical loading/unloading, hormones, and aging [74]. These fiber types can be categorized using histochemical, biochemical, morphological, or physiological characteristics; however, classification of muscle fibers by different techniques do not always agree. Initially, muscles were classified as being fast twitch (type I) or slow twitch (type II), based on speed of shortening of individual fibers [75]. This classification also corresponded to a morphological difference, with the fast-twitch muscles appearing white in some species and the slow-twitch muscles appearing red. The redness of the fiber correlates with high amounts of myoglobin, which provides a ready source of oxygen in support of oxidative metabolism. The slow-twitch, red fibers generally contain greater amounts of mitochondria and lipids as fuel for oxidative metabolism, whereas fast-twitch fibers are more equipped for anaerobic metabolism fueled by carbohydrates. Later, the type I and type II muscle fibers were reclassified with a bifunctional nomenclature based on the contractile properties and oxidative capacity of the fibers. More specifically, "fast-twitch oxidative" (FOG) muscle contracts with a faster contractile speed compared with "slow-twitch oxidative" (SO), but has a higher oxidative capacity than "fast-twitch glycolytic" (FG) muscle.

With the advent of immunohistochemical staining methods capable of differentiating myosin heavy chain isoforms, fiber types could be further categorized into type I "slow–red" and type IIa "fast–red," while type IIb "fast–white" was categorized into types IIx and IIb in rodents, and IIx, but not IIb, is expressed in humans [76]. A good correlation exists between type I and SO fibers. However, the correlations between type IIa and FOG fibers and type IIb and FG fibers are more varied. The type IIb fibers do not always rely primarily on anaerobic/glycolytic metabolism, nor do the type IIa fibers always rely primarily on aerobic/oxidative metabolism [77]. With the development of new molecular methods, it is evident that more specific classifications of muscle fiber types will be developed.

BOX 15.2 SEQUENCE OF EVENTS IN SKELETAL MUSCLE EXCITATION–CONTRACTION COUPLING

1. Motor neuron triggers depolarization of the voltage across the SL.
2. Action potential moves along the SL to the T-tubules.
3. Depolarization is detected by the α subunit of the DHPR (voltage sensor), resulting in a conformational change.
4. Conformational change in the DHPR is transmitted to RyR1.
5. RyR1 channel opens allowing diffusion of Ca^{2+} from inside the SR to the sarcoplasm.
6. In nonmammalian skeletal muscle, Ca^{2+} released via RyR1 binds to RyR3, triggering further Ca^{2+} release from the SR.
7. Ca^{2+} concentration levels in the sarcoplasm rise to >10 μM; low-affinity Ca^{2+} binding sites on TnC are saturated causing a conformational change in the troponin complex.
8. The conformational change in troponin is transmitted to tropomyosin, resulting in shifting of the tropomyosin molecule from its steric blocking position along the actin backbone to a position deeper in the actin–actin groove.
9. The shift in position of tropomyosin exposes myosin binding on the actin filament enabling crossbridge formation with myosin heads. Phosphate ion is released from the myosin head.
10. Myosin heads release ADP and undergo a conformational change pulling the actin filament toward the center of the sarcomere.
11. ATP binds to myosin head resulting in detachment of myosin head from actin.
12. Myosin heads hydrolyze ATP and undergo conformational change to reorient perpendicular position relative to the filament.
13. Steps 9 through 12 continue as long as ATP is available and Ca^{2+} concentration is high enough to maintain saturation of the troponin's Ca^{2+} binding sites.
14. When neural stimulation ceases, voltage across the SL is restored.
15. The DHPR undergoes a conformational change back to its resting-state structure.
16. The DHPR conformational change causes RyR1 channel to close, thereby ending Ca^{2+} release.
17. With the cessation of Ca^{2+} release, the activity of the Ca^{2+} pump protein now dominates by transporting Ca^{2+} against the concentration gradient from the sarcoplasm to the lumen of the SR. The sarcoplasmic Ca^{2+} concentration decreases below 100 nM.
18. Decrease in sarcoplasmic Ca^{2+} concentration results in removal of Ca^{2+} from troponin.
19. Troponin undergoes a structural change, reverting to resting-state conformation, which is transmitted to tropomyosin.
20. Tropomyosin reverts to resting-state position on the actin filament, thereby blocking actin–myosin crossbridge formation.
21. Muscle relaxes.
22. Additional note: in the conversion of muscle to meat, when ATP is depleted, the Ca pump can no longer function to restore resting-state Ca^{2+} concentrations, and the myosin–actin crossbridges cannot be broken (Step 11). This is the rigor state.

15.3.7 Summary

- Muscle fibers are classified as fast twitch, slow twitch, or intermediate, depending on the type of primary metabolism (glycolytic, oxidative) and protein isoforms comprising the fiber.
- Skeletal muscle as a contractile organ is built in a hierarchical pattern: myofilaments (thin and thick) < sarcomere < myofibril < myofiber (cell) < fiber bundle (fascicle) < organ.

- The muscle ECM or intramuscular connective tissue is likewise organized in a hierarchical pattern within the muscle. A myofiber is enveloped by the endomysium. A bundle of myofibers is surrounded by the perimysium. The entire organ is encased by the epimysium. The ECM provides mechanical strength to the tissue, serves as a framework in which blood vessels and nerves are embedded, and plays a dynamic role in signaling processes associated with cell growth and differentiation.
- Although Ca^{2+} is limiting as a nutrient in muscle, it plays a critical role in intracellular signaling to initiate the cascade of molecular events leading to the shortening of sarcomeres and thus muscle contraction.
- Muscle contraction occurs as a result of crossbridge formation between myosin heads and actin monomers, a conformational change in the crossbridge resulting in myosin "pulling" the thin filaments from opposite ends of the sarcomere toward the middle, thereby shortening the sarcomere. ATP plays a dual role in this process by (1) serving a source of energy for contraction and (2) binding to the myosin head to release it from actin.

15.4 CONVERSION OF MUSCLE TO MEAT

Live muscle tissue has an extensively developed metabolic system designed to support the specific function of muscle, which is to convert chemical energy to mechanical energy. The immediate source of chemical energy is ATP, and the supporting metabolic reactions are geared in large part toward sustaining levels of ATP needed for contraction as well as for maintaining cellular homeostasis. Circulation of blood to the muscle tissue to deliver oxygen and energy substrates and to remove carbon dioxide and metabolic end products is clearly critical to supporting this metabolic machinery.

Upon slaughter of an animal, the initial step in the conversion of muscle to meat is the cessation of blood flow to muscle that occurs at slaughter. Although physiological death of the animal occurs within moments after slaughter, the various organs of the body including muscle draw on reserve mechanisms in a futile effort to maintain cellular homeostasis. Without continuous oxygen delivery to the muscle, the myofiber utilizes its reserves of oxygen bound to myoglobin within the cell; quickly thereafter, anaerobic glycolysis becomes the dominant metabolic pathway for generation of ATP. As metabolic end products accumulate and the supply of substrates is exhausted, the synthesis of ATP can no longer match the rate of hydrolysis. Sarcoplasmic Ca^{2+} concentrations can no longer be sustained at resting-state levels, while other ion pumps requiring ATP, such as the Na^+-K^+-ATPase, become inoperative. The decline of ATP also results in the stiffening of muscle known as rigor mortis; this results from lack of sufficient ATP to dissociate myosin from actin during contraction. Eventually, endogenous enzymatic activity results in partial degradation of the structure of the myofilament framework. The time course of this series of reactions varies widely from species to species, and as will be seen later, even within a species.

There are a few primary reserves of energy that fuel the immediate needs of muscle in the early postmortem state. Creatine phosphate serves as a rapidly accessible reservoir of high-energy phosphate; as ATP is hydrolyzed to meet the demands of muscle contraction, creatine kinase transfers the phosphate moiety from creatine phosphate to ADP, thereby regenerating ATP. The enzyme adenylate kinase (described in Section 15.3.4.2) probably also contributes to generation of ATP at this point. However, in postmortem muscle, these sources of ATP synthesis are soon exhausted. Glycogen is generally a greater resource for synthesis of ATP. The enzyme phosphorylase cleaves monomeric glucose units from glycogen, yielding glucose-1-phosphate that subsequently enters the glycolytic pathway to generate ATP. In the absence of oxygen, pyruvate is further converted to lactate that accumulates in the muscle.

As can be seen in Figure 15.15, the initial generation of ATP from creatine phosphate and from glycogen is nearly equal to the rate of ATP utilization, thus sustaining the ATP concentration in the range of 5 mM. As long as ATP levels remain high, the physiological requirements of the muscle

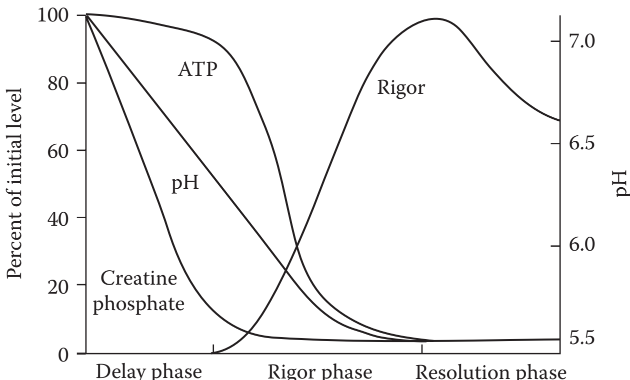


FIGURE 15.15 Schematic representation of postmortem changes in ATP concentration, creatine phosphate concentration, pH, and rigor development in muscle as a function of time. (Reprinted from Chiang, W. et al., in *Food Chemistry: Principles and Applications*, 2nd edn., Y.H. Hui, ed., Science Technology System, West Sacramento, CA, 2007. With permission.)

are generally supported and the physical characteristics and response to stimuli of the tissue during this early stage of conversion of muscle to meat (delay phase) remain similar to those of live muscle. Most notably, the muscle remains pliable and undergoes lengthening when subjected to stretch. However, even as ATP levels remain high, hydrolysis of ATP to support physiological needs results in generation and accumulation of hydrogen ions that reduce the pH of the muscle. The rate of pH decline closely reflects the accumulation of lactate, which serves as a marker of the rate of postmortem glycolysis [11]. As we shall see later, extreme variations in the rate of postmortem glycolysis are often associated with meat quality problems. The length of time associated with the delay phase is quite variable, depending on species, animal genetics, antemortem nutritional status, and management of the animal prior to slaughter, and possibly slaughter method. With red meats, this period may last up to 12 h, whereas with poultry, this period may be in the range of 30 min to 2 h [2].

When CrP is near depletion, the concentration of ATP begins to decline precipitously as utilization now exceeds the regenerative capacity, thus begins the rigor phase (Figure 15.15). As ATP levels fall, there is a corresponding increase in rigor development, measured by the increase in resistance of muscle to stretch. Depletion of ATP reduces the ability of the Ca^{2+} pump protein to maintain sarcoplasmic Ca^{2+} concentrations in the submicromolar or resting muscle concentration range. Likewise, there is a gradual increase in the number of myosin crossbridges with actin that remain locked because of a lack of ATP to dissociate the two proteins, leading eventually to a maximal stiffening or inextensibility of the muscle. As might be expected, some degree of sarcomere shortening may take place during this period, the magnitude of which is closely related to meat toughness.

The final phase of conversion of muscle to meat is referred to as ageing or rigor resolution. This period may last from a few days in poultry, pork, and lamb to 2 weeks for beef. During the resolution phase, there is a gradual increase in muscle extensibility and tenderness. The basis for these favorable changes is largely the result of proteolytic disruption of the myofiber ultrastructure, most notably at the Z-discs that lose structural integrity rapidly as a function of time. While development of tension during rigor has important implications in the eventual resolution of rigor, it is widely accepted that proteolysis is largely responsible for disruption of muscle structure and increase in meat tenderness [78].

15.4.1 Postmortem Degradation of Muscle Proteins

It is recognized that as muscle undergoes conversion to meat, the tenderness of meat tends to increase as a function storage time postmortem. This tenderization may be at least partially the result of degradation of both the myofibrillar and cytoskeletal proteins in muscle [79].

Among the muscle proteases, calpain has been studied most intensively with regard to its role in postmortem muscle protein degradation. Calpain is a Ca^{2+}-activated, cysteine-protease that is most active in the neutral pH range. Calpain is regulated by a variety of factors, including Ca^{2+}, phospholipids, and calpastatin, a widely distributed calpain-specific protein inhibitor [80]. Muscle tissue primarily expresses three different calpains: the two ubiquitous calpains and calpain 3 [81]. Ubiquitous calpains include μ-calpain, which requires 5–50 μM Ca^{2+} for its half-activation and m-calpain that requires 0.25–1 mM Ca^{2+} for its half-activation. These two isoforms are ubiquitously expressed in tissues, suggesting their involvement in basic and essential cellular functions mediated by the Ca^{2+} signaling pathway. Calpain 3 (also called p94, or CAPN3) is a skeletal muscle-specific calpain isoform, and it has a lower Ca^{2+} requirement for its activation. Ubiquitous calpains are composed of two subunits: a catalytic subunit of M_r ~ 80,000 and a regulatory subunit of M_r ~ 30,000. The regulatory subunits are homologous between the two isoforms, but the catalytic subunits are slightly different. Calpain 3 possesses the classical structure of a calpain except that it carries three unique sequences not found in any other calpains. Ubiquitous calpains tend to be concentrated in the Z-discs, and treatment of myofibrils with calpains causes rapid and complete loss of the Z-discs (Box 15.3). In skeletal muscle cell, calpain 3 binds specifically to certain regions of titin [82]; however, calpain 3 does not cut titin. As the Ca^{2+} concentration increases postmortem, calpains (mainly the ubiquitous calpains) are activated and initiate the degradation of muscle proteins such as TnT, titin, nebulin, MyBP-C, α-actinin, desmin, filamin, vinculin, and synemin [79]. Most of these proteins are either directly attached to (e.g., titin, nebulin), closely associated with (e.g., filamin, desmin, synemin), or near (vinculin) the myofibrillar Z-discs. When Z-discs are almost completely disrupted, actin and myosin are passively released together with other proteins from the sarcomere and become substrates for other proteolytic enzymes.

Cathepsins are lysosomal proteases that are maximally active at acidic pH, a condition that prevails for the remaining postmortem period, particularly the ageing period. At first, this suggests that cathepsin activity is likely to be more important than calpains for achieving the desired tenderization effects during ageing. However, when proteinases are incubated with myofibrils, it is the activity of the calpains that closely mimics the proteolytic events in postmortem tenderization. Furthermore, postmortem tenderization is Ca^{2+}-mediated [83], a characteristic only associated with the calpain proteolytic system in skeletal muscle. Still, the importance of cathepsins in ageing of meat cannot be overlooked. Their intracellular location and activity on numerous proteins in postmortem skeletal muscle make them logical candidates for postmortem proteolysis and tenderization [84].

The proteasome is a large, ubiquitous ATP- and ubiquitin-dependent proteolytic system that may also be involved in myofibrillar protein degradation during ageing of meat. The proteasome is able to degrade actin and myosin *in vitro* [85]; however, the proteasome is not able to degrade intact myofibrils. Muscle proteins such as actin and myosin are released from the sarcomere by a Ca^{2+}/calpain-dependent mechanism before they undergo ubiquitination and degradation by the proteasome. In animals overexpressing calpastatin, postmortem degradation by the proteasome is reduced, confirming the involvement of calpains [86]. Therefore, a calpain may be the initiator of myofibrillar degradation, and the proteasome may be responsible for proteolytic reactions that remove all myofibrillar fragments and hydrolyze them to amino acids.

15.4.2 Summary

- The conversion of muscle to meat following the death of an animal is a gradual process, taking place over several hours to days. The properties of muscle in the early (delay) phase resemble that of the live animal as the cell utilizes all available reserves to produce ATP. As ATP levels decline (rigor phase), crossbridge cycling declines. When ATP is depleted (rigor phase), myosin can no longer detach from actin, leading

BOX 15.3 MOLECULAR AND ULTRASTRUCTURAL CHANGES IN POSTMORTEM MUSCLE

Consumers make purchasing decisions regarding meat products based on several characteristics, one of the most important of which is tenderness. Products perceived as tough and difficult to chew as well as those that are soft and mushy are both likely to be rejected. Thus, scientists have focused on developing a detailed understanding of the biochemical and structural changes taking place in the conversion of muscle to meat so that the quality characteristics of the final product are most desirable.

Electron micrographs of muscle in the early postmortem stages show distinct changes, most notably in the Z-discs. At the point of death, the Z-discs of bovine muscle (centered in the I-bands) are dense, well demarcated zones that are structurally intact (Figure B15.3.1a). After 24 h at 25°C (Figure B15.3.1b), the Z-discs have broadened considerably and have lost much of their mass. The sarcomeres appear to have swollen, probably because of degradation of the cytoskeletal filaments linking adjoining myofibrils.

SDS-PAGE analysis of protein degradation has revealed a complex relationship between ultrastructural changes observed in muscle tissue and meat tenderness measured by Warner–Bratzler shear force measurements or sensory analysis. Titin, desmin, filamin, dystrophin, and TnT are some of the major proteins degraded postmortem (reviewed in [145]). Except for TnT, each of these proteins play an important role in maintaining structural integrity of muscle, so

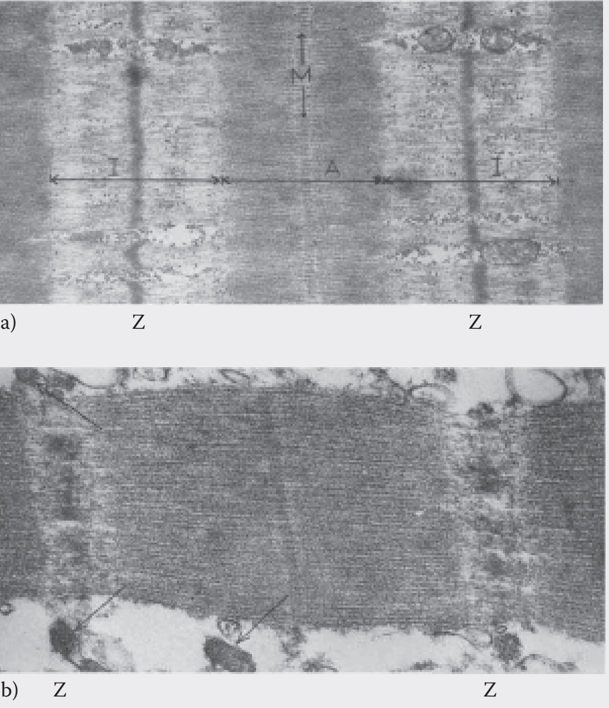


FIGURE B15.3.1 Use of electron microscopy and electrophoresis to analyze changes in postmortem muscle structure and function. (a) Electron micrograph of bovine muscle at time of death. The Z-discs (Z) located at the center of each I-band (I). Other structures: M-line (M), A-band (A). (b) Electron micrograph of bovine muscle after 24 h of storage at 25°C. ([a, b]: From Henderson, D.W., Goll, D.E., and Stromer, M.H. (1970). A comparison of shortening and Z line degradation in post-mortem bovine, porcine, and rabbit muscle. *Am. J. Anat.* 128, 117–135. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reprinted with permission.)

(*Continued*)

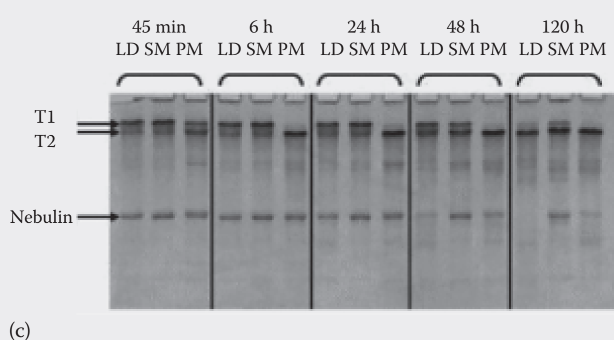


FIGURE B15.3.1 (*Continued*) Use of electron microscopy and electrophoresis to analyze changes in postmortem muscle structure and function. (c) SDS-PAGE gel showing degradation of titin and nebulin in whole muscle extracts of porcine longissimus dorsi (LD), semimembranosus (SM), and psoas major (PM) muscles. T1 indicates intact titin; T2 is a proteolytic fragment of titin. ([c]: Reprinted from *Meat Sci*., 86, Huff-Lonergan, E., Zhang, W., and Lonergan, S.M., 184–195, Copyright 2010, with permission from Elsevier.)

one may speculate that degradation of these proteins results in deterioration of the Z-disc and overall sarcomeric structure. Fragmentation of these proteins could trigger further enzymatic or oxidative processes that are associated with development of tenderness. TnT is a regulatory protein within the myofibril, and its degradation is thought to be more likely a marker of enzymatic activity in muscle than a contributor to the tenderization effect of ageing. Interestingly, the major myofibrillar proteins myosin and actin remain largely intact during ageing of meat.

Complicating the picture of muscle proteolysis and tenderization is the fact that different muscles display rates of degradation of proteins. Figure B15.3.1c shows losses of titin (T1) and nebulin in three different muscles as a function of time. While titin and nebulin are degraded, the rate and extent of loss varies significantly among the three muscles. At this time, the contribution of fragmentation of these proteins to tenderness is still unclear. Meat tenderness is most likely a multifactorial phenomenon, and much more work remains to be done in dissecting the relative contributions of each component.

to muscle stiffening or inextensibility. In the subsequent resolution phase, extensibility and tenderness increase as a result of enzymatic degradation of myofibrillar ultrastructure.

- Among the candidate proteolytic systems involved in tenderization of meat (calpains, cathepsins, proteasomes), calpains are primarily responsible for the enzymatic degradation of myofibrillar and cytoskeletal filaments leading to tenderization.

15.5 NATURAL AND INDUCED POSTMORTEM BIOCHEMICAL CHANGES AFFECTING MEAT QUALITY

Consumer choices in meat purchases are strongly influenced by various product attributes including water-holding capacity, color, fat content, and tenderness of the meat. Meat cuts displaying abnormally light or dark color or excessive loss of moisture within the package are more likely to be rejected by consumers, thereby downgrading the product value. Likewise, an unsatisfactory experience by a consumer with an unusually tough cut of meat may cause result in subsequent product rejection.

The quality attributes of meat are influenced by multiple interacting factors including animal species, breed, genotype, nutritional status, pre-slaughter handling, and postmortem chilling, processing, and storage. Some specific examples of quality problems and their underlying molecular bases are described in the following sections.

Following aging of meat, the characteristics of the tissue differ substantially from that of live muscle. Postmortem metabolism has led to a decrease in pH from the physiological value of ~7.4 in muscle tissue to an ultimate pH of ~5.5 to 5.9 in red meat and poultry. In addition, a degree of contraction has taken place in the tissue prior to formation of the rigor complex.

The consequences of reduced pH are simultaneously beneficial and detrimental to the value of the product. Clearly, the acidic pH of meat will retard microbial growth and thereby extend shelf life compared to the neutral pH of muscle. However, this advantage is offset by the economic loss to processors resulting from loss of water from the tissue as the pH becomes increasingly acidic. The isoelectric point of myosin (the dominant protein in muscle) is approximately 5.0; at this pH, the sum of positive and negative charges is zero, protein–protein interactions are maximal, and protein–water interactions are minimal. As a result, myofibrils shrink and lose much of their water-holding capacity. This loss of water during storage of fresh or cooked product (sometimes referred to as "purge") may be quite substantial, resulting in reduced value because the product bathed in its exudate is unattractive. Moreover, because meat is sold by weight, loss of water equates to loss of product weight and thus decreased profitability. A product that has lost a substantial amount of water will be perceived by the consumer as having both reduced juiciness and tenderness. It must be also be recognized that the watery exudate carries significant quantities of water-soluble vitamins, minerals, amino acids, and other nutrients that would be lost to the consumer. In addition to reduced water-holding capacity, visual defects may also be brought about by rapid postmortem glycolysis and a low ultimate pH being attained.

15.5.1 Pale, Soft, Exudative Meat

All muscle tissue undergoes a reduction in pH as a result of ATP hydrolysis during the conversion of muscle to meat. However, in aberrant cases, the rate of pH decline is unusually rapid such that most of the pH reduction takes place while the carcass temperature is high. For example, within 45 min postmortem, pig muscle pH is typically in the range of 6.5–6.7, while the temperature is approximately 37°C. In some carcasses, however, the pH may drop to less than 6.0 during the same time period. It is the latter combination of rapidly decreasing pH while the carcass temperature is still high that results in denaturation of some of the contractile proteins, with consequent loss of water-holding capacity, leading to the phenomenon known as "pale, soft, exudative (PSE) meat" [87]. While these attributes of the fresh cuts are likely to lead to rejection by consumers, the reduced protein functionality of the PSE product in processed meats also has serious economic consequences for the processing industry. The incidence of PSE pork has been estimated at 15.5% [88], although these results have been questioned.

The molecular basis for the PSE meat problem has been the subject of intense investigation over the last half-century. It is clear that antemortem stressors of the animal such as heat, transportation, physical exercise, mixing of unfamiliar animals, and animal handling are significant contributors to the problem of meat quality. The exact mechanism by which these stressors result in PSE meat is still unclear, but it is evident from a variety of studies that reduction of antemortem stress results in significant improvement in overall meat quality.

An animal's genotype may further increase its predisposition to an adverse response to stress. In the 1960s, it was noted that subsets of pigs within various breeds were particularly susceptible to stressors. The term "porcine stress syndrome" was coined to describe the inheritable muscle disorder of the pigs with low tolerance to the effects of stress [89]. These animals typically responded to stressors by developing a condition known as malignant hyperthermia, which is characterized by severe muscle contracture, respiratory distress, rapid-onset high fever, and

eventually death. Animals with this disorder that did not succumb to the effects of stress were much more likely than normal animals to yield PSE meat.

Studies over the next 30 years eventually led to the identification of a mutation in the RyR that was responsible for porcine stress syndrome. The substitution of thymine for cytosine at nucleotide 1843 in the RyR coding sequence leads to substitution of cysteine for arginine at residue 615 [90]. This mutation leads to excessive Ca^{2+} release from SR in the stressed animal, which, in turn, triggers severe muscle contracture and eventual malignant hyperthermia in the live pig. Excessive postmortem calcium release in the muscle of the PSS-susceptible pig triggers muscle contraction and associated anaerobic glycolysis with consequent hydrogen ion accumulation and heat production associated with development of PSE pork.

The problem of PSE meat has classically been associated with pork; however, in the early 1990s, increasing incidence of PSE meat became apparent in the turkey processing industry. The striking similarity in development of PSE pork and turkey led to the suggestion that a mutation in the RyR is responsible for the problem of PSE turkey [91]. As noted in Section 15.3.4.6, there is a significant difference in the excitation–contraction coupling mechanisms between mammals and birds. Thus, it is possible that if a mutation exists, it may be either in the RyR1 or RyR3 isoforms or in both. To date, no mutations have been identified in either turkey RyR isoform. However, there are intriguing indications that turkeys adapt to heat stress by expression of various alternatively spliced RyR transcript variants that may alter the tendency to produce PSE meat [92]. The advent of RNA deep sequencing methods to analyze the muscle transcriptome affords new opportunities to analyze normal and PSE meat samples for differences in gene expression and thus differences in protein abundance. The application of this method has revealed expression differences among several candidate genes in normal turkey meat compared to PSE samples, including pyruvate dehydrogenase kinase 4, the enzyme that regulates pyruvate dehydrogenase activity [93].

Another genetic abnormality that can lead to PSE meat from pigs is the Napole gene. The mutation in this case is a substitution of glutamine for arginine at residue 200 of the gamma subunit of AMP-activated protein kinase [94]. This enzyme plays a variety of roles in muscle including activation of ATP-producing pathways and inhibition of ATP-consuming pathways as well as inactivation of glycogen synthase.

Pigs possessing the dominant RN-allele tend to have much higher glycogen content than pigs with the recessive rn+ allele. The rate of postmortem pH decline in muscle from pigs with the Napole gene tends to be normal. However, the high levels of glycogen lead to an extended pH decline resulting in a very low ultimate pH, typically near 5.0. Because the isoelectric point of myosin is approximately 5.0, this low ultimate pH results in PSE meat with poor protein functionality. In fact, although the water-holding capacity is reduced compared to normal pork, the protein functionality of RN-pork in processed meats is even lower than pork possessing the RyR abnormality. The fact that 65%–80% of pork is consumed as processed meats demonstrates the importance of eliminating the Napole gene from pigs.

15.5.2 Dark, Firm, and Dry Meat

An occasional consequence of pre-slaughter stress is antemortem depletion of glycogen stores through stressors, exercise, or excessive fasting. This leads to a product that has the opposite characteristics of PSE meat and is referred to as “dark, firm, and dry” or DFD meat [2]. The meat color may vary from slightly dark red to extremely dark or nearly black in contrast to the normal cherry red appearance of normal red meat. The problem is most notable in beef, but has also been reported in pork. The lack of adequate glycogen reserves results in early termination of glycolysis, so the ultimate pH remains relatively high (>6.0). The incidence of DFD meat tends to be seasonal, with higher incidence when animals are exposed to sustained periods of cold, damp weather as compared to summer.

DFD meat has much higher water-holding capacity than normal because the ultimate pH is typically at least one pH unit higher than the isoelectric point of myosin. However, this advantage is strongly offset by the increased susceptibility of the product to growth of microorganisms and the rejection by consumers because of the abnormal color. The unusually dark color of this product is also a product of the high postmortem pH that keeps the charge on the muscle proteins high, thereby maximizing separation of muscle myofibrils and reducing light scattering. Active mitochondrial respiration at the higher pH also reduces the fraction of oxymyoglobin in the tissue.

15.5.3 Cold Shortening

In the early 1950s, the New Zealand lamb processing industry began receiving complaints from importers in Europe and North America that the meat was excessively tough. This was in contrast to lamb consumed in New Zealand that was not considered to be tough, suggesting that processing and/or storage factors may have been playing a role in the meat toughness [95].

A series of studies from the New Zealand Meat Research Institute elegantly demonstrated the complex relationship between pre-rigor chilling and meat toughness (Figure 15.16). When pre-rigor muscles are excised from the bones to which they attached, they undergo contraction. The extent of contraction depends on the type of muscle (red vs. white), amount of time postmortem, physiological state of the muscle, and temperature. At near-physiological temperatures, the degree of shortening is high. At lower temperatures, the extent of muscle contraction decreases progressively until a nadir is reached in the range of 10°C–20°C, temperatures at which minimal shortening takes place. If the same muscles are subjected pre-rigor to still lower temperatures, one notes a dramatic increase in the degree of shortening at temperatures below 10°C. The cold-induced muscle contraction is termed "cold shortening."

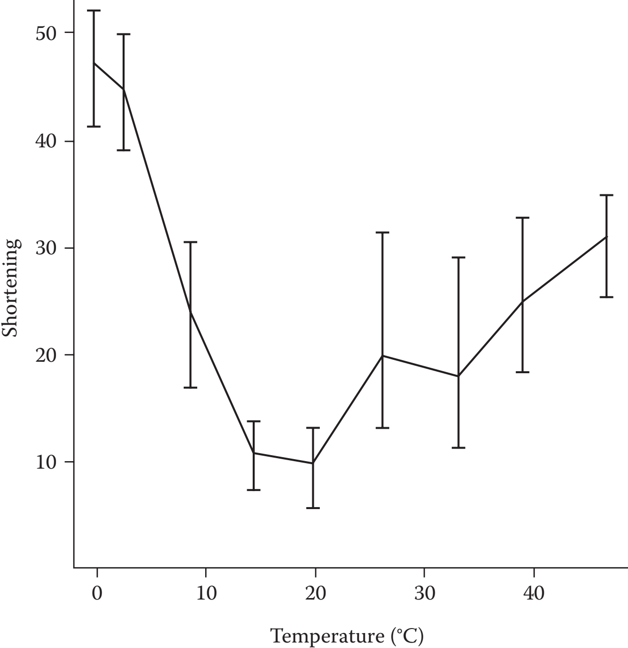


FIGURE 15.16 Shortening of excised pre-rigor beef muscle as a function of storage temperature. (Reprinted from Locker, H.H. and Hagyard, C.J., *J. Sci. Food Agric.*, 14, 787, 1963. With permission.)

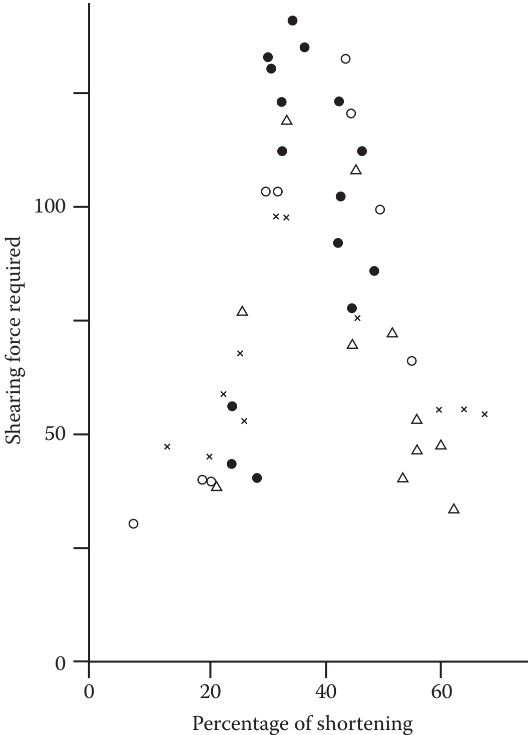


FIGURE 15.17 Meat toughness measured by shear force required to cut through a meat sample as a function of sarcomere shortening. (Reprinted from Marsh, B.B. and Leet, N.G., *Nature*, 211, 635, 1966. With permission.)

Marsh and Leet [96] subsequently demonstrated the relationship between pre-rigor cold shortening and toughness (Figure 15.17). The Warner–Bratzler shear test measures the amount of force required to cut through a fixed size of muscle tissue. Using this test, Marsh and Leet demonstrated that as the degree of shortening increased, the degree of toughness (measured by shearing force) increased, up to the point when muscle had contracted by about 40%. At this point, there is maximal overlap between the thin and thick filaments, and nearly all of the myosin heads are bound to actin filaments. Beyond this point, additional contraction may occur resulting in a decrease in toughness. The explanation for this observation is that under some circumstances such as lack of tension, muscle contraction may continue to the point that the thick filaments puncture the Z-line, causing extensive damage to the muscle ultrastructure and thus leading to an increase in tenderness [97]. Based on these observations, substantial modifications were made to the post-slaughter chilling of lamb carcasses to reduce pre-rigor chilling rate. Subsequent studies in the United States and Europe have validated the significance of cold shortening in the red meat industry and led to practices to minimize this problem.

The mechanism underlying the development of cold shortening is still not completely clear, but the following factors clearly play a role. Muscle must be in the pre-rigor state for contraction to occur; only during this period are there adequate levels of ATP to provide the energy for contraction as well as for dissociating myosin heads from actin for a subsequent contraction cycle. The temperature must be lower than 10°C, and the closer it is to 0°C, the greater the degree of shortening. Finally, the muscles most susceptible to cold shortening are those with the highest percentage of red fibers, for example, beef and lamb or sheep muscles. Cold shortening occurs to a lesser extent in pork because most pig muscles have higher percentage of white muscle fibers. Relatively little cold shortening occurs in poultry muscle.

It appears that altered postmortem calcium regulation and temperature-dependent changes in enzyme activities are responsible for the cold shortening phenomenon. As the temperature of the muscle goes below 10°C, anoxic mitochondria and SR lose their ability to retain Ca^{2+}. The Ca^{2+} pump protein would normally keep sarcoplasmic Ca^{2+} concentrations at resting muscle levels. However, at lower temperatures, the rate of SR reaccumulation of Ca^{2+} is suppressed; thus, sarcoplasmic Ca^{2+} concentrations increase, thereby triggering muscle contraction. Red muscle fibers are more likely to exhibit cold shortening. They rely primarily on oxidative metabolism to support energy requirements; thus, they have a higher amount of mitochondria than white fibers. Moreover, red fibers tend to have a less developed SR network, thereby reducing their ability to reaccumulate Ca^{2+} [98].

Strategies to prevent cold shortening were initially built on the relationship between the rate at which the carcass was chilled and development of toughness. Keeping carcass temperatures in the range of 10°C–20°C until rigor mortis is established prevents cold shortening. However, this approach is in conflict with the desire of lowering carcass temperatures quickly to minimize microbial growth on the surface of the meat. In the 1970s, experiments with electrical stimulation of carcasses demonstrated that the time for development of rigor mortis could be dramatically shortened [99]. This procedure is described in Section 15.5.5 and has now become standard industry practice for most beef and lamb slaughter operations.

15.5.4 Thaw Rigor

A phenomenon closely related to cold shortening is the severe muscle contracture that takes place upon thawing muscle that had been frozen while still in the pre-rigor condition. Thaw rigor was first described by Sharp and Marsh [100] who noted that thawing of pre-rigor whale muscle resulted in shortening of the muscle by as much as 60% and was accompanied by substantial loss of water-holding capacity. Thaw rigor likely results from structural damage to the SL and SR membranes from ice crystal formation. The loss of membrane integrity results in Ca^{2+} influx into the sarcoplasm triggering muscle contraction because ATP levels are still sufficiently high for shortening to occur. Likewise, membrane damage results in excessive drip loss from the muscle fibers upon thawing. The same approaches to preventing cold shortening, electrical stimulation or holding muscle on the carcass at temperatures above freezing point until the onset of rigor mortis, will serve to prevent thaw rigor.

15.5.5 Electrical Stimulation

Electrical stimulation refers to the application of an alternating electrical current to carcasses of meat animals following slaughter. When applied to carcasses during the early postmortem period, electrical stimulation induces extensive muscle contraction and relaxation that consequently accelerates the rate of muscle metabolism, ATP turnover, and rigor development. Depending on the voltage, frequency, and duration of the stimulus, this procedure reportedly improves the tenderness, flavor, color, quality grade, retail case life, and processing characteristics of meat and meat products [101].

Improvements in color and quality grade have been primarily responsible for the adoption of electrical stimulation by the processing industry in the United States. In other countries, the primary reason has been improvement in tenderness. Use of electrical stimulation was initially adopted in New Zealand, where the practice of rapid chilling and freezing of lamb carcasses for export led to cold shortening, thaw shortening, and increased meat toughness [99]. However, electrical stimulation is also effective in improving meat tenderness in the absence of cold shortening. Pearson and Dutson [102] discussed mechanisms for tenderization. Lysosomal disruption with consequent release of endogenous proteases has been suggested as a mechanism, based on an increase in the free activity of lysosomal enzymes after electrical stimulation. Thus, increased proteolytic activity prior to and during the ageing period could account for a portion of the increased tenderness. Another mechanism appears to be the physical disruption of myofiber integrity caused

by the extensive contractions induced with electrical stimulation [103]. Zones of supercontraction (contracture bands) and excessive stretching of the myofilaments adjacent to contracture bands are evident in photomicrographs of meat from electrically stimulated carcasses. Based on the physical dimensions of thick and thin filaments, contractions beyond 40% of resting sarcomere length would result in the thick filaments penetrating Z-lines and interacting with thin filaments of adjacent sarcomeres [97]. Other areas must stretch and/or tear to accommodate these zones of supercontraction. An increase in tenderness would ensue. Although conclusive support is still lacking for the case of physical disruption, the proposed mechanism is consistent with other instances of tenderization associated with excessive contraction.

The beneficial effects of electrical stimulation are thought to occur because of the acceleration in the postmortem conversion of muscle to meat. In essence, stimulated carcasses reach their maximum quality grade sooner, which allows the packer to either increase production at similar quality grade levels or increase quality grade levels at similar production [101]. Where quality grade influences the pricing system, either case is financially more favorable for the packer and accounts for the extensive use of electrical stimulation in the beef industry.

15.5.6 Summary

- Water-holding capacity, color, texture, and toughness are important meat quality indices that strongly influence consumer choice. These attributes are influenced to some degree by management practices impacting the live animal (e.g., minimize transportation stress, feed withholding prior to slaughter) as well as processing protocols (e.g., control chill rate, use of electrical stimulation).
- Identification of genes and gene × environment interactions associated with some meat quality problems (PSE meat, DFD meat, etc.) will be necessary to reduce the incidence of these quality defects.

15.6 CHEMICAL CHANGES IN MEAT DURING PRESERVATION

The susceptibility of muscle tissue to microbial spoilage entails that meat be preserved by physical or chemical methods. Refrigeration and freezing provide the most effective means to retard microbial growth as well as to minimize deleterious chemical and biochemical processes in meat and meat products. Traditionally, fresh meat is preserved by salting and partial dehydration that raise the osmotic pressure and lower the water activity thereby suppressing the growth of microorganisms. Irradiation and high-pressure treatments are relatively new preservation interventions that are becoming acceptable in the meat industry. On the other hand, nontraditional packaging systems, such as modified atmosphere packaging, are gaining popularity to extend meat shelf life. All these preservation techniques affect not only the ecology of microorganisms on meat but also the chemical properties of meat and meat products.

15.6.1 Chilling and Refrigeration

In a typical packing plant, animal carcasses are rapidly chilled in a 2°C–5°C chill cooler to minimize microbial growth; for chicken broilers and fish, the carcasses are usually chilled by immersion in ice slurries or by blasting cold air. The time duration for a warm carcass to reach its final chilled temperature varies, depending on its size, thickness of subcutaneous fat, and the chilling methods. The chilling time can be as short as 1 h for an ice-chilled broiler carcass and up to 24 h for a 300 kg beef carcass. At some commercial packing plants, cold-water showering and high-velocity air are used to facilitate the chilling process.

The rate of chilling affects the enzymatic reactions in postmortem muscle tissue, which in turn, affects the quality of aged meat. The major biochemical changes occurring during the early stage of muscle to meat conversion—the pH fall (glycolysis), the depletion of ATP, and muscle

contraction—are enzymatic processes. As the carcasses are rapidly chilled, these biochemical changes are retarded due to the inhibition of enzyme activities. Lipid oxidation also proceeds more slowly at refrigeration temperatures due to reduced activity of oxidative enzymes. Inhibition of lipid oxidation would preserve the fresh meat flavor and minimize myoglobin oxidation. In spite of its general benefits, chilling could also produce undesirable quality characteristics of meat. For example, when a lean beef carcass is chilled rapidly pre-rigor, a dark discoloration band with a sunken appearance, commonly referred to as the "heat ring," sometimes forms near the lumbodorsal fascia of longissimus muscle. This phenomenon seems to be caused by cold shortening (muscle fiber supercontraction) of the edge of pre-rigor muscle but can be eliminated by carcass electrical stimulation immediately postmortem.

As discussed previously, exposing pre-rigor muscle to cold temperatures could have an adverse effect on meat tenderness due to increased muscle fiber contraction or myofibril shortening, notably for red meat. To minimize cold shortening, it is imperative that muscle remain attached to the skeleton during chilling, especially in the early stage of postmortem storage. Because the activity of the major endogenous proteases involved in meat tenderization during postmortem aging, for example, calpain and cathepsins, is greatly reduced at low temperatures, it is necessary that the carcass or meat be adequately aged to achieve an acceptable level of tenderness.

Super chilling or deep chilling is also used to inhibit microbial growth and prolong meat shelf life. In this process, meat is chilled to 1°C–2°C below the freezing point of water. While a very thin layer of ice may form on the surface, the interior of meat remains unfrozen but supercooled [104]. Chicken broilers and fish are particularly suitable for super chilling as they can be readily submerged in a super chilling aqueous media. This relatively new technology is highly desirable because spoilage microorganisms and enzymes do not easily adapt to subzero temperatures. However, super chilling does not always curtail, and sometimes actually accelerates, chemical reactions. Furthermore, technologies have been developed to store meat and meat products at temperatures between 0°C and −0.8°C to maintain the product in an unfrozen status while achieving a substantially prolonged microbiological shelf life.

15.6.2 Freezing

Freezing is one of the most effective methods of preserving meat. When meat and meat products are stored at temperatures below −10°C, microbial growth and enzyme reactions are essentially curtailed, and hence, quality loss is minimized. However, physical and chemical reactions can still occur in meat during freezing, storage, and subsequently thawing. Chemical changes in frozen meat during storage include discoloration and development of oxidative rancidity, which result from oxidation of myoglobin and unsaturated lipids, respectively, and texture hardening due to protein denaturation and aggregation. These adverse changes are influenced by the rate of freezing and thawing, the duration of frozen storage, fluctuations of the freezer temperature during storage, and the atmospheric condition of the frozen meat. In the case of processed meat, the ingredients added to meat (e.g., NaCl) and the specific processing procedures, such as grinding, chopping, emulsification, and restructuring, can influence the quality and shelf life of the frozen products. Antioxidants are often added to inhibit salt-induced oxidation in frozen meat products.

Freeze-induced protein denaturation, a main side effect of frozen meat, is attributed to physical damage resulting from the formation and accretion of ice crystals and from chemical processes associated with dehydration and concentration of solutes in the muscle tissue. Freeze-induced protein denaturation is especially notable under slow freezing conditions. At a slow freezing rate, the exterior fluid of muscle cells cools more rapidly than the interior fluid, and when the supercooled extracellular fluid reaches a critical temperature, water separates from solutes and forms ice crystals. As crystallization proceeds, extracellular salt becomes more concentrated, creating an osmotic pressure gradient across the cell membrane. These processes can lead to protein denaturation and disruption of the cell membrane [105]. To prevent protein

denaturation, cryoprotectants, such as polyphosphate and polyols (sorbitol, sucrose, polydextrin, etc.), can be incorporated into meat prior to freezing.

The rate of freezing is dictated by freezing methods employed and follows the order of cryogenic freezing > blast-freezing > still-freezing. Cryogenic freezing, which uses condensed gases such as liquid nitrogen (−195°C) and solid carbon dioxide or dry ice (−98°C), rapidly chills meat to below 0°C and transforms liquid water into ice crystals in a matter of minutes. Blast-freezing air (e.g., −50°C) also enables a rapid heat transfer thereby crystallizing intramuscular water in a very efficient manner. On the other hand, still-freezing allows slow heat dissipation from meat and can cause damage to muscle cell and proteins. In general, fast freezing promotes the formation of small ice crystals that are uniformly distributed inside and outside the muscle cells, while slow freezing favors the formation of large ice crystals that are much fewer in quantity and are prevalent extracellularly.

A relatively new freezing technology, known as "pressure-shift freezing," has been introduced as a potential meat quality preservation method. During pressure-shift freezing process, meat samples are chilled to subfreezing temperatures (e.g., −20°C) and will not freeze under a certain high pressure. When the pressure is suddenly released, instantaneous and homogeneous microcrystallization occurs throughout the muscle tissue. Meat processed with pressure-shift freezing reportedly has a minimally altered ultrastructure, reduced protein denaturation, and an improved product quality [106,107].

15.6.3 Pressurization

High-pressure treatment is potentially useful for meat preservation as well as for processing to improve product quality. Hydrostatic pressures ranging from 100 to 800 MPa have been used to destroy pathogenic microorganisms and inactivate spoilage enzymes in fresh meat prior to storage. Nonthermal, high-pressure processing is also used to process post-packaging ready-to-eat meats, such as deli slices, to eliminate potential contamination by *Listeria monocytogenes*. Because the compression energy is low (e.g., 19.2 kJ for 1 L of water under a 400 MPa pressure), covalent bonds are usually not affected. However, high-pressure treatment may disrupt electrostatic and hydrophobic interactions in proteins, thereby rendering them less stable. Because pressurization processes do not depend on additives or temperature, treated meat products will retain their original flavor and taste.

Physical modifications in muscle tissue under high pressures include the decrease in the volume of the aqueous phase and a drop in pH. These changes are reversible upon pressure release. However, even a brief exposure to these temporary changes could permanently alter the protein structure and its association with nonprotein compounds. Pressures at above 100 MPa can cause protein quaternary structure to dissociate into its subunits, monomeric structure to partially unfold, and can induce protein aggregation and gelation [108,109]. High-pressure treatment separates myosin heavy chains into one-headed monomers, which is followed by head-to-head interaction to form aggregates [110].

Pressure-induced changes in the hydration volume appear to play a major role in the unfolding, dissociation, aggregation, and gelation of muscle proteins. Pressure-treated muscle protein products, such as surimi, can spontaneously form a gel at mild temperatures. This results from increased exposures of hydrophobic side chain groups, allowing protein aggregation to readily occur. Due to protein structural modifications and improved solubilization, high-pressure-treated meat is suitable for the manufacture of low-salt meat products because water binding and gelation become less dependent on high ionic strengths [111]. The increased exposures of hydrophobic groups also allow pressure-processed muscle proteins to more effectively aid in the formation and stabilization of meat emulsions.

High-pressure treatments of pre-rigor muscle increase the rate of glycolysis and fiber contraction, which is attributed to multiple factors, including the disruption of SR where calcium is normally stored and the loss of Ca-ATPase activity. The high cytosolic concentration of Ca^{2+} activates enzymes involved in glycolysis (e.g., phosphorylase kinase) and muscle contraction (e.g., myosin

ATPase). When the applied pressure is sufficiently high (e.g., >400 MPa), discoloration occurs in raw meat as indicated by an increased L* value and a decreased a* value [108]. Indeed, exposures of raw meat to high pressures result in an increased metmyoglobin (brown) content as the expense of myoglobin (red) due to oxidation of heme iron and the denaturation of globin.

A pressure at above 150 MPa can induce extensive changes in sarcomere structure as well, for example, the disappearance of the M-line and H-zone and the loss of integrity of I-band filaments. Surprisingly, these structural changes do not seem to lead to meat tenderness improvement. This may be due to thickening of the Z-lines in costameres and the loss of protease activity [110]. On the other hand, collagen structure is not affected by high-pressure processing.

In high-pressure-treated beef, the level of μ-calpain is markedly reduced during aging [112]. Both μ-calpain and m-calpain are partly inactivated at 200 MPa and completely inactivated at 400 MPa. High-pressure-induced denaturation and the enhanced autolysis of calpains due to the high concentration of cytosolic Ca^{2+} released from SR are responsible for the negative effect. On the other hand, high-pressure application increases catheptic activity (B, D, L, H, and peptidases) due to the disruption of lysosomal membrane. The increased release of cathepsins from lysosomes apparently is sufficient to overcome the pressure-induced denaturation. Nevertheless, the enhanced catheptic activity is not able to compensate for the reduced tenderness resulting from the loss of calpain and structural changes in myofibrils. For this reason, softening of muscle tissue in fish during storage due to excessive proteolytic activities can be overcome by high-pressure treatments that inactivate endogenous proteases, especially lysosomal enzymes.

Extremely high pressures generated with pyrotechnic devices have been invented to tenderize meat. A particular example of such technologies is Hydrodyne® [113]. In this method, encapsulated fresh meat is placed in a sealed, water-filled container situated below the ground level. A small amount of an explosive, consisting of a liquid and a solid, generates a shock wave that is in acoustic match with water in muscle. The shock wave produces an extraordinarily high pressure measuring about 680 atm or 10,000 psi at the contact surface with the meat. Meat exposed to such a high pressure exhibits remarkable tenderness improvements and requires less aging time to achieve desirable tenderness. The tenderizing effect is attributed to the disruption of the myofibrils, including the Z-discs. Because Hydrodyne also inactivates microorganisms, it has the additional benefits of preserving fresh meat and enhancing meat safety.

15.6.4 Irradiation

Irradiation as a means to inactivate pathogenic microorganisms has gained acceptance in the meat industry. There are two types of radiations: ionizing radiation and nonionizing radiation. In nonionizing radiation, such as microwave and infrared frequencies, the energy of radiation is not high enough to cause atoms to ionize. Instead, it relies on the heat it generates to destroy microorganisms and, hence, is suitable for heat-processed meat products. In ionizing radiation, a radiation generated by high-speed electrons or radioactive isotopes (gamma radiation) strike atoms to produce ions, and destruction of microorganisms is therefore more effective. The permissible dosages of irradiation are 1.5–3.0 kilogray (kGy) for poultry and 7.0 kGy for beef.

γ-Irradiation is a proven method of radiation for fresh or raw meat. Although it is effective in reducing microbial contamination, adverse chemical changes to muscle tissue due to radiolysis do occur. For example, γ-irradiation of fresh meat can produce superoxide and hydroxyl radicals. These primary radicals are highly reactive and can react with muscle lipids and proteins to generate secondary radicals and lipid and protein degradation products. Differing from small radicals, which are short lived in the aqueous environment of meat, protein radicals can be relatively long lived and can cross-link with one another causing the muscle tissue to harden. On the other hand, degradation of unsaturated lipids in muscle following γ-irradiation leads to the production of various hydrocarbons, particularly alkenes and carbonyl compounds, which contribute to off-flavor of treated products. Volatile sulfur compounds are also produced by γ-irradiation due to radiolytic degradation of

side chains of methionine and cysteine residues, and they are the main off-odor volatiles produced in irradiated, vacuum-packaged red meat and poultry [114].

Discoloration is another major consequence of γ-irradiation. Irradiated meat can develop unattractive greenish or brownish gray colors, which appear to be caused by the breakdown of the porphyrin structure of the heme or the formation of sulfmyoglobin [115]. For light-colored meat such as poultry breast, an intense pink color can form that has been attributed to formation of a carbon monoxide–myoglobin complex [116]. Because chemical changes caused by γ-irradiation are usually radical-driven processes, the use of vacuum packaging or incorporation of proper water- and lipid-soluble antioxidants can minimize the negative impact on muscle food quality.

15.6.5 Summary

- Chilling improves the shelf stability of fresh and processed meats through the retardation of biochemical reactions (microbial and enzymatic) and chemical processes that are dependent on thermal energy, while freezing (conversion of water to ice) imparts the product stability additionally through the deprival of the aqueous medium required for microbial metabolism and chemical reactions.
- The achievement of an extended shelf stability of meat by high-pressure treatments is due to the disruption of microbial cells as well as inactivation of some endogenous metabolic enzymes.
- Ionizing irradiation provides the most efficient nonthermal pasteurization or sterilization of meat because it generates radicals through ionization that cause damage to microbial DNA and other vial cellular components. However, undesirable flavor changes can result from high doses of radiation exposures.

15.7 CHEMISTRY OF PROCESSED MEATS

Processing of meat refers to the application of physical, chemical, and thermal treatments of muscle tissue to increase the product variety, to offer convenience, and to extend meat shelf life. It involves extensive modifications of the physicochemical properties of fresh meat. Processed meats may be separated into three main categories: (1) those in which the structural characteristics of muscle are minimally altered, for example, cured ham and bacon and corned beef; (2) those with moderately altered muscle structure, for example, sectioned and then restructured roasts and steaks; and (3) those that are extensively comminuted and then reformed, for example, sausage, frankfurters, and many luncheon meats. The chemical changes in the muscle tissue depend on the specific modification procedures and ingredients employed. For example, the development of a stable, pinkish red color in cured ham is due to the chemical reaction of nitric oxide with myoglobin; the formation of stable, fat globules in emulsion-type products is largely attributed to protein–lipid interaction at the water–oil interface; and the adhesiveness and smooth texture of boneless turkey ham result from interaction and gelation of myofibrillar proteins extracted by salt and phosphate.

15.7.1 Curing

The term "curing" refers to the treatment of fresh meat with salt and nitrite (or nitrate) for the purpose of preservation and obtaining desirable color and flavor. The origin of the curing technology is lost in antiquity, but is generally believed to be around 3000 BC. Cured meats have a characteristic pinkish color and a distinct aroma. They include traditional products such as ham, bacon, and summer sausage and a variety of ready-to-eat products available in the deli or refrigeration section at retail outlets, for example, restructured and sliced turkey ham and deli-type bologna.

Sodium chloride (NaCl) is the common salt used in cured meats. The main functions of salt, other than imparting flavor, are to extract myofibrillar proteins and to increase osmotic pressure, thus, inhibiting bacterial growth and subsequent product spoilage. Although salt is an indispensable ingredient in cured products, the actual curing agent is nitrite (NO_2^-) or nitrate (NO_3^-). Nitrate was

originally approved for color fixation in cured meats, but now it has largely been replaced by nitrite, because the latter is the immediate precursor of nitric oxide (NO), the ultimate curing compound. Nitrate is now restricted to dry-cured products, such as country-cured hams and dry sausages. In making these products, nitrate is slowly converted to nitrite by microorganisms or by reducing compounds, allowing slow curing reactions that presumably produce more desirable flavors and a stable color. Alternatively, cured meats can be prepared with the extracts of nitrate-containing vegetables and fruits, such as celery and cherry. The extracts are generally treated with bacterial cultures, including *Staphylococcus xylosus* and *Staphylococcus carnosus*, which contain nitrate reductase to convert nitrate to nitrite for subsequent curing reaction [117].

Nitrite is a multifunctional chemical. It induces and stabilizes the pinkish color of lean meat, contributes to the characteristic flavor of cured meat, inhibits the growth of spoilage and pathogenic microorganisms (particularly *Clostridium botulinum*), and retards development of oxidative rancidity. The pinkish red color characteristic of cooked, cured meats results from the reaction of the myoglobin heme with nitric oxide forming the nitrosylmyoglobin pigment. Nitric oxide is derived from nitrite in the presence of reducing compounds such as erythorbic acid. Part of nitrite dissolved in water can form nitrous acid (HNO_2). Under reducing conditions, nitrous acid decomposes to nitric oxide. When nitric oxide binds to the heme iron, it changes the electron distribution in the heme structure, thereby producing a pinkish color. Upon heating, nitrosylmyoglobin is converted to nitrosylhemochromogen, which is more stable due to globin denaturation.

$$NaNO_2 + H_2O \longrightarrow HNO_2 + NaOH$$

$$3HNO_2 \longrightarrow HNO_3 + 2NO + H_2O$$

Reducing compounds are added in meat curing mixtures to hasten color development via converting nitrite to nitric oxide and ferric ion of the heme to ferrous ion. The most commonly used reducing compound is sodium erythorbate (an isomer of ascorbate). Muscle itself also contains endogenous reductants and enzymatic reducing activity, for example, cytochromes, quinines, and NADH, but the reducing power of these factors is relatively small. In addition to reducing metmyoglobin (Fe^{3+}) to myoglobin (Fe^{2+}), and nitrite to nitric oxide, erythorbate also serves as an antioxidant to stabilize both color and flavor and to decrease the formation of nitrosamines. Phosphates, such as sodium pyrophosphate, tripolyphosphate, and hexametaphosphate, are other curing adjuncts. Phosphates do not directly enter the curing reactions, but they function to increase water-holding capacity of muscle and contribute to oxidative stability by chelating pro-oxidative metal ions.

15.7.2 Hydration and Water Retention

As discussed in previous sections, water accounts for 70%–80% of the weight in fresh meat. In injected or pumped meat, the water content can exceed 85%. The amount of moisture present in cooked meat determines the product juiciness and influences its tenderness. Water in meat is either bound or in a free form. Bound water is tightly associated with proteins through hydrogen bonds, which is influenced by the surface charge and polarity of protein. Free water is held via capillary forces in different compartments of the muscle tissue, for example, in the

spaces between myofilaments, between myofibrils, and outside the fibers. This form of water makes up the bulk of the water in meat (70%–90%). In comminuted meats, a large portion of water is also retained via entrapment in the matrix of myofibrillar protein gels. Denaturing conditions, such as frozen storage, oxidation, and rapid acid accumulation postmortem while the muscle temperature remains high, lead to reduced water binding in meat. An example of meat with poor water-binding ability is PSE pork and turkey, which has been discussed in previous sections.

The ability to bind, immobilize, and retain indigenous as well as exogenous water in processed meat is largely attributed to myofibrillar proteins, which is influenced by meat ingredients. High concentrations of monovalent salt (NaCl or KCl) solutions, that is, brine, are commonly incorporated into meat through marination or injection. Hydration and retention of added water are made possible through the NaCl-induced myofibril expansion due to increased electrostatic repulsion, which results in transverse swelling [118]. A variety of phosphate compounds, including sodium pyrophosphate, sodium tripolyphosphate, and sodium hexametaphosphate, are used in conjunction with salt to further improve the moisture-retention capability of meat. Injected fresh meat usually contains both salt (0.5%–2.0%) and phosphate (0.25%–0.40%). When an alkaline phosphate is used, it confers an additional benefit by raising the meat pH from around 5.5–5.6 (which is close the isoelectric point of actomyosin) to 5.8–6.0 where myosin and most other muscle proteins will bind water more strongly due to increased net charges. A pH elevation would also allow the inter-filamental spaces to further expand via electrostatic repulsions for additional water to be immobilized.

The mechanism NaCl- and phosphate-induced meat hydration extends beyond their simple electrostatic repulsion effect. In addition to increasing charge repulsions between adjacent myofilaments, high concentrations of NaCl (e.g., >2.5%) are able to dissociate myosin filaments, creating a bulky polypeptide matrix for moisture retention [119]. Furthermore, at elevated NaCl concentrations, the isoelectric point of myosin shifts to a lower pH due to screening of positive charges ($-NH_3^+$) in proteins by Cl^-. As a result, myosin (or actomyosin) within the normal pH range of meat will carry more surface charges (Figure 15.18). The increased inter-peptide electrostatic repulsion enables a stronger protein–water interaction and a greater water-retaining capacity of meat. On the other hand, low concentrations of pyrophosphate and tripolyphosphate (<0.5% or 5–15 mM) are capable of dissociating the

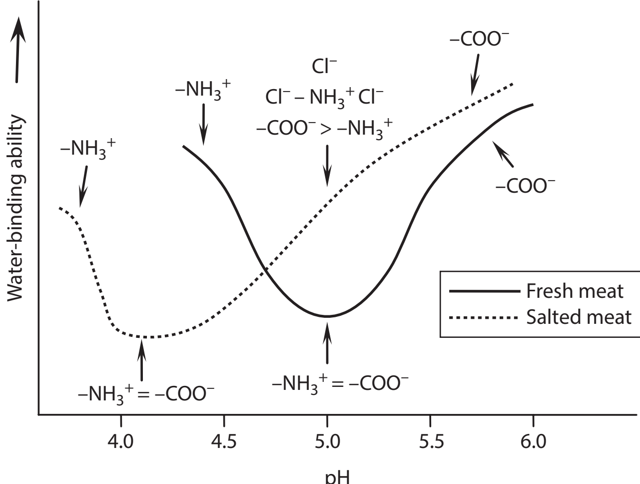


FIGURE 15.18 Schematic representation of the relationship between pH and water binding by proteins in fresh and salted meat.

actomyosin complex. In the presence of magnesium, the dissociation effect of pyrophosphate is very similar to that exerted by ATP. The detachment of actin from myosin filaments allows water to more readily diffuse into the inter-filamental spaces. The dissociation also improves meat tenderness. Significant muscle fiber swelling occurs as the NaCl concentration is raised from 0.1 M to about 0.6 M in the absence of phosphate or to 0.4 M in the presence of phosphate [120]. The extent of swelling and hydration continues until 1.0 M NaCl (approximately 4.0% of muscle weight) is incorporated where the swollen fiber will start to shrink due to a salt-out effect.

Hydration of salt- and phosphate-treated meat is accompanied by partial extraction of myofibrillar proteins. Selective removal of proteins from the myofibril backbone may be necessary for the transverse expansion of the protein filaments. Phase contrast microscopy shows that myofibril "swelling" and removal of proteins from the thick filaments (myosin) in the 0.6–1.0 M NaCl solution occur concurrently [118]. The addition of 10 mM pyrophosphate or tripolyphosphate greatly facilitates the hydration process and causes myosin to be extracted from the ends of the A-band where myosin cross-links with actin. Moreover, the extraction of transverse structural polypeptides, for example, M-protein, X-protein, and myosin-binding protein-C, by the presence of salt and phosphate, seems to promote loosening of myofibril lattices, thereby allowing water pickup by muscle fibers [120,121].

15.7.3 Formation of Protein Gel Matrix

Gelation of proteins is a physicochemical process involved in the production of restructured and comminuted meat products. The gel formation not only is responsible for adhesion of meat pieces and particles, but it also plays an important role in water, flavor, and fat binding in cooked products. Gelation in heated muscle foods takes place as a three-step sequential process. The initial unfolding (denaturation) of individual protein molecules is followed by their aggregation, largely through hydrophobic interactions; and at the final step, small protein aggregates or oligomers are cross-linked to form fine strands that eventually lead to a continuous viscoelastic network [122]:

$$\chi P_N \xrightarrow[\text{(denaturation)}]{\text{Heating}} \chi P_D \xrightarrow[\text{(aggregation)}]{\text{Heating}} (P_D)_\phi, (P_D)_\psi, \ldots \xrightarrow[\text{(cross-linking)}]{\text{Heating/cooling}} (P_D)_\phi - (P_D)_\psi - \cdots$$

where

χ is the total number of protein molecules

ϕ and ψ ($\phi + \psi + \cdots = \chi$) are the number of molecules that are aggregated at certain point of the gelation process

P_N is the native protein

P_D is the denatured protein

Examples of gel-type products are bolognas, frankfurters, and various luncheon meats made from comminuted muscle. Because of its adhesion ability, the gel formed at the junction of meat chunks in restructured products (e.g., boneless ham and turkey rolls) is largely responsible for the product integrity and sliceability.

Sarcoplasmic and connective tissue (stromal) proteins play only a minor role in the overall gelation phenomenon in processed meat. Most sarcoplasmic proteins are readily coagulated when salted meat is cooked to 40°C–60°C and they do not form an ordered, functional gel structure. Partially hydrolyzed collagen (gelatin) is the best-known gelling protein, and its gelation is relatively insensitive to ionic strength. Gelatin forms reversible, cold-set gels, which are stabilized by hydrogen bonds. However, dissociation and degradation of collagen into soluble gelatin (the gelling component) requires moist, prolonged heating, a condition that is not commonly employed in producing muscle foods. On the other hand, myofibrillar proteins as a whole are superior gelling proteins, playing a vital role in producing desirable textural characteristics in processed muscle foods.

In particular, myosin (pre-rigor) or actomyosin (post-rigor) accounts for most of the gel-forming capacity of the myofibril protein system [123].

In order to form a gel, myofibrillar proteins must be extracted first, and this is usually initiated by mixing meat with salt (NaCl or KCl) and phosphates. The gelling properties of myofibrillar proteins are influenced by the proteins' structure and size, their concentrations, the source or type of meat, and the various processing conditions such as pH, ionic strength, and heating rate. Thus, myosin, which has a large length-to-diameter ratio (approximately 100 nm in length and 1.5–2 nm in diameter), can form a highly viscoelastic gel, whereas actin, which is a globular protein of about one-tenth of the myosin size, is a poor candidate for gelation [124] although it may reinforce myosin gels at a myosin-to-actin ratio (w/w) of about 24 [125]. Myofibrillar proteins from muscle white (fast-twitch glycolytic) fibers form more rigid gels than those from red (slow-twitch oxidative) fibers, and this is attributed to the different physicochemical characteristics existing between myosin isoforms [126]. This explains why chicken pectoralis major, which consists exclusively of white fibers, and its myosin or mixed myofibrillar proteins, form stronger gels than chicken gastrocnemius (preponderantly red fibers) or its proteins under equal meat processing conditions. Another unique property of myofibrillar proteins is that they tend to form the strongest gel at pH around 6.0, although the exact pH optima vary slightly depending on muscle types and animal species.

The mechanism of heat-induced myofibrillar protein gelation is largely accounted for by myosin, the major gelling component in the salt extract of processed meat. Under a typical meat processing condition (pH 6.0, 0.6 M or 2.5% NaCl), the gelation begins with unfolding of S1 region of HMM when the protein sol is heated to about 35°C, leading to hydrophobic association through head–head interactions (Figure 15.19). The oligomers then coalesce at about 48°C, producing a somewhat elastic characteristic. When the temperature approaches 50°C–60°C, conformational changes in LMM (rod) occur, creating an open structure that exposes hydrophobic regions and specific side chain groups. The structural change results in a temporary decline in the elastic characteristic of the

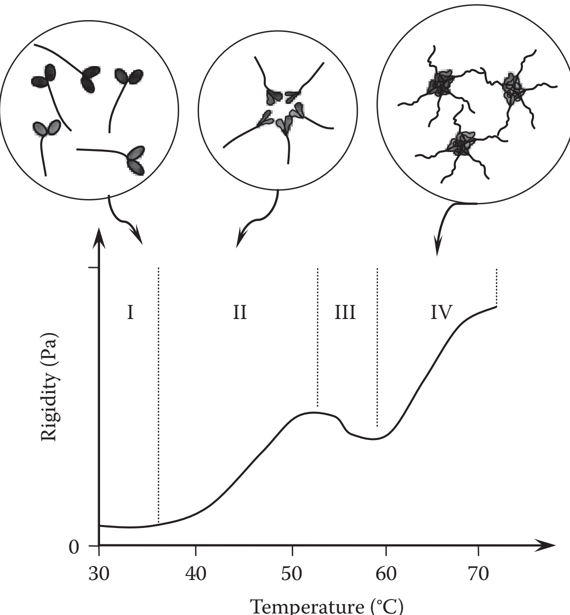


FIGURE 15.19 Schematic representation of heat-induced gelation of myosin in a 0.6 M NaCl, pH 6.0 solution. The four temperature zones show, respectively, (I) no change in myosin, (II) head–head association, (III) structural rearrangement of myosin aggregates owing to unfolding of light meromyosin, and (IV) cross-linking of myosin agglomerates via tail–tail association.

semi-gel. For actomyosin, the drop in the gel rigidity in this intermediate temperature region is also related to the detachment of actin. However, the ensuing association of LMM via tail–tail interactions upon further heating leads to the formation of permanent strands and filamentous gel networks with high elasticity and water-binding capacity.

Microbial transglutaminase, an enzyme that catalyzes acyl transfer reactions thereby cross-linking proteins through the glutamine-lysine bridge, has a remarkable effect on myofibrillar protein gelation. The incorporation of this enzyme into the gelling solution results in as much as 10-fold increases in myofibril gel strength and, hence, appears to be an excellent food ingredient for use in meat and surimi processing where meat binding is of major importance [127,128]. Furthermore, through promoting protein association, reactive oxygen species, oxidized ascorbic acid and flavonoids, and many other oxidizing agents are capable of increasing the gelling potential of muscle proteins. Interestingly, under oxidative stress conditions, myosin tail–tail (rod) cross-linking via disulfide bond formation is favored over head–head (S1) association, and this phenomenon is particularly notable for white muscle fibers and plays a role in improving myosin gelation in mildly oxidizing systems [129,130]. Box 15.4 illustrates the effects of protein oxidation of chicken breast muscle.

15.7.4 Fat Immobilization and Stabilization

Fat in processed meat, notably in emulsified products, is immobilized and stabilized by the formation of protein interfacial membrane and protein matrices. During comminution or emulsification, large fat particles or the adipose tissue are broken down to fine granules through shear. As the small fat globules are formed, they are coated by proteins, which are amphoteric in nature, that is, possessing both hydrophobic and hydrophilic groups. Specifically, the nonpolar groups are imbedded in fat (hydrophobic), while the polar groups extend into the aqueous phase, forming an interfacial film that separates the two immiscible phases (lipid and water). The adsorption of protein on the surface of fat globules results in a total reduction in free energy, which is complemented by the protein gel matrix, thereby further enhancing the emulsion stability. The relative emulsifying activity of muscle proteins follows the order of myosin > actomyosin > sarcoplasmic proteins > actin [131]. The excellent emulsifying capability of myosin is attributed to its unique structure (high length-to-diameter ratio) as well as to its bipolar nature (hydrophobic head and hydrophilic tail). A presumptive monolayer interfacial film, formed predominantly by myosin, is presented in Figure 15.20.

Fat globules in a well-comminuted meat, commonly referred to as "batter," are uniformly distributed in a continuous, yet complex, aqueous phase, which is comprised of salt-soluble proteins, segments of fibers, myofibrils, connective tissue fibers, collagen fragments, and various ingredients suspended in water. The two emulsion stabilization mechanisms—formation of a protein coating on the surface of fat particles to reduce the interfacial tension and immobilization of fat particles in protein matrices largely through physical entrapment—are applicable to meat batters. The coating, that is, protein membrane that surrounds fat globules, is not homogeneous but multilayered in nature. Three distinct layers of proteinaceous structure have been observed in thick interfacial protein film of meat batters [132]. They are described as a thin, internal layer coating the surface of the fat globule, probably resulting from depositing proteins onto the myosin or actomyosin monomolecular layer (Figure 15.20). This innermost layer is bound through a diffuse region to another layer of similar density. This second layer is bound to a very thick, diffuse protein coat, forming a stable protein membrane structure.

Similarly, stabilization of fat by protein gel matrix is a complex physicochemical process because the gelling solution, referred to as protein "sol," is not simply proteins suspended in the aqueous solution. Rather, the sol represents a heterogeneous matrix consisting of soluble proteins with some insoluble, hydrated myofibril or fiber fragments suspended in it. Often, nonmeat ingredients (soy proteins, starch, seasonings, etc.) are also present. Hence, the gel formed after cooking may be

BOX 15.4 OXIDATION ALTERS THE CROSS-LINKING PATTERN OF MYOSIN SUBFRAGMENTS

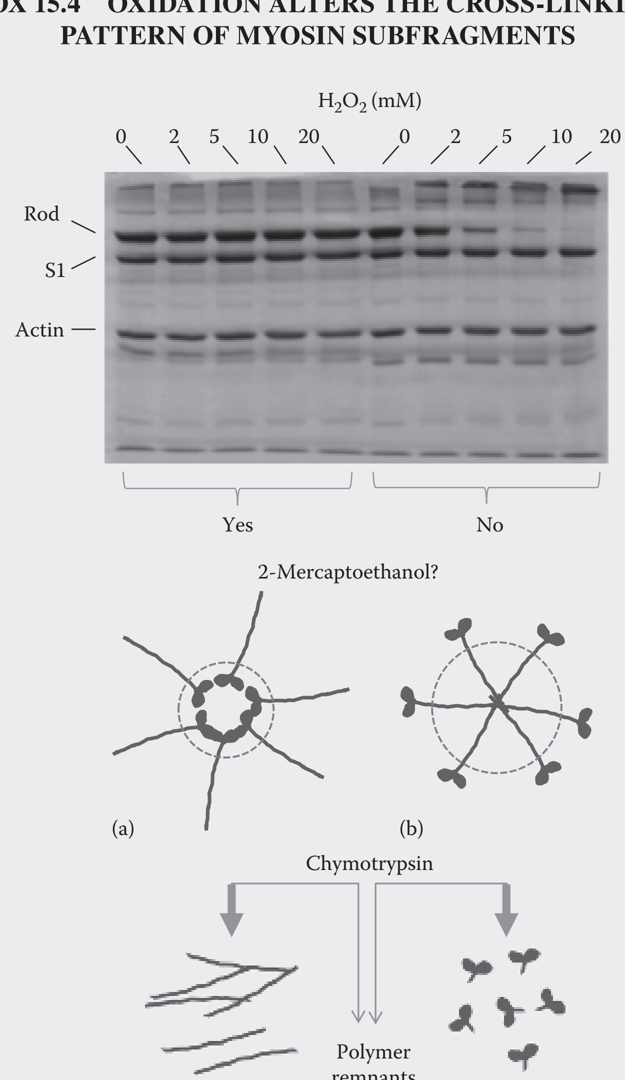


FIGURE B15.4.1 Myofibrillar protein extracted from chicken pectoralis major muscle is oxidatively stressed with hydroxyl radical generated with Fe^{2+}–H_2O_2 to induce partial unfolding of both the helical tail (rod) and globular head (S1). Differing from S1–S1 (a) association in nonoxidized myosin, polymers formed with increasing H_2O_2 concentrations are predominantly through rod–rod (b) cross-linking via disulfide bond. Chymotrypsin treatment at 0.1 M NaCl breaks down the polymers by the cleavage at the kink region of myosin neck to release rod (c), S1 (d), and remnants that remain polymerized but reducible by 2-mercaptoethanol. In agreement, at 0.6 M NaCl, light meromyosin is lost, while heavy meromyosin remains abundant after oxidation. The two experiments produce consistent evidence that hydroxyl radical promotes tail–tail interaction of myosin via disulfide linkages.

considered as a composite system where aggregates formed from extracted myofibrillar proteins, fragments of myofibrils, and protein-coated fat globules interact, leading to an interwoven network. Some of the insoluble fibrils may entangle with the gel scaffold, whereas the fat globules may act as fillers in the void spaces of the gel matrix, thereby reinforcing the gel. The protein matrix gel is

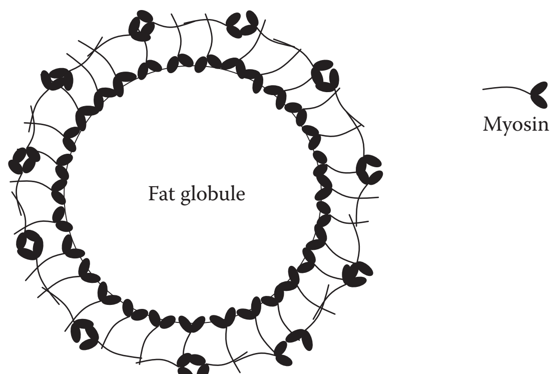


FIGURE 15.20 Schematic representation of a meat emulsion fat globule depicting a myosin monolayer.

stabilized by a combination of forces, including hydrophobic and electrostatic interactions, hydrogen bonds, and van der Waal's interactions. Covalent bonds, such as disulfide linkages, appear to be minor except when oxidation is involved.

The physicochemical and rheological properties of the fat globule membrane and the continuous protein matrices are the determinants of emulsion stability, and they are influenced by many factors, including pH, viscosity of the aqueous phase, time and temperature of chopping or emulsifying, and the lean meat-to-fat ratio. The composition and properties of both the fat globule membrane and the continuous aqueous phase can be modified by means of ingredients and additives to obtain a high emulsion stability against coalescence at high cooking temperatures. Hydrolyzed soy, gluten, and whey proteins may help stabilize the emulsion system by reinforcing the interfacial membrane and the protein matrix. Treatment of emulsifying proteins with microbial transglutaminase prior to emulsification also enhances the emulsion stability and, additionally, enables the amorphous emulsion to form a stable gel matrix system upon heating [133]. It appears that the enzyme effects by means of promoting the interaction and cross-linking of membranes of fat particles as well as the interaction of the fat globules with segments of the protein network in the gel matrix.

15.7.5 Meat Restructuring

Restructured meats are fabricated and then reshaped products, and they include many formed raw steaks, chops, and roasts that resemble the intact products in texture. They also include a variety of cooked, ready-to-eat meats. Most cooked, ready-to-consume luncheon meats more or less fall into the restructured meat category. Restructured meats can be classified into three main groups, based on the specific method used and meat particle size reduction, that is, the extent of comminution: sectioned (entire muscle or muscle groups) and formed, chunked (coarse meat particles) and formed, and flaked (flakes from frozen meat) and formed.

Regardless of the method of reducing meat particle size, the single most critical factor affecting the product manufacture is the application and the action of salt. Salt (NaCl) is required to extract myofibrillar proteins, particularly myosin and actomyosin. The protein extract is a highly viscous, tacky exudate that provides adhesion between meat particles. For the bind to be effective, however, the protein extract must be converted to a viscoelastic, semisolid matrix, that is, a gel or semi-gel. This is made possible by heat when the formed meat is cooked. Extraction of salt-soluble myofibrillar proteins is accomplished by mixing meat with at least 0.5 M salt in a mechanical mixer, such as a massager and a tumbler. Tumbling relies on gravitational impact and abrasion from dropping meat pieces (previously tossed) against other meat pieces to extract myofibrillar proteins, while massaging relies mainly on abrasion between meat particles and between meat and the rotating paddles of the massager to extract proteins. Polyphosphate is also used in conjunction with NaCl

to improve protein extraction and moisture retention. Other ingredients, for example, seasonings, nonfat dry milk, whey protein concentrate, soy flour, and nitrite, may also be blended into restructured meat to accentuate the flavor and to enhance binding strength, texture, and appearance.

Restructured fresh beef steaks and pork chops by the use of microbial transglutaminase have been successfully manufactured [134]. The enzyme forms covalent cross-links between glutamine and lysine side chains thereby bonding meat particles together. Meat products restructured without heating offer great flexibility and portion control and can be marketed in specific shapes and sizes.

15.7.6 Chemistry of Surimi

Surimi is a crude myofibrillar protein concentrate prepared by washing minced, mechanically deboned fish muscle to remove sarcoplasmic constituents and fat, followed by mixing with cryoprotectants (usually polyols) to prevent protein denaturation during frozen storage. Surimi is an "intermediate" product because it is further processed to make various kamaboko (fish cakes) and seafood analogs, such as imitation crab meat and lobster, or utilized as a functional ingredient in other products.

A main quality concern with many of the fish species used for surimi is that they contain significant proteolytic activity that is detrimental to the texture of surimi-based products. For example, cathepsins B, L, and an L-like protease are difficult to be completely removed by the washing process. These endogenous proteases exhibit high activities in the 45°C–55°C temperature range and, thus, can impair the textural properties of surimi-based cooked seafood analogs by degrading actomyosin [135]. Dry beef plasma protein, egg white, and potato extract are capable of preventing surimi gel softening and, hence, are blended into surimi before cooking is initiated. Small peptides (enzyme inhibitors) present in these additives may compete with fish muscle proteins as substrates for the proteases. Because the majority of the catheptic enzyme superfamily are cysteine proteases, cystatin (a general cathepsin inhibitor) is also used to prevent surimi gel weakening during cooking. This inhibitor can be efficiently produced using recombinant technology [136].

An alternative approach to the traditional surimi preparation method is acid or alkaline solubilization. Unlike the traditional surimi process, which employs repeated washing of the minced fish muscle tissue to concentrate myofibrillar protein by removing fat and sarcoplasmic constituents, the solubilization method involves either acid (pH 2.5–3.5) or alkaline (pH 9–10) treatment to solubilize both myofibrillar and sarcoplasmic proteins from homogenized muscle tissue. The soluble proteins are subsequently recovered by isoelectric precipitation (pH 5.0–5.5) [137]. This technique has the main advantage of high product yields (protein recovery >90% compared to 55%–65% from the traditional washing method). The acid solubilization–isoelectric precipitation method is particularly suitable for dark-muscle and fatty fish.

15.7.7 Summary

- Nitrite curing is a very important aspect of meat processing in which nitric oxide produced from the reduction of nitrite binds to the heme iron in myoglobin to produce the characteristic pinkish-red color of meat known as nitrosylmyoglobin (raw) or nitrosylhemochromogen (cooked).
- Hydration and water retention in processed meat are achieved by the chemical effect of salt and phosphate that promote electrostatic repulsion between myofilaments and the dissociation of the actomyosin complex to create expanded inter-myofilamental spaces for water entrapment.
- Gelation of myofibrillar proteins generally occurs during cooking when structurally unfolded, soluble proteins start to associate predominantly via hydrophobic forces to form aggregate. These aggregates subsequently interact to produce a three-dimensional matrix capable of immobilizing water and fat and exerting an adhesive force to bind meat

particles. Gel formation plays an essential role in the processing of restructured meat and cooked surimi products.

- Meat emulsions are comminuted products formed by the encapsulation of dispersed fat particles with an interfacial protein membrane, and the emulsion particles with a reduced surface tension are further stabilized by physical entrapments in the protein gel matrix that has high viscosity and elasticity.
- In meat processing with salt and phosphate, actomyosin is split into its constituting myosin and actin by the action of pyrophosphate or tripolyphosphate that binds to the myosin head. The general functionality of mixed muscle proteins, including water holding, gelation, emulsification, and adhesion, is largely attributed to a single protein: myosin.

QUESTIONS

15.1 Compare and contrast the variation in fatty acid composition among beef, pork, chicken, and fish.
15.2 Although meats are generally considered nutrient dense, they are generally poor sources of which vitamins and minerals?
15.3 Describe the roles of the endomysium, perimysium, and endomysium in skeletal muscle.
15.4 What proteins comprise the thin filament and thick filament, respectively?
15.5 What feature of primary structure gives rise to the unique tertiary structure of fibrous collagens?
15.6 What types of cross-links are formed in collagen and how do they contribute to both collagen function and meat tenderness?
15.7 Explain the importance of creatine phosphate in both living muscle and in the conversion of muscle to meat.
15.8 How is one troponin molecule able to regulate the activity of seven actin monomers?
15.9 What is a rigor bond and what is its importance to meat tenderness?
15.10 If one muscle has average sarcomere lengths of 1.8 μm and another has an average sarcomere length of 1.4 μm, which muscle would likely be more tough and why?
15.11 Describe the step-by-step process of muscle contraction. How is this cycle related to the conversion of muscle to meat?
15.12 Describe the key biochemical events taking place during the three phases of conversion of muscle to meat.
15.13 What is the role of glycogen in development of PSE and DFD meats?
15.14 Describe the mode of action of freezing, high-pressure treatment, and irradiation for the quality preservation of fresh meat and meat products.
15.15 Outline the chemical reactions for meat curing with nitrite, and indicate the adjunct chemicals and additives that are commonly applied to facilitate the curing process.
15.16 Describe the specific chemical roles of NaCl and pyrophosphate in the gelation of myofibrillar proteins during cooking and the texture formation in cooked meat as a result.
15.17 List and describe both intrinsic and extrinsic factors that affect the stability of a meat emulsion.

REFERENCES

1. Rollo, F., Ubaldi, M., Ermini, L., and Marota, I. (2002) Otzi's last meals: DNA analysis of the intestinal content of the Neolithic glacier mummy from the Alps. *Proc Natl Acad Sci USA* **99**, 12594–12599.
2. Lawrie, R. A. and Ledward, D. A. (2006) *Lawrie's Meat Science*, 7th edn., Woodhead Publishing, Cambridge, U.K.
3. U.S. Department of Agriculture, Agricultural Research Service (2013). National Nutrient Database for Standard Reference, Release 26, https://ndb.nal.usda.gov/ndb/, Accessed October 4, 2016.
4. Allen, C. E. and Foegeding, E. A. (1981) Some lipid characteristics and interactions in muscle foods—A review. *Food Technol* **35**, 253–257.

5. Gray, J. I. and Pearson, A. M. (1998) Lipid-derived off-flavors in meat formation and inhibition. In *Flavor of Meat and Meat Products* (Shahidi, F. ed.), Chapman and Hall, London, U.K., pp. 116–139.
6. Daley, C. A., Abbott, A., Doyle, P. S., Nader, G. A., and Larson, S. (2010) A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutr J* **9**, 10.
7. Bodwell, C. and Anderson, B. (1986) Nutritional composition and value of meat and meat products. In *Muscle as Food* (Bechtel, P. ed.), Academic Press, Inc., Orlando, FL.
8. Ryan, J. T., Ross, R. P., Bolton, D., Fitzgerald, G. F., and Stanton, C. (2011) Bioactive peptides from muscle sources: Meat and fish. *Nutrients* **3**, 765–791.
9. Faustman, C., Chan, W. K., Schaefer, D. M., and Havens, A. (1998) Beef color update: The role for vitamin E. *J Anim Sci* **76**, 1019–1026.
10. Knight, S. and Winterfeldt, E. A. (1977) Nutrient quality and acceptability of mechanically deboned meat. *J Am Diet Assoc* **71**, 501–504.
11. Bendall, J. (1973) Post-mortem changes in muscle. In *The Structure and Function of Muscle* (Bourne, G. ed.), 2nd edn., Academic Press, Inc., London, U.K., pp. 243–309.
12. Aberle, E. D., Forrest, J. C., Gerrard, D. E., and Mills, E. W. (2001) *Principles of Meat Science*, 4th edn., Kendall/Hunt, Dubuque, IA.
13. Rossi, A. E. and Dirksen, R. T. (2006) Sarcoplasmic reticulum: The dynamic calcium governor of muscle. *Muscle Nerve* **33**, 715–731.
14. Dulhunty, A. F., Haarmann, C. S., Green, D., Laver, D. R., Board, P. G., and Casarotto, M. G. (2002) Interactions between dihydropyridine receptors and ryanodine receptors in striated muscle. *Prog Biophys Mol Biol* **79**, 45–75.
15. Huxley, H. and Hanson, J. (1954) Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature* **173**, 973–976.
16. Bers, D. M. (2001) *Excitation-Contraction Coupling and Cardiac Contractile Force*, 2nd edn., Kluwer Academic Publishers, Dordrecht, the Netherlands.
17. Hill, J. A. and Olxon, E. N. (2012) *Muscle Fundamental Biology and Mechanisms of Disease*, Elsevier Science, Amsterdam, the Netherlands.
18. Barr, L. and Christ, G. J. (2000) *A Functional View of Smooth Muscle*, JAI Press, Stamford, CT.
19. Scopes, R. (1970) Characterization and study of sarcoplasmic proteins. In *The Physiology and Biochemistry of Muscle as a Food* (Briskey, E. J., Cassens, R. G., and Marsh, B. B. eds.), The University of Wisconsin Press, Madison, WI, pp. 471–492.
20. Yates, L. D. and Greaser, M. L. (1983) Quantitative determination of myosin and actin in rabbit skeletal muscle. *J Mol Biol* **168**, 123–141.
21. Stefansson, G. and Hultin, H. (1994) On the solubility of cod muscle proteins in water. *J Agric Food Chem* **42**, 2656–2664.
22. McCormick, R. J. (1999) Extracellular modifications to muscle collagen: Implications for meat quality. *Poult Sci* **78**, 785–791.
23. McCormick, R. J. (2009) Collagen. In *Applied Muscle Biology and Meat Science* (Du, M. and McCormick, R. J. eds.), CRC Press, Boca Raton, FL, pp. 129–148.
24. Resurreccion, A. V. A. (1994) Cookery of muscle foods. In *Muscle Foods* (Kinsman, D. M., Kotula, A. W., and Breidenstein, B. C. eds.), Chapman and Hall, New York, pp. 406–429.
25. Kjaer, M. (2004) Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiol Rev* **84**, 649–698.
26. Velleman, S. G. (2012) Meat science and muscle biology symposium: Extracellular matrix regulation of skeletal muscle formation. *J Anim Sci* **90**, 936–941.
27. Lewis, M. P., Machell, J. R., Hunt, N. P., Sinanan, A. C., and Tippett, H. L. (2001) The extracellular matrix of muscle—Implications for manipulation of the craniofacial musculature. *Eur J Oral Sci* **109**, 209–221.
28. Osses, N. and Brandan, E. (2002) ECM is required for skeletal muscle differentiation independently of muscle regulatory factor expression. *Am J Physiol Cell Physiol* **282**, C383–C394.
29. Velleman, S. G., Liu, X., Eggen, K. H., and Nestor, K. E. (1999) Developmental regulation of proteoglycan synthesis and decorin expression during turkey embryonic skeletal muscle formation. *Poult Sci* **78**, 1619–1626.
30. Velleman, S. G. (2001) Role of the extracellular matrix in muscle growth and development. *J Anim Sci* **80**, E8–E13.
31. Lawson, M. A. (2004) The role of integrin degradation in post-mortem drip loss in pork. *Meat Sci* **68**, 559–566.
32. Fratzl, P. (2008) *Collagen: Structure and Mechanics*, Springer Science+Business Media, LLC, New York.

33. Purslow, P. P. (2014) New developments on the role of intramuscular connective tissue in meat toughness. *Annu Rev Food Sci Technol* **5**, 133–153.
34. Ushiki, T. (2002) Collagen fibers, reticular fibers and elastic fibers. A comprehensive understanding from a morphological viewpoint. *Arch Histol Cytol* **65**, 109–126.
35. Purslow, P. P. (2005) Intramuscular connective tissue and its role in meat quality. *Meat Sci* **70**, 435–447.
36. Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K., and Eppenberger, H. M. (1992) Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: The 'phosphocreatine circuit' for cellular energy homeostasis. *Biochem J* **281**(Pt 1), 21–40.
37. Foth, B. J., Goedecke, M. C., and Soldati, D. (2006) New insights into myosin evolution and classification. *Proc Natl Acad Sci USA* **103**, 3681–3686.
38. Clark, K. A., McElhinny, A. S., Beckerle, M. C., and Gregorio, C. C. (2002) Striated muscle cytoarchitecture: An intricate web of form and function. *Annu Rev Cell Dev Biol* **18**, 637–706.
39. Lowey, S., Slayter, H. S., Weeds, A. G., and Baker, H. (1969) Substructure of the myosin molecule. I. Subfragments of myosin by enzymic degradation. *J Mol Biol* **42**, 1–29.
40. Highsmith, S., Kretzschmar, K. M., O'Konski, C. T., and Morales, M. F. (1977) Flexibility of myosin rod, light meromyosin, and myosin subfragment-2 in solution. *Proc Natl Acad Sci USA* **74**, 4986–4990.
41. Rayment, I., Rypniewski, W. R., Schmidt-Bäse, K., Smith, R., Tomchick, D. R., Benning, M. M., Winkelmann, D. A., Wesenberg, G., and Holden, H. M. (1993) Three-dimensional structure of myosin subfragment-1: A molecular motor. *Science* **261**, 50–58.
42. Kabsch, W., Mannherz, H. G., Suck, D., Pai, E. F., and Holmes, K. C. (1990) Atomic structure of the actin:DNase I complex. *Nature* **347**, 37–44.
43. Carlier, M. F. (1991) Actin: Protein structure and filament dynamics. *J Biol Chem* **266**, 1–4.
44. Brown, J. H. and Cohen, C. (2005) Regulation of muscle contraction by tropomyosin and troponin: How structure illuminates function. *Adv Protein Chem* **71**, 121–159.
45. Lees-Miller, J. P. and Helfman, D. M. (1991) The molecular basis for tropomyosin isoform diversity. *Bioessays* **13**, 429–437.
46. Grabarek, Z., Tao, T., and Gergely, J. (1992) Molecular mechanism of troponin-C function. *J Muscle Res Cell Motil* **13**, 383–393.
47. Potter, J. D. and Gergely, J. (1975) The calcium and magnesium binding sites on troponin and their role in the regulation of myofibrillar adenosine triphosphatase. *J Biol Chem* **250**, 4628–4633.
48. Wang, K., McClure, J., and Tu, A. (1979) Titin: Major myofibrillar components of striated muscle. *Proc Natl Acad Sci USA* **76**, 3698–3702.
49. Labeit, S. and Kolmerer, B. (1995) Titins: Giant proteins in charge of muscle ultrastructure and elasticity. *Science* **270**, 293–296.
50. Granzier, H. L. and Labeit, S. (2004) The giant protein titin: A major player in myocardial mechanics, signaling, and disease. *Circ Res* **94**, 284–295.
51. Tskhovrebova, L. and Trinick, J. (2003) Titin: Properties and family relationships. *Nat Rev Mol Cell Biol* **4**, 679–689.
52. Trinick, J. (1994) Titin and nebulin: Protein rulers in muscle? *Trends Biochem Sci* **19**, 405–409.
53. McElhinny, A. S., Kazmierski, S. T., Labeit, S., and Gregorio, C. C. (2003) Nebulin: The nebulous, multifunctional giant of striated muscle. *Trends Cardiovasc Med* **13**, 195–201.
54. Blanchard, A., Ohanian, V., and Critchley, D. (1989) The structure and function of alpha-actinin. *J Muscle Res Cell Motil* **10**, 280–289.
55. Cooper, J. A. and Schafer, D. A. (2000) Control of actin assembly and disassembly at filament ends. *Curr Opin Cell Biol* **12**, 97–103.
56. Fischer, R. S. and Fowler, V. M. (2003) Tropomodulins: Life at the slow end. *Trends Cell Biol* **13**, 593–601.
57. Lazarides, E. (1980) Desmin and intermediate filaments in muscle cells. *Results Probl Cell Differ* **11**, 124–131.
58. van der Ven, P. F., Obermann, W. M., Lemke, B., Gautel, M., Weber, K., and Fürst, D. O. (2000) Characterization of muscle filamin isoforms suggests a possible role of gamma-filamin/ABP-L in sarcomeric Z-disc formation. *Cell Motil Cytoskeleton* **45**, 149–162.
59. Bennett, P., Craig, R., Starr, R., and Offer, G. (1986) The ultrastructural location of C-protein, X-protein and H-protein in rabbit muscle. *J Muscle Res Cell Motil* **7**, 550–567.
60. Koretz, J. F., Irving, T. C., and Wang, K. (1993) Filamentous aggregates of native titin and binding of C-protein and AMP-deaminase. *Arch Biochem Biophys* **304**, 305–309.
61. Schoenauer, R., Lange, S., Hirschy, A., Ehler, E., Perriard, J. C., and Agarkova, I. (2008) Myomesin 3, a novel structural component of the M-band in striated muscle. *J Mol Biol* **376**, 338–351.

62. Hoffman, E. P., Knudson, C. M., Campbell, K. P., and Kunkel, L. M. (1987) Subcellular fractionation of dystrophin to the triads of skeletal muscle. *Nature* **330**, 754–758.
63. Michele, D. E. and Campbell, K. P. (2003) Dystrophin-glycoprotein complex: Post-translational processing and dystroglycan function. *J Biol Chem* **278**, 15457–15460.
64. Ervasti, J. M. (2003) Costameres: The Achilles' heel of Herculean muscle. *J Biol Chem* **278**, 13591–13594.
65. Leong, P. and MacLennan, D. H. (1998) Complex interactions between skeletal muscle ryanodine receptor and dihydropyridine receptor proteins. *Biochem Cell Biol* **76**, 681–694.
66. Catterall, W. A., Perez-Reyes, E., Snutch, T. P., and Striessnig, J. (2005) International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. *Pharmacol Rev* **57**, 411–425.
67. Catterall, W. A. (1988) Structure and function of voltage-sensitive ion channels. *Science* **242**, 50–61.
68. Sutko, J. L. and Airey, J. A. (1996) Ryanodine receptor Ca^{2+} release channels: Does diversity in form equal diversity in function? *Physiol Rev* **76**, 1027–1071.
69. Block, B. A., Imagawa, T., Campbell, K. P., and Franzini-Armstrong, C. (1988) Structural evidence for direct interaction between the molecular components of the transverse tubule/sarcoplasmic reticulum junction in skeletal muscle. *J Cell Biol* **107**, 2587–2600.
70. Felder, E. and Franzini-Armstrong, C. (2002) Type 3 ryanodine receptors of skeletal muscle are segregated in a parajunctional position. *Proc Natl Acad Sci USA* **99**, 1695–1700.
71. MacLennan, D. H., Rice, W. J., and Green, N. M. (1997) The mechanism of Ca^{2+} transport by sarco(endo) plasmic reticulum Ca^{2+}-ATPases. *J Biol Chem* **272**, 28815–28818.
72. Rayment, I. and Holden, H. M. (1994) The three-dimensional structure of a molecular motor. *Trends Biochem Sci* **19**, 129–134.
73. Onishi, H., Mochizuki, N., and Morales, M. F. (2004) On the myosin catalysis of ATP hydrolysis. *Biochemistry* **43**, 3757–3763.
74. Pette, D. and Staron, R. S. (2001) Transitions of muscle fiber phenotypic profiles. *Histochem Cell Biol* **115**, 359–372.
75. Spangenburg, E. E. and Booth, F. W. (2003) Molecular regulation of individual skeletal muscle fibre types. *Acta Physiol Scand* **178**, 413–424.
76. Scott, W., Stevens, J., and Binder–Macleod, S. A. (2001) Human skeletal muscle fiber type classifications. *Phys Ther* **81**, 1810–1816.
77. Pette, D., Peuker, H., and Staron, R. S. (1999) The impact of biochemical methods for single muscle fibre analysis. *Acta Physiol Scand* **166**, 261–277.
78. Maltin, C., Balcerzak, D., Tilley, R., and Delday, M. (2003) Determinants of meat quality: Tenderness. *Proc Nutr Soc* **62**, 337–347.
79. Huff-Lonergan, E., Mitsuhashi, T., Beekman, D. D., Parrish, F. C., Olson, D. G., and Robson, R. M. (1996) Proteolysis of specific muscle structural proteins by mu-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. *J Anim Sci* **74**, 993–1008.
80. Goll, D. E., Thompson, V. F., Li, H., Wei, W., and Cong, J. (2003) The calpain system. *Physiol Rev* **83**, 731–801.
81. Bartoli, M. and Richard, I. (2005) Calpains in muscle wasting. *Int J Biochem Cell Biol* **37**, 2115–2133.
82. Sorimachi, H., Kinbara, K., Kimura, S., Takahashi, M., Ishiura, S., Sasagawa, N., Sorimachi, N., Shimada, H., Tagawa, K., and Maruyama, K. (1995) Muscle-specific calpain, p94, responsible for limb girdle muscular dystrophy type 2A, associates with connectin through IS2, a p94-specific sequence. *J Biol Chem* **270**, 31158–31162.
83. Koohmaraie, M. (1992) Role of neutral proteinases in postmortem muscle protein degradation and meat tenderness. *Proc Recip Meat Conf* **45**, 63–71.
84. Zeece, M. G., Woods, T. L., Keen, M. A., and Reville, W. J. (1992) Role of proteinases and inhibitors in postmortem muscle protein degradation. *Proc Recip Meat Conf* **45**, 51–61.
85. Solomon, V. and Goldberg, A. L. (1996) Importance of the ATP-ubiquitin-proteasome pathway in the degradation of soluble and myofibrillar proteins in rabbit muscle extracts. *J Biol Chem* **271**, 26690–26697.
86. Kent, M. P., Spencer, M. J., and Koohmaraie, M. (2004) Postmortem proteolysis is reduced in transgenic mice overexpressing calpastatin. *J Anim Sci* **82**, 794–801.
87. Wismer-Pedersen, J. (1959) Quality of pork in relation of rate of pH change postmortem. *Food Res* **24**, 711–727.
88. McKeith, F. K. and Stetzer, A. J. (2003) Benchmarking value in the pork supply chain: Quantitative strategies and opportunities to improve quality: Phase I. American Meat Science Association, Savoy, IL.
89. Mitchell, G. and Heffron, J. J. (1982) Porcine stress syndromes. *Adv Food Res* **28**, 167–230.

90. Fujii, J., Otsu, K., Zorzato, F., de Leon, S., Khanna, V. K., Weiler, J. E., O'Brien, P. J., and MacLennan, D. H. (1991) Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* **253**, 448–451.
91. Sosnicki, A., Greaser, M., Pietrzak, M., Pospiech, E., and Sante, V. (1998) PSE-like syndrome in breast muscle of domestic turkeys: A review. *J Muscle Foods* **9**, 13–23.
92. Chiang, W., Allison, C. P., Linz, J. E., and Strasburg, G. M. (2004) Identification of two alphaRYR alleles and characterization of alphaRYR transcript variants in turkey skeletal muscle. *Gene* **330**, 177–184.
93. Malila, Y., Carr, K. M., Ernst, C. W., Velleman, S. G., Reed, K. M., and Strasburg, G. M. (2014) Deep transcriptome sequencing reveals differences in global gene expression between normal and pale, soft, and exudative turkey meat. *J Anim Sci* **92**, 1250–1260.
94. Andersson, L. (2003) Identification and characterization of AMPK gamma 3 mutations in the pig. *Biochem Soc Trans* **31**, 232–235.
95. Marsh, B. B. and Thompson, J. F. (1958) Rigor mortis and thaw rigor in lamb. *J Sci Food Agric* **9**, 417–424.
96. Marsh, B. B. and Leet, N. G. (1966) Resistance to shearing of heat-denatured muscle in relation to shortening. *Nature* **211**, 635–636.
97. Marsh, B. B. (1985) Electrical-stimulation research: Present concepts and future directions. *Adv Meat Res* **1**, 277–305.
98. Pearson, A. M. and Young, R. B. (1989) *Muscle and Meat Biochemistry*, Academic Press, San Diego, CA.
99. Carse, W. A. (1973) Meat quality and the acceleration of postmortem glycolysis by electrical stimulation. *J Food Technol* **8**, 163–166.
100. Sharp, J. G. and Marsh, B. B. (1953) Whale meat, production and preservation. Food Invest. Board, G.B. Dep. Sci. Ind. Res. Spec. Rep. 58.
101. Smith, G. C. (1985) Effects of electrical stimulation on meat quality, color, grade, heat ring and palatability. *Adv Meat Res* **1**, 121–158.
102. Pearson, A. M. and Dutson, T. R. (1985) Scientific basis for electrical stimulation. *Adv Meat Res* **1**, 185–218.
103. Sorinmade, S. O., Cross, H. R., Ono, K., and Wergin, W. P. (1982) Mechanisms of ultrastructural changes in electrically stimulated beef Longissimus muscle. *Meat Sci* **6**, 71–77.
104. Zhou, G. H., Xu, X. L., and Liu, Y. (2010) Preservation technologies for fresh meat—A review. *Meat Sci* **86**, 119–128.
105. Fennema, O. (1982) Behavior of proteins at low-temperatures. *ACS Symp Series* **206**, 109–133.
106. Fernandez-Martin, F., Otero, L., Solas, M., and Sanz, P. (2000) Protein denaturation and structural damage during high-pressure-shift freezing of porcine and bovine muscle. *J Food Sci* **65**, 1002–1008.
107. Zhu, S., Le Bail, A., Ramaswamy, H., and Chapleau, N. (2004) Characterization of ice crystals in pork muscle formed by pressure-shift freezing as compared with classical freezing methods. *J Food Sci* **69**, E190–E197.
108. Cheftel, J. and Culioli, J. (1997) Effects of high pressure on meat: A review. *Meat Sci* **46**, 211–236.
109. Heremans, K., Van Camp, J., and Huyghebaert, A. (1997) High-pressure effects on proteins. In *Food Proteins and Their Applications* (Damodaran, S. and Paraf, A. eds.), Marcel Dekker, Inc., New York, pp. 473–502.
110. Jung, S., Ghoul, M., and de Lamballerie-Anton, M. (2000) Changes in lysosomal enzyme activities and shear values of high pressure treated meat during ageing. *Meat Sci* **56**, 239–246.
111. Sikes, A. L., Tobin, A. B., and Tume, R. K. (2009) Use of high pressure to reduce cook loss and improve texture of low-salt beef sausage batters. *Innov Food Sci Emerg Technol* **10**, 405–412.
112. Ouali, A. (1990) Meat tenderization: Possible causes and mechanisms: A review. *J Muscle Foods* **1**, 129–165.
113. Solomon, M., Long, J., and Eastridge, J. (1997) The Hydrodyne: A new process to improve beef tenderness. *J Anim Sci* **75**, 1534–1537.
114. Ahn, D. U., Jo, C., Du, T., Olson, D. G., and Nam, K. C. (2000) Quality characteristics of pork patties irradiated and stored in different packaging and storage conditions. *Meat Sci* **56**, 203–209.
115. Brewer, S. (2004) Irradiation effects on meat color—A review. *Meat Sci* **68**, 1–17.
116. Nam, K. and Ahn, D. (2003) Effects of ascorbic acid and antioxidants on the color of irradiated ground beef. *J Food Sci* **68**, 1686–1690.
117. Sebranek, J. G. and Bacus, J. N. (2007) Cured meat products without direct addition of nitrate or nitrite: What are the issues? *Meat Sci* **77**, 136–147.
118. Offer, G. and Trinick, J. (1983) On the mechanism of water holding in meat—The swelling and shrinking of myofibrils. *Meat Sci* **8**, 245–281.

119. Hamm, R. (1986) Functional properties of the myofibrillar system and their measurements. In *Muscle as Food* (Bechtel, P. ed.), Academic Press, New York, NY, pp. 135–199.
120. Xiong, Y., Lou, X., Wang, C., Moody, W., and Harmon, R. (2000) Protein extraction from chicken myofibrils irrigated with various polyphosphate and NaCl solutions. *J Food Sci* **65**, 96–100.
121. Parsons, N. and Knight, P. (1990) Origin of variable extraction of myosin from myofibrils treated with salt and pyrophosphate. *J Sci Food Agric* **51**, 71–90.
122. Xiong, Y. L. (2004) Muscle proteins. In *Proteins in Food Processing* (Yada, R. ed.), Woodhead Publishing, Cambridge, U.K., pp. 100–122.
123. Asghar, A., Samejima, K., and Yasui, T. (1985) Functionality of muscle proteins in gelation mechanisms of structured meat-products. *CRC Crit Rev Food Sci Nutr* **22**, 27–106.
124. Samejima, K., Hashimoto, Y., Yasui, T., and Fukazawa, T. (1969) Heat gelling properties of myosin, actin, actomyosin and myosin-subunits in a saline model system. *J Food Sci* **34**, 242–245.
125. Morita, J. and Ogata, T. (1991) Role of light-chains in heat-induced gelation of skeletal-muscle myosin. *J Food Sci* **56**, 855–856.
126. Xiong, Y. L. (1994) Myofibrillar protein from different muscle fiber types: Implications of biochemical and functional properties in meat processing. *Crit Rev Food Sci Nutr* **34**, 293–319.
127. Kuraishi, C., Sakamoto, J., Yamazaki, K., Susa, Y., Kuhara, C., and Soeda, T. (1997) Production of restructured meat using microbial transglutaminase without salt or cooking. *J Food Sci* **62**, 488–490.
128. Ramirez-Suarez, J. and Xiong, Y. (2003) Effect of transglutaminase-induced cross-linking on gelation of myofibrillar/soy protein mixtures. *Meat Sci* **65**, 899–907.
129. Ooizumi, T. and Xiong, Y. L. (2006) Identification of cross-linking site(s) of myosin heavy chains in oxidatively stressed chicken myofibrils. *J Food Sci* **71**, C196–C199.
130. Xiong, Y. L., Blanchard, S. P., Ooizumi, T., and Ma, Y. (2010) Hydroxyl radical and ferryl-generating systems promote gel network formation of myofibrillar protein. *J Food Sci* **75**, C215–C221.
131. Galluzzo, S. and Regenstein, J. (1978) Role of chicken breast muscle proteins in meat emulsion formation—Myosin, actin and synthetic actomyosin. *J Food Sci* **43**, 1761–1765.
132. Gordon, A. and Barbut, S. (1992) Mechanisms of meat batter stabilization—A review. *Crit Rev Food Sci Nutr* **32**, 299–332.
133. Ramirez-Suarez, J. and Xiong, Y. (2003) Rheological properties of mixed muscle/nonmuscle protein emulsions treated with transglutaminase at two ionic strengths. *Int J Food Sci Technol* **38**, 777–785.
134. Motoki, M. and Seguro, K. (1998) Transglutaminase and its use for food processing. *Trends Food Sci Technol* **9**, 204–210.
135. An, H. J., Seymour, T. A., Wu, J. W., and Morrissey, M. T. (1994) Assay systems and characterization of Pacific whiting (*Merluccius productus*) protease. *J Food Sci* **59**, 277–281.
136. Tzeng, S. and Jiang, S. (2004) Glycosylation modification improved the characteristics of recombinant chicken cystatin and its application on mackerel surimi. *J Agric Food Chem* **52**, 3612–3616.
137. Hultin, H. O. (2000) Surimi processing from dark muscle fish. In *Surimi and Surimi Seafood* (Park, J. W. ed.), Marcel Dekker, Inc., New York, pp. 59–77.
138. Tortora, G.J. and Derrickson B. (2014) *Principles of Anatomy and Physiology* 14th Ed. John Wiley and Sons, Inc., Hoboken, NJ.
139. Junqueira, L.C., Carneiro, J., and Kelley R.O. (1989) *Basic Histology.* Appleton & Lange, Norwalk, CT.
140. Chiang, W., Strasburg, G.M., and Byrem, T.M. (2007) Red meats. Ch. 23, pp. 23-1–23-21. In *Food Chemistry: Principles and Applications,* 2nd Ed. Y.H. Hui (Ed.), Science Technology System, West Sacramento, CA.
141. Craig, R. and Woodhead, J.L. (2006) Structure and function of myosin filaments. *Curr Opin Struc Biol* 16, 204–212.
142. Rayment, I., Smith, C., and Yount, R.G. (1996) The active site of myosin. *Annu Rev Physiol* **58**, 671–702.
143. Weber, K. and Osborn, M. (1969) The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J Biol Chem* **244**, 4406–4412.
144. Greaser, M.L. and Gergely, J. (1971) Reconstitution of troponin activity from three protein components. *J Biol. Chem* **246**, 4226–4233.
145. Huff-Lonergan, E., Zhang, W., Lonergan, S.M. (2010). Biochemistry of postmortem muscle—Lessons on mechanisms of meat tenderization. *Meat Sci* **86**, 184–195.
146. Henderson, D.W., Goll, D.E., and Stromer, M.H. (1970) A comparison of shortening and Z line degradation in post-mortem bovine, porcine, and rabbit muscle. *Am. J. Anat.* **128**, 117–135.
147. Locker, R.H. and Hagyard, C.J. (1963) A cold shortening effect in beef muscles. *J Sci Food Agric* **14**, 787–793.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

16 Postharvest Physiology of Edible Plant Tissues

Christopher B. Watkins

CONTENTS

16.1 Introduction 1018
16.2 Quality and Postharvest Physiology 1020
16.3 Nature and Structure of Edible Plant Tissues 1021
 16.3.1 Morphology 1021
 16.3.2 Physiological Stage of Development at Harvest 1022
 16.3.3 Ripening and Senescence 1024
 16.3.4 Preharvest Factors 1025
16.4 Primary Metabolism 1026
16.5 Hormones 1032
 16.5.1 Ethylene 1032
  16.5.1.1 Ethylene Biosynthesis 1032
  16.5.1.2 Non-Climacteric and Climacteric Fruits 1034
 16.5.2 Auxins 1037
 16.5.3 Gibberellins 1037
 16.5.4 Abscisic Acid 1037
 16.5.5 Cytokinins 1037
 16.5.6 Other Hormones 1038
  16.5.6.1 Polyamines 1038
  16.5.6.2 Nitric Oxide 1038
  16.5.6.3 Jasmonic Acid, Brassinosteroids, and Salicylic Acid 1039
16.6 Composition 1039
 16.6.1 Water 1039
 16.6.2 Carbohydrates 1040
  16.6.2.1 Structural Carbohydrates 1040
  16.6.2.2 Soluble Carbohydrates 1043
  16.6.2.3 Storage Carbohydrates 1044
 16.6.3 Organic Acids 1047
 16.6.4 Phenolics 1048
 16.6.5 Proteins and Amino Acids 1049
 16.6.6 Lipids 1051
 16.6.7 Pigments 1052
 16.6.8 Volatile Organic Compounds 1053
 16.6.9 Vitamins and Health-Promoting Substances 1055
  16.6.9.1 Ascorbic Acid (Ascorbate, Vitamin C) 1055
  16.6.9.2 Vitamin A 1056
  16.6.9.3 Phytochemicals 1056
  16.6.9.4 Sulfur Compounds 1057

16.7 Postharvest Technologies 1057
16.7.1 Storage Temperature 1057
16.7.2 Relative Humidity 1062
16.7.3 Modified and Controlled Atmosphere Storage 1062
16.7.4 Edible Coatings 1065
16.7.5 Ethylene 1069
16.7.5.1 Ethylene Avoidance 1070
16.7.5.2 Ethylene Adsorbers, Oxidation, and Catalytic Decay 1071
16.7.5.3 Inhibitors of Ethylene Action 1071
16.7.6 Heat Treatments 1073
16.7.7 Ionizing Radiation 1073
16.7.8 Other Technologies 1074
16.8 Transgenic Plant Products 1074
16.8.1 Genetically Modified Organisms 1074
16.8.2 Nutritionally Enhanced Food Crops 1075
16.8.3 Modification of Ripening and Senescence Processes 1076
16.9 Commodity Requirements 1076
16.9.1 Cereals, Nuts, and Seeds 1076
16.9.2 Whole Fruits and Vegetables 1077
16.9.3 Fresh-Cut (Minimally Processed) Fruits and Vegetables 1077
16.10 Conclusions 1079
References 1080

16.1 INTRODUCTION

Edible plant tissues include cereal grains, nuts, seeds, fruits, vegetables, and even flowers. Depending on the product, they may be consumed whole, fresh-cut, and/or processed. Except when processed, these products are metabolically active. O_2 is used and CO_2 is produced during respiration, the process by which carbohydrate and other substrates, such as organic acids, proteins, and fats, are metabolized to provide the energy necessary for cells to maintain structure and function, while also producing heat ("vital heat") and water. The substrates cannot be replenished once the product has been removed from the plant. Therefore, faster respiration rates will result in loss of food nutritional value, loss of saleable weight, poorer flavor and texture, and, thus, reduced product quality.

Tuber or root crops, grains, legumes, and other seeds are considered staple crops and are major contributors to the food supply (Table 16.1), and of these, rice, maize, and wheat contribute about two-thirds of human food consumption. The cereal grains have lower water content (averaging 6.8-fold less), corresponding to higher energy, protein, and carbohydrate contents (3-, 6.5-, and 3.1-fold more, respectively) than tuber and root crops [1]. The staple crops derived from cereals (rice, maize, wheat, barley, sorghum), tubers (potato, sweet potato, yam), storage roots (cassava, taro), and seeds of beans and peas are major starch-storing organs, and much of our agricultural land is devoted to their growth [2]. Most of the estimated 2500 million tons of starch crops harvested annually are consumed directly as food or used as animal feed [3].

These staples make significant contributions of minerals and vitamins to human health, but the importance of fruits and vegetables in reducing risk of cardiovascular disease, stroke, diabetes, and cancers has become increasingly recognized. In addition to the major constituents of acids, sugars, and aroma volatiles that contribute to eating quality for any given product expected by the consumer, the contribution of antioxidants (vitamins A, C, and E), phenolics, and other phytochemicals is critical. Many fruits and vegetables also have aesthetic value (along with flowers and ornamental products) that contributes to human well-being.

TABLE 16.1
Nutrient Content per 100 g Portion of Major Staple Foods

	Maize/ Corn	Rice (White)	Rice (Brown)	Wheat	Potato	Cassava	Soybean (Green)	Sweet Potato	Sorghum	Yam	Plantain
Water (g)	10	12	10	13	79	60	68	77	9	70	65
Energy (kJ)	1528	1528	1549	1369	322	670	615	360	1419	494	511
Protein (g)	9.4	7.1	7.9	12.6	2.0	1.4	13.0	1.6	11.3	1.5	1.3
Fat (g)	4.7	0.7	2.9	1.5	0.1	0.3	6.8	0.05	3.3	0.2	0.4
Carbohydrate (g)	74	80	77	71	17	38	11	20	75	28	32
Fiber (g)	7.3	1.3	3.5	12.2	2.2	1.8	4.2	3	6.3	4.1	2.3

Source: USDA Nutrient Data Laboratory, ndb.nal.usda.gov/ndb/search/list, 2014.

Reliable estimates for losses of edible plant tissues after harvest are not easily obtained, but roughly one-third or about 1.3 billion tons of the food produced in the world goes to waste each year [4]. In general, grains, nuts, and seeds have high product stability associated with low moisture levels in the tissues. Postharvest management of these products is usually focused therefore on control of germination, mycotoxins, and insect infestations, for example, by appropriate drying after harvest. Storage periods for grains can be over 12 months under appropriate conditions. Nevertheless, losses of cereal grains in Africa average about 15% per year, due to factors such as grain shattering during harvesting and handling, spillage during transport, and quality loss at all steps in the postharvest chain, including storage, especially under warm and humid climatic conditions. The principal agents of quality loss are molds, insects, rodents, and birds. For fruits and vegetables, losses average about 32% [5]. These losses are similar in both developing and developed countries, but reasons for loss are different: in developing counties, lack of infrastructure leads to greater losses between production and retail (22%) compared with losses at retail, food service, and consumer sites (10%), while in developed countries greater losses occur at retail and beyond (20%) because of factors such as product deterioration, excess production, poor home storage, and “plate waste” (dissatisfaction, food preferences, “full stomachs”).

Fruits and vegetables tend to be much less stable than cereal crops because their higher water content results in continuation of active metabolism after harvest, resulting in both desirable and undesirable changes during storage. Desirable changes include development of pigments: for example, lycopene synthesis in tomato, anthocyanin synthesis in apple and strawberry, and development of carotenoids (yellow and orange colors) in apricots and peaches. Other changes include softening to edible ripeness, loss of chlorophyll (de-greening), and development of aroma and flavor characteristics. The same processes can be positive in some situations and negative in others: loss of chlorophyll is desirable in tomatoes but undesirable in cucumbers and broccoli. Conversion of starch to sugars is desirable for apples but undesirable for potatoes (excessive browning during frying), whereas conversion of sugars to starch is desirable for potatoes but undesirable for peas and sweet corn (loss of sweetness). Depending on the physiological stage of the harvested product, growth processes may continue, such as undesirable sprouting of potato and geotropic curvature of asparagus.

Fresh-cut or minimally processed fruits and vegetables have been an increasing component of the market in developed countries due to convenience, healthiness, attractive appearance, and flavor. Preparation of these products requires processes such as cutting, shredding, grating, and disinfecting prior to packaging, which results in injury to the plant tissues, evoking wound responses that typically shorten storage periods compared with the whole product.

Accordingly, utilization of postharvest technologies to slow down metabolic processes associated with senescence and ripening of perishable plant tissues, whether whole or fresh-cut, is essential to maintain quality after harvest. An understanding of the chemistry of postharvest physiology of these tissues is fundamental to the successful application of these technologies to preserve edible plant tissues.

16.2 QUALITY AND POSTHARVEST PHYSIOLOGY

Postharvest technologies help maintain quality of edible plant tissues by linking production with consumption, adding value, extending marketing periods, and enabling new markets to be accessed [6]. However, different perspectives exist on what "quality" is, how it can be measured, and how it relates to consumer acceptability [7]. Definitions have included "fitness for use" and "meeting expectations of the consumer," and "the degree of excellence of a product or its suitability for a particular use." On a global scale, quality is increasingly integrated with economic, social, and environmental issues, to include factors such as worker-protection standards, chemical use, irradiation, cultural preferences, and genetic modification [6].

Many consumers believe that term "fresh" refers to products that are freshly harvested and sent directly to the market place, rather than stored. However, storage is a fundamental aspect of ensuring food supply year-round, and can vary in length from a few days in a cooler to many months under controlled atmosphere conditions. Any product that is "intact" and/or as marketed after harvest prior to the end of its natural shelf-life is "fresh." The quality and health-promoting substances of a product after storage can be identical to that at harvest if the cultivar selection and storage conditions are optimal. However, as discussed in Section 16.3.4, factors such as cultivar selection and harvest timing can influence the degree of dissatisfaction with the quality of fruits and vegetables in the marketplace.

The primary objectives of postharvest technologies are to maintain quality by:

1. Reducing metabolic rates that result in undesirable changes in color, composition, texture, flavor, and nutritional status, and undesirable growth such as germination, sprouting, or rooting
2. Reducing water loss that results in loss of saleable weight, wilting, shriveling, softening, and loss of crispness
3. Minimizing bruising, friction damage, and other mechanical injuries
4. Reducing spoilage caused by microbial decay, especially of damaged or wounded tissues
5. Preventing development of freezing injuries, or physiological disorders such as chilling injury or senescent disorders
6. Ensuring that handling, storage, and transportation methods minimize the risk of chemical or microbial contaminations that affect food safety

These objectives can be met by understanding that maintaining quality from the time of harvest to when it is eaten by the consumer is an integrated process of handling, storage, transport, and retail display, which recognizes the biology of each type of edible plant tissue. Products must be harvested at optimum maturity or quality, handled carefully to avoid mechanical injury, cooled quickly to remove field heat, treated with postharvest chemicals if necessary and/or stored in modified atmospheres appropriate for the product, and maintained at acceptable temperatures during storage, transport, and marketing. Contaminating chemicals must be avoided or removed, and attention should be paid to avoid injurious effects of naturally occurring chemicals such as ethylene. All freshly harvested produce has naturally occurring bacteria, yeast, and molds, and these "contaminants" are associated with dust, insects, soil, rainfall, and sometimes human activity. Food poisoning outbreaks have been associated with contamination of produce by animals and by human contact. Therefore, attention to food safety protocols, such as Good Agricultural Practices (GAP), Hazard Analysis, and Critical Control Point (HACCP) programs, is becoming an increasingly important requirement for marketing of edible plant products.

16.3 NATURE AND STRUCTURE OF EDIBLE PLANT TISSUES

16.3.1 Morphology

The range of plant tissues that are harvested for use by humans is vast, but may be classified by the plant part [8]. This classification includes intact plants, detached plant parts, aboveground structures (leaves, petioles, stems, spikes, flowers, dried and fleshy fruits, and other structures such as mushrooms [fungi]), and belowground structures (roots, rhizomes and tubers, bulbs, corms, and non-storage organs such as root cuttings and crowns). These tissues are discussed in detail elsewhere [8] and are summarized in Table 16.2. The structure of harvested products can further be subdivided into general tissue types of which the products are composed—dermal, ground, vascular, support, and meristematic (Table 16.3). The characteristics of these plant parts and tissues types are diverse but include differences in metabolic rates, presence or absence of carbohydrate reserves, and susceptibility to water loss and injury. Postharvest management protocols may also vary greatly from a focus on ripening and senescence-related processes to those associated with factors such as growth, for example, sprouting, rooting and germination, lignifications, or wound

TABLE 16.2
Plant Parts Harvested for Use by Humans Based on Morphology

Type of Plant Part		Examples
Intact plants		Bean or alfalfa sprouts; bare root seedlings and rooted cuttings
Detached plant parts—above ground	Leaves	Spinach, collards, Chinese cabbage, lettuce, cabbage
	Petioles	Celery, rhubarb, pak-choi
	Stems, shoots, and spikes	Asparagus, bamboo shoots, gladiolus, flowering ginger spikes
	Flowers	Cauliflower, broccoli, lily blossoms
	Fruits—fleshy	Apple, banana, fig, orange, peach, pineapple, strawberry, tomato
	Fruits—dried	Wheat, rice, soybean, Brazil nut, poppy, soybean, walnut
	Other structures	Mushroom, truffles
Detached plant parts—below ground	Roots	Beet, carrot
	Rhizomes and tubers	Ginger, lotus, potato, sweet potato
	Bulbs	Onion
	Corms	Chinese water chestnut, taro

Source: Kays, S.J., *Postharvest Physiology of Perishable Plant Products*, Exon Press, Athens, Greece, 1997.

TABLE 16.3
General Tissue Types and Cell Types of Edible Plant Tissues

Tissue Type	Cell Type
Dermal	Epidermis (stomata, trichomes, nectaries, hydathodes), periderm
Ground	Parenchyma cells
Vascular	Xylem, phloem
Support	Collenchyma, sclerenchyma
Meristematic	Meristematic

Source: Kays, S.J., *Postharvest Physiology of Perishable Plant Products*, Exon Press, Athens, Greece, 1997.

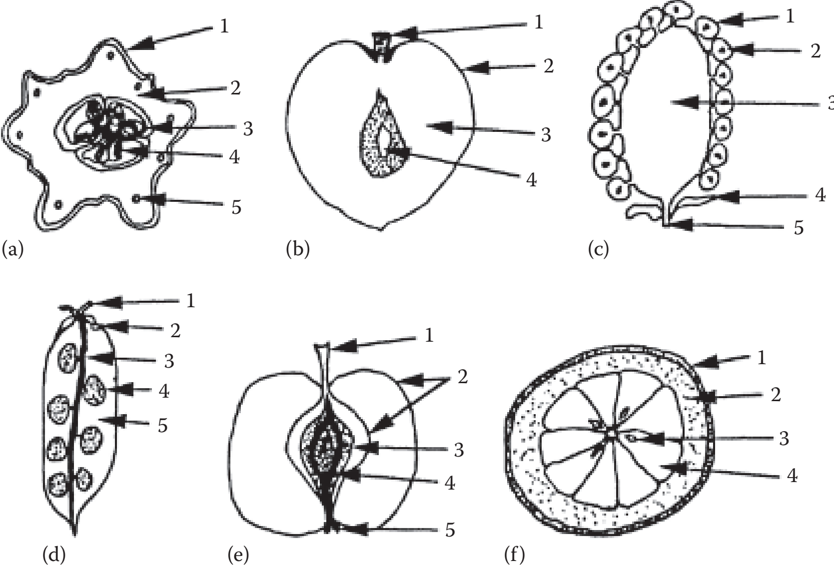


FIGURE 16.1 Diagrammatic illustrations of anatomical structures of different types of fruit. (a) Pepo (cucumber, squash, and pumpkin) in cross section: (1) rind (receptacular), (2) flesh (ovary wall), (3) placenta, (4) seed, and (5) vascular bundle. (b) Drupe (cherry, peach, and plum) in longitudinal section: (1) pedicel, (2) skin (ovary wall), (3) flesh (ovary wall), pit (stony ovary wall), and (5) seed. (c) Aggregate (raspberry, strawberry, and blackberry) in longitudinal section: (1) fleshy ovary wall, (2) seed (stony ovary wall plus seed), (3) fleshy receptacle, (4) sepal, and (5) pedicel. (d) Legume (pea, soybean, and lima bean) in longitudinal section: (1) pedicel, (2) sepal, (3) vascular bundles, (4) seed, and (5) pod (ovary wall). (e) Pome (apple and pear) in longitudinal section: (1) pedicel, (2) skin and flesh (receptacle), (3) leathery carpel (ovary wall), (4) seed, and (5) calyx (sepals and stamens). (f) Hespiridium (citrus) in cross section: (1) collenchymatous exocarp (the flavedo), (2) parenchymatous mesocarp (the albedo), (3) seed, and (4) endocarp of juice sacs formed by breakdown of groups of parenchyma-like cells.

periderm formation. Classification by plant part, and an understanding of the contribution of tissues types, provides important distinctions for the food chemist to conceptualize the physical and physiological characteristics that influence postharvest behavior and impact harvest, handling, and storage management of products for whole, fresh-cut, or processing uses.

The morphology of edible plant products can also differ greatly by type [9]. For fruits, the fleshy part that is eaten is derived from many plant parts (Figure 16.1). The fruit can be derived from the pistil or accessory parts. Whatever its origin, fruit are largely composed of parenchymatous tissue. Cereals are examples of dried fruits or ovules of dried fruits. Rice, wheat, and barley grains are indehiscent fruits that are classed as caryopses: one-seeded fruits in which the thin pericarp and the seed are adherent (Figure 16.2).

16.3.2 Physiological Stage of Development at Harvest

The physiological stage that horticultural products are harvested for commercial purposes ranges from sprouts and seedlings, which are harvested when the plant is in the very early stages of growth, to seeds and dry beans that are harvested at the senescent stage of development (Figure 16.3). The physiology of most product types is affected primarily by the degree of development, which affects factors such as the rate of respiration, epidermal development, and susceptibility to injury and pathogens, and also by postharvest factors such as storage temperatures.

Decisions associated with how the commodity is harvested, handled, and stored can greatly affect the storage life of even the most long-lived fruit or vegetable. For fully developed fruits, two competing factors often exist. On one hand, characteristics of the products such as sweetness and

flavor that are desired by the consumer increase as they mature and ripen. At the same time, the storability of the product continues to decrease (Figure 16.4). The outcome of these competing factors is that fruit destined for storage should be harvested earlier than that suitable for immediate consumption. Examples include apples, which if picked at full ripeness will have a low starch content and a high concentration of soluble solids and be highly aromatic, but will have a short storage life. In contrast, apples destined for long-term storage must be harvested less mature when the fruit has high starch content and a less developed aromatic character. Strawberries that are fully red and fully flavored will have a much shorter storage life than when harvested at the white tip stage of maturity. However, the fruit harvested at the white tip stage will have less intense flavor and aroma profiles than the fully ripened fruit. Therefore, the perceived quality of fruits in the supermarket during off-season is often lower than the locally grown ones because of the need to balance postharvest transit time to market with the rate of product deterioration.

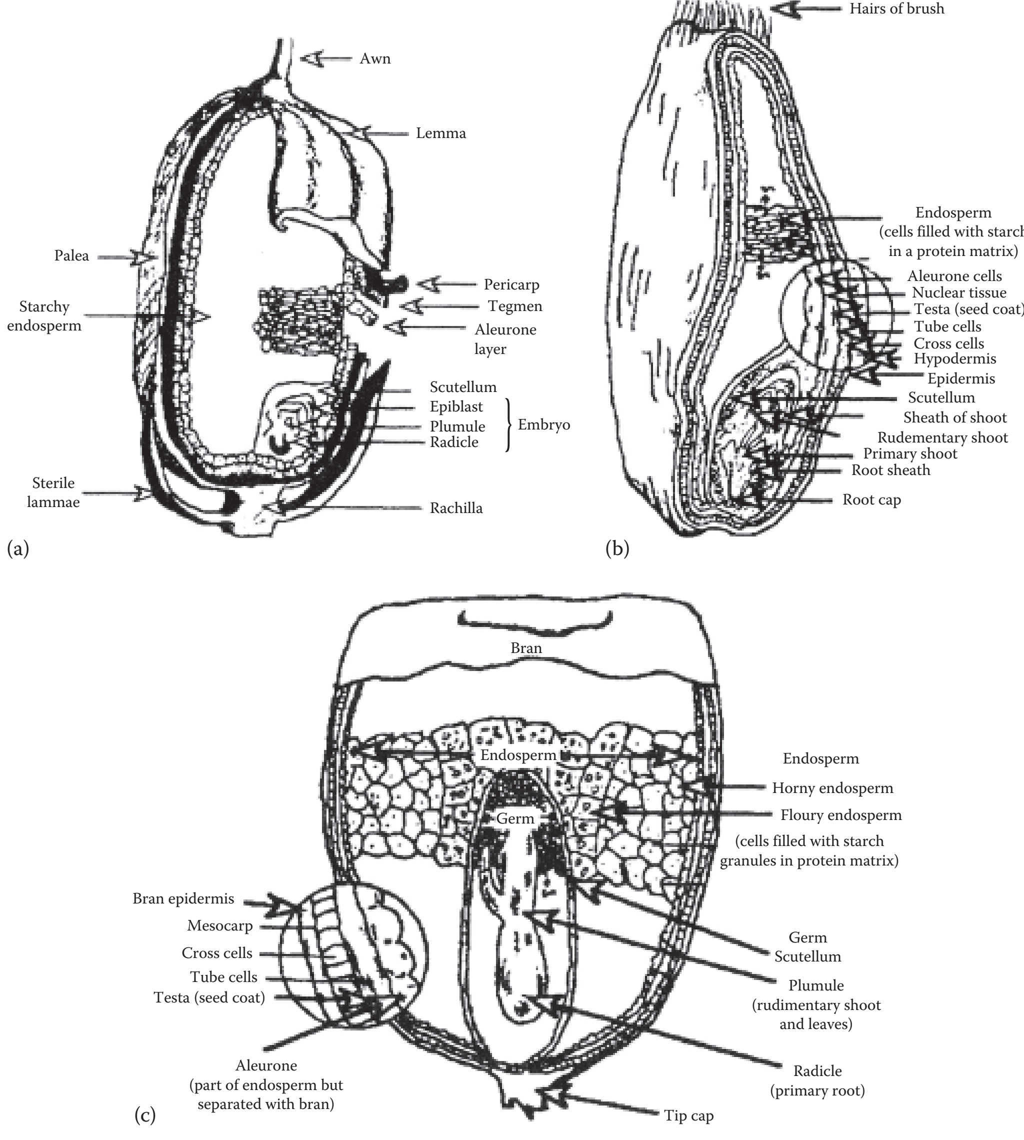


FIGURE 16.2 Diagrammatic illustrations of cereal grains called caryopsis fruit: (a) rice, (b) wheat, (c) corn. *(Continued)*

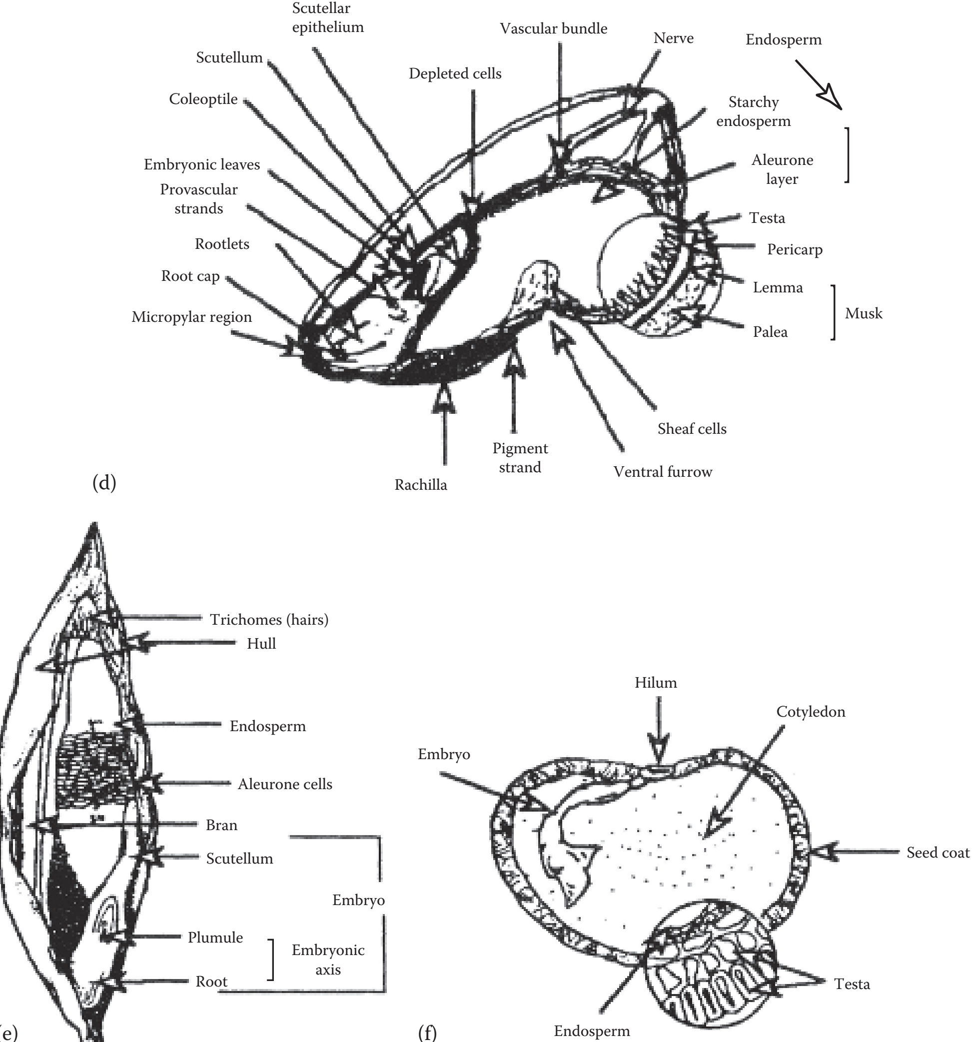


FIGURE 16.2 (*Continued*) Diagrammatic illustrations of cereal grains called caryopsis fruit: (d) barley, and (e) oat. An example of a true seed crop that does not contain a thin fruit shell is soybean (f).

16.3.3 Ripening and Senescence

Harvested edible plant tissues typically undergo senescence processes that lead to cell death. Senescence may be regarded as the final stage of plant development during which the plant exhausts the organic resources accumulated during growth and development to sustain life processes or, in other words, the final stage of ontogeny* that leads to death and decrease of functional capacity. However, ripening is an additional developmental stage in fruits. Ripening involves distinct metabolically active processes, both anabolic and catabolic, that results in cell-wall softening and texture changes, color changes, and aroma production, which make the fruit more desirable for animals as food, or more susceptible to decay by the action of microorganisms, to ensure seed dispersal. By definition, a fruit is "mature" only if it can complete its normal ripening after it has been harvested. Fruits exhibit a wide range of developmental changes during the course of their development from

* Ontogeny: course of development of an organism.

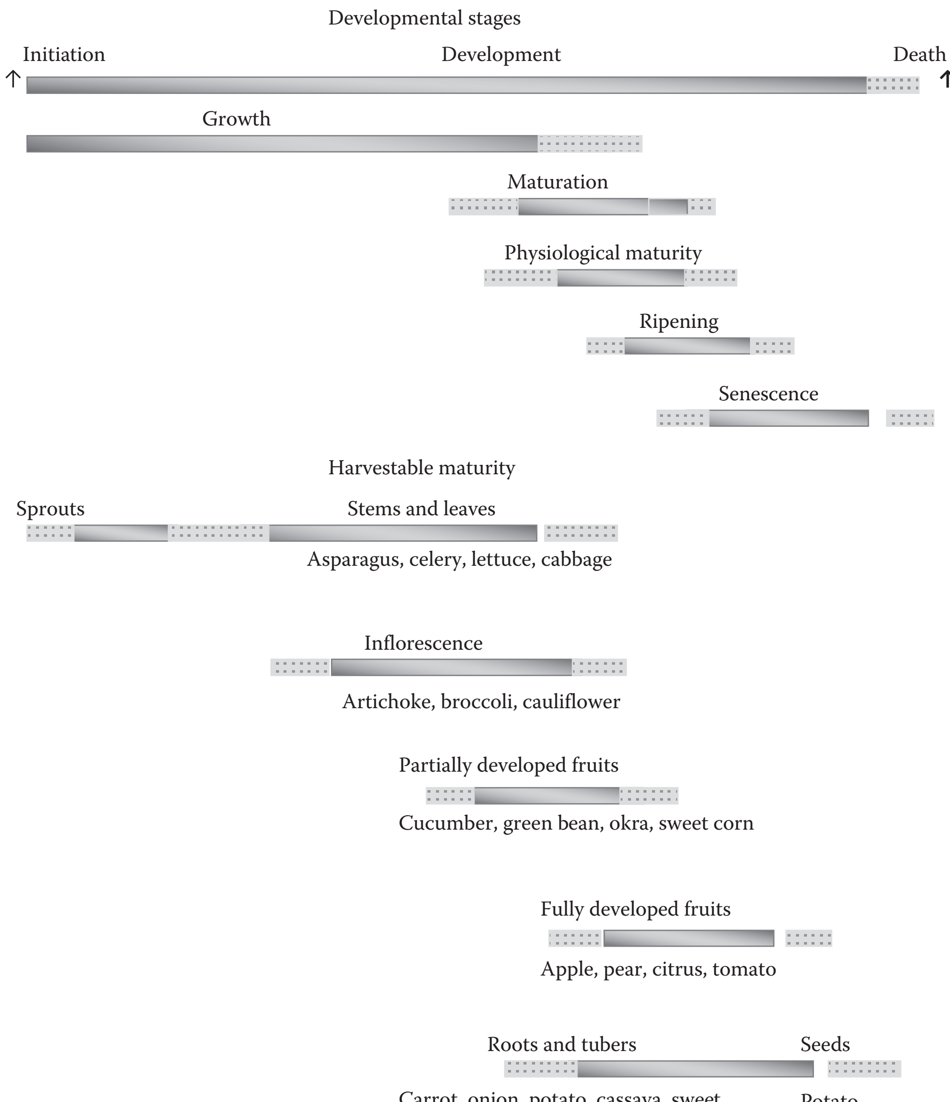


FIGURE 16.3 Horticultural maturity in relation to developmental stage of the plant. (Modified from Watada, A.E. et al., *HortScience*, 19, 20, 1984.)

the ovary to the mature fruit, even though their ripening patterns may be very different, for example, climacteric or non-climacteric (Section 16.5.1.2).

16.3.4 Preharvest Factors

Although this chapter is concerned with postharvest physiology, many of the responses of edible plant tissues are greatly affected by cultivar selection and preharvest management. Growers usually select cultivars on the basis of marketability (visual qualities specific to the market of choice) and yield, because these factors directly affect economic sustainability. However, cultivars can vary greatly in storage and shelf-life, including resistance to postharvest diseases and physiological disorders. Differences in storage potential within specific products result from different physiologies and biochemistries of each cultivar. Also, breeders have sometimes favored selections with better resistance to the handling abuses during harvest,

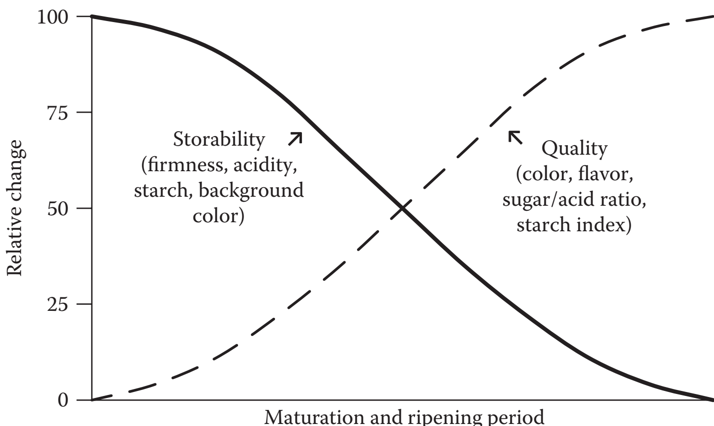


FIGURE 16.4 The relationship between the storability of a ripening horticultural product and the quality characteristics desired by the consumer. As a fruit ripens, the quality attributes such as color and flavor increase, but the potential storage period decline.

handling, and transport, which result in bruising and skin damage. The strategy has sometimes yielded the development of cultivars that have tougher skins and sometimes reduced eating quality. In some cases, genes that control processes such as low ethylene production, low respiration, and slower softening have been bred into the commodity. For example, the *rin* mutant has been incorporated into most commercial tomato cultivars, resulting in firmer fruits with slower softening rates but with less aroma and flavor.

The storability of fruits and vegetables is affected by the mineral composition at harvest [10]. High calcium levels are usually associated with good storability, and, conversely, low calcium with shorter storage life and susceptibility to storage disorders and pathogen infection. In tomato, for example, a disorder known as "blossom end rot" is associated with low calcium concentrations in the fruit. Nitrogen is often applied to increase yield, but it can negatively affect postharvest quality because respiration rates are higher, and calcium levels are lower because product size is often larger and therefore mineral levels may become limiting. Another example is onion, where high nitrogen improves yield but increases storage rots. High nitrogen causes the onions to develop thick necks that are prone to wounding when topped, and therefore greater decay development. High levels of potassium, magnesium, and boron, and low levels of phosphorus, can also lower storability. Other factors such as pest and pathogen management in the field or orchard affect the disease potential of the produce after harvest.

16.4 PRIMARY METABOLISM

Photosynthesis is the source of carbohydrates for edible plant tissues either directly as in green leafy products or translocated as in fruits and tubers, but typically harvested products are removed from environments in which photosynthesis can occur. Many products that continue to have potential to photosynthesize, such as ornamentals and leafy cuttings, are usually not edible. Edible detached plant parts with photosynthetic potential, such as leaves, shoots, petioles, and chlorophyll-containing fruit such as apples and peppers, are stored in low-light environments. Low storage temperatures further eliminate any contribution of photosynthetic sources of carbon. Therefore, the carbohydrate in the product at the time of harvest is the sole source of energy for maintenance of cellular function.

The primary metabolic process for utilization of carbohydrate is respiration, which is essential for the maintenance of adequate supplies of the high-energy compounds adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NADH), and pyrophosphate (PPi), which are necessary to maintain cellular organization of living cells. In plants, substrates such as starches, sugars, and organic acids are catabolized by glycolysis and associated pathways to simpler molecules such as CO_2 and H_2O, together with the release of heat (energy).

TABLE 16.4
Examples of Linkages between Glycolytic Pathway and TCA Cycle with Other Pathways within the Cell

Glycolytic Pathway Intermediate	Derived Metabolite(s)
Glucose-6-phosphate	Nucleotides
Fructose-6-phosphate	Amino acids, glycolipids, glycoproteins
Dihydroxyacetone phosphate	Lipids
3-Phosphoglycerate	Serine
Phosphoenolpyruvate	Amino acids, pyrimidines
Pyruvate	Alanine
TCA cycle	
Citrate	Amino acids, cholesterol, fatty acids, isoprenoids
α-Ketoglutarate	Glutamate, other amino acids, purines
Succinyl CoA	Heme, chlorophyll
Oxaloacetate	Aspartate, other amino acids, purines, pyrimidines

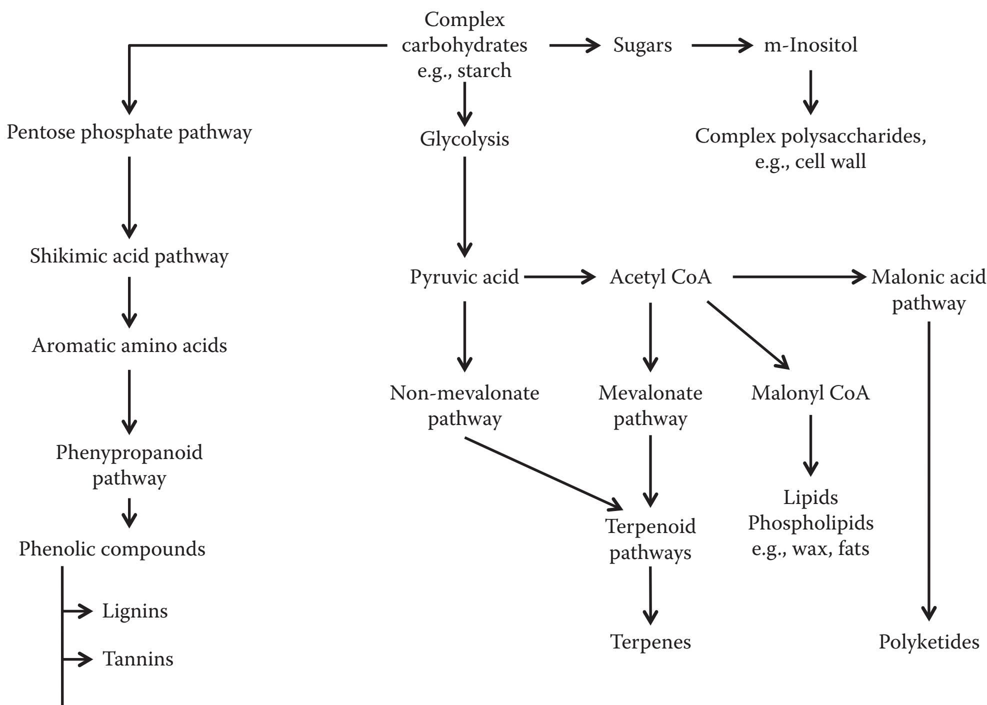


FIGURE 16.5 Overview of primary and secondary pathways in edible plant tissues.

Intermediates formed during glycolysis provide the carbon skeletons that are utilized by the cell to synthesize amino acids, nucleotides, pigments, lipids, and flavor compounds (Table 16.4). In addition, carbohydrate is utilized through an intricate linkage of pathways (Figure 16.5) to produce many important compounds that impact quality and storability of harvested plant tissues.

In glycolysis, if glucose is used as substrate, the overall equation for respiration can be written as follows:

$$C_6H_{12}O_6 + 6\ O_2 + 38\ ADP + 38\ Pi \rightarrow 6\ CO_2 + 6\ H_2O + 38\ ATP + 686\ kcal$$

Glucose can come from simple sugars including sucrose, or complex carbohydrates such as starch. When the substrate is carbohydrate and respiration is aerobic, the respiratory quotient (RQ), which is the ratio of CO_2 being produced to O_2 consumed, is near 1. If the substrate is lipid, the RQ will be less than 1 (e.g., for palmitic acid it is 0.36), and for organic acid the RQ will be greater than 1 (e.g., for malic acid it is 1.33). O_2 used in respiration diffuses from the surrounding atmosphere, while CO_2 diffuses out of the tissue. Of the 686 kcal produced per mole of glucose, about 281 kcal (41%) is used to produce 38 ATP molecules, 13 kcal (2%) accounts for the increased entropy as glucose is converted to oxidized end products, and 392 kcal (57%) is lost as "vital heat."

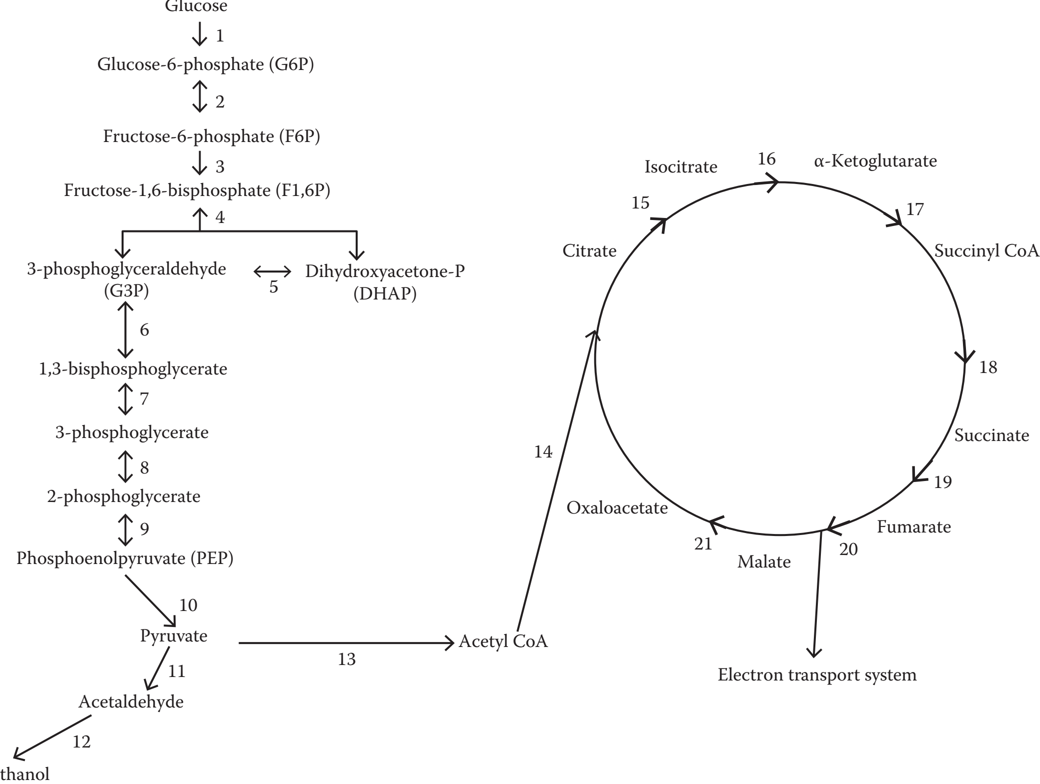


FIGURE 16.6 The glycolysis pathway, TCA cycle, and electron pathway, with associated enzymes and reactants. Each molecule of glucose produces two molecules of ATP and two molecules of NADH. Each molecule of pyruvate produces one molecule of $FADH_2$ and four molecules of NADH. Through the electron transport system, one NADH molecule produces three ATP molecules, while one $FADH_2$ molecule produces two ATP molecules. (1) Hexokinase (glucose + ATP → G6P + ADP), (2), phosphohexose isomerase (G6P → F6P), (3) phosphofructokinase (F6P + ATP → F1,6P + ADP), (4) aldolase (F1,6P → DHAP + G3P), (5) isomerase (DHAP → G3P), (6) 3-phosphoglyceraldehyde dehydrogenase (G3P + NAD^+ → 1,3-bisphosphoglyerate + NADH, (7) phosphoglycerokinase (1,3-bisphosphoglycerate + ADP + Pi → 3-phosphoglycerate + ATP), (8) phosphoglyceromutase (3-phosphoglycerate → 2-phosphoglycerate), (9) enolase (2-phosphoglycerate → PEP + H_2O), (10) pyruvate kinase (PEP + ADP → pyruvate + ATP), (11) pyruvate decarboxylase (pyruvate → acetaldehyde + CO_2), (12) alcohol dehydrogenase (acetaldehyde + NADH → ethanol + NAD^+), (13) pyruvate dehydrogenase complex (pyruvate + CoA-SH + NAD^+ → acetyl CoA + CO_2 + NADH + H^+), (14) citrate synthase (acetyl CoA + oxaloacetate + H_2O→ citrate + CoA), (15) aconitase (citrate + H_2O → isocitrate + H_2O), (16) isocitrate dehydrogenase (isocitrate + NAD → α-ketoglutarate + NADH + CO_2), (17) α-ketoglutarate dehydrogenase (α-ketoglutarate + NAD → succinyl CoA + NADH + CO_2), (18) succinate thiokinase (succinyl CoA + Pi + nucleoside diphosphate (either GDP or ADP) → succinate + CoA + nucleoside triphosphate (either GTP or ATP)), (19) succinate dehydrogenase (succinate + H_2O → fumarate), (20) fumarase (fumarate + FAD → malate + $FADH_2$), (21) malate dehydrogenase (malate + NAD → oxaloacetate + NADH + H^+).

Respiration involves a series of three complex and interconnected metabolic pathways—glycolysis, tricarboxylic acid (TCA) cycle, and electron transport system [11] (Figure 16.6).

Glycolysis, which occurs in the cytoplasm, results in the production of two molecules of pyruvate from each molecule of glucose. Each of the 10 distinct, sequential reactions in glycolysis is catalyzed by a specific enzyme that performs one of the following actions: add an energy-containing phosphate group to the substrate molecule, rearrange the molecule, or break down the molecule to a simpler one. The two key enzymes in glycolysis are phosphofructokinase (PFK) and pyruvate kinase (PK). Cells can control their rate of energy production by altering the rate of glycolysis, primarily through controlling PFK and PK activity. One of the products of respiration, namely ATP, is used as a negative feedback inhibitor to control the activity of PFK. Glycolysis produces two molecules of ATP and two molecules of NADH from the breakdown of each molecule of glucose.

The TCA cycle, which occurs in the mitochondrial matrix, involves the breakdown of pyruvate into CO_2 in nine sequential enzymatic reactions. Pyruvate is decarboxylated to form acetate, which condenses with a coenzyme to form acetyl CoA (Figure 16.6). This compound then enters the cycle by condensation with oxaloacetate to form citric acid. Citric acid has three carboxyl groups from which the cycle derives its name. Through a series of seven successive rearrangements, oxidations, and decarboxylations, citric acid is converted back into oxaloacetate, which is then ready to accept another acetyl CoA molecule. In addition to producing the many intermediates that are used in the synthetic reactions of the cell, the TCA cycle also produces one molecule of flavin adenine dinucleotide ($FADH_2$) and four molecules of NADH for each molecule of pyruvate metabolized.

The electron transport system, which occurs on membranes in the mitochondria, involves the production of ATP from the high-energy intermediates $FADH_2$ and NADH. In a series of reactions, one NADH molecule produces three ATP molecules, while one $FADH_2$ molecule produces two ATP molecules. The production of ATP depends not only on the energy derived from NADH and $FADH_2$ but also on the chemical environment (pH and ion concentrations) within the cell and mitochondria.

In the absence of O_2, NADH and $FADH_2$ accumulate in the reduced form. As the oxidized forms (NAD+ and FAD) are consumed, the TCA cycle comes to a halt and glycolysis becomes the sole source of ATP production. Regeneration of NAD+ is absolutely essential for the survival of the anaerobic cell and occurs through the reductive decarboxylation of pyruvate to ethanol in fermentative metabolism.

Fermentation, or anaerobic respiration, involves the conversion of hexose sugars into alcohol and CO_2 in the absence of O_2. Pyruvate produced through glycolysis (reactions that do not require O_2) can be converted to lactic acid, malic acid, acetyl CoA, or acetaldehyde. The dominant pathway(s) engaged depends on cellular pH, prior stresses, and the metabolic needs of the cell. Acidification of the cytoplasm enhances the activity of pyruvic decarboxylase, which then shunts pyruvate to form CO_2 and acetaldehyde. The acetaldehyde is converted by the enzyme alcohol dehydrogenase to ethanol with the regeneration of NAD+. Two molecules of ATP and 21 kcal of heat energy are produced in anaerobic respiration (alcoholic fermentation) from each molecule of glucose. To maintain the supply of ATP at the aerobic rate, 19 times as many glucose molecules would be needed, and glycolysis would increase 19-fold. However, only two molecules of CO_2 are produced during glycolysis, instead of six during aerobic respiration, and therefore the rate of CO_2 production increases by only 6.3-fold.

The O_2 concentration at which a shift from predominantly aerobic to predominantly anaerobic respiration occurs varies among tissues and is known as the anaerobic compensation point (ACP) (Figure 16.7) [12]. ACP represents the O_2 concentration below which anaerobic conditions occur. Storage of edible plant tissues just above ACP will typically result in maximum storage periods, but exposure of tissues to extended periods at O_2 concentrations below ACP will cause cell death.

The oxidative pentose phosphate pathway can also break down sugars to CO_2. The first step of the pathway is the irreversible oxidation of glucose-6-P from glycolysis to 6-phosphogluconic acid [8]. The pathway provides a source of ribose-5-phosphate for nucleic acid production, a source of reduced NADP for synthetic reactions, and a means of interconversion of sugars to provide three, four, five, six, or seven carbon skeletons for biosynthetic reactions. Both the pentose phosphate and TCA cycle pathways appear to be operative in harvested plant tissues, but the precise contribution

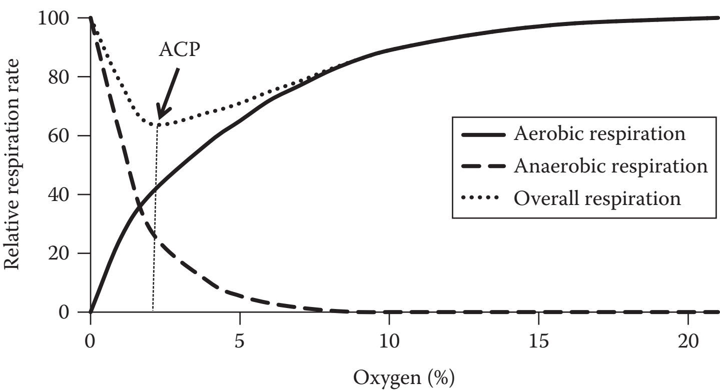


FIGURE 16.7 Aerobic, anaerobic, and overall respiration rate of a generalized plant product in response to oxygen in the storage environment. The anaerobic compensation point (ACP) represents the lowest oxygen concentration achievable without injurious fermentation and represents the concentration at which the maximum storage potential can be obtained.

of each is not well known. However, while in tomato fruit the pentose phosphate pathway accounts for about 16% of the total carbohydrate utilized, in storage root tissues between 25% and 50% of carbohydrate may be oxidized via this pathway [8].

The respiration rate is tightly coupled to the rate of metabolism, and therefore its measurement provides an important method of monitoring the metabolic and physiological state of edible plant tissues. Respiration rates can be very low to very high depending on the product (Table 16.5), and

TABLE 16.5
Selected Edible Plant Tissues Classified according to Their Relative Respiration Rates

Class	Range Respiration (mg CO_2/kg·h) at 5°C	Intact	Fresh-Cut
Very low	<5	Dates, dried fruits and vegetables, nuts	
Low	5–10	Apple, beet, celery, cranberry, garlic, grape, honeydew melon, onion, papaya, potato (mature), sweet potato, watermelon	Diced pepper, grated red beet, potato slices
Moderate	11–20	Apricot, banana, blueberry, cabbage, cantaloupe, carrot (topped), celeriac, cherry, cucumber, fig, gooseberry, lettuce (head), nectarine, olive, peach, pear, pepper, plum, potato (immature), radish (topped), summer squash, tomato	Cantaloupe cubes, carrot sticks and slices, onion rings, Peeled garlic, shredded cabbage and head lettuce, squash slices
High	21–30	Blackberry, carrot (with tops), cauliflower, leeks, lettuce (leaf), lima bean, radish (with tops), raspberry, strawberry	Cauliflower florets, leek rings, cut-salad mixes of leafy lettuces, chicory, endive, arugula, and/or radiccio
Very high	>30	Artichoke, asparagus, bean sprouts, broccoli, Brussels sprouts, endive, green onions, kale, mushroom, okra, parsley, peas, snap bean, spinach, sweetcorn, watercress	Broccoli florets, slice mushrooms, shelled peas

Source: Modified from Kader, A.A., *Postharvest Technology of Horticultural Crops*, Regents of the University of California, Division of Agricultural and Natural Resources, Oakland, CA, 2002; Kader, A.A. and Saltveit, M.E., Respiration and gas exchange, in *Postharvest Physiology and Pathology of Vegetables*, Bartz, J.A. and Brecht, J.K., Eds, Marcel Dekker, New York, pp. 7–30, 2003.

TABLE 16.6
Selected Edible Plant Tissues Classified according to Relative Perishability and Potential Storage Life in Air at Near-Optimal Temperature and RH

Relative Perishability	Potential Storage Life (weeks)	Edible Plant Tissue
Very low	>16	Tree nuts, dried fruits and vegetables, grains
Low	8–16	Apple and pear (some cultivars), potato (mature), dry onion, garlic, pumpkin, winter squash, sweet potato, taro; bulbs and other propagules of ornamental plants
Moderate	4–8	Apple and pear (some cultivars), grape (SO_2-treated), pummelo, table beet, carrot, radish, potato (immature)
High	2–4	Grape (without SO_2 treatment), melons, nectarine, papaya, peach, pepino, plum; artichoke, green beans, Brussels sprouts, cabbage, celery, eggplant, head lettuce, okra, pepper, summer squash, tomato (partially ripe)
Very high	<2	Apricot, blackberry, blueberry, cherry, fig, raspberry, strawberry; asparagus, bean sprouts, broccoli, cauliflower, cantaloupe, green onion, leaf lettuce, mushroom, pea, spinach, sweet corn, tomato (ripe); most cut flowers and foliage; fresh-cut (minimally processed) fruits and vegetables

Source: Modified from Kader, A.A., *Postharvest Technology of Horticultural Crops*, Regents of the University of California, Division of Agricultural and Natural Resources, Oakland, CA, 2002.

respiration rates are sometimes related to the growth stage at harvest (Figure 16.3). While the range of average respiration rates for each product type can be affected by seasonal growing conditions, stage of development at which the product is harvested, and cultivar and postharvest management, the storage life of products is broadly consistent. As a general rule, the rate of deterioration of the many different edible plant tissues after harvest is associated with their respective respiration rates (Table 16.6), as higher respiration rates result in faster use of carbohydrate and other energy reserves in the tissues, thereby resulting in hastened loss of product quality. Products with very high respiration rates such as asparagus, mushroom, parsley, peas, spinach, and sweet corn deteriorate much more rapidly than those such as apple, beet, celery, garlic, grape, honeydew melon, and onion, with low respiration rates. Dried products have extremely low respiration rates and therefore long storage potential under proper storage conditions. Rice, for example, has a very low respiration rate of <1 mg $CO_2/kg \cdot h$ at optimum conditions, but rates increase markedly under high moisture and temperature conditions [13].

Importantly, the relationship between respiration rates and maintenance of quality sets inherent limitations to increase storage periods within any particular product type. A product that has a storage life of a week, for example, cannot be extended appreciably compared with one with a storage life of several months. Nevertheless, controlling the rate of respiration is a critical aspect of all postharvest technologies, as any treatments that reduce respiration rates will increase the storage potential. Decreasing the storage temperature is the major technology used to control the respiration of many edible plant tissues. Low O_2 and elevated CO_2 concentrations will also decrease respiration rates, but O_2 levels must remain adequate to support aerobic respiration, and CO_2 concentrations should be low enough to prevent injury development. If a fresh fruit or vegetable is kept in a sealed plastic bag, for example, cell death can occur as a result of inadequate O_2 and excessive CO_2 concentrations accumulating in the atmosphere around the product. Also, “vital heat” resulting from respiration will result in higher temperatures around the commodity and will reduce storage life if not removed by refrigeration or ventilation. Water is also a product of respiration, and uncontrolled loss can result in loss of quality, for example, wilting. The way in which these factors are incorporated into postharvest technologies to maintain quality of edible plant tissues is described in Section 16.7.

16.5 HORMONES

Plant hormones are chemical compounds that potentiate a signaling network and regulate the metabolic systems involved in plant growth and development, and plant responses to biotic and abiotic stresses. The five "classic" plant hormones are ethylene, auxins, gibberellins, abscisic acid, and cytokinins (Figure 16.8), and are commonly regarded as plant growth regulators (PGRs). Many studies on postharvest quality of edible plant tissues have focused on ethylene because of its direct effects on ripening and senescence, and in part because of its ease of measurement. Relative to ethylene, less is known about the involvement of other hormones in ripening and senescence. Another confounding factor in interpreting hormones and their effects is that, while concentrations of hormones are important, the sensitivity of tissues to the hormones is also critical [14]. In this section, the roles of key hormones and their function are briefly outlined. However, it should be recognized that these divisions, while useful for understanding of postharvest processes, are artificial. It has become well recognized that metabolic processes are regulated in a complex way by the crosstalk of several hormones, including the more recently recognized families of plant hormones such as polyamines, nitric oxide, jasmonic acid, brassinosteroids, and salicylic acid [15–17]. Receptor mechanisms have been conclusively identified or discounted, hormone transport processes have been largely elucidated, and the cellular processes downstream of hormone signaling have been painstakingly dissected. The classification of hormones into developmental or environmental response categories has been replaced by mapping of hormonal signaling into transcriptional and post-transcriptional response networks [18].

16.5.1 Ethylene

Ethylene is a naturally occurring plant growth regulator that affects many aspects of growth and development of plants. It is a gas that can exert its effects at very low concentrations, from parts per billion (ppb; nL/L) to parts per million (ppm; μL/L).

16.5.1.1 Ethylene Biosynthesis

Ethylene biosynthesis, perception, signal transduction, and regulation at the genetic and biochemical levels have been well documented [19]. In higher plants, ethylene is produced from methionine, which is first converted to *S*-adenosyl-L-methionine (*S*-AdoMet, or SAM) by the addition of adenine and utilization of ATP catalyzed by SAM synthetase (Figure 16.9). SAM is an important

Ethylene Auxin (indoleacetic acid) Gibberellic acid

Abscisic acid Cytokinin (zeatin)

FIGURE 16.8 Structures of major plant hormones: ethylene, auxin (indoleacetic acid), gibberellic acid, abscisic acid, and cytokinin (zeatin).

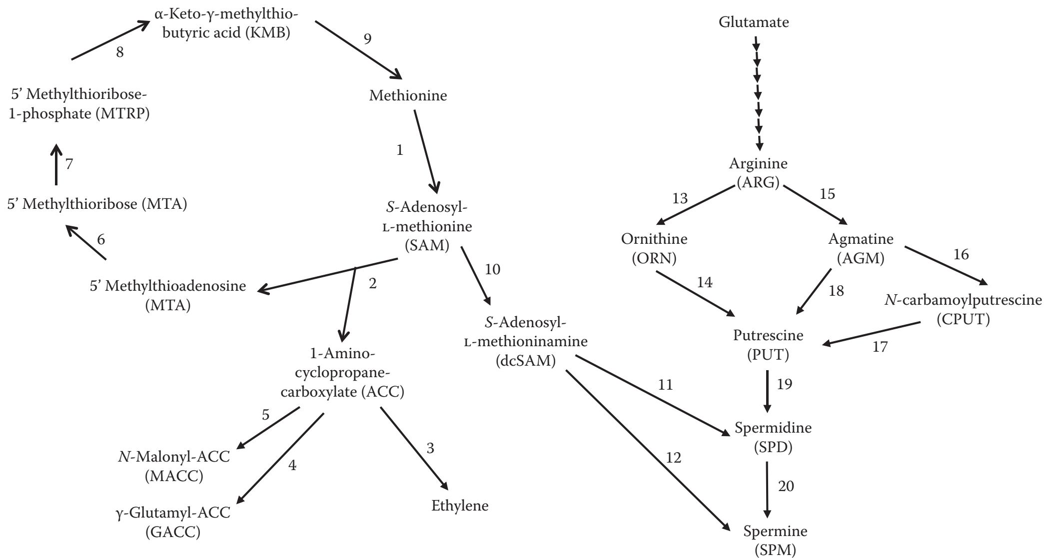


FIGURE 16.9 The ethylene and polyamine biosynthetic pathways, with associated enzymes and reactants. (1) SAM synthase (MET + ATP → SAM + diphosphate (PPi) + phosphate (Pi)), (2) ACC synthase (SAM → ACC + MTA), (3) ACC oxidase (ACC + ½ O_2 → Ethylene + CO_2 + HCN + H_2O), (4) γ-glutamyl-transpeptidase (ACC + glutathione → GACC + Cys-Gly), (5) ACC *N*-malonyl-transferase (ACC + malonyl coenzyme A → MACC + coenzyme A-SH), (6) MTA nucleosidase (MTA + H_2O → MTR + adenine), (7) MTR kinase (MTR + ATP → MTRP + ADP), (8) spontaneous reaction, (9) transaminase (KMB + amino acid → Methionine + 2-oxo acid), (10) SAM decarboxylase (SAM → dSAM + CO_2), (11) SPD synthase (dSAM → SPD + MTA), (12) SPM synthase (dSAM → SPM + MTA), (13) arginase (ARG + H_2O → ORN + urea), (14) ORN decarboxylase (ORN → PUT + CO_2), (15) ARG decarboxylase (ARG → AGM + CO_2), (16) AGM iminohydrolase (agmatine deiminase) (AGM + H_2O → CPUT + NH_3), (17) *N*-carbamoylputrescine amidohydrolase (amidase) (CPUT+ $2H_2O$ → PUT + CO_2 + NH_3), (18) agmatinase (AGM + H_2O → PUT + urea), (19) SPD synthase (PUT + dSAM → SPD + SAM), (20) SPM synthase (SPD + dSAM → SPM + SAM).

metabolite, as it is a propylamine group donor in polyamine biosynthesis (Section 16.6.2.1) and a methyl group donor in transmethylation of lipids, nucleic acids, and polysaccharides. In addition, SAM in involved in the first dedicated step of ethylene biosynthesis, the conversion of SAM to aminocyclopropane-1-carboxylic acid (ACC). The enzyme involved in this step, ACC synthase, is normally rate-limiting. The by-product of this step, 5′-methylthioadenosine (MTA), is recycled to methionine via the Yang cycle, thereby allowing the production of ethylene to occur with a small pool of free methionine. ACC is then converted to ethylene by ACC oxidase (formerly known as the ethylene-forming enzyme). ACC can also be metabolized by the action of *N*-malonyltransferase and γ-glutamyltranspeptidase, to produce malonyl ACC (MACC) and γ-glutamyl-ACC (GACC), respectively. MACC cannot be metabolized back to ACC under physiological conditions. The significance of the conversion of ACC to MACC and GACC remains unclear, but conjugation of ACC may contribute to the regulation of ethylene formation. ACC itself may have an important role as a signaling molecule [20].

Ethylene has to be perceived by the plant cell to exert its action. As a result of the binding, signal transduction occurs via a series of gene expression regulators, resulting in the expression of genes and synthesis of proteins, many of which are important in senescence and ripening. Phenotypic*

* Phenotype: visible characteristics.

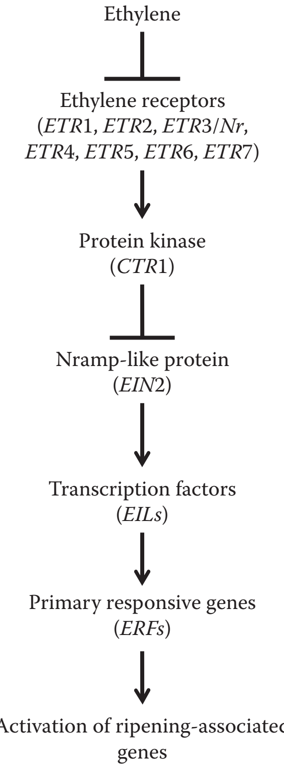


FIGURE 16.10 Ethylene perception and signaling transduction pathway for the model fruit system: Tomato. Perception of ethylene is via binding to seven ethylene receptor (ethylene response) genes (*ETR*, *ETR2*, *ETR3/ Never ripe* (*Nr*), *ETR4-7*). The ethylene signaling cascade ends with transcriptional activation ethylene response factors (ERFs).

changes in response to ethylene are determined by (1) the perception of the hormone, (2) the transduction of the signal through gene expression regulators, and (3) the expression of genes and synthesis of proteins sensitive to the received ethylene signal [21].

Perception of ethylene is via binding to receptors (Figure 16.10) on the endoplasmic reticulum. In tomato fruit, seven ethylene receptors (ethylene response) genes (*ETR, ETR2, ETR3/ Never ripe* (*Nr*)*, ETR4–7*) have been identified, while there are five in *Arabidopsis* [19,22]. Differential expression of these genes may regulate ethylene receptivity in different tissues and mediate different biological processes including ripening. Ethylene signaling downstream of the receptors is mediated through a negative regulator constitutive triple response1 (*CTR1*) MAP kinase kinase kinase gene. The positive regulator ethylene insensitive 2 (EIN2) protein, negatively regulated by *CTR1*, mediates later steps of ethylene signaling via the EIN3 transcription factors. There is one *EIN3* gene in *Arabidopsis* and four *EIL* (*EIN3-like*) genes in tomato (*EIL1–4*). The ethylene signaling cascade ends with transcriptional activation ethylene response factors (ERFs). The ERFs then activate secondary response genes that are responsible for ripening.

16.5.1.2 Non-Climacteric and Climacteric Fruits

Fruits can be divided into two categories, those that do not produce ethylene as part of ripening and senescence, and those where ethylene production is critical for normal ripening to occur. The two types of fruits have different respiration patterns during maturation and ripening (Figure 16.11).

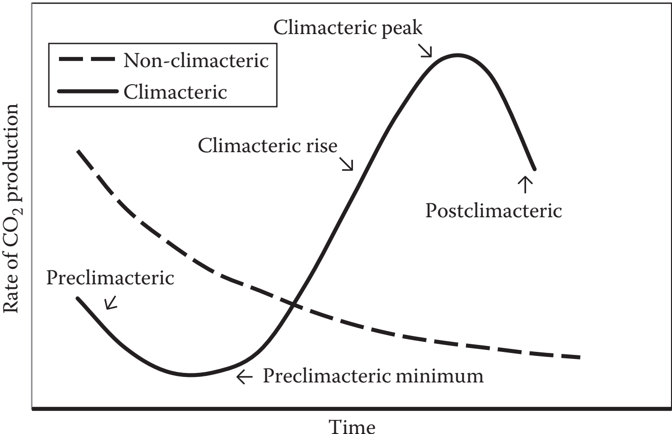


FIGURE 16.11 Patterns of respiration in a non-climacteric fruit compared with a climacteric fruit. In a non-climacteric fruit, the respiration rate declines over time, while in a climacteric fruit the rate declined from a pre-climacteric rate to a preclimacteric minimum before increasing to a peak rate.

The respiration rates of non-climacteric fruits gradually decrease over time and they do not produce appreciable amounts of ethylene. In contrast, climacteric fruits produce a surge of respiration that is associated with autocatalytic ethylene production. The distinction between non-climacteric and climacteric fruits was originally based on differences in patterns of respiration, but is now often identified by differences in ethylene production.

Two ethylene systems are thought to operate. System I ethylene exists in both non-climacteric and climacteric plant tissues as basal ethylene production. It is also responsible for the basal ethylene production in vegetables as well as for ethylene production that occurs as a result of wounding and other (a)biotic stresses. System II ethylene is responsible for the autocatalytic ethylene production that occurs in climacteric fruits. During ripening of climacteric fruit, System II ethylene production is associated with the upregulation of *ACC synthase* and *ACC oxidase* genes, as well as increased activities of these enzymes. Transcriptional regulation of *ACC synthase* is the major control point of ethylene biosynthesis; while *ACC oxidase* activity is not thought to be rate-limiting during normal ripening, repression of *ACC oxidase* gene activation in transgenic mutants reduces ethylene production and prevents or impedes ripening [19]. While the broad separation between climacteric and non-climacteric fruits is accepted, there is increasing evidence that such categorization is not absolute. Increases of ethylene production, albeit small, have been detected in the non-climacteric strawberry fruit [15]. Also, both ethylene-dependent and -independent ripening events have been described for climacteric fruits; for example, in melon, ethylene-independent expression of genes encode for flesh color, sugars, acidity, some cell-wall degrading enzymes, and some softening, and ethylene-dependent expression of genes encode for chlorophyll degradation, abscission, aromas, climacteric respiration, some cell-wall degrading enzymes, and the majority of softening [23].

Table 16.7 provides examples of climacteric and non-climacteric fruits. Many popular fruits are listed in the climacteric category, including apples, peaches, plums, and tomatoes. Ensuring low ethylene levels around these fruits can help delay the start of ripening, but normal ripening cannot occur in the absence of ethylene. For example, mature green tomatoes will not develop red color or soften without endogenous or exogenous ethylene. While non-climacteric fruits do not need increased ethylene production in order to ripen, they can be affected, usually negatively, by exposure to exogenous ethylene from ethylene-producing fruits and vegetables, damaged commodities, and contamination. Climacteric fruit can be relatively short- and long-lived, for example, peach and apple, respectively, as can non-climacteric fruits, for example, strawberries and lemons,

TABLE 16.7
Selected Fruits (Including Vegetable Fruits) Classified according to Climacteric and Non-Climacteric Respiratory Behavior during Ripening

Climacteric	Non-Climacteric
Apple	Blackberry
Apricot	Cacao
Avocado	Cherry (sweet, sour)
Banana	Cranberry
Bitter melon	Cacao
Blueberry	Cranberry
Breadfruit	Cucumber
Cantaloupe	Eggplant
Cherimoya	Grape
Feijoa	Lemon
Kiwifruit	Loquat
Mango	Mandarin
Muskmelon	Olive
Nectarine	Pepper
Peach	Pineapple
Pear	Raspberry
Plum	Strawberry
Tomato	Summer squash
Watermelon	Tamarillo

respectively. Therefore, while fruits and vegetables vary greatly in the rates of ethylene production (Table 16.8), no clear association with these rates and storage life exists. However, within a fruit type there may be relationships between ethylene production and rate of ripening, with high production associated with rapid softening, for example, McIntosh apples.

Stimulated ethylene production can also occur as a result of disease and decay, exposure to chilling temperatures, and wounding (including during fresh-cut processing). In addition, ethylene is produced by internal combustion engines, smoke, and other sources of pollution, and special care has to be taken

TABLE 16.8
Fruits and Vegetables Classified according to Ethylene Production Rates

Class	Production Rate at 20°C (μL C_2H_4/kg · h)	Edible Plant Tissue
Very low	Less than 0.1	Artichoke, asparagus, cauliflower, cherry, citrus fruits, grape, jujube, strawberry, pomegranate, leafy vegetables, root vegetables, potato
Low	0.1–1.0	Blackberry, blueberry, casaba melon, cranberry, cucumber, eggplant, okra, olive, pepper (sweet and chili), persimmon, pineapple, pumpkin, raspberry, tamarillo, watermelon
Moderate	1.0–10.0	Banana, fig, guava, honeydew melon, lychee, mango, plantain, tomato
High	10.0–100.0	Apple, apricot, avocado, cantaloupe, feijoa, kiwifruit (ripe), nectarine, papaya, peach, pear, plum
Very high	More than 100.0	Cherimoya, mammee apple, passion fruit, sapote

Source: Modified from Kader, A.A., *Postharvest Technology of Horticultural Crops*, Regents of the University of California, Division of Agricultural and Natural Resources, Oakland, CA, 2002.

to avoid contamination of sensitive products such as kiwifruit in the handling and storage environment. Other gaseous analogs such as propylene, carbon dioxide, and acetylene can exert similar effects to those of ethylene but only at much higher concentrations. Exposure of fruits and vegetables to ethylene can stimulate respiration and thereby increase use of carbohydrate reserves. Incorporating knowledge about responses of plant tissue to ethylene into postharvest technologies is described in Section 16.7.

16.5.2 Auxins

Auxins (Figure 16.8) are the main hormones responsible for cell elongation in phototropism* and gravitropism,† and they control the differentiation of meristem into vascular tissue and promote leaf development and arrangement [24,25]. Auxins affect flowering, fruit set and ripening, and inhibition of abscission.‡ Indoleacetic acid (IAA) is the common form, being derived from tryptophan. Auxin is active at very low concentrations is plant cells; precise control of these concentrations are modulated by the rates of its synthesis, catabolism pathways (IAA oxidase), degradation (e.g., H_2O_2, light, direct oxidation), conjugation, and transport.

Application of synthetic auxins is used to prevent premature drop of fruit such as apples, but can negatively affect fruit quality by stimulating ethylene production. However, in tomatoes, elevated auxin concentrations can delay ripening. Ripening in the non-climacteric strawberry occurs when the auxin concentration decreases to below a threshold level [26]. In strawberries, auxins are produced by the achenes,§ and anthocyanin accumulation and softening is accelerated by achene removal.

16.5.3 Gibberellins

Gibberellins (GAs) (Figure 16.8) are a group of about 125 closely related plant hormones that stimulate shoot elongation, seed germination, and fruit and flower maturation. GAs are synthesized in the root and stem apical meristems, young leaves, and seed embryos. GAs also break dormancy in the seeds of plants that require exposure to cold or light to germinate. Other effects of GAs include gender expression, seedless fruit development, and the delay of senescence in leaves and fruits [27]. Maturing grapes are routinely treated with GA to promote larger fruit size as well as looser bunches (longer stems).

16.5.4 Abscisic Acid

Abscisic acid (ABA) (Figure 16.8) is associated with responses of whole plants to different kinds of abiotic stresses such as drought, high temperature, chilling, and salinity; however, involvement of ABA in regulating abiotic stress in fruits has rarely been reported [28]. The evidence that ABA is involved in control of ripening is strong [15,28]: (1) a sharp increase in ABA accumulation is observed during the onset of fruit ripening and/or the ripening process in both climacteric and non-climacteric fruits; (2) ABA application enhances the production of several metabolites that promote ripening; and (3) inhibition of ABA signaling in RNAi-silenced strawberry fruit impedes fruit ripening. Abscisic acid is a strong antagonist of GA action.

16.5.5 Cytokinins

Cytokinins (Figure 16.8) are most abundant in growing tissues, such as roots, embryos, and fruits, where cell division (cytokinesis) is occurring. Cytokinins delay senescence and maintain the green color and freshness of many leafy vegetables, and can also delay fruit ripening [27].

---

* Phototropism: orientation in response to light.
† Gravitropism: orientation in response to gravity.
‡ Abscission: natural detachment of plant parts.
§ Achenes: small, dry one-seeded indehiscent fruit.

16.5.6 Other Hormones

16.5.6.1 Polyamines

Polyamines (PAs) are small aliphatic amines positively charged at physiological pH in the cell. The diamine putrescine (PUT), triamine spermidine (SPD), and tetraamine spermine (SPM) are present in all living organisms (Figure 16.12). PAs are regarded as PGRs and have been implicated in a wide range of metabolic processes during plant growth and development, including senescence and ripening as well as abiotic and biotic plant stress responses [29].

The importance of PAs is related to their central role in multiple signaling pathways that drive various cellular functions. The intracellular levels of PAs in plants are mostly regulated by anabolic and catabolic processes, as well as by their conjugation with hydroxycinnamic acids. PUT is produced by two alternative pathways (Figure 16.9): from ornithine by the action of ornithine decarboxylase (ODC), or from arginine by the action of arginine decarboxylase (ADC) to agmatine and then by the actions of agmatine iminohydrolase and *N*-carbamoylputrescine amidohydrolase. The two pathways may be explained by differential compartmentalization of ADC and ODC in the chloroplast and cytoplasm, respectively. PUT is converted to SPD by the symmetrical addition of an aminopropyl residue from decarboxylated *S*-adenosylmethionine (dcSAM), by the action of SPD synthase. dcSAM is used in the same reaction, but catalyzed by SPM synthase to produce SPM. The free PA pools are modulated by catabolic pathways, degradation, conjugation, and transport.

The utilization of SAM represents an important linkage to ethylene biosynthetic pathway, as this substrate also produces ACC (Figure 16.9) and exerts opposite effects on ripening and senescence. PA concentrations are often the highest in the early phases of fruit growth, and decreases in concentration may be signals for fruit ripening, but these relationships are not always consistent [29].

16.5.6.2 Nitric Oxide

Nitric oxide (NO) is a gaseous free radical with a relatively long half-life (3–5 s) compared with other free radicals in biological systems. In mammalian systems, NO is metabolized via the conversion of arginine to citrulline by nitric oxide synthase. It is an important signaling molecule with diverse physiological functions in plant growth and development, including ripening and senescence [16,30]. NO can provoke both beneficial and harmful effects in plant cells, depending on factors such as the local concentration of NO, the rate of synthesis, translocation, effectiveness of removal of this reactive nitrogen species, suppression of reactive oxygen species, as well as its ability to directly interact with other molecules and signals [16,31]. Exogenous NO can increase the storage and shelf-life of climacteric and non-climacteric fruits and vegetables, as well as fresh-cut products by delaying ripening and senescence, and inhibiting chilling injury development [30].

H_2N — NH_2

Putrescine

H_2N — $\overset{H}{N}$ — NH_2

Spermidine

H_2N — $\underset{H}{N}$ — $\overset{H}{N}$ — NH_2

Spermine

FIGURE 16.12 Structures of the diamine putrescine, triamine spermidine and tetraamine spermine.

Jasmonic acid Methyl jasmonate Salicylic acid Methyl salicylate

Brassinosteroids

FIGURE 16.13 Structures of jasmonic acid, methyl jasmonate, salicylic acid, methyl jasmonate and brassinosteroids.

16.5.6.3 Jasmonic Acid, Brassinosteroids, and Salicylic Acid

Jasmonates such as jasmonic acid (JA) and methylJA (Figure 16.13) are important cellular regulators of biological processes including senescence and ripening [15]. Postharvest application of jasmonates to fruits can increase sugar and anthocyanin concentrations, lignin biosynthesis, and ethylene production, and may regulate cell-wall degradation.

Salicylic acid (SA), a monohydroxybenzoic acid, and methyl salicylate (MeSA) (Figure 16.13) are found in plants with roles in plant growth and development such as photosynthesis, transpiration, ion uptake and transport, and induction of disease and stress resistance. SA treatments can delay ripening of fruits such as apples, bananas, kiwifruit, mango, peach, persimmon, and tomato, probably by inhibiting ethylene production. However, there is insufficient evidence about endogenous SA changes to prove a role of the hormone during fruit ripening.

Brassinosteroids (BRs) are growth-promoting steroids (Figure 16.13) that are now recognized as plant hormones; mutant analysis has demonstrated that the ability to synthesize, perceive, and respond to BRs is essential to normal plant growth and development [32]. While information about their role in ripening and senescence is limited, BRs have been implicated in ripening of grapes, strawberries, and tomatoes.

16.6 COMPOSITION

16.6.1 Water

Water is the major component of most fruits and vegetables. Fruits and vegetables can be regarded as "water inside pleasing packages" or "water with a mechanical structure"! Therefore, water loss or transpiration is a major factor affecting the quality of fruits and vegetables. In addition to lower saleable weight, loss of water can affect quality in many ways, including wilting, shriveling, flaccidness, soft texture, and loss of nutritional value. The rate of water loss and the impact of this loss varies from product to product. For example, maximum permissible losses can range from 3% for lettuce

to 10% for onions. Products vary in potential for water loss by morphological differences such as cuticle* thickness and composition and presence or absence of stomata† and lenticels,‡ which are structures that allow gases and moisture to move in or out of the plant. For some products, these differences are affected by developmental stage.

16.6.2 Carbohydrates

Carbohydrates are a major constituent of edible plant tissues, making up 50%–80% of the dry matter. They can be classified into three general groups: structural, soluble, and storage carbohydrates. Carbohydrates are the predominant energy sources for cells and are the major translocated products of photosynthesis. Once an edible plant tissue is removed from the parent plant, or photosynthesis is interrupted in any way, carbohydrates represent the reserves necessary to maintain cellular function. The carbohydrate composition, especially the balance between complex carbohydrates and simple sugars (and acids), can have a major influence on product acceptability by the consumer. The roles of carbohydrates and their metabolism have been detailed by others [8,29,33–35] and summarized here.

16.6.2.1 Structural Carbohydrates

Plant cell walls make up most of the dietary fiber in edible plant tissues. Structural carbohydrates form the foundation of the cell wall that surrounds the cell membrane and provides cells with structural support and protection. The primary cell wall generally is a thin, flexible, and extensible layer formed while the cell is growing. The secondary cell wall forms as a thick layer inside the primary cell wall after the cell is fully grown. The middle lamella forms at the interface between adjacent plant cells and promotes cell-to-cell adhesion.

The cell wall is comprised of cellulose and a range of hemicellulosic and pectic polysaccharides. Collectively, these compounds represent approximately 90% of the dry matter in the cell wall. Other components include cell-wall proteins, both structural and enzymatic, mineral ions, and phenolic compounds, such as lignin, in secondary walls. Most cell types in edible plant tissues have non-lignified primary walls, and usually only small amounts of the secondary wall are lignified. Examples of exceptions are the outer layers of wheat grains and parts of wheat bran, in mature asparagus where sclerenchyma fibers are responsible for the tough, stringy texture, and stone cells in pear and feijoas that give the fruits a gritty texture. Cell-wall proteins include extensins, a family of hydroxyproline-rich glycoproteins, which are necessary for cell wall expansion to occur. Extensins are highly abundant and form cross-linked networks in the cell wall.

Cellulose, a linear polymer of β-1,4-linked D-glucose residues, is deposited in the wall in the form of crystalline/semicrystalline microfibrils, which result from lateral associations of individual cellulose chains by extensive hydrogen bonding. The resulting crystalline structure is resistant to chemical and enzymatic degradation. The microfibrils are laid down in helical arrays around the cell, and the pattern of their deposition provides control over the direction of cell expansion.

Hemicelluloses are mainly composed of several neutral sugars, especially xylose but also include mannose, galactose, rhamnose, and arabinose. Also, in contrast to cellulose, hemicelluloses are branched. The most common matrix glycan in most fruits and vegetables is xyloglucan, a polymer made up of β-1,4-D-glucose with regularly spaced xylose side chains (mostly α-1,6-linked), to which other sugars are attached. Hemicelluloses associate with and noncovalently cross-link cellulose microfibrils.

Pectic polysaccharides, or pectins, are linear or branched polymers that are rich in galacturonic acid and can contain up to 17 different monosaccharide types, including substituted species. These polymers are dispersed throughout the primary cell wall, and can form a gel matrix that is co-extensive with the cellulose–hemicellulose network. Pectic polysaccharides include homogalacturonic acid (HGA),

* Cuticle: outer layer of cutin and wax secreted by epidermis.
† Stomata: openings in epidermis allowing for gas/vapor exchange.
‡ Lenticels: aggregates of cells forming pores at surface allowing for gas/vapor exchange.

rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), and xylogalacturonan, the latter three of which are branched heteroglycans. HGA is a contiguous 1,4-linked α-D-galacturonic acid polymer that is synthesized with a high degree of methyl-esterification at the C-6 position and carries acetyl groups on O-2 and O-3. HGA is found in the primary cell wall, and is also a major component of the middle lamella, where it is less esterified. The degree of esterification affects the ionic charge and calcium binding capacity, as well as availability of sites for enzyme action. The low esterification of HGA in the middle lamella promotes gel formation, and this is thought to be important for cell–cell adhesion. RG-I pectins contain a backbone of the repeating disaccharide of α-D-(1,4)-galacturonic acid and α-(1,2)-rhamnose. Side chains of various neutral sugars branch from many of the rhamnose residues. The neutral sugars are mainly D-galactose, L-arabinose, and D-xylose, with the types and proportions of neutral sugars varying with the origin of pectin. RG-I is also found in the primary cell wall and, generally, to a lesser extent in the middle lamella. The highly branched sidechains have conferred the term "hairy region." The linkages that integrate the pectin superstructure in the wall include calcium bridges between uronic acid carboxyl groups creating the so-called pectin egg-box, and borate diester bonds spanning two RG-II monomers. There is some evidence that the RGI side chains can be covalently linked to hemicelluloses, forming a super-macromolecular polymeric network. RG-II is a complex, highly branched polysaccharide that is a minor component of primary cell wall and is absent from the middle lamella. Cell walls also contain non-polysaccharide constituents including structural proteins and numerous enzymes, and some cells have specialized walls, such as those with lignified secondary walls, or the walls of epidermal cells, which include large amounts of structural lipids, in the form of a cuticle.

The composition of cell walls varies among plant taxa, tissues, cell types, and during development, and can influence the quality of the edible plant tissue. For example, cell walls of various grain tissues and their components have important impacts on the end uses of the grain—in milling, water uptake in the conditioning step and the breakage pattern is influenced by the cell-wall organization and composition in the pericarp-seed coat tissues [36]. The profiles of major components also are generally known. In contrast, the architecture of the cell wall is less understood, although a number of models of their structure have been proposed. These models typically represent the wall structural components, their orientations, interactions, and often their relative abundances, which collectively provide a static view of the overall architecture [37]. While several models have common components, they differ in their bonding interactions and polysaccharide distributions. The most frequently cited models describe the cell wall as composed of two polysaccharide networks [38]. One comprises cellulose microfibrils cross-linked with hemicelluloses (most often xyloglucans or xylans): a simple analogy of this network is that of the steel-and-wire grids in a reinforced concrete slab, while the other network, the pectin polysaccharides, would be the concrete (Figure 16.14). A weakness of all models is that

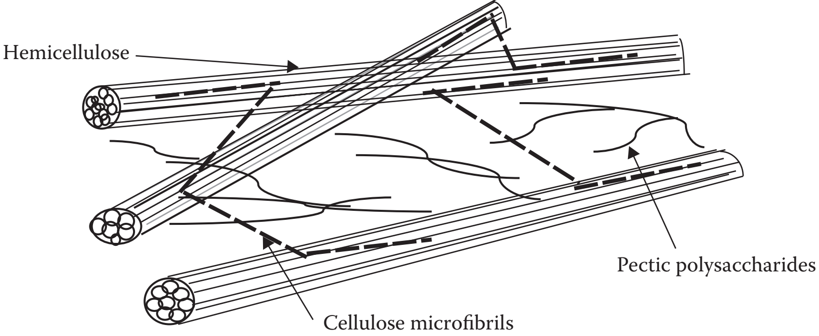


FIGURE 16.14 Idealized cell wall model showing the cellulose microfibrils, hemicellulose and pectic polysaccharides.

they do not reflect the dynamic nature of the cell wall where biosynthetic and degradative processes are occurring simultaneously, especially during plant growth.

While changes in the cell-wall composition occur during normal plant growth and senescence processes, the most dramatic changes are probably those associated with softening and texture changes that occur during the ripening of many fleshy fruits [39]. These changes are often associated with those of color, aroma, and nutritional attributes, which make ripened fruits attractive as food for humans, to animals for seed dispersal, and more subject to decay. Development of a soft edible texture in fruit is often desired by consumers, with examples including avocados, kiwifruit, and pears, although softening is more limited in fruit such as apples. How cells fail in response to applied stresses such as mastication can greatly affect sensory perception, a good example being a mealy versus crisp apple. If the middle lamella is weaker than the cell wall, the cells will separate from each other, while if the middle lamella is stronger than the cell wall, the cell walls will fail. In the former case, the tissue will taste mealy compared with juicy in the latter case.

Fruit texture is influenced by the structural integrity of the primary cell wall and the middle lamella, accumulation of storage polysaccharides such as starch, and the turgor pressure within cells resulting from osmosis. Changes in turgor pressure can have major effects on texture of some fruits (e.g., citrus), and hydrolysis of starch in mango and banana is associated with loosening of the cell wall structure. However, ripening-related changes in wall architecture generally involve degradation of the primary wall and middle lamella polysaccharides and alterations in the bonding between polymers, resulting in cell separation and wall swelling. Most polysaccharides undergo depolymerization, although the relative extent of pectin and hemicellulose degradation varies considerably between species, and it is not clear to what degrees cellulose is degraded. Similarly, there is some debate regarding which, if any, components of the wall are the first to undergo modification. Some reports suggest that the earliest events to be initiated are the loss of pectic galactan side chains and the depolymerization of matrix glycans, which may occur in the early ripening stages, followed by a loss of pectic arabinan side chains and pectin solubilization from the wall. The depolymerization of pectins may begin during early to mid-ripening, but is usually most pronounced late in ripening [39]. However, some of these events may be absent or occur to limited extents in some species. During the ripening of various fruits, pectin depolymerization is absent to low in apple, banana, melon, pepper, strawberry, papaya, and watermelon, but moderate to high in avocado, peach, and tomato. Pectin solubilization is absent to low in apple and watermelon, but moderate to high in avocado, banana, blackberry, kiwifruit, plum, and tomato. Loss of pectic galactan and arabinan side chains also vary by the fruit type [39].

In addition, cell-wall swelling may be related to a loosening of the hemicellulose–cellulose network and to pectin solubilization, and these processes combined with the loss of pectic side chains increase wall porosity. Indeed, it has also been proposed that one of the early events in ripening is a decrease in the hydrogen bonding between hemicelluloses and cellulose microfibrils. An increase in porosity of the walls as a result of pectin depolymerization may allow increased access of degradative enzymes to their substrates. The importance of cuticle properties as an additional factor affecting fruit firmness has become increasingly recognized [40,41]. Cuticles can influence water loss, and hence cellular turgor, as well as play a central role in sensing and interaction of the edible plant product with the surrounding environment.

A wide range of enzymes and nonenzymatic proteins mediate cell-wall changes during fruit ripening. One of the best studied in this regard is polygalacturonase (PG), which hydrolyzes demethylated HGA. Manipulation of activity by gene silencing or overexpression of the *PG* gene in tomato fruit has shown that it is not solely responsible for softening [19]. It is now clear that a suite of cell-wall-modifying enzymes is likely involved, including pectin methylesterases, pectate lyase, arabinanases and galactanases, and a spectrum of glycosidases, as well as the wall-modifying ("loosening") protein expansin, which appears to act nonenzymatically. The exact contribution of

each enzyme, or how they act synergistically, is not well defined. Moreover, their relative importance probably varies in accordance with the specific compositions of the cell walls and diverse array of textural states in fruits of different species, which in turn is associated with the difference in texture qualities of different fruits.

16.6.2.2 Soluble Carbohydrates

Soluble carbohydrates in edible plant tissues are primarily the monosaccharides glucose and fructose and the disaccharide sucrose, which are found in different amounts and proportions in fruits and vegetables (Table 16.9). Other soluble carbohydrates found in varying amounts in certain species include xylose, mannose, arabinose, galactose, maltose, stachyose, and raffinose. Sugar alcohols such as sorbitol, can occur in significant amounts in fruits of the Rosaceae family such as apple, peach, and cherry. Sucrose is the primary soluble carbohydrate transported into the cells from leaves and other photosynthetic tissues. Other translocated compounds include sorbitol in the Rosaceae, mannitol in celery, and raffinose and stachyose in squash and muskmelon. Soluble carbohydrates are also derived from storage carbohydrates and processes such as cell-wall breakdown. The soluble carbohydrates are used in metabolic processes such as glycolysis or for secondary metabolic pathways including accumulation of storage compounds such as starch. In addition, low molecular weight carbohydrates contribute to the characteristic flavor and quality attributes of many edible plant products.

The enzymes associated with metabolism of soluble carbohydrates are invertases (acid, alkaline, and neutral), sucrose synthase, and sucrose phosphate synthase. Invertases convert sucrose into glucose and fructose, sucrose synthase converts UDP-glucose and fructose into sucrose and UDP, while sucrose phosphate synthase converts UDP-glucose and fructose-6-P to sucrose-6-P and UDP. The role of these enzymes can shift depending on species, stage of development, and during postharvest storage (discussed in following section) [34].

TABLE 16.9
Major Soluble Sugars (mg/g FW) in Selected Fruits and Vegetables

Product	Sucrose	Glucose	Fructose
Asparagus	0.3–3.0	5.5–10	8.2–14
Cabbage	0.2–4.0	14–17	9–22
Carrot	34–45	1.0–11	3.9–15
Cucumber	*tr–1.0	6.7–12	8.0–12
Eggplant	*tr–4.2	14–20	14–20
Muskmelon	24–90	7.0–25	8.0–22
Onion – sweet	8.0–29	13–25	9.4–24
Potato	0.4–2.4	0.2–3.0	0.05–1.4
Spinach	*tr–1.0	0.1–1.2	0.1–5.1
Strawberry	5.0–16	20–22	23–26
Sweet cherry	4.4–20	61–161	54–102
Sweet potato	19–47	0.5–2.3	0.9–4.0
Table grape	0.7–29	55–77	68–85
Tomato	*tr–1.0	8.9–22	11–16

Sources: Modified from Maness, N. and Perkins-Veazie, P., Soluble and storage carbohydrates, in *Postharvest Physiology and Pathology of Vegetables*, Bartz, J.A. and Brecht, J.K., Eds, Marcel Dekker, New York, pp. 361–382, 2003; Lee, C.Y. et al., *NY Food Life Sci. Bull*, New York State Agricultural Experiment Station, Geneva, 1, 1–12, 1970; Paul, A.A. et al., *J. Human Nutr.*, 32, 335, 1978; Li, B.W. et al., *J. Food Comp. Anal.*, 15, 715, 2002.

* tr = trace.

16.6.2.3 Storage Carbohydrates

Starch is an insoluble homoglucan composed of two polymers of glucose, amylopectin and amylose, the major storage carbohydrates in edible plant tissues. Amylopectin and amylose (Figure 16.15) together form semicrystalline, insoluble granules with an internal lamellar structure [42] (Figure 16.16). Amylopectin is the major component of starch, typically making up 75% or more of the starch granule and is responsible for its granular nature. Amylopectin is a large, branched molecule, with glucosyl residues linked by α-1,4-bonds to form chains of between 6 and >100 glucosyl residues in length. The α-1,4-linked chains are connected by α-1,6-bonds (branch points). The lesser abundant amylose is a linear structure comprised only of α-1,4-D-glucose units. The semicrystalline structure makes up the bulk of the matrix of the starch granule and is highly conserved in higher plant starches [2].

As the principal storage carbohydrate, starch plays important roles during the life cycle of the plant. In leaves, a fraction of the carbon assimilated through photosynthesis is retained in the chloroplasts as starch rather than being converted to sucrose for export to the sites of growth or organs that serve as sinks to accumulate sugars and/or storage carbohydrate. This transitory starch is degraded at night to provide substrates for leaf respiration and for continued sucrose synthesis for export to the rest of the plant. In non-photosynthetic organs such as stems, roots, tubers, and

Amylose Amylopectin

FIGURE 16.15 Structures of amylose and amylopectin.

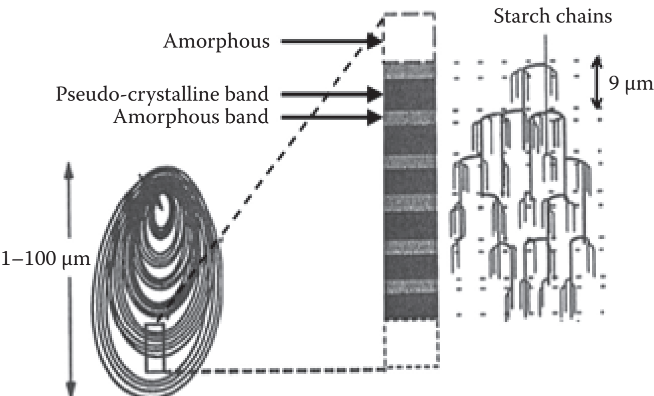


FIGURE 16.16 Organization of starch within a starch granule. (From Mishra, S. et al., Food structure and carbohydrate digestibility, in *Biochemistry, Genetics and Molecular Biology* "*Carbohydrates - Comprehensive Studies on Glycobiology and Glycotechnology*," C-F. Chang, Ed, InTech, Rijeka, Croatia, 2012.)

seeds, sucrose may be converted to starch for longer term storage, often to high levels, in specialized plastids termed amyloplasts. Many climacteric fruits such as apples and bananas also accumulate starch during maturation, and hydrolysis of this starch to sugars is an important part of the ripening process. Postharvest technologies can also lead to undesirable changes in sugar–starch balance. Potato storage at less than 10°C can cause transformation of some starch to soluble sugar, which results in undesirable darkening of chips and French fries via the Maillard reaction [43]. In sweet corn, warm storage temperatures can lead to net starch biosynthesis and diminution of soluble sugar levels, resulting in loss of desired sweetness.

In higher plants, starch is synthesized in plastids in both photosynthetic and nonphotosynthetic cells. Biosynthesis of both transitory and reserve starch is regulated by events involving interactions between metabolites and enzymes present in both the cytosol and plastids [2,44]. While aspects of starch biosynthesis are still being debated, classic models for photosynthetic and heterotrophic* cells are available [44,45].

In photosynthetic plant tissues, starch is the end product of a pathway directly linked to the Calvin cycle by means of plastid phosphoglucose isomerase (PGI), which exclusively takes place in the chloroplast. Photosynthetically synthesized triose-phosphate is exported to the cytosol and converted to sucrose, which is transported to heterotrophic parts of the plant. The mechanism of starch biosynthesis in both chloroplasts and amyloplasts has generally been considered to be a unidirectional and

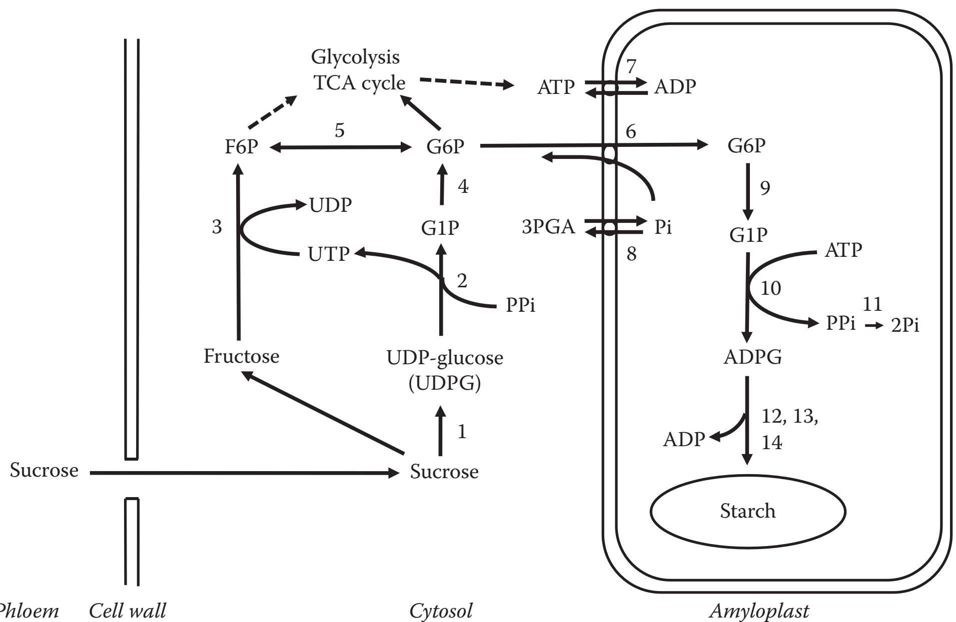


FIGURE 16.17 Schematic representation of the conversion of sucrose to starch in heterotrophic tissues of dicotyledonous plants. (1) Sucrose synthase (sucrose + UDP → UDP-glucose + fructose), (2) UDP-glucose pyrophosphorylase (UDP-glucose + PPi → G1P + UTP), (3) Fructokinase (fructose + UTP → F6P + UDP), (4) Cytosolic phosphoglucomutase (F1P → G6P), (5) Phosphoglucoisomerase (G6P → F6P), (6) Hexose phosphate translocator, (7) Adenylate translocator, (8) Triose phosphate translocator, (9) Plastidic phosphoglucomutase (G6P → G1P), (10) ADP-glucose pyrophosphorylase (ATP + G1P → PPi + ADP-glucose), (11) Alkaline pyrophosphate (PPi → 2Pi), (12) Granule-bound starch synthase (ADP-glucose + $(1,4\text{-}\alpha\text{-d-glucosyl})_n$ → ADP + (1,4-α-d-glucosyl)n+1), (13) Soluble starch synthase (ADP-glucose + (1,4-α-d-glucosyl)n → ADP + $(1,4\text{-}\alpha\text{-D-glucosyl})_{n+1}$), (14) Branching enzyme.

* Heterotrophic: requiring complex sources of N and C for metabolic synthesis from other tissues because it cannot synthesize its own.

vectorial process wherein ADPG pyrophosphorylase (AGPase) exclusively catalyzes the synthesis of ADPG and PPi, and acts as the major rate-limiting step of the gluconeogenic process.

In heterotrophic tissues, sucrose and UDP are transformed by sucrose synthase (SS) to produce UDPglucose (UDPG) and fructose. UDPG is then converted to glucose-1-P (G1P) by UDPG pyrophosphorylase (UGPase), and G1P is subsequently metabolized to glucose-6-phosphate (G6P) by means of the cytosolic phosphoglucomutase (PGM). G6P then enters the amyloplast, where it is converted to starch by the sequential activities of plastid PGM, AGPase, and starch synthase [44,45] (Figure 16.17).

Starch hydrolysis involves the conversion of the insoluble semicrystalline matrix formed by amylopectin to glucose and G1P, which can feed into intermediary metabolism. Degradative reactions take place in photosynthetic tissues where biosynthesis occurs and therefore requires metabolic coordination, while in heterotrophic tissues biosynthesis and hydrolysis processes are often temporally separated. Starch hydrolysis requires the coordinated action of several enzymes at the starch granule surface, the major ones being α-amylase, β-amylase, phosphorylase, and α-glucosidase (maltase) (Figure 16.18). α-Amylases hydrolyze the α-(1–4) linkages of amylose to release oligosaccharide fragments of ~10 glucose subunits (maltodextrins), which are more slowly hydrolyzed exhaustively to maltose [8]. α-Amylases also hydrolyze α-(1–4) linkages of amylopectin, but is not active in regions of the α-(1–6) branch points, leaving limit dextrins (2–10 glucosyl units). β-Amylase hydrolyzes maltose units (two glucose units) from the nonreducing end of starch chains to yield maltose and limit dextrins. Starch phosphorylase attacks the α-(1–4) linkages to form G1P, incorporating P_i. α-Glucosidase catalyzes the hydrolysis of maltose to glucose.

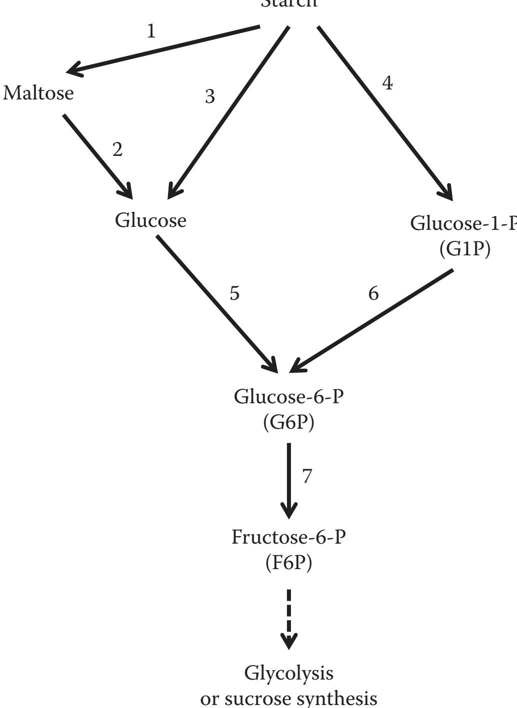


FIGURE 16.18 Pathways of starch breakdown. (1) β-amylase (starch + nH_2O → n maltose), (2) α-glucosidase syn. maltase (maltose + H_2O → 2 glucose), (3) α-amylase (starch → glucose), (4) starch phosphorylase (starch + n Pi → n G1P), (5) hexose kinase (glucose + ATP → G6P + ADP), (6) phosphoglucomutase (G1P → G6P), (7) phosphohexose isomerase (G6P → F6P).

Malic acid Tataric acid Citric acid Isocitric acid Oxalic acid

FIGURE 16.19 Structures of major organic acids found in edible plant tissues.

16.6.3 Organic Acids

The most common organic acids in edible plant tissues are mono-, di-, or tri-carboxylic acids (Figure 16.19). Organic acids may be in the free acid or conjugate base (anionic) forms, as salts or chelates with cations, or covalently combined with other metabolites to form esters or glycosides. Metabolic functions of organic acids are many, including as intermediates in the TCA cycle (Section 16.3.1), as metabolites in photosynthesis (phosphoglyceric acid), and as respiratory substrate where they are found in high concentrations to serve as a source of energy for postharvest cellular function.

Organic acids also accumulate in the vacuoles of edible plant tissues, especially in fruits where they contribute to flavor profiles and turgor pressure. In general, the accumulation of acids in vegetables is less pronounced. Organic acid accumulation is typically associated with less mature fruits, and decreasing acidity is a common feature of ripening. Exceptions exist, an example being the accumulation of acidity during on-tree ripening of sweet cherries [46]. The type of acid(s) and their relative proportion varies widely among and within fruit species, for example, a range from 0.4% to 1.7% and 0.7% to 1.6% in cultivars of tomato and plum, respectively [29]. Predominant acids in some edible plant tissues include malic acid in apples, apricot, artichoke, broccoli, cauliflower, onion, plum, nectarines, peach, loquat, pomegranate, and sweet cherry; citric acid in citrus fruits, tomato, persimmon, blueberry, strawberry, and mango; isocitric acid in blackberry; tartaric acid in avocado and grape; and oxalic acid in spinach and rhubarb. Tartaric acid is important in table and wine grapes, and the organoleptic properties and aging potential of wines are intimately linked to its concentration in the fruit, as well as that added during vinification. Other important organic acids include chlorogenic acid and ascorbic acid, discussed in relation to phenolics (Section 16.6.4) and antioxidative metabolism (Section 16.6.9.1), respectively.

Organic acids are largely responsible for the sensory perception of tartness and sourness in both fruits and vegetables. However, the balance between sugars and acids is often a more important factor in taste perception rather than absolute concentrations of either set of compounds alone. If sugar concentrations are high, the perception of acidity can be diminished. Citric acid can mask the perception of sucrose and fructose, while malic acid enhances sucrose perception [47].

Organic acids also exist as esters with alcohols to yield characteristic tastes and aromas of fruits and vegetables. Esters are especially responsible for the aroma of many fruits, including apples, pears, bananas, pineapples, and strawberries. An extensive range of esters are found, and the predominant forms in some fruits are ethyl, propyl butyl, amyl, hexyl, and isobutyl acetates, butyl and amyl butyrates, and butyl propionates in apple, and methyl and ethyl butanoates and hexanoates in strawberry. Some of these alcohol and organic acid components are derived from amino acid catabolism.

Malic acid synthesis occurs by the sequential action of phosphoenolpyruvate carboxylase and NAD-malate dehydrogenase, while degradation to pyruvate plus CO_2 is catalyzed by the NADP-malic enzyme. Interestingly, the patterns of malic acid accumulation and degradation do not correspond with the typical classification into climacteric or non-climacteric fruits or the overall changes

in respiration rates [15]. Certain types of climacteric fruits use malate during the respiratory burst, while others continue accumulating malate throughout ripening. Malic acid metabolism is important for transitory starch metabolism during fruit development [48].

Citric acid is synthesized by the condensation of acetyl CoA with oxaloacetate, catalyzed by citrate synthase in the mitochondria via the TCA cycle and stored in vacuole. Degradation of citric acid occurs mainly in the cytosol, catalyzed by a cascade of enzymes, including aconitase, isocitrate dehydrogenase, glutamate decarboxylase, and glutamine synthase. Citrate is sequentially metabolized to isocitrate, 2-oxoglutarate and glutamate, and glutamate utilized for glutamine production and catabolized through the γ-aminobutyrate (GABA) shunt (Section 16.6.5) [49]. In addition to isomerization by cytosolic aconitase, citrate can be degraded by ATP-citric lyase to oxaloacetate and acetyl-CoA in cell cytosol to support synthesis of amino acids, fatty acids, isoprenoids, and other metabolites [50].

Tartaric acid synthesis from ascorbic acid is a catabolic reaction that proceeds via the conversion of ascorbic acid to 2-keto L-idonic acid, with successive reduction to L-idonic acid and oxidation to 5-keto D-gluconic acid. In the penultimate step, 5-keto D-gluconic acid is cleaved between carbons 4 and 5 to yield the four-carbon L-threo-tetruronate, which is oxidized spontaneously to form tartaric acid [51].

16.6.4 Phenolics

Phenolic compounds are plant secondary metabolites that affect the appearance (color), taste, and flavor of edible plant tissues. Phenolics are also strong antioxidants due to the electron delocalization over the aromatic ring and their high redox potential, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [29]. Consequently, phenolics are recognized for their health-promoting properties such as anti-platelet, antioxidant, and anti-inflammatory activities [52].

There is a large variety in phenolic structure and occurrence, including very simple phenolics such as hydroxybenzoic acids, as well as large polymers such as condensed tannins and hydrolysable tannins with high molecular weights. Phenolic compounds include oleuropein and related compounds, hydroxybenzoic acid derivatives, cinnamates, flavonoids (flavones and isoflavones, flavanones,

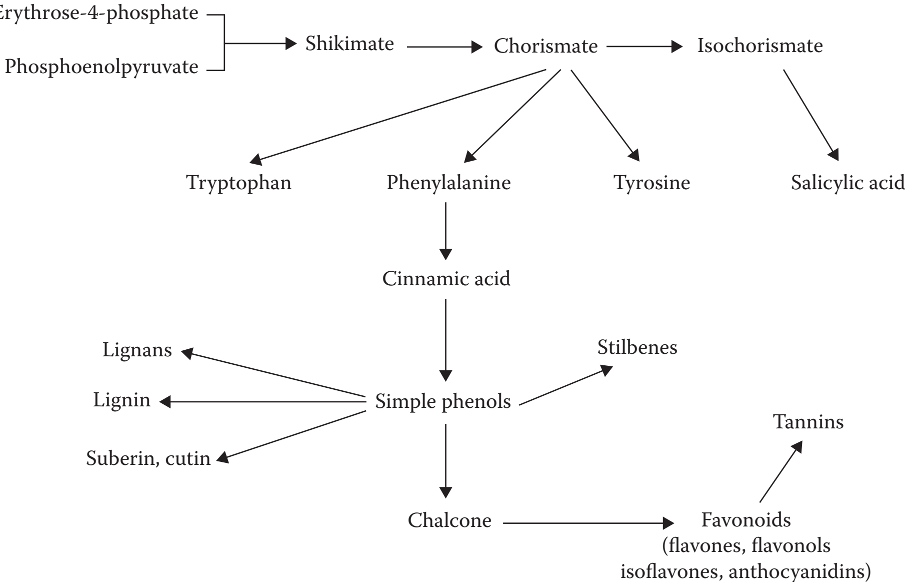


FIGURE 16.20 Simplified pathway of phenolic acid synthesis and derived compounds.

flavonols, and flavanols), lignans and stilbenes, anthocyanins, chalcones and dihydrochalcones, proanthocyanidins and tannin-like compounds, and ellagitannins.

Most phenolic compounds are synthesized from phosphoenolpyruvate (from glycolysis) and erythrose-4-phosphate (from the pentose phosphate pathway) through shikimate in the shikimic acid pathway. 3-Deoxyarabino-heptulosonate 7-phosphate is biosynthesized by the corresponding synthase, which is the key enzyme that controls the carbon flow into the phenolic metabolite pathway (Figure 16.20). The aromatic amino acid phenylalanine is deaminated by the enzyme phenylalanine ammonia-lyase (PAL), the key enzyme in phenolic biosynthesis. PAL catalyzes the non-oxidative deamination of L-phenylalanine to form *trans*-cinnamic acid and a free ammonium ion. This reaction is the first step in the biosynthesis of a large range of phenylpropanoid-derived secondary products in plants, such as flavonoids and isoflavonoids, coumarins, lignins, wound-protective hydroxycinnamic acid esters, and other phenolic compounds. Regulation of PAL activity therefore is important in modulating phenylpropanoid biosynthesis in plants.

The involvement of phenolics in postharvest physiology of edible plant tissues is extensive because of the aforementioned roles in appearance, taste, and flavor, and is discussed in Sections 16.6.7 through 16.6.9. However, an important additional involvement of phenolic compounds is in browning in fruits and vegetables. Enzymatic oxidation of phenolic compounds occurs by the action of polyphenol oxidase (PPO), which is mainly located in plastids in higher plants. PPO catalyzes two different reactions in the presence of molecular oxygen: the hydroxylation of monophenols to *o*-diphenols (monophenolase, cresolase, or hydroxylase activity) and the oxidation of *o*-diphenols to *o*-quinones (diphenolase, catecholase, or oxidase activity). The *o*-quinones nonenzymatically polymerize and give rise to heterogeneous black, brown, or red pigments commonly called melanins.

Melanins are usually detrimental to product quality. Examples include browning of cut surfaces by wounding either by inappropriate handling (e.g., bruising) or deliberate activities (fresh-cut products), physiological disorders such as senescent breakdown of apples, or injuries by inappropriate postharvest temperature or atmosphere management.

16.6.5 Proteins and Amino Acids

Proteins are critical to plant function as they regulate metabolism via their enzymatic functions in the cytoplasm, membranes, and cell walls. These enzymes are synthesized, activated, and/or degraded during the many normal cellular functions, as well as mediating responses to environmental changes, and changes in growth and development such as ripening and senescence. In addition to these catalytic proteins, others can have structural, regulatory, or storage functions. Proteins can also be lipoproteins (lipid prosthetic group), nucleoproteins (nucleic acid), metalloproteins (metal), or glycoproteins (carbohydrate).

Free amino acids (protein and non-protein) and their water-soluble derivatives can play a major role in the storage of nitrogen for subsequent growth and development by plants. For example, they can account for 5% or more of the dry weight of a legume seed, for example, broad bean (arginine), or as in the seeds of lentils (γ-hydroxyarginine). Plants store proteins in the embryo and vegetative cells to provide carbon, nitrogen, and sulfur resources that are critical to the life cycle of plants. Mechanisms for protein storage and mobilization serve many different developmental and physiological functions.

Storage proteins in cereals and grains are major contributors to human diets [53], but high-protein nuts such as peanuts, walnut, almonds, cashews, and Brazil nuts can also be important. While fruits typically have low protein concentrations, vegetables such as lentils, edamame, chickpeas, beans, green peas, corn, asparagus, and potatoes can have higher levels, though still relatively small percentages of the product composition.

Several types of non-protein nitrogen compounds are found in edible plant tissues, where, in addition to being a storage protein, they can act as a growth inhibitor and in defense against insects and herbivores. These compounds include

- Canavanine (2-amino-4-guanidinoxybutanoic acid) in beans [54].
- Glycine betaine and proline, which are two major organic osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity, and extreme temperatures [55]. Both compounds are thought to have positive effects on maintaining enzyme and membrane integrity along with adaptive roles in mediating osmotic adjustment in plants.
- Dopamine is found in a variety of food plants, with the highest concentrations in bananas, where the fruit pulp of red and yellow bananas contains concentrations of 40–50 ppm by weight [56]. Potatoes, avocados, broccoli, and Brussels sprouts may also contain dopamine at levels of 1 ppm or more, while oranges, tomatoes, spinach, beans, and some other plants contain less than 1 ppm.
- Alkaloids are nitrogenous organic compounds, the most well known in edible plant tissues being solanine [57]. This glycoalkaloid is toxic and contained in leaves, fruits, and tubers of nightshade plants (Solanaceae) such as tomato, potatoes, and goji berries.

Another non-protein amino acid, γ-aminobutyric acid (GABA), is found in a wide range of organisms. GABA has been characterized as a neurotransmitter or neuromodulator in the central nervous system of animals, but plays different roles in plant metabolism including carbon–nitrogen metabolism, energy balance, signaling, and development. GABA can accumulate under both biotic and

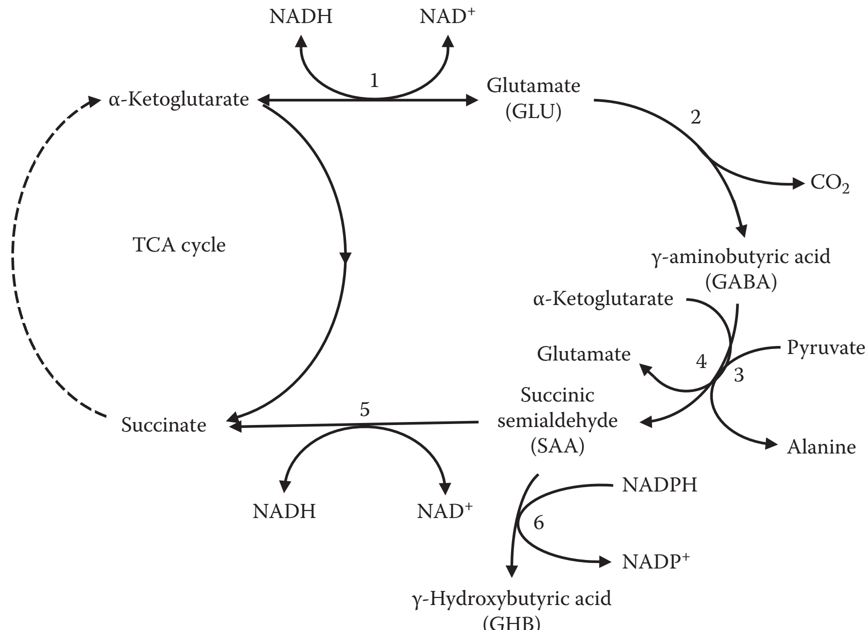


FIGURE 16.21 The γ-aminobutyric acid (GABA) shunt. (1) Glutamate dehydrogenase (α-ketoglutarate + NH_4^+ + NADH → GLU + NAD^+), (2) glutamate decarboxylase (GAD) (GLU → GABA + CO_2), (3) GABA transaminase-TP (pyruvate dependent; GABA + pyruvate → SAA + alanine), (4) GABA transaminase-TK (α-ketoglutarate dependent; GABA + α-ketoglutarate → SAA + glutamate), (5) succinic semialdehyde dehydrogenase (SAA + NAD^+ → succinate + NADH), (6) succinic semialdehyde reductase (SAA + NADPH → GHB + $NADP^+$).

abiotic stresses including anoxia, chilling, drought, salinity, and mechanical damage [58]. Recently, it has been found that GABA accumulates in fruits in response to postharvest treatments such as elevated CO_2 and during development of physiological disorders. Also, exogenous GABA treatments may inhibit physiological disorders such as chilling injury.

GABA is produced primarily via the GABA shunt (Figure 16.21). The GABA shunt is so intimately related to the TCA cycle that it might be considered a single entity with a major role in primary C/N metabolism [59]. Glutamate derived from the TCA cycle is decarboxylated to produce GABA and CO_2 via pH- and calmodulin-dependent glutamate decarboxylase (GAD) activity in the cytosol. GABA is then transported to the mitochondria. GABA is then catabolized first by the activity of GABA transaminase, which can use either pyruvate (-TP) or α-ketoglutarate (-TK) to produce succinic semialdehyde (SAA) and alanine or glutamate, respectively. SAA is then catabolized by the NAD^+-dependent enzyme succinic semialdehyde dehydrogenase to produce succinate and NADH, which are used for the mitochondrial respiratory pathway. Succinic semialdehyde dehydrogenase is highly sensitive to mitochondrial energy status, and activity would be inhibited under stress conditions in which the NAD^+: NADH ratios are low. SAA would accumulate, resulting in feedback inhibition of GABA transaminase activity. SAA is also catabolized to γ-hydroxybutyric acid (GHB) through SAA reductase activity. Production of SAA may function in stress tolerance through detoxification of SAA. Another pathway for the production of GABA is from polyamines by the oxidation of putrescine [58].

16.6.6 Lipids

Lipids are a diverse group of hydrophobic compounds comprised of long hydrocarbon chains and often ester linkages in the molecule. Lipids have critical functions in whole plants and detached tissues where they have structural and metabolic roles as components of cellular membranes, oil bodies, and cuticular waxes. There are four general types of lipids in plants: triacylglycerols, phospholipids, waxes, and isoprene-derived lipids (See Chapter 4). Triacylglycerols are comprised of three fatty acids esterified to a glycerol molecule, are the most energy-rich form of food reserve, and exist in tissues as oil droplets or lipid bodies. Membrane lipids are comprised primarily of diacylglycerol esters with the third OH group of glycerol esterified to a polar group such as a carbohydrate/sugar (yielding a glycolipid) or (organo) phosphate compound (yielding a phospholipid). The galactosyl-glycolipids are major and sometimes dominant lipids in the membranes of plastids (chloroplast, chromoplasts, amyloplasts); they are particularly abundant in grains such as wheat, and are believed to contribute to gluten development in doughs. Sterols and other isoprene-derived lipids such as tocopherols and carotenoids are found as part of membranes to provide necessary functionality for different tissue types. Cuticular lipids are a complex mix of hydrocarbons and esters of long-chain aliphatic acids (including diacids and oxygenated acids) and alcohols embedded in a lipid polymer called cutin. Waxes and cutin make up the cuticle, which regulates water and gas transmission between the plant and the environment. Also, while lipid concentrations are low in most edible plant tissues (Table 16.1), lipids represent storage compounds that can be used as a source of energy in fruits such as avocados and olives, cereal grains, and in seeds such as peanut and walnut [8]. Fats are more highly reduced than starch and provide almost twice the energy on a per-weight basis. When fatty acids are removed from glycerol, they can undergo β-oxidation to yield energy.

The lipid composition of membranes determines their physical properties and functionality, and has a major role in quality maintenance of edible plant tissues. Phospholipids, mainly phosphatidylcholine and phosphatidylethanolamine, comprise most of the lipid matrix in many plant cell membranes. The fluidity of the lipid bilayer is determined largely by the fatty acid composition and positional distribution of the phospholipids. Membranes have barrier properties to water and ions, maintain compartmentalization of various cellular functions, and contain enzymes specific to these cellular functions. Changes in the quality of harvested plant tissues are greatly affected by

membrane function, as catabolism of membranes is a critical part of senescence. Water loss and the consequent loss of turgor pressure, which results in wilting and undesirable texture changes, are in part attributable to increased permeability of the plasmalemma (cell membrane) and tonoplast (vacuolar membrane). Responses of tissues to low storage temperatures, and especially susceptibility to chilling injury, are strongly associated with lipid composition of membranes. Generally, plant tissues with more fluid membranes (greater degree of unsaturation) are able to withstand lower, nonfreezing temperatures.

Lipid peroxidation of membranes appears to be an integral part of ripening and senescence, and under stress conditions such as chilling-injury-inducing temperatures. Peroxidation can occur both as a part of the cascade of phospholipid catabolism and as a result of free-radical-mediated reactions initiated by reactive oxygen species. Lipid peroxidation and membrane deterioration occurs if the antioxidative defense systems are compromised, resulting in adverse effects on enzymatic activity and physical barrier properties of the membranes. These processes are similar for normal ripening, senescence, and in response to imposed stress conditions, and include one or more of the following: (1) a general decline in the glycerolipid fatty acid unsaturation due to lipid peroxidation and/or decreased unsaturation, (2) a change in the content and proportions of the phospholipid and galactolipid classes, (3) an accumulation of destabilizing lipid catabolites and peroxidation products, and (4) an increase in the level and/or changes in composition and conjugation of sterol lipids [60].

As components of waxes, cutin, and suberin, lipids also are critical components of plants and affect the storage behavior and shelf-life of many edible plant tissues. Waxes and cutin are found in the cuticle, provide protection from dehydration and pathogens, and influence gas diffusion from air to the inside of the product. Suberin is a lipid-derived polymeric material found on underground plant parts and on healed surface wounds. Like cutin, suberin is often embedded with waxes [8], and also provides protection from dehydration and pathogens.

16.6.7 Pigments

The pigments found in edible plant tissues include a wide array of compounds that have essential roles in photosynthesis and/or provide visual attraction for animals and insects. Four primary classes of pigments based on their chemistry are chlorophylls, carotenoids, flavonoids (particularly anthocyanins), and betalains.

Chlorophylls are the primary pigments in plants, the function of which is to capture light energy and convert it into chemical energy (carbon assimilation). It is the presence and relative abundance of chlorophyll that gives plants their green color. The green color of many leafy vegetables and some fruit, for example, green apples, is a major indicator of freshness and quality for the consumer. Therefore, postharvest technologies are designed to prevent loss of greenness. On the other hand, loss of green color and development of red, yellow, and orange coloration is a quality indicator for other products such as tomato, banana, pears, and citrus.

Carotenoids, which are yellow, orange, or red tetraterpenoids, function as accessory pigments in plants, helping to fuel photosynthesis by gathering wavelengths of light not readily absorbed by chlorophyll. Carotenoids also protect the chlorophylls from photooxidation and have other important functions in plants, such as precursors to abscisic acid (ABA). Plants, in general, contain six ubiquitous carotenoids: neoxanthin, violaxanthin, antheraxanthin, zeaxanthin, lutein, and ß-carotene, together with the main two chlorophylls (Chl) Chl a and Chl b. The most familiar carotenoids in plants are ß-carotene (an orange pigment), lutein (a yellow pigment found in fruits and vegetables and the most abundant carotenoid in plants), and lycopene (the red pigment responsible for the color of tomatoes). The pigment composition and the resulting color are characteristic of the species and cultivar, but carotenoid composition and relative abundance in green leaf tissues of plants is somewhat conserved, probably because of the need for optimal function of photosynthesis.

Flavonoids include the water-soluble red or blue anthocyanins and pale yellow compounds such as rutin, quercitin, and kaempferol. Anthocyanins (see Chapter 10), the largest and most diverse group of

TABLE 16.10
Major Anthocyanins and Carotenoids in Various Edible Plant Tissues

Anthocyanin	Fruit or Vegetable
Pelargonidin 3-glucoside	Strawberry, sarsaparilla
Cyanidin 3-rutinoside	Sweet cherry
Cyanidin 3-glucoside	
Cyanidin 3-glucoside	Plum, blackberry, pomegranate
Peonidin 3-glucoside	Grapes
Cyanidin 3-glucoside	
Malvidin 3-glucoside	
Malvidin 3-glucoside	Blueberry
Carotenoid	
Capsanthin	Red pepper
Lycopene	Tomato, watermelon, papaya, guava, Deep Red and Star Ruby pummelo
β-Carotene	Peach, nectarine, plum, loquat, apricot, Mexican lime, citron, carrot, sweet potato
β-Cryptoxanthin	Mandarin, lemon, Rangpur lime
Violaxanthin	Chandler pummelo, orange

Source: Modified from Valero, D. and Serrano, M., *Postharvest Biology and Technology for Preserving Fruit Quality*, CRC Press, Boca Raton, FL, 2010.

plant pigments, are derived from the phenyl propanoid pathway. The color of anthocyanins is affected by pH in the host tissue. Anthocyanins occur in all tissues of higher plants, providing color in leaves, plant stem, roots, flowers, and fruits, though not always in sufficient quantities to be noticeable.

Betalains are red or yellow pigments. Like anthocyanins they are water-soluble, but unlike anthocyanins the base structure is synthesized primarily from tyrosine and other amino acids. This class of pigments is found only in the Caryophyllales, and never co-occurs in plants with anthocyanins. Betalains are responsible for the deep red color of beets, and are used commercially as food-coloring agents.

Examples of major carotenoids and anthocyanin species in edible plant tissues are shown in Table 16.10. Breeding and selection by humans have had a major influence on the composition of edible plant tissues. Examples include carrot, which had white roots, lacking carotenoids and having only traces of lutein and other carotenoids before domestication. Intensive breeding has generated the currently known carotenoid-rich varieties, including the widely popular orange carrots that accumulate high levels of the pro-vitamin A carotenoids β-carotene and, to a lesser extent, α-carotene [61]. The color of purple carrots is caused by the accumulation of anthocyanins, although these cultivars also accumulate carotenoids. A large diversity of citrus types and external color from the green of limes to the yellow of lemons, orange in mandarins and sweet oranges, and pink in red grapefruits exist. These colors typically reflect different compositions of chlorophylls and carotenoids, although anthocyanins provide a red to purple tint, in the flesh of a specific group, blood oranges [62]. Traditional and biotechnological approaches are being taken to increase concentrations of pigments such as anthocyanin, betalain, and lycopene in a variety of fruits and vegetables [63,64].

16.6.8 Volatile Organic Compounds

Plant volatile organic compounds (VOCs) are secondary metabolites that play important roles in biotic interactions and in abiotic stress responses. These volatile compounds contribute to the aroma of many vegetables, but are mostly recognized for their role in fruits. In climacteric fruits, VOCs typically increase during the onset of ripening and peak either at or shortly before full ripening, and

biosynthesis of many volatiles is regulated by ethylene. Accumulation of VOCs in fruits is presumably associated with seed dispersal in a similar manner to color development, but is an important facet of quality for human consumers where they interact with the olfactory epithelium in the nose to produce species-characteristic aroma sensations.

Fruits and vegetables can contain hundreds of volatiles, but these are not necessarily perceived by the consumer. Differences in number and concentration of VOCs can be cultivar-specific within a genotype (e.g., apple) [65]. There are complex interactions between nonvolatile and volatile compounds, other taste sensations (sweet, sour, salty, bitter), and the balance of reactions that produce and degrade them when macerated and/or cooked. A relatively few VOCs are flavor-impact compounds, and the aroma thresholds (the concentration below which no aroma is perceived) vary enormously. For example, the aroma threshold for butyl ethanoate is 5000 ppb, but is only 0.13 ppb for its isomer ethyl butanoate.

Important VOCs in vegetables include phthalides in celery, thiopropanal *S*-oxide in fresh onions, 2-propenyl isothiocyanate in cabbage, and 2,5-nonadienal in cucumber [66]. Apples produce a complex mixture of over 200 volatile compounds, including alcohols, aldehydes, ketones, sesquiterpenes, and esters. Esters are associated with "fruity" attributes of fruit flavor and typically increase to high levels late in the ripening process. Butyl acetate, hexyl acetate, and 2-methylbutyl acetate dominate the flavor of ripe fruit, with the latter two being identified by analytical sensory panels as having the greatest impact on the attractiveness of the fruit.

The biosynthesis of VOCs is variable across different fruits and vegetables, but tomato can be used to illustrate several key pathways that have been identified and are shared among most edible fruits. The concentration of most, although not all, of the flavor volatiles in a tomato increases at the onset of ripening and peak either at or shortly before full ripening, suggesting that synthesis of flavor volatiles is highly regulated [67]. VOCs can be derived from carotenoids, fatty acids, terpenoids, and amino acids.

1. *Carotenoid-derived volatiles*: Among the most important VOCs in tomato fruits are apocarotenoids, which are derived from carotenoids such as β-ionone, 6-methyl-5-hepten-2-one, geranylacetone, and β-damascenone. Those carotenoid-derived VOCs are known to be produced by nonenzymatic oxidative cleavage of linear and cyclic carotenoids or by the cleavage action of carotenoid dioxygenase. Given their linkage to carotenoids, their abundance is well correlated with fruit ripening and they are highly abundant in red tomato fruits.
2. *Fatty acid–derived volatiles*: The odorous lipid breakdown products, such as *cis*-2-penten-1-ol, *trans*-2-pentenal, *cis*-3-hexanal, *trans*-2-hexanal, and *trans*-2-heptenal, are among the most abundant aroma volatiles in tomato fruits. Hexanals are formed from the 13-hydroperoxides of linoleic-related fatty acids and generated by the coordinated and sequential reactions of a lipoxygenase, lyase, and isomerase. *cis*-3-Hexanal is associated with the fresh green aroma of tomato and it has an exceptionally low sensory threshold of 0.25 ppb.
3. *Terpenoid volatiles*: The major class of terpenoid VOCs is represented by lipophilic mono-, sesqui-, and diterpenoids, which are derived either from geranyl diphosphate or *trans*-farnesyl diphosphate. These compounds are generally volatile. Tomatoes are rich in terpenoids. During early fruit development, the cytosolic pathway of terpenoid biosynthesis is operational, producing the glycoalkaloids and sterols, and during ripening, the activity of the plastidic terpenoid pathway increases. However, ripe tomato fruits contain only minute quantities of monoterpenes and sesquiterpenes. Citrus essential oils are particularly rich in terpenoids.
4. *Amino acid–derived volatiles*: Tomato fruit volatiles are also derived from amino acids. Several branched chain amino acids and aromatic amino acids are associated with senescence, while those derived from phenylalanine, such as guaiacol, MeSA, and eugenol, also contribute to the aroma of tomato fruits.

16.6.9 Vitamins and Health-Promoting Substances

The importance of vitamins in edible plant tissues in promoting good health and preventing or alleviating disease has long been known. The nutritional factors provided in significant amounts to human diets by edible plant tissues are water-soluble vitamins B_1 (thiamine), B_2 (riboflavin), B_6 (pyridoxine), B_{12}, niacin, biotin, folic acid, pantothenic acid, and vitamin C (ascorbic acid). All of these are not produced by humans (except some niacin) and therefore plant-based diets are essential for maintaining human health. In addition, there has been increasing emphasis of other antioxidants such as tocopherols (nutritionally required), flavonoids, carotenoids, and glucosinolates. Edible plant foods are also the exclusive source of dietary fiber for humans (see Section 3.4 for a detailed account). Fruits and vegetables have received recognition as "chemopreventors" and "functional foods."

16.6.9.1 Ascorbic Acid (Ascorbate, Vitamin C)

L-Ascorbic acid is a water-soluble antioxidant that is structurally related to C_6 sugars ($C_6H_8O_6$), being an aldono-1,4-lactone of a hexonic acid (Figure 16.22) [68]. It is one of the most important nutritional quality factors in many horticultural crops and has many biological functions in the human body. It is widely accepted that the predominant pathway for the biosynthesis of ascorbic acid is via GDP-mannose and L-galactose. D-Galacturonic acid, D-glucuronic acid, and GDP-L-gulose could be minor ascorbate precursors [69]. However, humans and some other vertebrae are unable to synthesize ascorbic acid due to the lack of the l-glucono-1,4-lactone oxidase enzyme. Around 90% of vitamin C in the human diet is derived from fresh vegetables and fruits to meet needs such as maintenance of cartilage, bones, gums, skin, and teeth, and as powerful reducing

Ascorbic acid Dehydroascorbic acid

FIGURE 16.22 Structures of ascorbic acid and dehydroascorbic acid.

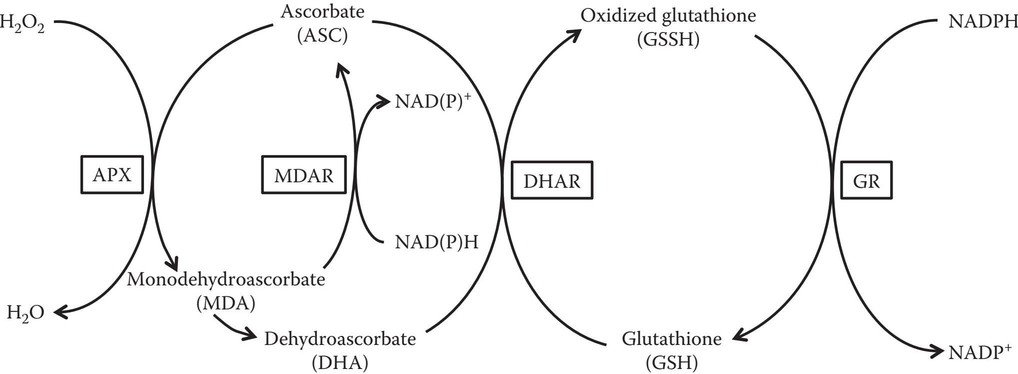


FIGURE 16.23 The ascorbate–glutathione cycle. Hydrogen peroxide (H_2O_2) is reduced to H_2O by ascorbate peroxidase (APX) activity using ascorbate (ASC) as the electron donor to produce monodehydroascorbate (MDA). ASC is regenerated from MDA by monodehydroascorbate reductase (MDAR) activity using NAD(P)H. Any MDA that is not reduced disproportionates to ASC plus dehydroascorbate (DHA). DHA is reduced to ASC by dehydroascorbate reductase (DHAR) activity at the expense of glutathione (GSH), yielding oxidized glutathione (GSSG). Finally GSSG is reduced by glutathione reductase (GR) using NADPH as the electron donor.

agent against oxidative stress–related diseases including cancers, cardiovascular disease, aging, and cataract formation [68].

In plants, ascorbic acid functions as an enzyme cofactor, a radical scavenger, and a donor/acceptor in electron transport either at the plasma membrane or in the chloroplasts, and it serves as the substrate for oxalate and tartrate biosynthesis in species such as grapes. The primary function of ascorbic acid is to detoxify hydrogen peroxide (H_2O_2) produced by metabolism during photosynthesis and especially in stress conditions. An intrinsic feature during fruit ripening is the increased production of reactive oxygen species (ROS). Recent studies also reveal that ROS are central players in the complex signaling network of cells, and acquire dynamic and specific roles in signaling. ROS homeostasis is maintained by a system of redox system enzymes. Ascorbic acid contributes ROS detoxification via a four-step biochemical pathway, known as the ascorbate–glutathione cycle or the Foyer, Halliwell, Ashada cycle (Figure 16.23) [70]. Several enzymes are involved in the cycle, such as ascorbate peroxidase oxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). Under normal metabolic conditions and in response to survivable stresses, these reactions maintain a high redox state of ascorbate (ASA/DHA) and glutathione (GSH/GSSG). Other critical antioxidant enzymes include peroxidases, superoxide dismutase, and catalase.

Concentrations of ascorbic acid in grain staples are low, especially in comparison with fruits and vegetables. Ascorbic acid concentrations in edible plant tissues can be influenced by various factors such as genotypic differences, preharvest climatic conditions and cultural practices, maturity and harvesting methods, and postharvest handling procedures [71]. In general, postharvest treatments that maintain quality, such as low storage temperatures and avoidance of water loss, slow down the loss of ascorbic acid.

16.6.9.2 Vitamin A

Vitamin A is the generic descriptor for lipid-soluble compounds with the qualitative biological activity of retinol (retinols and some carotenoids). Vitamin A is an isoprenoid with a 6-carbon cyclic ring and an 11-carbon side chain (see Section 8.7.1 for more details). It is extremely important to human nutrition, as its synthesis is dependent on plant-based sources of carotene. Vitamin A is formed in the intestinal mucosa by the cleavage of carotene, and because retinol is not present per se in plants, the provitamin A carotenoids are considered to be the vitamin A functional group recognized as international units (IU) of α- and β-carotene, 1.2 and 0.9 μg, respectively [8,72]. Deficiency of this vitamin is common, afflicting millions of children each year with xerophthalmia, blindness, or death. Genetic engineering of the β-carotene biosynthetic pathway in rice endosperm to develop Golden Rice has the potential to greatly improve human health but is the subject of regulatory concerns (see Section 16.8.2).

Leafy vegetables and fruits average 5000 IU and 100–500 IU vitamin A per 100 g fresh weight, respectively, but mango and papaya contribute greater levels (3000 and 2500 IU per 100 g fresh weight, respectively) [8]. With the exception of Golden Rice and sweet potato (2500 IU per 100 g fresh weight), staple crops have negligible levels. The stability of carotenoids is generally greater than ascorbic acid [72] during postharvest storage, but can be affected by genotype, prestorage conditions, and nonoptimal storage temperature and humidity.

16.6.9.3 Phytochemicals

Phenolic compounds are strong antioxidants (Section 16.6.4), and they are recognized for their health-promoting activity for antioxidant and other biological effects. In fruits such as the apple, the contribution of ascorbic acid to overall antioxidant activity is small (0.4%). It has been suggested that most of the antioxidant activity of some fruits and vegetables may come from phenolics and flavonoids and that the additive and synergistic effects of phytochemicals in fruit and vegetables are responsible for their potent antioxidant and anticancer activities [73]. In general, phenolic compounds are stable during storage and processing, unless PPO activity results in their oxidation.

16.6.9.4 Sulfur Compounds

Onions and other *Allium* vegetables have flavor precursor compounds that are secondary metabolites involved in the biosynthesis and metabolism of cysteine and glutathione in essential pathways for the uptake of sulfur and detoxification. The major compounds are γ-glutamyl-*S*-alk(en)yl-L-cysteines and *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs). Hydrolysis of the cytoplasmic-located ACSOs with vacuolar alliinase activity during mastication or homogenization produces pungent sulfur compounds and the by-products pyruvate and ammonia [10]. Cruciferous vegetables (*Brassicaceae*, syn. *Cruciferae*) contain glucosinolates that are transformed to isothiocyanates and indoles, which are thought to be health-promoting compounds. The active compounds are not present in intact products, but mastication or homogenization results in hydrolysis by myrosinase. ACSOs and glucosinolates may increase during storage, but generally decrease as the product quality deteriorates [6].

16.7 POSTHARVEST TECHNOLOGIES

16.7.1 Storage Temperature

The most fundamental postharvest tool available to maintain quality of edible plant tissues is temperature control. Temperature has a profound effect on the rates of biological reactions [11]. Therefore, for each edible plant tissue, it is critical to cool these products as quickly as possible after harvest to slow down metabolic activity. Benefits of lower temperatures include reduced respiration rates, reduced water loss, reduced sensitivity to ethylene, and decreased susceptibility of products to physiological and microbial decay. A major exception to low storage temperatures is curing, which is used for onions to dry the neck and outer scales, and for potatoes to develop wound periderm over damaged surfaces. Curing is usually carried out at ambient conditions in the field or at temperatures from 7°C to 16°C in curing rooms.

Low storage temperature can maintain the appearance of edible plant tissues, including the stability of nutritionally important compounds such as anthocyanins, carotenoids, and ascorbic acid, as well as preserve color and retard the rate of undesirable softening and texture changes [6,74]. Flavor and aroma compounds are often higher in freshly eaten products rather than cold-stored (e.g., tomato) [75], but the interplay of flavor compounds with sugars, acids, and tissue matrices is complex and results in varied responses to storage.

Cooling methods are customized to the product type and scale of operation. These methods include room or passive cooling, forced-air cooling, hydro-cooling, package icing, and vacuum cooling. Full details of each are available from many books and web-based sources [76,77] and will not be described here. However, regardless of cooling method, the cooling rate of products follows standard laws of physics in that it becomes progressively slower to cool products down over time as the temperature differential between product and cooling medium diminishes. The typical cooling curve for an edible plant tissue in a cold storage room (Figure 16.24) illustrates the concept of "half-cooling" or "seven-eighths-cooling" times. Half-cooling refers to the time taken to reduce the initial product temperature from that when first placed in the cold room halfway to the set temperature in the room. For example, if the product temperature was 20°C when placed in the room and air temperature in the room was set at 0°C or 10°C, half cooling would refer to the time that it took for the product temperature to reach 10°C and 15°C, respectively. It will then take the same time period to reduce the product temperature by half again, and so on. So, seven-eighths cooling is three times as long as half-cooling.

Decreasing temperatures over the physiological range (0°C–30°C) of most crops causes an exponential decrease in respiration. The van't Hoff Rule states that the velocity of a biological reaction decreases two- to threefold for every 10°C decrease in temperature. The temperature quotient for a 10°C interval is called the Q_{10}, which is calculated by dividing the reaction rate at a higher temperature by the rate at a 10°C lower temperature: $Q_{10} = R_2/R_1$. The temperature quotient is useful

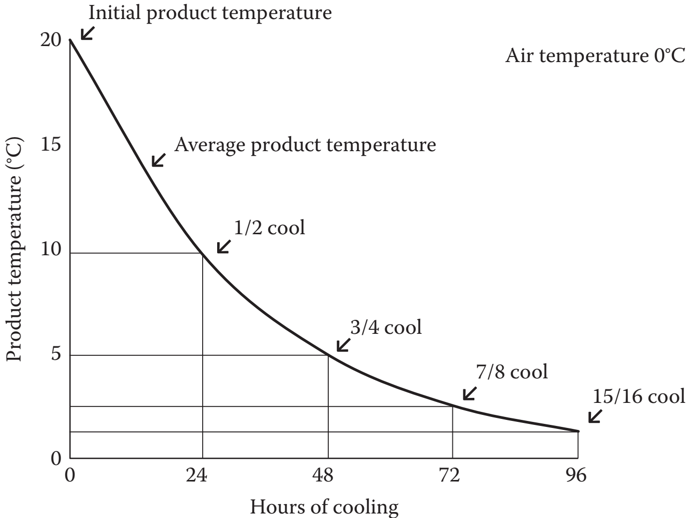


FIGURE 16.24 A typical cooling pattern for an edible plant tissue in a cold room. In this example, a product at 20°C is placed in a cold room with air temperature of 0°C. The time to reduce the temperature of the product by 50% (i.e., to 10°C) is known as the "half cooling."

because it allows us to estimate potential shelf-life gains by the extent of cooling based on reduction in respiration rates. However, the respiration rate does not follow ideal behavior, and the Q_{10} is usually smaller at higher than at lower temperature ranges.

Typical values for Q_{10} at various temperatures range 2.5–4.0 from 0°C to 10°C; 2.0–2.5 from 10°C to 20°C; 1.5–2.0 from 20°C to 30°C, and 1.0–1.5 from 30°C to 40°C [11]. The Q_{10} values can be used to demonstrate the effects of different temperatures on the rates of respiration or deterioration and relative shelf-life of a typical perishable commodity (Table 16.11). This table shows that a product with a storage life of 100 days at 0°C has a storage life of 13 days at 20°C, and only 4 days at 40°C.

TABLE 16.11
Effect of Temperature on Respiration Rate and Relative Storage Life of Edible Plant Tissues Based on Q_{10}

Temperature (°C)	Assumed Q_{10}	Relative Velocity of Respiration	Relative Storage Life
0		1.0	100
	3.0		
10		3.0	33
	2.5		
20		7.5	13
	2.0		
30		15.0	7
	1.5		
40		22.5	4

Source: Kader, A.A. and Saltveit, M.E., Respiration and gas exchange, in *Postharvest Physiology and Pathology of Vegetables*, Bartz, J.A. and Brecht, J.K., Eds, Marcel Dekker, New York, 2003, pp. 7–30.

TABLE 16.12
Edible Plant Tissues Classified according to Sensitivity to Chilling Injury

Non-Chilling-Sensitive	Chilling-Sensitive
Apples*	Avocado
Apricots	Banana
Asparagus	Bean, snap
Beans, Lima	Cantaloupe
Beets	Cranberry
Blackberries	Cucumber
Blueberries	Eggplant
Broccoli	Lemon
Cauliflower	Lime
Celery	Mango
Corn, sweet	Muskmelons
Cherries	Orange
Currants	Papaya
Garlic	Pepper
Grapes	Pineapple
Mushrooms	Potatoes
Onions	Pumpkins
Parsley	Squash
Peaches*	Sweet potatoes
Raspberries	Tomatoes
Spinach	Watermelons
Strawberries	Yams
Turnips	Zucchini

Source: Modified from Kader, A.A., *Postharvest Technology of Horticultural Crops*, Regents of the University of California, Division of Agricultural and Natural Resources, Oakland, CA, 2002.

* Some cultivars are chilling sensitive.

In general, the lower the storage temperature, the longer the storage life of a given commodity, as long as the freezing temperature for the product is not reached. However, the lowest safe storage temperature is not the same for all products, as many of them are sensitive to low temperatures and develop chilling injuries. Products can be categorized as chilling-sensitive or chilling-insensitive (Table 16.12). Chilling-sensitive products are often subtropical and tropical in origin, but the specific product, maturity and degree of ripeness at harvest, and length of exposure to specific low temperatures affect responses of different products to cold temperatures. Examples of differences among product types include bananas, which are extremely chilling-sensitive if stored below 12.5°C for a few days, while honeydew melons require weeks to show chilling symptoms at 5°C. Maturity and the degree of ripeness are also important factors in conferring chilling sensitivity in fruits such as avocados, honeydew melons, and tomatoes. Riper fruits are less sensitive to chilling injury, especially if a symptom of damage is failure to ripen. Also, damage may occur in a short time if the temperatures are considerably below the threshold level, but take a longer time to express if the product is just under the minimum safe temperature. For example, it is common for tomatoes to be kept in the refrigerator despite their chilling sensitivity, but unless the time period

TABLE 16.13
Selected Edible Plant Tissues Susceptible to Chilling Injury When Stored at Low but Nonfreezing Temperatures

Edible Plant Tissue	Lowest Safe Temperature (°C)	Injury Symptoms
Apple—certain cultivars	2–3	Internal browning, brown core, soggy breakdown
Asparagus	0–2	Dull, gray-green, limp tips
Avocado	4.5–13	Grayish-brown discoloration of flesh
Bananas	11.5–13	Dull color when ripened
Bean (lima)	1–4.5	Rusty brown specks, spots or areas
Bean (snap)	7	Pitting and russeting
Cranberries	2	Rubbery texture, red flesh
Cucumbers	7	Pitting, water-soaked spots, decay
Eggplant	7	Surface scald, Alternaria rot, blackening of seeds
Guavas	4.5	Pulp injury, decay
Grapefruit	10	Scald, pitting, watery breakdown
Lemons	11–13	Pitting, membranous staining, red blotch
Limes	7–9	Pitting, turning tan with time
Lychee	3	Skin browning
Mango	10–13	Grayish scald-like discoloration of skin, uneven ripening
Pineapple	7–10	Dull green when ripe, internal browning
Potato	3	Mahogany browning, sweetening
Pumpkins and hardshell squash	10	Decay, especially Alternaria rot
Sweet potato	13	Decay, pitting, internal discoloration, hardcore when cooked
Tomato—ripe	7–10	Water soaking and softening, decay
Tomato—mature green	13	Poor color when ripe, Alternaria rot
Watermelon	4.5	Pitting, objectionable flavor

Source: Modified from Kader, A.A., *Postharvest Technology of Horticultural Crops*, Regents of the University of California, Division of Agricultural and Natural Resources, Oakland, CA, 2002.

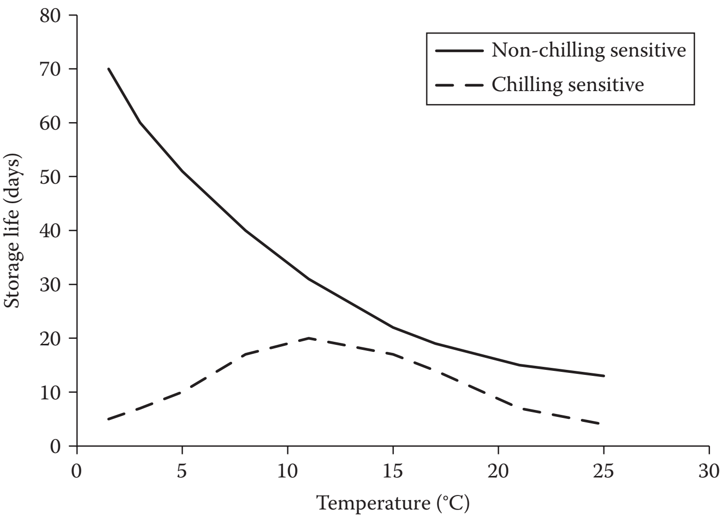


FIGURE 16.25 Relative storage lives of chilling and non-chilling sensitive products.

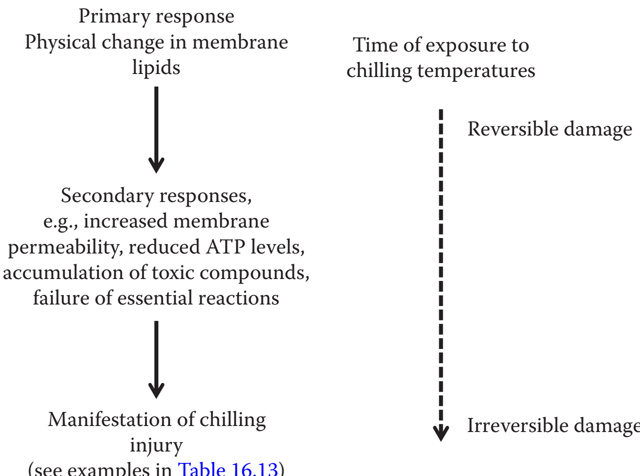


FIGURE 16.26 A simplified scheme of the responses of chilling sensitive plant tissues to chilling stress.

is extended, chilling injury is not detected. Also, storage recommendations can be complex; for example, fruits such as peach develop chilling injuries at a slower rate at 0°C than at temperatures between 4°C and 10°C, and therefore the lower storage temperature is recommended. Postharvest strategies that can alleviate chilling injury of edible plant tissues include modified and controlled atmosphere storage, heat treatments, temperature preconditioning, and intermittent warming [78].

The optimum temperature for storage is higher for chilling-sensitive than for non-chilling products. Examples of safe storage temperatures for several fruits and vegetables are provided in Table 16.13, as well as a range of chilling injury symptoms. Injury symptoms can be manifested in many ways, including irregular ripening, failure to ripen, water-soaked appearance, skin discoloration, mealiness, pits on the skin surface, and increased susceptibility to decay.

Figure 16.25 illustrates the differences between the responses of non-chilling-sensitive and chilling-sensitive fruits and vegetables to storage temperature. In non-chilling-sensitive products, the longest storage life is associated with the lowest nonfreezing storage temperature. However, for chilling-sensitive products, the storage life increases with decreasing storage temperature to reach a maximum from 7°C to 18°C depending on the product. At lower temperatures, the storage life decreases because of susceptibility to chilling injuries.

The physiological basis for chilling injury development has long centered on physical changes in membranes in which the molecular ordering of membrane lipids is altered (liquid to gel phase transition for some lipid species) in the temperature range where chilling effects become apparent. Subsequent secondary events occur and, if the chilling event is limited in intensity and exposure, the plant tissues can recover (Figure 16.26). Support for this hypothesis includes evidence that tropical species tend to have lipids with a higher proportion of saturated fatty acids (e.g., palmitic acid, which lacks double bonds and therefore have higher melting points), while cool-climate plants tend to have more unsaturated fatty acids such as oleic, linoleic, and linolenic acids. However, a consistent pattern of differences in lipid membrane composition between chilling-susceptible and chilling-resistant plants has yet to emerge, and no single physiological factor has been linked with plant susceptibility to chilling injury [79]. Mechanisms that may be involved in greater resistance to chilling injury, especially those resulting from postharvest strategies such as heat treatments, include enhancement of membrane integrity by the increase of unsaturated fatty acid/saturated fatty acid ratio; enhancement of heat shock protein gene expression and accumulation; enhancement of the antioxidant system activity; enhancement of the arginine pathways which lead to the accumulation of signaling molecules with pivotal roles in

improving chilling tolerance such as polyamines, nitric oxide, and proline; alteration in phenylalanine ammonia-lyase and polyphenol oxidase enzyme activities; and enhancement of sugar metabolism [80].

Another form of damage that can occur is freezing injury, which occurs if edible plants are exposed (usually inadvertently) to temperatures below the freezing point. Freezing injury results from the formation of ice crystals and the destruction of cell integrity during thawing. As a consequence, injury is expressed most commonly as a water-soaked appearance, associated with loss of cellular structure and turgor. The susceptibility of different fruits and vegetables to freezing injury varies widely [81]. The exact temperature below 0°C at which the product will freeze depends on the amount of sugars or other solutes present and the corresponding freezing point depression. For example, lettuce with a low sugar content may freeze at −0.2°C, while plums with a high sugar content may freeze at −1.7°C or lower. Some commodities may be frozen and thawed a number of times with little or no injury, whereas others are permanently injured by even a slight freezing. All fruits and vegetables can be categorized into three groups based on their sensitivity to freezing: most susceptible—likely to be injured by even one light freezing; moderately susceptible—will recover from one or two light freezing periods; and least susceptible—those that can be lightly frozen several times without serious damage.

The nature of freezing damage and the effects on metabolism and chemistry of edible plant tissues resemble outcomes conferred by other types of physical damage, which are not very important in the context of this chapter. However, slow warming of frozen tissues can sometimes result in partial recovery of the product, though typically the subsequent storage life of the products will be compromised.

16.7.2 Relative Humidity

Relative humidity (RH) is defined as the ratio of water vapor pressure in the air to the saturation vapor pressure at the same temperature. Water loss during postharvest handling and storage is a function of product permeability, temperature, and the vapor pressure deficit between the internal tissues of the product and the surrounding atmosphere [74]. The RH directly affects the storage quality of products. Cereal grains should be maintained under low humidity conditions, but for most products excessive water loss results in wilting, shriveling, flaccidness, soft texture, and loss of nutritional value as well as saleable weight. The RH around most fruits and vegetables should be kept high, and while RH approaching 100% can encourage growth of microorganisms and splitting of skin surfaces, it is usually more of a problem to maintain sufficiently high humidity than the opposite. Nevertheless, interactions between RH and temperature mean that recommended regimens for storage of edible plant tissues can represent compromises among their physical, physiological, and pathological responses.

16.7.3 Modified and Controlled Atmosphere Storage

Modified atmosphere (MA) storage refers to a change in the atmosphere around the product, typically a reduction of O_2 levels from 21% in ambient air, and an increase in CO_2 from 0.04% in ambient air. MA can be developed passively by product respiration or by active means where the desired gas composition is injected into a headspace, often in a package (MA packaging: MAP). Subsequently, the atmosphere in the bags is then a function of factors such as product type and temperature, which affect the respiration rates, permeability of the plastic film to oxygen and carbon dioxide diffusion, and the ratio of product mass to the bag volume. Controlled atmosphere (CA) is a subset of MA, but as the name suggests, the atmosphere around the product is continuously controlled. Control is conferred by use of equipment such as nitrogen generators and carbon dioxide scrubbers to maintain the desired gas composition.

Based on the net chemical changes for respiration (Section 16.4) it has long been assumed that low O_2 and high CO_2 would inhibit respiration and thereby increase storage life. However, the interaction between the gases and metabolism of edible plant tissues is more complex, especially in relation to ethylene perception and production. Ethylene action is inhibited by low O_2, 2.8% being the concentration

TABLE 16.14
O_2 Limits below Which Injury Can Occur for Selected Horticultural Crops Held at Typical Storage Temperatures

Minimum O_2 Concentration Tolerated (%)	Commodities
0.5	Tree nuts, dried fruits and vegetables
1	Some cultivars of apples and pears, broccoli, mushrooms, garlic, onion, most cut or sliced (minimally processed) fruits and vegetables
2	Most cultivars of apples and pears, kiwifruit, apricot, cherry, nectarine, peach, plum, strawberry, papaya, pineapple, olive, cantaloupe, sweet corn, green bean, celery, lettuce, cabbage, cauliflower, Brussels sprouts
3	Avocado, persimmon, tomato, peppers, cucumber, artichoke
5	Citrus fruits, green pea, asparagus, potato, sweet potato

Source: Modified from Kader, A.A., *Postharvest Technology of Horticultural Crops*, Regents of the University of California, Division of Agricultural and Natural Resources, Oakland, CA, 2002.

TABLE 16.15
CO_2 Limits above Which Injury Can Occur for Selected Horticultural Crops Held at Typical Storage Temperatures

Maximum CO_2 Concentration Tolerated (%)	Edible Plant Tissue
2	Asian pear, European pear, apricot, grape, olive, tomato, pepper (sweet), lettuce, endive, Chinese cabbage, celery, artichoke, sweet potato
5	Apple (most cultivars), peach, nectarine, plum, orange, avocado, banana, mango, papaya, kiwifruit, cranberry, pea, pepper (chili), eggplant, cauliflower, cabbage, Brussels sprouts, radish, carrot
10	Grapefruit, lemon, lime, persimmon, pineapple, cucumber, summer squash, snap bean, okra, asparagus, broccoli, parsley, leek, green onion, dry onion, garlic, potato
15	Strawberry, raspberry, blackberry, blueberry, cherry, fig, cantaloupe, sweet corn, mushroom, spinach, kale, Swiss chard

Source: Modified from Kader, A.A., *Postharvest Technology of Horticultural Crops*, Regents of the University of California, Division of Agricultural and Natural Resources, Oakland, CA, 2002.

at which ethylene action is halved [82], and by elevated CO_2 concentrations. The effects of the two gases can be interactive, and together they may slow down respiration to a greater extent than either alone.

Reducing O_2 concentrations around fruits and vegetables slows down respiration rates until the ACP is reached (Figure 16.7). This transition in respiration is associated with fermentation and injurious accumulations of acetaldehyde and ethanol, which result in damage.

Each type of edible plant tissue has tolerances to low O_2 and high CO_2 (Tables 16.14 and 16.15), although there is considerable variation conferred by variety, growing condition, and length of exposure to each gas. The safe concentration range of O_2 and CO_2 for most edible plant tissues during storage at optimal temperatures have been identified [83], and are well above the ACP to account for variability of product responses.

Atmospheres obtained within MA systems such as MAP are a function of respiration rates, which is affected by product weight and storage temperature, permeability of the films to O_2 and CO_2, and volume of the film bag. Applications of MAP are still relatively restricted because of the

TABLE 16.16
Overview of the General Effects of O_2 Levels below 5% and CO_2 Levels above 5% on Metabolism of Edible Plant Tissues

	General Effects on Metabolism	
	Reduced O_2	Elevated CO_2
Respiration		
1. Rate	↓	↓, NE, or ↑
2. Shift from aerobic to anaerobic	↑(<1%)	↑ (>20%)
3. Energy produced	↓	
Ethylene biosynthesis and action		
1. Methionine to S-AdoMet	NE	?
2. Synthesis of ACC synthase	↓	↓
3. ACC synthase activity	NE	↓
4. Synthesis of ACC oxidase	↓	↓
5. ACC oxidase activity	↓	↓ or ↑
6. Ethylene action	↓	↓
Compositional changes		
1. Pigments		
a. Chlorophyll degradation	↓	↓
b. Anthocyanin development	↓	↓
c. Carotenoids biosynthesis	↓	↓
2. Phenolics		
a. Phenylalanine ammonia lyase activity	↓	↑
b. Total phenolics	↓	↓
c. Polyphenol oxidase activity	↓	↓
3. Cell wall components		
a. Polygalacturonase activity	↓	↓
b. Soluble polyuronides	↓	↓
4. Starch to sugar conversion	↓	↓
5. Organic and amino acids		
a. Loss of acidity	↓	↓
b. Succinic acid	↓	↑
c. Malic acid	↑	↓
d. Aspartic and glutamic acid	?	↓
e. ϒ-Amino butyric acid	?	↓
6. Volatile compounds		
a. Characteristic volatile aroma compounds	↓	↓
b. Off-flavors (fermentation products)	↑(<1%)	↑(>20%)
7. Vitamins		
a. Provitamin A (carotene) loss	↓	↓
b. Ascorbic acid loss	↓	↓

Source: Modified from Kader, A.A. and Saltveit, M.E., Atmosphere modification, in *Postharvest Physiology and Pathology of Vegetables*, Bartz, J.A. and Brecht, J.K., Eds, Marcel Dekker, New York, 2003, pp. 229–246.

↓ = decrease or inhibit, NE = no effect, ↑ = stimulate or increase, ? = inadequate data for conclusion.

limited availability of suitable films that are cost effective, and difficulties in maintaining uniform temperatures through the entire marketing chain. The major exception is the wide use of MAP for fresh-cut produce (Section 16.9.3).

Beneficial effects at the optimal CA conditions include retardation of senescence (including ripening) and associated biochemical and physiological changes, particularly slowing down rates of respiration, ethylene production, softening, and compositional changes. An overview of the general effects of O_2 and CO_2 concentrations on metabolic processes is shown in Table 16.16. The specific concentrations at which these general responses occur and their magnitude differ among plant types and cultivars, maturity and ripening stages, storage temperatures and durations, and in some cases ethylene concentrations [84]. Stressful CA conditions decrease pH and ATP levels and reduce pyruvate dehydrogenase activity while activating pyruvate decarboxylase and alcohol dehydrogenase activities with the production of the fermentation products acetaldehyde and ethanol (Figure 16.6).

Commercial use of CA storage is greatest for preserving apples and pears, less on cabbages, sweet onions, kiwifruits, avocados, persimmons, pomegranates, nuts, dried fruits, and vegetables. Atmospheric modification during long-distance transport is used with apples, asparagus, avocados, bananas, broccoli, cane berries, cherries, figs, kiwifruits, mangos, melons, nectarines, peaches, pears, plums, and strawberries. Continued technological developments in the future to provide CA during transport and storage at a reasonable cost (positive benefit/cost ratio) are essential to greater applications on fresh horticultural commodities and their products. CA is limited for many products because it requires significant capital investment. Structures must be air-tight and refrigerated, with precise temperature control and equipment to modify the atmospheres. The volume of these storage rooms is also large to maximize the value of the equipment. Therefore, the return on investment requires long-lived commodities that are stored for months, not days or weeks.

The economic importance and long-term storage potential of apples have been drivers for the development of new CA technologies. Standard CA, in which O_2 and CO_2 concentrations are maintained in the 2%–3% range, has increasingly become replaced by ultralow O_2 (ULO). Some cultivars in certain growing regions can be routinely stored in low O_2 concentrations between 1% and 1.5% if high-quality storage rooms and computerized monitoring and maintenance of gas levels are available.

A new CA technology, known as dynamic CA (DCA), has been developed and commercialized. Rather than establishing a predetermined low O_2 concentration, fruit quality in storage can further be maximized by storing them at concentrations that are closer to the ACP (Section 16.4; Figure 16.7). O_2 concentrations in the storage room are lowered to the point where stress signals are measurable, and then the concentration is increased by about 0.2% above the O_2 concentration at which stress is observed to ensure that fermentation and damage to the product are avoided. It is possible, therefore, to follow fruit responses in real time and ensure that rates of fruit metabolism are maintained as low as possible throughout storage. Three methods to determine stress in the fruit are ethanol accumulation, RQ, and chlorophyll fluorescence [85,86]. DCA can, in principle, be applied to any fruit or vegetable tissue, but most use of this technology has been on apples.

Other atmosphere-based technologies such as hypobaric storage, in which commodities are stored at low atmospheric pressures, provide potential for long-term storage, but practical cost-affordable systems are not yet available.

16.7.4 Edible Coatings

Edible coatings are thin layers of edible material that are applied to surfaces of plant tissues in addition to or as a replacement for natural protective waxy coatings. Application to products can be by dipping, spraying, or brushing. Edible coatings can act in a similar manner as MAP in that internal gas concentrations may be modified as a result of coating applications. An ideal coating is defined as one that can extend the storage life of whole or minimally processed fresh fruits and vegetables without causing anaerobiosis or negatively affecting any desirable quality attribute.

The main reasons for use of edible coatings are improved appearance, reduced water loss, delayed ripening, and reduced incidence of decay and physiological disorders. Shellac and carnauba wax are the two most common coating materials because they are used alone or in combination on apples and oranges. Synthetic coatings have been used for decades; most consumers are probably aware that apples are waxed, but they may not be as aware for other products such as cucumbers, oranges, mangoes, papayas, and peppers. Nevertheless, recent consumer interest in nutrition, food safety, and environmental concerns have revitalized efforts in edible coating research [87–89]. There is increasing use of edible coatings of fresh-cut fruits and vegetables to reduce deterioration rates associated with cutting and processing. Edible coatings may also be used on nonfleshy products such as peanuts and roasted almonds. Another area of interest is the addition of active ingredients such as antioxidants, antimicrobials, and neutraceuticals to edible coatings [88].

The requirements of edible coatings vary according to product and purpose. However, they must not result in low O_2 or high CO_2 concentrations that would result in off-flavor development and deterioration, or interfere with product quality. Desirable characteristics of coatings include improved appearance, maintenance of structural integrity, improved mechanical handling properties, or carry active agents such as antioxidants and vitamins; water resistance, so that they remain intact and cover the product; reduced water permeability; melt above 40°C without decomposition; be easily emulsifiable, nonsticky or tacky, and have efficient drying performance; have low viscosity and be economical, and be translucent to opaque, and capable of tolerating slight pressure [88,89].

Materials used for coatings include lipids, polysaccharides, proteins, and combinations of these materials or composite coatings [90]. Any Generally Recognized As Safe (GRAS) materials that are approved for use in coatings without restriction are considered by the FDA to be "edible."

Lipids are used primarily because of their hydroscopic properties, which makes them good barriers to water loss [88]. Commonly used lipid coatings include the following:

- *Wax- and oil-based coatings*. These can be animal derived (e.g., shellac wax, bees wax), vegetable-derived (e.g., carnauba wax and candelilla wax), or mineral and synthetic waxes (e.g., paraffin wax).
- *Fatty acids and monoglycerides*. These are mainly used as emulsifiers and dispersing agents (fatty acids are extracted from vegetable oils and monoglycerides prepared by trans-esterification of glycerol and triacylglycerols).
- *Resins and rosins*. Shellac resin, composed of aleuritic and shelloic acids, is a secretion of the insect *Laccifer lacca*, while resins are obtained from the oleoresins of pine trees, residues that are left after distillation of volatiles from the crude resin.
- Emulsions (derivatives of glycerol and fatty acids, e.g., polyglycerols-polystearates).

Polysaccharides are hydrophilic and do not function well as physical moisture barriers. They can still interact with water and retard loss of moisture to the atmosphere. However, they can have excellent gas barrier properties, and the linear structure of many polysaccharides renders their films tough, flexible, and transparent [88]. Commonly used polysaccharides include the following:

- *Cellulose and derivatives*. Polymer chains of anhydroglucose are tightly packed, resulting in a highly crystalline structure (cellulose; Section 16.6.2.1), which requires treatment with alkali to increase its water solubility, followed by treatment with chloroacetic acid, methyl chloride, or propylene oxide to produce carboxymethyl cellulose (CMC), methyl cellulose (MC), hydroxypropyl methyl cellulose (HPMC), or hydroxypropyl cellulose (HPC). These coatings are water-soluble and transparent, and exhibit higher barrier capabilities to moisture and O_2 transmission than cellulose.
- *Starch and derivatives*. Amylose and amylopectin (Section 16.6.2.3) have been used to produce biodegradable films. However, starch typically needs to be treated either with plasticizers (e.g., glycerol, polyethylene glycol, mannitol, sorbitol) blended with other materials

and/or chemically modified to form films with good mechanical properties such as high percentage elongation as well as tensile and flexural strength.

- *Chitin and chitosans*. Chitin, structurally analogous to cellulose, is found in exoskeletons of crustaceans, fungal cell walls, and other biological materials. Chitosan is derived from chitin by deacetylation in the presence of alkali. It also has antimicrobial activity against a wide range of pathogenic and spoilage microorganisms, including Gram-positive and Gram-negative bacteria, and has been widely used in antimicrobial films [89].
- *Alginates and carrageenans*. Alginates, extracted from seaweeds, are salts of alginic acid, which is a linear copolymer of D-mannuronic and L-guluronic monomers. Alginate reacts with gelling agents such as calcium and magnesium to form coating materials. Carrageenan is a complex mixture of at least five water-soluble galactose polymers. These films are poor moisture barriers as they are hydrophilic films, although incorporation of calcium can reduce their water vapor permeability.
- *Pectin (Section 16.6.2.1)*. High methoxy pectin forms excellent films, and plasticized blends of citrus pectin and high amylose starch give strong, flexible films.
- *Aloe gel*. Aloe vera gel, obtained from the parenchyma cells of perennial *Aloe* spp. succulent plants, has been identified as a novel skin coating [89]. Aloe gels also have antifungal activity.

Protein. A variety of protein sources can be used as edible coatings, but protein coatings are the least developed materials [88]. However, they have received greater attention because of their abundance as agricultural by-products and food-processing residuals. The presence of reactive amino acid residues enables proteins to be modified and cross-linked through physical and chemical treatments to produce novel polymeric structures [91]. Proteins are generally hydrophilic and susceptible to moisture absorption, and therefore affected by temperature and RH. The most common edible coatings derived from proteins are the following:

- *Gelatins*. Gelatins are obtained by controlled hydrolysis from the fibrous insoluble protein, collagen, a major constituent of skin, bones, and connective tissues. Gelatins characteristically have high glycine, proline, and hydroxyproline contents, and contain mixtures of single and double unfolded chains of hydrophilic character.
- *Corn zein*. Corn zein is a prolamine protein of corn endosperm, dissolves in ethanol, and has excellent film-forming characteristics.
- *Wheat gluten* is a general term for water-insoluble proteins of wheat flour, and contains gliadin and glutenin. Gliadin is soluble in 70% ethanol, while glutenin is not, and edible films can be made by drying aqueous ethanol solutions of wheat gluten.
- *Soy protein*. Most of the protein in soybean is insoluble in water but soluble in dilute neutral salt solutions. Soy protein consists of two major protein fractions; 7S (conglycin, 35%) and 11S (glycinin, 52%), each containing cysteine residues leading to disulfide bridge formation. Edible coatings can be formed by surface film formation from heated soymilk or film formation from solutions of soy protein isolates [91].
- *Casein*. Casein is a milk-derived protein that is easily processable due to its random coil structure to produce materials that range from stiff and brittle to flexible and tough [88].
- *Keratin*. Keratin is extracted from waste materials such as hair, nails, and feathers, but is difficult to process due to its high cystine (disulfide) content and low aqueous solubility. After processing, a fully biodegradable, water-insoluble plastic is produced, but its mechanical properties are still poor compared with those of other proteins. Blending or lamination is required to overcome insensitivity to RH [88].

- *Whey*. Whey protein is a by-product of cheese and yogurt production, and can be processed to produce flexible but brittle films. Whey protein is hydrophilic, and lipids are added to film-forming solutions to reduce moisture migration.

The effects of edible coatings have been tested extensively on a range of whole and minimally processed fruits and vegetables [88–90]. While commonly used on apples, bell pepper, lemon, oranges, cucumbers, and other products, the actual commercial use of coatings is not easily quantified. Minimally processed products where edible coatings may be applied include apple, cantaloupe, carrot, lettuce, muskmelon, pear, peach, and potato. However, use of coatings requires labeling on packages, which might be perceived as detracting from the fresh image of their products [87].

The primary effects of edible coatings on product quality are exerted through modification of water loss and internal atmospheres. Fruits and vegetables lose water to the surrounding environment as a result of transpiration, and therefore storage under high RH conditions is often desirable. Loss of water from products can be aggravated by damage to the natural protective coatings by washing and other handling steps, and can be especially so in minimally processed products. In general, lipid materials (wax and oil) offer the most effective barrier to water vapor, followed by shellac, with carbohydrates and proteins being the least effective due to their hydrophilic characteristics [90].

Edible coatings can modify the internal atmosphere of treated products by creating a barrier to gas exchange. O_2 concentrations decrease while CO_2 concentrations increase in treated products, resulting in effects similar to those obtained using MAP; if concentrations of the respective gases are below 8% or higher than 5%, decreased respiration rates and ethylene production can result in slower ripening or senescence. High gas-permeable materials, such as polyethylene and carnauba wax, control water loss but do not cause much modification of internal atmospheres and the progress of ripening, while resins have low gas permeability and can control ripening more effectively. However, resin use has greater potential for development of injurious anaerobic gas concentrations under abusive temperature conditions that can occur commercially. Carbohydrate and protein coatings are generally hydrophilic but can modify internal atmospheres.

Quality characteristics affected by the inhibition of ripening and senescence are as follows:

- *Appearance*. High gloss and shine and reduced water loss (shriveling) result in better product appearance for consumers. Other O_2-dependent processes that can be inhibited by edible coatings include sprouting and chlorophyll and solanine synthesis in potatoes, degreening of limes and lemons, and white blush (haze) formation of baby carrots.
- *Physical factors*. Rates of loss of product firmness and acidity and increases of soluble solids concentrations are commonly reduced by application of edible coatings, but the magnitude of the effects is greatly dependent on product type and specific coating used [89].
- *Flavor and nutrition*. Coatings can affect flavor of products by influencing the metabolism of volatile biosynthesis and/or their entrapment in the treated product. Positive effects have been reported, but others may be negative, especially if ethanol production is increased due to anaerobiosis. Losses of phenolic concentrations and total antioxidant activities can be reduced by edible coatings, but this is also affected by product type and coating [87,89]. Higher carotenoid and ascorbic acid have been found in coated compared to uncoated peeled carrots and peppers, respectively.
- *Browning*. Browning and PPO activity can be reduced in longan, fresh-cut mushroom, pumpkin, and peach by the use of edible coatings [92].
- *Decay*. Edible coatings can reduce wounding (surface injury, scarring, abrasion), which can result in infection by opportunistic pathogens. Coatings carrying acidulants or preservatives can reduce decay in citrus, cucumber, cut potato, and strawberry [87].

16.7.5 Ethylene

Exposure to ethylene can be beneficial or detrimental, not only depending on the specific fruit or vegetable but also when the exposure occurs [93]. Responses of commodities to ethylene can be affected by the species, cultivar, cultural practices, prior exposure to hormones, and levels of past and current stresses. There is no set standard for ethylene concentrations at which detrimental effects will occur, and there are important differences in ethylene sensitivity among fruit and vegetable types (Table 16.17). Climacteric fruits such as apples and pears have high ethylene production and high sensitivity, while other products (e.g., broccoli, cabbage, carrots, and strawberries) can have low rates of ethylene production but are highly sensitive to ethylene. Most non-climacteric fruits, such as cherry, grape, berries and pepper, have low ethylene production and low sensitivity to ethylene.

Ethylene, released from a liquid chemical formulation ethephon (2-chloroethane phosphonic acid), can be used for preharvest treatment of apples and tomatoes to stimulate red color development. After harvest, ethylene is applied commercially to accelerate chlorophyll loss and ensure even ripening of bananas, and sometimes as part of "ready-to-eat" protocols for avocados and pears. Consumers can also use ethylene-producing fruits such as apples to ripen other fruit types by enclosing both fruits in a paper bag.

Detrimental effects of ethylene are often of more concern to growers and marketers. Exposure of unripe climacteric fruits to ethylene can cause earlier than desirable ripening, as well as undesirable yellowing of green vegetables such as cucumbers, parsley, and broccoli, and many other negative effects (Table 16.18). Exposure of vegetables and non-climacteric fruits also increases their respiration rates, meaning that the carbohydrate reserves are used more rapidly, as well as increasing water loss and hastening the onset of senescence. Typically, exposure occurs from mixing ethylene-producing and ethylene-sensitive commodities in the same storage room. The same response to ethylene, though biochemically identical for different commodities, can be beneficial or detrimental. Acceleration of chlorophyll loss, promotion of ripening, and stimulation of phenolics production can be beneficial or detrimental depending on the product (Table 16.19).

TABLE 16.17
Ethylene Production and Sensitivity of Selected Edible Plant Tissues

Commodity	Ethylene Production	Ethylene Sensitivity
Climacteric fruit		
Apple, kiwifruit, pear, cherimoya	High	High (0.03–0.1 ppm)
Avocado, cantaloupe melon, passion fruit	High	Medium (>0.4 ppm)
Apricot, banana, mango	Medium	High (0.03–0.1 ppm)
Nectarine, papaya, peach, plum, tomato	Medium	Medium (>0.4 ppm)
Vegetables and non-climacteric fruits		
Broccoli, Brussels sprouts, cabbage, carrot,	Low	High (0.01–0.02 ppm)
Cauliflower, cucumber, lettuce, persimmon	Low	High (0.01–0.02 ppm)
Potato, spinach, strawberry	Low	High (0.01–0.02 ppm)
Asparagus, bean, celery, citrus, eggplant	Low	Medium (0.04–0.2 ppm)
Artichoke, berries, cherry, grape, pineapple	Low	Low (>0.2 ppm)
Pepper	Low	Low (>0.2 ppm)

Source: Modified from Martinez-Romero, D. et al., *Crit. Rev. Food Sci. Nutr.*, 47, 543, 2007.

TABLE 16.18
Summary of Detrimental Effects of Ethylene on Quality of Edible Plant Tissues

Ethylene Effect	Symptom or Affected Organ	Commodity
Physiological disorders	Chilling injury	Persimmon, avocado
	Russet spotting	Lettuce
	Superficial scald	Pear, apple
	Internal browning	Pear, peach
Abscission	Bunch	Cherry tomato
	Stalk	Muskmelon
	Calyx	Persimmon
Bitterness	Isocoumarin	Carrot, lettuce
Toughness	Lignification	Asparagus
Off-flavors	Volatiles	Banana
Sprouting	Tubercle, bulb	Potato, onion
Color	Yellowing	Broccoli, parsley, cucumber
	Stem browning	Sweet cherry
Discoloration	Mesocarp	Avocado
Softening	Firmness	Avocado, mango, apple, Strawberry, kiwifruit, melon

Source: Modified from Martinez-Romero, D. et al., *Crit. Rev. Food Sci. Nutr.*, 47, 543, 2007.

TABLE 16.19
Examples of How the Same Physiological or Biochemical Response to Ethylene Can Be Beneficial in One System and Detrimental in Another

Ethylene Response	Example of Benefit	Example of Detriment
Accelerates chlorophyll loss	Degreening of citrus	Yellowing of green vegetables
Promotes ripening	Ripening of climacteric fruit	Overly soft and mealy fruit
Stimulates phenylpropanoid metabolism	Defense against pathogens	Browning and bitter taste

Source: Saltveit, M.E., *Postharvest Biol. Tec.*, 15, 279, 1999.

16.7.5.1 Ethylene Avoidance

Avoidance of exposure to ethylene begins with careful harvesting, grading, and packing to minimize damage to the commodities. In the case of climacteric products, it is difficult to reduce the internal levels of ethylene once autocatalytic production has started. Products should be cooled rapidly to their lowest safe temperature to reduce naturally occurring ethylene production and to decrease sensitivity to ethylene. Use of internal combustion engines around ethylene-sensitive commodities should be avoided by using electric forklifts or isolating vehicles from handling and storage areas. Natural sources of ethylene such as overripe and decaying produce should be removed from storage and handling areas. Ethylene-producing and ethylene-sensitive commodities should not be stored together for long periods. Retail displays should avoid placement of ethylene-producing fruits such as apples and tomatoes close to commodities such as lettuce and cucumbers, although good ventilation in such areas probably reduces the severity of ethylene exposure.

Ethylene concentrations in the storage environment can be reduced by ventilation with clean, fresh air. However, the fresh air has to be cooled, and increasing ventilation is therefore energy-intensive. Higher ventilation rates will also reduce the ability to maintain high RH in the cold room. Ventilation is also not suitable for CA storages or even packaged produce within normal storage environments because the atmospheres are tightly controlled.

16.7.5.2 Ethylene Adsorbers, Oxidation, and Catalytic Decay

Ethylene in storage rooms can be lowered by adsorption or oxidation [94]. Adsorbers ("scrubbers") such as activated carbon and zeolites (microporous aluminosilicate minerals) have been available for many years. Zeolites incorporated into plastic films can maintain sensory quality and reduce microbial storage. Ethylene can be oxidized using a number of strategies. Potassium permanganate ($KMnO_4$) is available in sachets, films, and filters, but its direct contact with edible products must be avoided because of its toxicity. Studies show effectiveness with some products, but effectiveness with high ethylene-producing products is commercially questionable. Because ethylene is absorbed by the potassium permanganate, its effectiveness is based on the presence of a large surface area, although systems have been developed where room air is drawn though the scrubber to increase efficiency.

Ozone (O_3) will also oxidize ethylene, and its use in slowing down ripening and as a disinfectant that lowers mold and bacterial contamination, has been documented. However, commodities vary in sensitivity to ozone exposure. Also, ozone is unstable, and therefore maintaining stable concentrations in storage can be difficult.

Catalytic decay of ethylene can be separated into two types. In the first, pure metallic elements can be used to increase the rate of chemical reactions, and in the case of ethylene, effectively oxidize it to CO_2 and water. Most work on ethylene removal has centered on Pd (palladium) and TiO_2 (titanium dioxide), using activated carbon as the catalyst support. Delayed ripening of tomatoes and avocados has been demonstrated using Pd-activated carbon. Another means of removing ethylene is light-activated catalysis (photocatalysis). The main compound used in photocatalysis is TiO_2, which is activated by UV light (300–370 nm wavelengths). The advantages of photocatalysis include destruction of ethylene where it is produced; Ti is cheap, photostable, and clean; RH in the storage room is unaffected; and ethylene destruction can be achieved at room temperature [94]. The main disadvantage is that the technology needs permanent UV light, and therefore it cannot be used inside packages.

16.7.5.3 Inhibitors of Ethylene Action

MA/CA storage inhibits ethylene perception and production by the action of low O_2 and high CO_2, as described on Section 2.6, but a powerful method to control ethylene perception has recently become available for fruits and vegetables. 1-Methycyclopropene (1-MCP) is a cyclopropene (Figure 16.27) that is a competitive inhibitor of ethylene perception, which acts by binding practically irreversibly to ethylene-binding sites, thereby preventing ethylene binding and the eliciting of subsequent signal transduction and translation (Section 16.5.1). 1-MCP is extremely active, but unstable in the liquid phase, but a process in which 1-MCP was complexed with α-cyclodextrin to maintain the stability of 1-MCP has been developed. The commercialization of 1-MCP as the SmartFresh^{SM} Quality System led to the rapid adoption of 1-MCP-based technologies for many horticultural industries. 1-MCP has undetectable residues, is a gas at physiological temperatures,

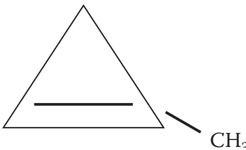


FIGURE 16.27 Chemical structure of 1-methylcyclopropene, an inhibitor of ethylene binding.

applied for a short time period (≤24 h) and is active at low concentrations (1 ppm). 1-MCP is typically applied to horticultural products as soon as possible after harvest, with the objective of quickly inhibiting ethylene action [95].

By 2016, regulatory approval for use of 1-MCP had been obtained in over 40 countries. 1-MCP is registered for use on a wide variety of fruits and vegetables, including apple, avocado, banana, broccoli, cucumber, date, kiwifruit, mango, melon, nectarine, papaya, peach, pear, pepper, persimmon, pineapple, plantain, plum, squash, and tomato. The specific products that are registered within each country vary greatly and according to the importance of the crop in that country.

As is the case for CA storage, most use of 1-MCP technology is for apples [96]. The focus on apples is in large part is due to the large volumes of fruit that are kept in CA storage for periods up to 12 months depending on the cultivar and growing region. In some cases, 1-MCP treatment at harvest can be used as an alternative to CA storage, but usually CA and 1-MCP are used in combination. The advantage of 1-MCP is that it prevents the rapid softening of fruit that can occur after removal from CA storage. Also, apple has been an ideal fruit for 1-MCP because the ideal product in the marketplace is one resembling that at harvest—one with a crisp fracturable texture, and an acid to sugar ratio appropriate to each cultivar.

Use of 1-MCP on other products is relatively limited. In contrast to apple, many other climacteric fruits such as the avocado, banana, pear, and tomato require a delay, not an inhibition of ripening, to ensure that the consumer receives high-quality products with the expected characteristics of color, texture, and flavor. Lower 1-MCP concentrations that do not inhibit ripening can be difficult to apply as a gas. However, new aqueous technologies for the application of 1-MCP in the field or as dips continue to be investigated [95]. Another factor that limits 1-MCP use is its cost relative to benefit, where for some products such as vegetables the cost of 1-MCP application may not justify its use. Yellowing of broccoli, which can result from storage and transport under abusive conditions of high temperature and exposure to ethylene, can be controlled by 1-MCP treatment, but such abuses are not common enough to warrant the treatment of a low-cost commodity.

Overall, several generalizations can be made about responses of fruits and vegetables to 1-MCP:

1. The primary features of ripening in climacteric fruits such as softening, color development, and volatile production of climacteric fruits are inexorably linked to ethylene production, but the specific effects of 1-MCP treatment are closely linked to the species, cultivars, and maturity. The capacity to interrupt the progression of ripening once initiated varies by the specific fruit and attributes studied. In general, fruits with faster rates of metabolism or at a riper physiological stage are less responsive to 1-MCP; if ethylene production has been initiated, inhibiting ethylene perception is less effective. The ripening of certain fruits such as guava, tomato, and banana can be completely inhibited or abnormal if the fruits are immature.
2. Non-climacteric fruits can be affected by 1-MCP, and this outcome provides insights about the occurrence of ethylene-dependent and ethylene-independent events during ripening including changes of gene expression (up and downregulation). Common benefits of 1-MCP treatment on non-climacteric products include delayed chlorophyll and protein losses.
3. Losses of health-promoting compounds such as vitamin C are usually slower in 1-MCP-treated products, whereas effects on phenolic compounds are often smaller.
4. The quality of treated products, including the levels of health-promoting compounds, is usually close to that of untreated fruit if ripening is delayed but not inhibited by 1-MCP.
5. Physiological disorders that are associated with senescence or induced by ethylene (endogenous and exogenous) are inhibited by 1-MCP treatment, but others such as those associated with elevated CO_2 in the storage environment are increased. Chilling injury is increased or decreased depending on whether ethylene production enhances or alleviates this disorder.

16.7.6 Heat Treatments

The use of heat treatments as a non-chemical means of controlling insect pests, preventing decay, increasing storage life, and preventing development of physiological disorders has been investigated in a number of edible plant tissues [97]. The three methods used to treat these products are (1) hot water treatments either by dips or sprays; (2) vapor heat (water-saturated air); and (3) hot air, either static or forced. Hot water treatments may be supplemented with other treatments such as brushing of the fruit [98].

Each product is treated with these methods at specific temperature and time period combinations (from seconds to days in length), which result in the desired response without injury to the tissues or an inability to recover metabolically from the treatment. The response of a particular fruit or vegetable will result from a combination of factors: preharvest environmental conditions, thermo-physiological age of the product, the time and temperature of exposure, and whether the product is transferred from heat to storage or ripening temperature. The responses of many plant products to heat treatments have been investigated (e.g., apples, asparagus, carrots, celery, lettuce, mangoes, peaches, papaya, potatoes, strawberries, and tomatoes), but commercial acceptance of the technology is limited by factors such as high energy costs.

Heat treatments can decrease decay by washing spores off products, by inflicting direct lethal effects on decay-causing organisms or pests, or by altering the wax structure and composition. Improved storage quality occurs through inhibition of the metabolic processes involved in ripening and senescence. An important feature of heat treatments on ripening fruits is inhibition of ethylene biosynthesis, largely because ACC oxidase activity is inhibited, but thermal effects may also evoke desensitized ethylene perception and diminished protein synthesis [97]. Respiration rates may initially increase during treatment but then decrease to lower levels than in control fruit. Other ripening factors inhibited by heat treatment include cell-wall disassembly, synthesis of carotenoids such as lycopene in tomato fruit that is mediated by ethylene, and flavor and volatiles evolution. Undesirable degreening of fruit has been observed in apple, cucumber, plantain, and tomato. If fruits are treated with inappropriate temperature/time combinations, then fruit will not recover from ripening inhibition.

Heat treatments are associated with a thermal stress response, involving the upregulation of a specific set of genes coding for heat shock proteins (HSPs); this response often corresponds to a downregulation of many ripening genes. Thermotolerance is thought to require transcription and translation of these HSPs, which leads to cellular protection. If a product is treated with incorrect temperature/time combinations, synthesis of cytoprotective proteins may be attenuated, leading to heat damage. However, products can be preconditioned using a moderate heat stress to provide tolerance to higher temperature stresses, and this process may be mediated by induction of HSPs [97]. Other changes to heat-treated products include greater fatty acid saturation in heated than unheated fruits.

16.7.7 Ionizing Radiation

Food irradiation involves exposing the products to gamma rays from a radioisotope source or to X-rays or electrons generated from an electron accelerator. The technology is considered safe and effective by the WHO, FAO, and the International Atomic Energy Agency, although some consumer resistance exists [99]. The potential of ionizing radiation is based on the fact that DNA of undesirable microorganisms is damaged, or that desirable physiological responses can be obtained without damaging or reducing the quality of the treated product. Radiation has no residues and can reduce the need for the use of chemicals on edible plant products.

Irradiation can protect product quality and reduce postharvest losses in a number of ways, including reducing microbial loads of pathogens such as *Escherichia coli* and *Listeria monocytogenes*; inhibition of carrot, onion, and potato spouting; and extending the shelf-life of whole and fresh-cut

fruits and vegetables. The effects of irradiation on the quality of edible plant products, including those on ethylene production, respiration, appearance, texture, flavor, and nutritional composition, are generally small [99,100]. Products vary in sensitivity, but a limiting factor for the use of irradiation is loss of product quality in the range of 1–2 kGy and above. Also, undesirable effects have been found at doses lower than 1 kGy. These include greater softening, loss of ascorbic acid, and interference with wound healing at doses that prevent potato sprouting. Fresh-cut fruits and vegetables appear less sensitive to irradiation than whole products. The use of hurdle technology, where a combination of methods is used to maintain quality, continues to be investigated to reduce effective dose rates. These additional methods include MAP, hot water treatments, chemical sanitizers, calcium salts, and antioxidants.

16.7.8 Other Technologies

Research is continuing to identify new technologies to maintain quality and increase the storage potential of edible plant tissues. These include the following:

1. Polyamines, which decrease during ripening and interact with the ethylene biosynthetic pathway (Sections 16.5.1.1 and 16.5.6.1). Postharvest treatment of fruit with polyamines can increase their endogenous levels, inhibit ethylene production, maintain quality, and protect against mechanical damage [29,94].
2. Nitric oxide (Section 16.5.6.2), which can delay senescence of several non-climacteric fruit and vegetables, in part by suppressing ethylene generation [16]. NO gas is applied as a fumigant or released from solutions of sodium nitroprusside, S-nitrosothiols, or diazeniumdiolates, and future development of the technology requires a smart carrier/controlled release system for NO [30,101]. An alternative treatment option is to apply compounds such as arginine, a precursor of NO biosynthesis, to stimulate NO production [30].

Application of these treatments and others may continue as modes of their action are better understood and application technologies developed. However, limitations to commercialization are not always solely related to effectiveness. Factors such as limited opportunities for patent control and small markets for many fruits and vegetables result in a lack of financial incentives to bear the cost of meeting the required regulatory approvals.

16.8 TRANSGENIC PLANT PRODUCTS

16.8.1 Genetically Modified Organisms

Plant breeding has paralleled human civilization, being the basis of the shift from hunting and gathering to agriculture. Domestication of crops for agricultural production for the human diet has resulted in many of the staples such as rice, wheat, maize, and potatoes, and selection for desirable traits of quality, yield, and disease and pest resistance continues. Whatever the edible plant tissue, farmers usually select cultivars on the basis of marketability (visual qualities specific to the market of choice) and yield, because these factors directly affect economic sustainability. As discussed in Section 16.3.4, desirable characteristics can be in conflict with quality. Breeders have sometimes favored fruit and vegetable selections with better resistance to the handling abuses but yielding cultivars that have tougher skins and sometimes reduced eating quality.

Many approaches have been used in plant breeding in addition to simple selection of plants with desirable attributes, including deliberate hybridization and mutation breeding [102]. Many fruit and vegetable crops have been generated by hybridization and selection (e.g., apple, strawberry, tomato, and squash) but the technology is limited by the requirement of two compatible plants in the same or closely related genus/species. Also, the possibility of transfer of undesirable traits

along with desired traits is high. Mutation breeding relies on spontaneous variations of species, for example, semi-dwarf cereal crops, and apple strains with red coloration, or by exposure of seeds, cuttings, pollen, or tissue-cultured cells to physical or chemical mutagens. Mutation breeding is a random, nonspecific process, and can produce mutations that revert to the original phenotype and are chimeras.*

More recently, transgenic technology, where a gene with desirable traits can be inserted into a host genome, has been used. Commonly known as genetically modified (GM) or genetically modified organisms (GMOs), the technology involves the insertion, or the upregulation and downregulation, of genes with specific functions. Genetic modification can be classified as "transgenic" where genes from other species are introduced into plants, or "cisgenic," where only genes within the same species or closely related ones are used for transformation. Most commercial application has been on field crop production, especially resistance to herbicides, for example, glyphosate (Roundup), stress, and insect and disease resistance. This technology has also been used for fruits such as papaya, where a gene that resists ringspot disease virus (PRSV) has been inserted into the fruit [103]. The field is moving very rapidly, with new technologies such as "clustered regularly interspaced short palindromic repeats" (CRISPRs) being employed to carry out gene editing with unprecedented precision, efficiency, and flexibility [104]. The technique is in early stages, but can potentially be used to modify metabolic processes of edible plant tissues.

Safety of GM food—principally concern about risks to human health, environmental impact, and perceptions of naturalness—has been elevated by groups opposed to its commercial development [105,106]. Relative hostility to GM foods in the EU, and the subsequent legislative barriers for their approval, is greater than in the United States [107]. Factors that affect public attitudes to GM foods include socioeconomic variables, individuals' knowledge and scientific background, and parents' education in science and religion [105]. Nevertheless, at least 36 countries have granted regulatory approval for GM crops since 1994, and more than 300 million acres of GM crops are grown by 17 million farmers in more than 25 countries.

Safety evaluation of transgenic food is based on the "Principle of Substantial Equivalence," in which the composition of the transgenic product is compared with that of the traditionally cultivated counterpart [108,109]. The objective of such comparisons is to detect unintended changes resulting from genetic modification. Examples of potential changes are toxicity, allergenicity, possible antibiotic resistance from GM crops, carcinogenicity from consuming GM foods, and alteration of nutritional quality (macro-, micro-, and anti-nutrients) [110]. All comparative studies on nutrients and natural toxicant composition of products such as potato, papaya, red pepper tomato, wheat, corn, and rice have found "substantial equivalence" in typical measurements including sugars, organic acids, carotenoids, alkaloids, VOCs, antioxidants, and minerals [103,110,111].

For edible plant products, however, most focus has been on gene modification that results in increases in nutritional quality or modification of the senescence and ripening processes to improve the maintenance of quality.

16.8.2 Nutritionally Enhanced Food Crops

Biofortification of crops can take place by adding appropriate minerals or inorganic compounds to the fertilizer or by conventional plant breeding, but biotechnology allows direct cost-effective and sustainable methods to improve product attributes [103,112]. An example is biofortified rice in which the gene for β-carotene, the precursor molecule for vitamin A, has been inserted to provide higher vitamin A concentrations [113]. GM rice, known as Golden Rice, was the first crop specifically designed to combat malnutrition; vitamin A efficiency causes eye degeneration in three million children each year. Biofortification with β-carotene has been extended to maize and cassava.

* Chimera: when cells of more than one genotype (genetic makeup) are found growing adjacent in the tissues of that plant.

Other GM crops include rice where gene insertions have been carried out to increase iron bioavailability and lower levels of phytic acid (an inhibitor of zinc absorption), and wheat to increase zinc content. A triple-vitamin-fortified maize expresses high amounts of β-carotene, ascorbate, and folate. Levels of celiac-disease-causing gliadins have been lowered in wheat.

An interesting area of research is the development of "designer crops" where the levels of bioactive compounds that are important to human health are increased. Examples include increased omega-3 fatty acids in plant seed storage oils, and expression of anthocyanins and resveratrol in tomatoes.

16.8.3 Modification of Ripening and Senescence Processes

Genetic modification of edible plant products, especially tomato, is commonplace in many laboratories and has led to increased understanding of ripening and senescence processes.

The first GM food available for human consumption was the Flavr Savr tomato. This tomato, produced by Calgene, was genetically engineered by inserting an antisense gene for the cell wall softening enzyme PG (Section 16.6.2.1). While the shelf-life of the fruit was increased, positive effects on firmness were not realized, and production lasted only between 1994 and 1997 [114]. A similar GM tomato with downregulated PG gene expression, produced in England by Zeneca, resulted in tomato paste that was 20% cheaper. This product, labeled as genetically engineered, was popular in the market, but increased anti-GMO sentiment resulted in production being stopped [115].

Recently, transgenic apples and potatoes have received regulatory approval in the United States [116]. Apples with reduced PPO activity and associated low browning, trademarked as Artic apples, and GM "Innate" potatoes, produced by J.R. Simplot Co., are designed to resist blackspot bruising and browning and contain less asparagine. Lower asparagine concentrations reduce the potential for the formation of acrylamide, a possible carcinogen, during the frying of potatoes.

16.9 COMMODITY REQUIREMENTS

16.9.1 Cereals, Nuts, and Seeds

Cereals, nuts, and seeds can typically be stored for extended periods provided that there is no insect infestation and water activity is low enough to prevent microbial growth. In contrast with fruits and vegetables, therefore, manipulations of the storage conditions for grains, nuts, and seeds is focused less on the product than on conditions that affect pests and microbial growth.

Components of successful storage of these products include the following [117]:

1. *Appropriate storage structures*. Storages should protect grains, nuts, and seeds from external environmental factors such as rain and groundwater, minimize the effects of environmental temperature and humidity, and exclude insects, rodents, and birds.
2. *Temperature control*. Temperature does not directly affect the product quality, but affects activity of insects and populations of molds, yeast, and bacteria.
3. *Humidity control*. Humidity in the intergranular air reaches equilibrium with the moisture of the grains, nuts, and seeds within the storage. RH should be maintained ≤70% to prevent losses due to molds, yeast, and bacteria.

Alternatives to synthetic pesticides include manipulation of temperature using forced aeration to modify the grain bulk microclimate to minimize pests and contamination while maintaining product quality; chilling of grain using refrigeration; and heat treatments. The gas composition within grain storages, which comprises about 50% of the volume of the storage structure, has lower O_2

and higher CO_2 concentrations than air depending on the levels of aeration. These atmospheres can be further modified (decreased O_2 and/or elevated CO_2 concentrations) to kill insects and inhibit pathogen growth. Inert dusts (e.g., clays, sands, ash, diatomaceous earth, synthetic silica) and mineral dusts (e.g., dolomite, lime), which function as desiccants, can be used to kill insects through abrasion of their cuticles and subsequent water loss.

16.9.2 Whole Fruits and Vegetables

Each fruit and vegetable, and sometimes the cultivar within a species, has specific storage requirements that represent an integration of the factors discussed in Sections 16.3 and 16.7. Factors that impact commodity requirements include the following:

1. The maximum storage life that can be obtained, which is usually a function of the genetics of the cultivar and stage of maturity and/or ripening at the time of harvest. For example, tomatoes have much shorter storage potential than apples, but even within each group potential can vary from days to weeks and weeks to months, respectively.
2. The optimum storage temperatures based on sensitivity to chilling and freezing injury. Subtropical and tropical fruits, for example, tend to have higher rates of metabolism and are more susceptible to chilling injury than temperate fruits.
3. RH: Generally high for fruits and vegetables, as moisture loss results in adverse effects on appearance, texture, flavor, and weight. Rates of moisture loss depend on the inherent properties of the product such as cuticular and periderm properties; presence or absence of stomata, lenticels, trichomes, and hairs; and storage temperature, which affects transpiration rates.
4. Tolerances of the product to low O_2 and CO_2 concentrations.
5. Sensitivity of products to ethylene.

Responses of edible plant products to these factors form the basis of published recommendations that are available from many sources including those easily accessible on the web [83]. The degree to which these recommendations are followed will depend on the specific industry involved and the level of sophistication available. A local market retail operation, for example, might store several products together and at temperatures that are inappropriate for some of them. Loss of quality can be negligible because of the limited time periods at these temperatures. In contrast, an apple storage facility that aims to store the fruit for 10 months must pay greater attention to choosing suitable cultivars, ensuring rapid cooling to optimum storage temperatures, utilizing supplementary technologies such as 1-MCP, and rapidly establishing the optimum CAs.

16.9.3 Fresh-Cut (Minimally Processed) Fruits and Vegetables

The growth of the market for fresh-cut or minimally processed fruits and vegetables due to the convenience of ready-to-eat products that are perceived as healthy has been an exciting development in recent years. Fresh-cut processing affects food chemistry of edible plant tissues is many ways [118,119]. The most significant difference between fresh-cut and whole products is obviously the extensive cutting of tissues and the associated physiological changes to the former, including wound responses. Cutting of the products removes the natural protection of the epidermis and causes major tissue disruption, which results in the contact between enzymes and substrates and exposes tissue surfaces to microbes. Fresh-cut processing increases respiration rates, wound-induced ethylene, water activity, and surface area per unit volume, the latter of which may accelerate water loss. These physiological changes may be accompanied by flavor loss, cut surface discoloration, color loss, decay, increased rate of vitamin loss, rapid softening, shrinkage, and a shorter storage life.

Production of fresh-cut products involves a series of processes that are designed to minimize the microbial load of incoming raw materials through efficient preparation in clean temperature- and humidity-controlled environments [119]. Unit operations involved in preparation include the following:

1. Receiving and storage
2. Preliminary washing and sorting of product for appropriate maturity and ripeness stage that is suitable for cutting
3. Precutting and processing treatments
4. Peeling (if necessary)
5. Size reduction and cutting
6. Washing and cooling
7. Dewatering
8. Packaging

Of these steps, common factors among whole fruits and vegetables that affect quality are cultivar selection appropriate for desired purposes, preharvest crop management, proper postharvest temperature and storage regimes, and the balance between harvest timing and quality (see Figure 16.4). A less mature fruit, for example, may be firmer and have better handling, shipping, and storage qualities, but may have lower aroma and flavor attributes. As with whole products, removal from the parent plant limits the energy resources available to continue "normal" postharvest metabolic activity.

In contrast to whole products, the application of MAP is a common and often essential feature of quality maintenance in fresh-cut produce [120]. Because of the removal of epidermal barriers that provide resistance to gas diffusion, it is common to find optimal O_2 and CO_2 concentrations that are lower and higher, respectively, in fresh-cut than whole products. Also, fresh-cut products from chilling-sensitive fruits and vegetables are often stored at lower temperatures than the whole product because the part of the tissue that visually exhibits injury has been removed and/or storage periods are not long enough for CI symptoms to develop.

Specific effects of fresh-cut processing that require good management are [118] as follows:

1. *Mechanical damage*. Sharp knives for cutting of fresh-cut products result in reduced damage and lower respiration rates compared with blunt knives. The smaller the cut product size, the higher the rates of respiration and ethylene production, and the greater the stimulation of PAL activity by ethylene. Wound-induced responses can include production of lignins (fibrous) and coumarins (bitter). Nutritional quality, especially vitamin C, might be decreased by water loss, exposure of tissue to light and air, enzymatic or chemical degradation, and sanitation chemicals such as chlorine. However, stability of vitamins is dependent on commodity type and temperature. The application of MAP, often a critical component of maintaining quality of fresh-cut products, can maintain nutritional compounds, but high CO_2 in packages can result in more rapid degradation. Phenolic concentrations and antioxidant capacity of fresh-cut products can increase as a result of wounding, from 26% to 191% and 51% to 442%, respectively [121].
2. *Enzymatic browning*. Reactions due to mixing phenolics and PPO activity can result in rapid browning after cutting, especially with products with high concentrations of preformed phenolic compounds (apple, artichokes, peach, pear, potato). Also, synthesis and accumulations of phenolics in products such as lettuce that have low concentrations at time of cutting can be stimulated by injury. Treatments applied to reduce enzymatic browning include ascorbic acid and other acidulants and/or sulfites, and O_2 and high CO_2 atmospheres (MAP).

3. *Undesirable changes in coloration*. Loss of chlorophyll and exposure of yellow or colorless carotenoids in green vegetables leads to unacceptable yellowing, while pheophytin formation can result in tissue browning. Cut carrots can develop whitening on the surface, which is associated with desiccation and sloughing of the outer cell layers and/or lignin formation. Pink or brown stains ("russet spotting") on lettuce are associated with the exposure of tissues to ethylene. Depending on the disorder, control measures include low temperature, MAP, humidity control, edible coatings, and antioxidants.
4. *Softening*. Pectic enzymes released during cutting can cause tissue softening, though mainly in parts of the product in contact with the cut surface. Texture changes also may occur because of dehydration. Control of these disorders can be minimized by appropriate temperature and humidity control. Ethylene production can accelerate softening, and in part can be controlled by MAP. Additional treatments of cut products with calcium salts are frequently employed to maintain firmness.
5. *Pithiness*. Development of airspaces in cortical* tissues of celery and radishes, known as aerenchyma,† is an undesirable feature. The disorder is controlled by low temperature and MAP.
6. *Off-flavors and off-odors*. Most typically, undesirable flavors and odors are associated with MAP in which O_2 concentrations are too low and or CO_2 concentrations are too high for the product. Appropriate selection of packaging films and avoidance of temperature fluctuations that result in changes in respiration rates are important control strategies. Another cause of off-flavors and off-odors results from wound-stimulated increases in secondary metabolites, such as chlorogenic acid in grated carrots and sesquiterpenes in fresh-cut pineapple.
7. *Translucency*. Translucency, a physiological disorder in which liquid accumulates in cellular free spaces, occurs in fresh-cut tomato and melon. A preprocessing factor that causes this defect is calcium deficiency in the tissues, although the disorder can be alleviated by maintenance of low temperature and MAP, and 1-MCP treatment to slow ethylene-mediated responses.

16.10 CONCLUSIONS

Edible plant products in the form of staple crops, fruits, and vegetables provide major sources of energy, proteins, carbohydrates, vitamins, and other health-promoting compounds for the world's population. This population, about 7.5 billion in 2016, is predicted to reach over 9 billion by 2050. Food availability and security is an important part of political stability across the globe, and represents a huge societal challenge. Increased production of edible plant products is needed for feeding the world population, but at the same time we face diminished utilizable arable land, problems with food distribution, increased use of plant resources for animal production, environmental concerns, and climate change. Furthermore, the more affluent the consumer, the more critical he or she is about wanting products that are blemish-free and of uniform size and color, safe from infectious pathogens and without pesticide residues, and often with increasing emphasis on sustainability.

These challenges will primarily be addressed at the field level, with emphasis on plant breeding and production practices that will result in higher yields of uniform products with reduced losses due to cosmetic factors. However, a significant improvement in the world food supply can be obtained by reducing the high rates of product losses after harvest in both developed and developing countries. Many of the staple crops have low perishability, but most edible plant products have relatively short storage potential. The topics covered in this chapter have outlined the underlying food (bio)chemistry

* Cortical: relating to cortex, unspecialized cells lying between the epidermis and vascular tissues.
† Aerenchyma: soft, spongy tissue containing large intercellular air spaces.

that affects the quality of edible plant products, both whole and fresh-cut, and the technologies that can be imposed to reduce the rates of metabolism that result in unacceptable product quality for the consumer. Application of these technologies is uneven, sometimes because of basic requirements for electricity. Others, such as genetic modification, have incredible potential to improve the nutritional quality and increase the storage potential of edible plant products but remain controversial.

REFERENCES

1. USDA Food Composition Databases, USDA Nutrient Data Laboratory, ndb.nal.usda.gov/ndb/search/list, 2014. Accessed September 30, 2016.
2. Zeeman, S.C., J. Kossmann, and A.M. Smith, Starch: Its metabolism, evolution, and biotechnological modification in plants. *Annual Review of Plant Biology*, 2010. 61:209–234.
3. Food And Agriculture Organization Of The United Nations, FAOSTAT, faostat.fao.org.proxy.library.cornell.edu, 2015. Accessed September 30, 2016.
4. FAO, Global food losses and food waster - Extent, causes and prevention. http://www.fao.org/docrep/014/mb060e/mb060e00.pdf, 2011.
5. Kader, A.A., Increasing food availability by reducing postharvest losses of fresh produce. *Acta Horticulturae*, 2005. 682:2169–2175.
6. Watkins, C.B. and J.H. Ekman, How postharvest technologies affect quality, in *Environmentally Friendly Technologies for Agricultural Produce Quality*, S. Ben-Yoshua, Ed. 2005, CRC Press: Boca Raton, FL, pp. 333–396.
7. Shewfelt, R.L., What is quality? *Postharvest Biology and Technology*, 1999. 15:197–200.
8. Kays, S.J., *Postharvest Physiology of Perishable Plant Products*. 1997, Athens, Greece: Exon Press.
9. Brecht, J.K., M.A. Ritenour, N.F. Haard, and G.W. Chism, Postharvest physiology of edible plant tissues, in *Fennema's Food Chemistry*, S. Damodaran, K.L. Parkin, and O.R. Fennema, Eds. 2008, pp. 975–1049, CRC Press: Boca Raton, FL.
10. Sams, C.E. and W.S. Conway, Preharvest nutritional factors affecting postharvest physiology, in *Postharvest Physiology and Pathology of Vegetables*, J.A. Bartz and J.K. Brecht, Eds. 2003, Marcel Dekker: New York, pp. 161–176.
11. Kader, A.A. and M.E. Saltveit, Respiration and gas exchange, in *Postharvest Physiology and Pathology of Vegetables*, J.A. Bartz and J.K. Brecht, Eds. 2003, Marcel Dekker: New York, pp. 7–30.
12. Ben-Yehoshua, S., R.M. Beaudry, S. Fishman, J. Jayanty, and N. Mir, Modified atmosphere packaging and controlled atmosphere storage, in *Environmentally Friendly Technologies for Agricultural Produce Quality*, S. Ben-Yoshua, Ed. 2005, CRC Press: Boca Raton, FL, pp. 61–112.
13. Dillahunty, A.L., T.J. Siebenmorgen, R.W. Buescher, D.E. Smith, and A. Mauromoustakos, Effect of moisture content and temperature on respiration rate of rice. *Cereal Chemistry*, 2000. 77:541–543.
14. Trewavas, A.J., Growth substances in context: A decade of sensitivity. *Biochemical Society Transactions*, 1992. 20:102–108.
15. Cherian, S., C.R. Figueroa, and H. Nair, 'Movers and shakers' in the regulation of fruit ripening: A cross-dissection of climacteric versus non-climacteric fruit. *Journal of Experimental Botany*, 2014. 65:4705–4722.
16. Manjunatha, G., V. Lokesh, and N. Bhagyalakshmi, Nitric oxide in fruit ripening: Trends and opportunities. *Biotechnology Advances*, 2010. 28:489–499.
17. Munné-Bosch, S. and M. Müller, Hormonal cross-talk in plant development and stress responses. *Frontiers in Plant Science*, 2013. 4: 529.
18. Murphy, A., Hormone crosstalk in plants. *Journal of Experimental Botany*, 2015. 66:4853–4854.
19. Gapper, N.E., R.P. McQuinn, and J.J. Giovannoni, Molecular and genetic regulation of fruit ripening. *Plant Molecular Biology*, 2013. 82:575–591.
20. Van de Poel, B. and D. Van der Straeten, 1-Aminocyclopropane-1-carboxylic acid (ACC) in plants: More than just the precursor of ethylene! *Frontiers in Plant Science*, 2014. 5:640.
21. Cara, B. and J.J. Giovannoni, Molecular biology of ethylene during tomato fruit development and maturation. *Plant Science*, 2008. 175:106–113.
22. Ju, C. and C. Chang, Mechanistic insights in ethylene perception and signal transduction. *Plant Physiology*, 2015. 169:85–95.

23. Pech, J.C., M. Bouzayen, and A. Latche, Climacteric fruit ripening: Ethylene-dependent and independent regulation of ripening pathways in melon fruit. *Plant Science*, 2008. 175:114–120.
24. Tromas, A. and C. Perrot-Rechenmann, Recent progress in auxin biology. *Comptes Rendus Biologies*, 2010. 333:297–306.
25. Su, L.Y., G. Diretto, E. Purgatto, S. Danoun, M. Zouine, Z.G. Li, J.P. Roustan, M. Bouzayen, G. Giuliano, and C. Chervin, Carotenoid accumulation during tomato fruit ripening is modulated by the auxin-ethylene balance. *BMC Plant Biology*, 2015. 15: 114.
26. Given, N.K., M.A. Venis, and D. Grierson, Hormonal regulation of ripening in the strawberry, a non-climacteric fruit. *Planta*, 1988. 174:402–406.
27. Ludford, P.M., Hormonal changes during postharvest, in *Postharvest Physiology and Pathology of Vegetables*, J.A. Bartz and J.K. Brecht, Eds. 2003, Marcel Dekker: New York, pp. 31–77.
28. Leng, P., B. Yuan, Y. Guo, and P. Chen, The role of abscisic acid in fruit ripening and responses to abiotic stress. *Journal of Experimental Botany*, 2014. 65:4577–4588.
29. Valero, D. and M. Serrano, *Postharvest Biology and Technology for Preserving Fruit Quality*. 2010, CRC Press: Boca Raton, FL.
30. Wills, R.B.H., Potential of nitric oxide as a postharvest technology, in *Advances in Postharvest Fruit and Vegetable Technology*, R.B.H. Wills and J. Golding, Eds. 2015, CRC Press: Boca Raton, FL, pp. 191–210.
31. Arasimowicz, M. and J. Floryszak-Wieczorek, Nitric oxide as a bioactive signalling molecule in plant stress responses. *Plant Science*, 2007. 172:876–887.
32. Haubrick, L.L. and S.M. Assmann, Brassinosteroids and plant function: Some clues, more puzzles. *Plant, Cell and Environment*, 2006. 29:446–457.
33. Caffall, K.H. and D. Mohnen, The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. *Carbohydrate Research*, 2009. 344:1879–1900.
34. Maness, N. and P. Perkins-Veazie, Soluble and storage carbohydrates, in *Postharvest Physiology and Pathology of Vegetables*, J.A. Bartz and J.K. Brecht, Eds. 2003, Marcel Dekker: New York, pp. 361–382.
35. Smith, A.C., K.W. Waldron, N. Maness, and P. Perkins-Veazie, Vegetable texture: Measurement and structural implications, in *Postharvest Physiology and Pathology of Vegetables*, J.A. Bartz and J.K. Brecht, Eds. 2003, Marcel Dekker: New York, pp. 297–330.
36. Stone, B., Cell walls of cereal grains. The Regional Institute, http://www.regional.org.au/au/cereals/2/12stone.htm, 2005.
37. Vicente, A.R., M. Saladie, J.K.C. Rose, and J.M. Labavitch, The linkage between cell wall metabolism and fruit softening: Looking to the future. *Journal of the Science of Food and Agriculture*, 2007. 87:1435–1448.
38. Carpita, N.C. and D.M. Gibeaut, Structural models of primary-cell walls in flowering plants - Consistency of molecular-structure with the physical-properties of the walls during growth. *Plant Journal*, 1993. 3:1–30.
39. Brummell, D.A., Cell wall disassembly in ripening fruit. *Functional Plant Biology*, 2006. 33:103–119.
40. Saladie, M., A.J. Matas, T. Isaacson, M.A. Jenks, S.M. Goodwin, K.J. Niklas, X.L. Ren, J.M. Labavitch, K.A. Shackel, A.R. Fernie, A. Lytovchenko, M.A. O'Neill, C.B. Watkins, and J.K.C. Rose, A reevaluation of the key factors that influence tomato fruit softening and integrity. *Plant Physiology*, 2007. 144:1012–1028.
41. Lara, I., B. Belge, and L.F. Goulao, The fruit cuticle as a modulator of postharvest quality. *Postharvest Biology and Technology*, 2014. 87:103–112.
42. Mishra, S., A. Haracre, and J. Monro, Food structure and carbohydrate digestibility, in *Carbohydrates—Comprehensive Studies on Glycobiology and Glycotechnology*, C-F. Chang, Ed. 2012, Chapter 13, pp. 289–316, InTech: Rijeka, Croatia. http://www.intechopen.com/books/carbohydrates-comprehensive-studies-on-glycobiology-and-glycotechnology/food-structure-and-carbohydrate-digestibility.
43. Blenkinsop, R.W., R.Y. Yada, and A.G. Marangoni, Metabolic control of low-temperature sweetening in potato tubers during postharvest storage, J. Janick, Ed. in *Horticultural Reviews*. 2004, John Wiley & Sons, Inc: New York, Volume 30, pp. 317–354.
44. Munoz, F.J., E. Baroja-Fernandez, M.T. Moran-Zorzano, A.M. Viale, E. Etxeberria, N. Alonso-Casajus, and J. Pozueta-Romero, Sucrose synthase controls both intracellular ADP glucose levels and transitory starch biosynthesis in source leaves. *Plant and Cell Physiology*, 2005. 46:1366–1376.

45. Tiessen, A., J.H.M. Hendriks, M. Stitt, A. Branscheid, Y. Gibon, E.M. Farre, and P. Geigenberger, Starch synthesis in potato tubers is regulated by post-translational redox modification of ADP-glucose pyrophosphorylase: A novel regulatory mechanism linking starch synthesis to the sucrose supply. *Plant Cell*, 2002. 14:2191–2213.
46. Serrano, M., F. Guillen, D. Martinez-Romero, S. Castillo, and D. Valero, Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. *Journal of Agricultural and Food Chemistry*, 2005. 53:2741–2745.
47. Lobit, P., M. Genard, B.H. Wu, P. Soing, and R. Habib, Modelling citrate metabolism in fruits: Responses to growth and temperature. *Journal of Experimental Botany*, 2003. 54:2489–2501.
48. Centeno, D.C., S. Osorio, A. Nunes-Nesi, A.L.F. Bertolo, R.T. Carneiro, W.L. Araujo, M.C. Steinhauser, J. Michalska, J. Rohrmann, P. Geigenberger, S.N. Oliver, M. Stitt, F. Carrari, J.K.C. Rose, and A.R. Fernie, Malate plays a crucial role in starch metabolism, ripening, and soluble solid content of tomato fruit and affects postharvest softening. *Plant Cell*, 2011. 23:162–184.
49. Degu, A., B. Hatew, A. Nunes-Nesi, L. Shlizerman, N. Zur, E. Katz, A.R. Fernie, E. Blumwald, and A. Sadka, Inhibition of aconitase in citrus fruit callus results in a metabolic shift towards amino acid biosynthesis. *Planta*, 2011. 234:501–513.
50. Hu, X.M., C.Y. Shi, X. Liu, L.F. Jin, Y.Z. Liu, and S.A. Peng, Genome-wide identification of citrus ATP-citrate lyase genes and their transcript analysis in fruits reveals their possible role in citrate utilization. *Molecular Genetics and Genomics*, 2015. 290:29–38.
51. DeBolt, S., D.R. Cook, and C.M. Ford, L-Tartaric acid synthesis from vitamin C in higher plants. *Proceedings of the National Academy of Sciences of the United States of America*, 2006. 103:5608–5613.
52. Tomas-Barberan, F.A. and J.C. Espin, Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture*, 2001. 81:853–876.
53. Shewry, P.R. and N.G. Halford, Cereal seed storage proteins: Structures, properties and role in grain utilization. *Journal of Experimental Botany*, 2002. 53:947–958.
54. Ekanayake, S., K. Skog, and N.G. Asp, Canavanine content in sword beans (*Canavalia gladiata*): Analysis and effect of processing. *Food and Chemical Toxicology*, 2007. 45:797–803.
55. Larher, F.R., D. Gagneul, C. Deleu, and A. Bouchereau, The physiological functions of nitrogenous solutes accumulated by higher plants subjected to environmental stress. *Acta Horticulturae*, 2007. 279:33–41.
56. Pereira, A. and M. Maraschin, Banana (*Musa* spp) from peel to pulp: Ethnopharmacology, source of bioactive compounds and its relevance for human health. *Journal of Ethnopharmacology*, 2015. 160:149–163.
57. Petersson, E.V., U. Arif, V. Schulzova, V. Krtkova, J. Hajslova, J. Meijer, H.C. Andersson, L. Jonsson, and F. Sitbon, Glycoalkaloid and calystegine levels in table potato cultivars subjected to wounding, light, and heat treatments. *Journal of Agricultural and Food Chemistry*, 2013. 61:5893–5902.
58. Shelp, B.J., G.G. Bozzo, C.P. Trobacher, G. Chiu, and V.S. Bajwa, Strategies and tools for studying the metabolism and function of gamma-aminobutyrate in plants. I. Pathway structure. *Botany-Botanique*, 2012. 90:651–668.
59. Fait, A., H. Fromm, D. Walter, G. Galili, and A.R. Fernie, Highway or byway: The metabolic role of the GABA shunt in plants. *Trends in Plant Science*, 2008. 13:14–19.
60. Whitaker, B.D., Chemical and physical changes in membranes, in *Postharvest Physiology and Pathology of Vegetables*, J.A. Bartz and J.K. Brecht, Eds. 2003, Marcel Dekker: New York, pp. 79–110.
61. Rodriguez-Concepcion, M. and C. Stange, Biosynthesis of carotenoids in carrot: An underground story comes to light. *Archives of Biochemistry and Biophysics*, 2013. 539:110–116.
62. Rodrigo, M.J., B. Alquezar, E. Alos, J. Lado, and L. Zacarias, Biochemical bases and molecular regulation of pigmentation in the peel of citrus fruit. *Scientia Horticulturae*, 2013. 163:46–62.
63. Seymour, G.B., N.H. Chapman, B.L. Chew, and J.K.C. Rose, Regulation of ripening and opportunities for control in tomato and other fruits. *Plant Biotechnology Journal*, 2013. 11:269–278.
64. Simon, P.W., Progress toward increasing intake of dietary nutrients from vegetables and fruits: The case for a greater role for the horticultural sciences. *HortScience*, 2014. 49:112–115.
65. Dixon, J. and E.W. Hewett, Factors affecting apple aroma/flavour volatile concentration: A review. *New Zealand Journal of Crop and Horticultural Science*, 2000. 28:155–173.
66. Sims, C.A. and R. Golaszewski, Vegetable flavor and changes during postharvest storage, in *Postharvest Physiology and Pathology of Vegetables*, J.A. Bartz and J.K. Brecht, Eds. 2003, Marcel Dekker: New York, pp. 7–30.
67. Klee, H.J. and J.J. Giovannoni, Genetics and control of tomato fruit ripening and quality attributes. *Annual Review of Genetics*, 2011. 45:41–59.

68. Davey, M.W., M. Van Montagu, D. Inze, M. Sanmartin, A. Kanellis, N. Smirnoff, I.J.J. Benzie, J.J. Strain, D. Favell, and J. Fletcher, Plant L-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture*, 2000. 80:825–860.
69. Smirnoff, N., Vitamin C: The metabolism and functions of ascorbic acid in plants, in *Advances in Botanical Research*, R.B. Fabrice and D. Roland, Eds. 2011, Academic Press: Cambridge, MA, pp. 107–177.
70. Foyer, C.H. and G. Noctor, Ascorbate and glutathione: The heart of the redox hub. *Plant Physiology*, 2011. 155:2–18.
71. Lee, S.K. and A.A. Kader, Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, 2000. 20:207–220.
72. Zhuang, H. and W.S. Barth, The physiological roles of vitamins in vegetables, in *Postharvest Physiology and Pathology of Vegetables*, J.A. Bartz and J.K. Brecht, Eds. 2003, Marcel Dekker: New York, pp. 341–360.
73. Liu, R.H., Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition*, 2003. 78:517S–520S.
74. Paull, R.E., Effect of temperature and relative humidity on fresh commodity quality. *Postharvest Biology and Technology*, 1999. 15:263–277.
75. Renard, C., C. Ginies, B. Gouble, S. Bureau, and M. Causse, Home conservation strategies for tomato (*Solanum lycopersicum*): Storage temperature vs. duration - Is there a compromise for better aroma preservation? *Food Chemistry*, 2013. 139:825–836.
76. Kader, A.A., *Postharvest Technology of Horticultural Crops*. 2002, Oakland, CA: Regents of the University of California, Division of Agricultural and Natural Resources.
77. Thompson, J.F., Precooling and storage facilities, in *The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks*, in Agricultural Handbook Number 66, Revised, K.C. Gross, C.Y. Wang, and M.E. Saltveit, Eds. 2016. pp. 11–21.
78. Wang, C.Y., Alleviation of chilling injury in tropical and subtropical fruits. 2010. 279:267–273.
79. Lukatkin, A.S., A. Brazaityte, C. Bobinas, and P. Duchovskis, Chilling injury in chilling-sensitive plants: A review. *Zemdirbyste (Agriculture)*, 2012. 99:111–124.
80. Aghdam, M.S. and S. Bodbodak, Postharvest heat treatment for mitigation of chilling injury in fruits and vegetables. *Food and Bioprocess Technology*, 2014. 7:37–53.
81. Wang, C.Y., Chilling and freezing injury, in *The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks*, in Agricultural Handbook Number 66, Revised, K.C. Gross, C.Y. Wang, and M.E. Saltveit, Eds. 2016. pp. 62–67.
82. Burg, S.P. and E.A. Burg, Molecular requirements for biological activity of ethylene. *Plant Physiology*, 1967. 42:144–152.
83. USDA, *The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks*, in Agricultural Handbook Number 66, Revised, K.C. Gross, C.Y. Wang, and M.E. Saltveit, Eds. 2016.
84. Kader, A.A. and M.E. Saltveit, Atmosphere modification, in *Postharvest Physiology and Pathology of Vegetables*, J.A. Bartz and J.K. Brecht, Eds. 2003, Marcel Dekker: New York, pp. 229–246.
85. Gasser, F., T. Eppler, W. Naunheim, S. Gabioud, and E. Hoehn, Control of the critical oxygen level during dynamic CA storage of apples by monitoring respiration as well as chlorophyll fluorescence. *Acta Horticulturae*, 2008. 796:69–76.
86. Zanella, A. and O. Rossi, Post-harvest retention of apple fruit firmness by 1-methylcyclopropene (1-MCP) treatment or dynamic CA storage with chlorophyll fluorescence (DCA-CF). *European Journal of Horticultural Science*, 2015. 80:11–17.
87. Baldwin, E.A., Coatings and other supplementary treatments to maintain vegetable quality, in *Postharvest Physiology and Pathology of Vegetables*, J.A. Bartz and J.K. Brecht, Eds. 2003, Marcel Dekker: New York, pp. 413–436.
88. Dhall, R.K., Advances in edible coatings for fresh fruits and vegetables: A review. *Critical Reviews in Food Science and Nutrition*, 2013. 53:435–450.
89. Serrano, M., D. Martinez-Romero, P.J. Zapata, F. Guillen, J.M. Valverde, H.M. Diaz-Mula, S. Castillo, and D. Valero, Advances in edible coatings, in *Advances in Postharvest Fruit and Vegetable Technology*, R.B.H. Wills and J. Golding, Eds. 2015, CRC Press: Boca Raton, FL, pp. 147–166.
90. Baldwin, E.A., Edible coatings, in *Environmentally Friendly Technologies for Agricultural Produce Quality*, S. Ben-Yehoshua, Ed. 2005, Taylor & Francis: Boca Raton, FL, pp. 301–315.
91. Gennadios, A., *Protein-Based Films and Coatings*. 2002, CRC Press: Boca Raton, FL.

92. Falguera, V., J. Pablo Quintero, A. Jimenez, J. Aldemar Munoz, and A. Ibarz, Edible films and coatings: Structures, active functions and trends in their use. *Trends in Food Science & Technology*, 2011. 22:292–303.
93. Saltveit, M.E., Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biology and Technology*, 1999. 15:279–292.
94. Martinez-Romero, D., G. Bailen, M. Serrano, F. Guillen, J.M. Valverde, P. Zapata, S. Castillo, and D. Valero, Tools to maintain postharvest fruit and vegetable quality through the inhibition of ethylene action: A review. *Critical Reviews in Food Science and Nutrition*, 2007. 47:543–560.
95. Watkins, C.B., Advances in the use of 1-MCP, in *Advances in Postharvest Fruit and Vegetable Technology*, R.B.H. Wills and J. Golding, Eds. 2015, CRC Press: Boca Raton, FL, pp. 117–146.
96. Watkins, C.B., Overview of 1-methylcyclopropene trials and uses for edible horticultural crops. *HortScience*, 2008. 43:86–94.
97. Lurie, S., Postharvest heat treatments. *Postharvest Biology and Technology*, 1998. 14:257–269.
98. Fallik, E., Prestorage hot water treatments (immersion, rinsing and brushing). *Postharvest Biology and Technology*, 2004. 32:125–134.
99. Arvanitoyannis, I.S., A.C. Stratakos, and P. Tsarouhas, Irradiation applications in vegetables and fruits: A review. *Critical Reviews in Food Science and Nutrition*, 2009. 49:427–462.
100. Fan, X.T., Irradiation of fresh and fresh-cut fruits and vegetables: Quality and shelf-life, in *Food Irradiation Research and Technology*, X. Fan and C.H. Sommers, Eds. 2012, Blackwell: Oxford, U.K., pp. 271–293.
101. Mahajan, P.V., O.J. Caleb, Z. Singh, C.B. Watkins, and M. Geyer, Postharvest treatments of fresh produce. *Philosophical Transactions of the Royal Society A-Mathematical Physical and Engineering Sciences*, 2014. 372: DOI: 10.1098/rsta.2013.0309.
102. Xiong, J.S., J. Ding, and Y. Li, Genome-editing technologies and their potential application in horticultural crop breeding. *Horticulture Research*, 2015. 2:(May 13, 2015).
103. Jiao, Z., J.C. Deng, G.K. Li, Z.M. Zhang, and Z.W. Cai, Study on the compositional differences between transgenic and non-transgenic papaya (*Carica papaya* L.). *Journal of Food Composition and Analysis*, 2010. 23:640–647.
104. Ledford, H., CRISPR, the disruptor. *Nature*, 2015. 522:20–24.
105. Hudson, J., A. Caplanova, and M. Novak, Public attitudes to GM foods. The balancing of risks and gains. *Appetite*, 2015. 92:303–313.
106. Konig, A., A. Cockburn, R.W.R. Crevel, E. Debruyne, R. Grafstroem, U. Hammerling, I. Kimber, I. Knudsen, H.A. Kuiper, A. Peijnenburg, A.H. Penninks, M. Poulsen, M. Schauzu, and J.M. Wal, Assessment of the safety of foods derived from genetically modified (GM) crops. *Food and Chemical Toxicology*, 2004. 42:1047–1088.
107. Ceccoli, S. and W. Hixon, Explaining attitudes toward genetically modified foods in the European Union. *International Political Science Review*, 2012. 33:301–319.
108. Commission, C.A., Codex principles and guidelines on foods derived from biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standard Programme. Food and Agriculture Organization of United Nations, Rome, Italy, 2003.
109. OECD, Safety evaluation of foods derived from modern biotechnology, concepts and principles. Organization for Economic Cooperation and Development, Paris, France, 1993.
110. Venneria, E., S. Fanasca, G. Monastra, E. Finotti, R. Ambra, E. Azzini, A. Durazzo, M.S. Foddai, and G. Maiani, Assessment of the nutritional values of genetically modified wheat, corn, and tomato crops. *Journal of Agricultural and Food Chemistry*, 2008. 56:9206–9214.
111. Bhandari, S.R., S. Basnet, K.H. Chung, K.H. Ryu, and Y.S. Lee, Comparisons of nutritional and phytochemical property of genetically modified CMV-resistant red pepper and its parental cultivar. *Horticulture Environment and Biotechnology*, 2012. 53:151–157.
112. Hefferon, K.L., Nutritionally enhanced food crops; Progress and perspectives. *International Journal of Molecular Sciences*, 2015. 16:3895–3914.
113. Xudong, Y., S. Al-Babili, A. Kloeti, Z. Jing, P. Lucca, P. Beyer, and I. Potrykus, Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science*, 2000. 287:303–305.
114. Weasel, L.H., *Food Fray*, 2009. Amacom Publishing: New York.
115. Grierson, D., Identifying and silencing tomato ripening genes with antisense genes. *Plant Biotechnology Journal*, 2016. 14:835–838.
116. FDA, FDA concludes arctic apples and innate potatoes are safe for consumption, http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm439121.htm, U.S.F.a.D. Administration, Ed. 2015.

117. Navarro, S. and J. Donahaye, Innovative environmentally friendly technologies to maintain quality of durable agricultural produce, in *Environmentally Friendly Technologies for Agricultural Produce Quality*, S. Ben-Yoshua, Ed. 2005, CRC Press: Boca Raton, FL, pp. 205–262.
118. Artes, F., P.A. Gomez, and F. Artes-Hernandez, Physical, physiological and microbial deterioration of minimally fresh processed fruits and vegetables. *Food Science and Technology International*, 2007. 13:177–188.
119. Barrett, D.M., J.C. Beaulieu, and R.L. Shewfelt, Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: Desirable levels, instrumental and sensory measurement, and the effects of processing. *Critical Reviews in Food Science and Nutrition*, 2010. 50:369–389.
120. Oliveira, M., M. Abadias, J. Usall, R. Torres, N. Teixido, and I. Vinas, Application of modified atmosphere packaging as a safety approach to fresh-cut fruits and vegetables—A review. *Trends in Food Science & Technology*, 2015. 46:13–26.
121. Reyes, L.F., J.E. Villarreal, and L. Cisneros-Zevallos, The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. *Food Chemistry*, 2007. 101:1254–1262.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Index

A

Abscisic acid (ABA), 1032, 1037
Absorption, 474
Accelerated solvent extraction (ASE), 888
Acesulfame K, 831
Acetic acid (CH_3COOH), 821
Acid–base properties
  amino acids, 241–244
  minerals
    Bronsted theory, 629–630
    Lewis theory, 630–632
Acidic microbial inhibitors, 805
Acid-modified starches, 141
Acrylamide formation, 110–113, 897–898
Actin, 966, 1005
α-Actinin, 978, 980
Activated charcoal, 849
Activation energy barrier–limited reactions, 77
Acylation, of proteins, 348–349
Adequate Intake (AI), 636–637
Adsorption, 474–475
Adsorption isotherm, 474
Adverse effects, of bioactive food components
  amygdalin, 891, 895
  carcinogens, 891, 894
  chymotrypsin, 893
  ciguatoxin, 893
  dhurrin, 891, 895
  garlic, 879
  goitrin, 893, 896
  HCN, 891, 896
  linamarin, 891, 895
  oxalates, 893
  pyropheophorbide-A, 893
  pyrrolizidine alkaloids, 891, 895
  α-solanine and α-chaconine, 891, 895
  tetrodotoxin, 893
  toxins and antinutrients, 891–893
Aglycon, 99
AIAs, *see* Aminoimidazoazaarenes
Ajoenes, 879
Aldohexose, 95
Alginates, 1067
Algins, 157–158
Alitame, 833
Alkylation, of proteins, 347
Alkylphenols, 787–788
Allergenicity, 324
Allicin, 879
Allyl methyl trisulfide, 879
Aloe vera gel, 1067
Alumina, 682
Amadori rearrangement, 106
Amino acid-derived volatiles, 1054
Amino acids
  acid–base properties, 241–244
  chemical reactivity, 247, 251
  hydrophobicity, 244–246
  optical properties, 246
  stereochemistry, 240
  structure and classification, 238–240
Aminoimidazoazaarenes (AIAs), 896–897
Ammonium carbonate [$(NH_4)_2CO_3$], 807
Amphiphiles, 475–477
Amygdalin, 891, 895
α-Amylase, 142
Amylopectin, 134–135, 1044
Amylose, 133–134
Animal foods, 660
Anisidine, 227
Annotated temperature composition state diagram, 81
Anthocyanidins, 709–710
Anthocyanins, 1053
  acylation, 711
  anthocyanidin, 709–710
  chemical structures, 711–712
  color and stability, 711
  copigmentation, 718–719
  enzyme reactions, 719–720
  flavonoids, chemical structure of, 708–709
  flavylium cation, 708–710
  glycosylations, 710–711
  light, 716–717
  metals, 717–718
  as natural food colorants, 720
  oxygen and ascorbic acid, 716
  structural transformations and pH levels, 712
  sugars and degradation products, 717
  sulfur dioxide, 718
  temperature, 712–716
Anthoxanthin, 721
Antibiotics, in food additives, 827–828
Antimicrobial agents, in food additives
  acetic acid, 821
  antibiotics, 827–828
  benzoic acid, 825–826
  diethyl pyrocarbonate, 828–829
  epoxides, 826–827
  glyceryl esters, 824
  lauric arginate, 824–825
  medium-chain fatty acids, 821–823
  natamycin, 825
  nitrate salts, 820–821
  nitrite, 820–821
  p-hydroxybenzoate alkyl esters, 826
  propionic acid, 821
  sorbic acid, 821–823
  sulfites, 818–820
  sulfur dioxide, 818–820
Antioxidants
  ascorbic acid and thiols, 221
  chemical mechanisms, 216
  food additives, 816–818
  free radicals, 217
  lipoxygenases, 222

metals, prooxidative activity of, 221–222
oxidation intermediates, 222
peroxides, 223
physical location, 224
plant phenolics, 220–221
prooxidants, 221
singlet oxygen, 222
superoxide anion, 222
synergistic activity, 223–224
synthetic phenolics, 220
tocopherols, 217–220
Apoptosis, 883
Aqueous solutions
bound water, 43–46
charge–dipole interaction, 35–37
colligative properties, 46–47
dipole–induced-dipole interaction, 39–40
hydrophobic effect, 40–43
water–solute interactions, 34–35
Aroma extract dilution analysis, 756
Ascorbic acid, 640, 1055–1056
analytical method, 581
bioavailability, 581
degradation, stability and modes of
chemical degradation, 575
environmental variables, 579–580
mechanism of, 576–579
metal ions, catalytic effects of, 575–576, 578
products of, 579–580
food additives, 816
functions, 580–581
in peas, 556–557
in potatoes, 557–558
structure and general properties, 573–575
and thiols, 221
in tomatoes, 554
ASE, *see* Accelerated solvent extraction
Ash, 657
Aspartame, 831–833
Astringency, 771–772
Auxins, 1032, 1037

B

Bancroft's rule, 517
Bentonite, 847
Benzoic acid (C_6H_5COOH), 825–826
Benzoyl peroxide [$(C_6H_5CO)_2O_2$], 849–850
Betaine, 616
Betalains, 1053
betacyanins, 726
betaxanthins, 726–727
chemical properties
conversion and stability, 730–731
enzymatic degradation, 730
heat and pH, 727–729
oxygen and light, 729–730
general structures and building blocks, 725–726
physical properties, 726–727
Bioactive food components
adverse effects, food components with
amygdalin, 891, 895
carcinogens, 891, 894
chymotrypsin, 893
ciguatoxin, 893
dhurrin, 891, 895
goitrin, 893, 896
HCN, 891, 896
linamarin, 891, 895
oxalates, 893
pyropheophorbide-A, 893
pyrrolizidine alkaloids, 891, 895
α-solanine and α-chaconine, 891, 895
tetrodotoxin, 893
toxins and antinutrients, 891–893
botanical dietary supplements, 886–888
extraction technologies, for botanicals
ASE, 888
EAE, 889
MAE, 889
PEF, 889
SFE, 888–889
Soxhlet extraction, 888
UAE, 889
health effects of, 865–866
nutraceuticals
anticancer effects, 883
anti-inflammatory effects, 882
antioxidant protection, 882–883
apoptosis, 883
bioavailability of, 885–886
carotenoids, 866–869
combined dietary treatment, 884
flavonoids (*see* Flavonoids)
immune system, 884
indoles, 879–882
isothiocyanates, 879–882
organosulfur compounds, 878–879
phytoestrogens, 884
polyphenols, 875–878, 884–885
proanthocyanidins, 874–875
process-induced, 890–891
process-induced toxicants, 893, 896–898
Bioavailability, of minerals
antagonists, 640–642
enhancers, 637, 640
Biocytin, 610
Biofortification, 1075
Biological scientists *vs.* food chemists, 1
Biological tissues, water content of, 20
Biotin
analytical methods, 611
bioavailability, 611
stability, 610
structure and general properties, 610
Bitter peptides, 324
Bitter taste substances
amino acids, DG values for, 762–764
caffeine, in coffee, tea, and cola nuts, 760
humulone to isohumulone, thermal isomerization, 760–761
limonin, 762–763
molecular structure–receptor relationship, 759
naringin, in grapefruit juices, 761–762
peptides, 762–764
phenylthiocarbamide, 763–765
PROP, 764–765

quinine, in soft drink beverages, 759–760
theobromine in cocoa, 760
Blair process, 699
Blanching, 556–557
Boiling point elevation, 47
Born self-energy of ion, 45
Bound water, 43–46
Bovine milk, 909, 913, 921–923, 929
Brassinosteroids (BRs), 1039
Brazzein, 836
Brominated vegetable oils, 846–847
Bromophenols, 791–792
Bronsted theory of acids and bases, 629–630
Browning flavors, 792–793
Brunauer, Emmett, and Teller (BET) monolayer water, 54, 62–65
β-scission reaction, 214–216

C

CA, *see* Controlled atmosphere
Cadmium, 656–657
Caffeic acid, 890
Caffeine, 760
Calcium, 644
bioavailability, 645–646
functional role, 666
Calcium alginate, 158
Calcium silicate ($CaSiO_3 \cdot XH_2O$), 851
Calcium stearate, 852
Calpain, 989
Camembert, 935
Camphor, 771
Capillary rise, 480
Caprenin, 843
Capsaicinoids, 769
Caramelization, 109–110
Caramellike flavor, 795–796
Carbohydrates, 164–165
dietary fiber, 163–165
food processing and handling, 9
monosaccharides, 92–113
oligosaccharides, 113–119
polysaccharides, 119–163
transforming enzymes, 389–391
enzymic pectin, 401–404
starch, 391, 394–398
sugar, 398–401
Carbonation, of food additives, 852–853
Carbon–carbon crosslinks, 216
Carbonyl iron, 663
Carbonyls, 226–227
Carboxymethylcelluloses (CMCs), 150–151
Cardiac muscle, structure of, 965
Carminic acid, 741
Carnitine, 616–617
Carnosic acid, 878
β-Carotene, 817, 867–868, 1076
Carotenoid-derived volatiles, 1054
Carotenoids, 229, 866–869, 1052–1053
and apocarotenoids, structures and formulas of, 702–704
carotenoid-chlorophyll-protein complexes, 705
chemical properties
antioxidant activity, 707
cis/trans isomerization, 707
oxidation, 706–707
commonly consumed foods, content in, 705
degradation, 565–566
glycosides, 705
isoprene units, 704
methylerythritol 4-phosphate pathway, 704
occurrence and distribution, 705
in ocean algae population, 702
oxidative cleavages of, 797
photoprotective role, 702
in photosynthetic organisms, 702
physical properties, extraction, and analysis, 706
stability, 708
structures and provitamin A activities, 559–561
vitamin A activity, 561–562, 702
Carrageenans, 123, 155–157, 851, 1067
Caryopsis fruit, 1023–1024
Caseins
acid casein, 914
bioactive peptides, 941–942
caseinates, 938
coagulation, 935
coprecipitates of, 938
edible plant tissues, 1067
essential amino acids, 945
families, 914
γ-caseins, 915
κ-caseins, 916–917
lysine, 945
micelles, 914–918
phosphoserine, 916
rennet, 938
WPIs, 938–941
Caseinate gels, 508–509
Caspase-3, 883
Catalytic power, of enzymes, 360–361
active sites, 364–365
approximation, 366
binding energy, 365–366
collision theory, 362
covalent catalysis, 366–371
net effects, 373
strain and distortion, 372
transition-state theory, 362–364
Cathepsins, 989
Cellulose, 149–151, 1040
Certified colors, 682, 732
properties of, 735–739
use of, 739–741
α-Chaconine, 891, 895
Chaotropes, 37
Charge–dipole interaction, 35–37
Chelate effect, 632–633
Chelating agents, 805, 814–817
Chemesthesis, 757
Chemical denaturing agents, 279–284
Chemical deterioration, of lipids
hydrolytic reactions, 205
oxidation reaction
antioxidants, 216–224
chemical pathway, 205–209

decomposition products, 213–216
measurement, 225–227
oxidation rates, influencing factors of, 224
prooxidants, 209–213
oxidative reactions, 205
Chemical leavening systems
ammonium salts, 807
baking mixes, 810
baking powders, 809–810
carbon dioxide, 807
evolution, 809
fast acting, 808
production, 808–809
slow acting, 808
dicalcium phosphate, 809
gluconic acid, 808
phosphates and potassium acid tartrate, 808
potassium bicarbonate, 807
properties, 809–810
refrigerated doughs, 810–811
sodium aluminum sulfate, 808
sodium bicarbonate, 807
Chemically modified starches, 143, 148
Chemical reactivity, of amino acids, 247, 251
Chemophobia, 14
Chili peppers, 769
Chitin, 1067
Chitosans, 1067
Chloride, 646–647
Chlorine, 852
Chlorine dioxide, 850
Chlorophyllase, 692–693
Chlorophylls, 1052
allomerization, 698
color preservation
acid neutralization, 699–700
HTST processing, 700
metallocomplexes, commercial application of, 700
thermal-processed vegetables, regreening of, 700–702
enzyme activity
chlorophyllase, 692–693
pheophytinase, 693
light-harvesting pigments, 689
metallocomplex formation, 696–698
nomenclature of, 690–691
pheophytins and pyropheophytins
in commercially canned vegetables, 696
and pyrochlorophylls, 696–697
in spinach, 694–696
photodegradation, 698–699
physical characteristics and analysis, 691–692
structural relationships, 691
structures of porphin, Fischer numbering scheme, 690–691
thermal processing, color loss, 699
Chlorosaccharides, 833–835
Chlortetracycline, 828
Cholecalciferol, 566–567
Cholesterol oxidation, 216
Choline, 616
Chymotrypsin, 893
Ciguatoxin, 893
Citric acids
acidulants, 816
edible plant tissues, 1047–1048
Citrus flavors, 780–781
Citrus oils, 847
CLA, *see* Conjugated linoleic acid
Clausius–Clapeyron equation, 66–67
Cocoa, 796–797
Coenzyme Q_{10}, 617–618
Cold-water-swelling starch, 148
Collagen, 966
cross-linking reactions, 970–971
groups, 967–968
HP and LP, 970, 972
polypeptides, 969–970
primary structure, 969
procollagen, 970
telopeptide regions, 969
tropocollagen, 968–969
Type I, 969
Type III, 968
Type IV, 968
Type V, 968
Colligative properties, 46–47
Collision theory of reaction catalysis, 362
Colloidal interaction
depletion interaction, 489
DLVO theory, 487–488
electric double layers, 485–487
hydrophobic interactions, 490
net interaction force, consequences, 485
steric repulsion, 488–489
van der Waals forces, 485
Color Additive Amendment, 731–732
Color additives, 732–733
Colorants
certified dye/colors
properties of, 735–739
use of, 739–741
classification of, 682–683
colors exempt from certification, 741–743
consumers, effects on, 682–683
definition, 682
dye/substratum, 682
electromagnetic spectrum, 682
energy range, visible light, 682
flavor perception, 683
nature identical, 682
plant and animal tissue pigments (*see* Pigments)
regulatory issues
synthetic and natural colorants, 734–735
United States, 731–734
sweetness perception, 683
Commodity milk products
butter, 936
cheese, 934–936
condensed milk, 936–937
consumption, 930–931
cultured products, 934
evaporated milk, 936–937

liquid milks
fat and protein, 931
heat treatment, effects of, 932–934
homogenization, 931–932
light exposure, 931
pasteurization, 932
temperature, 930
membrane separation processing, 937–938
milk powder, 936–937
Conjugated double bonds, 225–226
Conjugated linoleic acid (CLA), 229
Contact angles, 478–479
Contractile proteins
actin, 966, 976
myosin, 966, 973–975
Controlled atmosphere (CA), 1062–1065
Cook-up starches, *see* Modified food starches
Cooling substances, 770–771
Copper, 670–671
Corn starch, 133
Corn syrup solids, 142–143
Corn zein, 1067
Covalent catalysis, 366–371
Cretinism, 650
Critical micellization concentration (CMC), 476–477
Cross-linked starches, 144, 146–147
Cruciferae flavors, 774, 879
Culinary herbs, 782–784
Curcumin, 875–876
Cured meat pigments, 683, 685, 688
Cyanocobalamin, 613–615
Cyclamate, 830–831
Cyclamic acid, 830–831
Cyclodextrin glucanotransferase, 143
Cyclodextrins, 117–119
Cyclohexyl sulfamic acid, 830–831
Cysteine, chemical structure of, 651
Cytokinins, 1032, 1037

D

Daily reference values (DRVs), 550
Daily value (DV), 550
Damar gums, 846
Danish agar, 157
Dark, firm, and dry meat (DFD), 993–994
Degree of polymerization (DP), 119, 122
L-Dehydroascorbic acid (DHAA), 573–575
Deoxymyoglobin, 686
Deryagin–Landau, Verwey–Overbeek (DLVO) theory, 487–488
Desmin, 981
Dextrins, 141
Dextrose equivalency (DE), 142
DFD, *see* Dark, firm, and dry meat
DHPR, *see* Dihydropyridine receptor
Dhurrin, 891, 895
Diallyl thiosulfinate, 879
Dicalcium phosphate ($CaHPO_4$), 809
Dietary fiber, 163–164
2010 Dietary Guidelines for Americans, 647
Dietary lipids, 228
Dietary reference intakes (DRIs), 636–639
Dietary Supplement Health and Education Act (DSHEA), 886
Diethyl pyrocarbonate, 828–829
Digestibility, of proteins
antinutritional factors, 327–328
processing, 328
protein conformation, 325, 327
Dihydropyridine receptor (DHPR), 982–984
Dilauryl thiodipropionate, 817
Dipole–dipole interaction, 38–39
Dipole–induced-dipole interaction, 39–40
Disaccharides, 114
Dispersed systems
colloidal interaction
depletion interaction, 489
DLVO theory, 487–488
electric double layers, 485–487
hydrophobic interactions, 490
net interaction force, consequences, 485
steric repulsion, 488–489
van der Waals forces, 485
colloidal system, 470
emulsions, 526–527
Bancroft's rule, 517
coalescence, 520–523
continuous phase, 515
droplet breakup, 515–516
droplet-size distribution, 514
partial coalescence, 523–526
physical instability, types of, 519–520
protein, 517–519
recoalescence, 516–517
surface layer, 515
types, 469, 514
volume fraction, 514
fabricated foods, 468
foams
and emulsions, quantitative differences, 527
mechanical forces, 528–529
stability, 530–533
structure evolution, 529–530
supersaturation, 527–528
foods, 468–469
liquid dispersions
aggregation, 493–494
changes in, 491
sedimentation, 491–493
reaction rates, effects on, 472
size/scale, effects of
external forces, effect of, 471
pore size, 471
separation, ease of, 471
size distribution, 472
structural elements in foods, 470–471
surface area, 470
time scales involved, 471
visual appearance, 470
soft solids
biopolymers mixtures, phase separation, 495–497
cellular materials, 495
closely packed systems, 495
eating characteristics of foods, 510–513
gels, 495, 497–510, 512–513

solid dispersion, 470
surface phenomena
adsorption, 474–475
amphiphiles, 475–477
contact angles, 478–479
curved interfaces, 479–481
functions, 483–484
interfacial rheology, 481–482
interfacial tension, 473–474
polymers, 477–478
surface tension gradients, 482–483
Disulfide bonds, 265–267
Docosahexaenoic acid (DHA), 787
Dough formation, of proteins, 318–321
DP, *see* Degree of polymerization
Dye, 682
Dysregulated cellular signaling pathways, 883
Dystroglycan complex, 981
Dystrophin, 981

E

EAE, *see* Enzyme-assisted extraction
EC, *see* Epicatechin
ECM, *see* Extracellular matrix
Edible muscle tissues
cardiac muscle, 965
excitation-contraction coupling, 983–986
meat (*see* Meat)
muscle fiber types, 985
proteins
actin, 966, 976
α-actinin, 978, 980
CapZ, 980–981
desmin, 981
dystroglycan complex, 981
dystrophin, 981
ECM, 966–970
filamins, 981
integrins, 981–982
M-line, 981
MyBP-C, 981
MyBP-H, 981
myofibrillar, 966
myomesin, 981
myosin, 966, 973–975
nebulin, 978
sarcolemma, 982–983
sarcoplasmic, 966, 970, 972–973
SR, 982–983
stromal, 966
titin, 978
tropomodulin, 981
tropomyosin, 976–977
troponin, 977–978
skeletal muscle (*see* Skeletal muscle)
smooth muscles, 965–966
Edible plant tissues
carbohydrates
soluble, 1043–1044
storage, 1044–1046
structural, 1040–1043
commodity requirements
cereals, nuts, and seeds, 1076–1077
fresh-cut processing, 1077–1079
whole fruits and vegetables, 1077
desirable and undesirable changes, 1019
hormones
ABA, 1032, 1037
auxins, 1032, 1037
BR, 1039
cytokinins, 1032, 1037
ethylene (*see* Ethylene)
GAs, 1032, 1037
JA, 1039
NO, 1038
PAs, 1038
PGR, 1032
SA, 1039
structures of, 1032
lipids, 1051–1052
morphology, 1021–1024
organic acids, 1047–1048
phenolic compounds, 1048–1049
physiological stage, 1022–1023, 1025–1026
pigments, 1052–1053
postharvest technologies (*see* Postharvest technologies)
preharvest factors, 1025–1026
primary metabolism
aerobic respiration, 1029–1030
anaerobic respiration, 1029–1030
electron transport system, 1029
glycolytic pathway, 1027–1029
oxidative pentose phosphate pathway, 1029–1030
photosynthesis, 1026
potential storage life, 1031
relative perishability, 1031
respiration rate, 1030–1031
TCA cycle, 1027, 1029
proteins and aminoacids, 1049–1051
quality and postharvest physiology, 1020
ripening, 1024–1025
senescence, 1024–1025
staple foods, 1018–1019
storage period, 1019
transgenic plant products
GMO, 1074–1075
nutritionally enhanced food crops, 1075–1076
ripening, modification of, 1076
senescence processes, modification of, 1076
vitamins and health-promoting substances
ascorbic acid, 1055–1056
phytochemicals, 1056
sulfur compounds, 1057
Vitamin A, 1056
VOCs, 1054–1055
water composition, 1039–1040
EDTA, *see* Ethylenediaminetetraacetic acid
EGC, *see* Epigallocatechin
EGCG, *see* Epigallocatechin-3-gallate
Eicosapentaenoic acid (EPA), 787
Electrolytic iron, 663
Electromagnetic spectrum, 682
Electronic nose devices, 755
Electron transport system, 1029
Electrostatic interactions, 263–264

Emulsions, 526–527
  Bancroft's rule, 517
  coalescence
    centrifugation, 523
    external stress, 521
    film rupture, 520–521
    greater interfacial tension, 522
    proteins, 522–523
    smaller droplets, 522
    small-molecule surfactants, 523
    thicker film between droplets, 522
    Weber number, 521
  continuous phase, 515
  droplet breakup, 515–516
  droplet-size distribution, 514
  partial coalescence
    droplets, volume fraction of, 524
    fat crystallization, 524–525
    fat solid, proportion of, 523–524
    globule diameter, 525
    ice cream, 526
    shear rate, 524
    surfactant type and concentration, 525
  physical instability, types of, 519–520
  proteins, 299–304, 517–519
  recoalescence, 516–517
  surface layer, 515
  types, 469, 514
  volume fraction, 514
Endogenous enzymes
  cellular and tissue effects, 434–436
  color quality, 436–444
Enzyme-assisted extraction (EAE), 889
Enzymes
  biocatalysts, 359
  catalytic power, 360–361
    active sites, 364–365
    approximation, 366
    binding energy, 365–366
    collision theory, 362
    covalent catalysis, 366–371
    net effects, 373
    strain and distortion, 372
    transition-state theory, 362–364
  endogenous enzymes
    cellular and tissue effects, 434–436
    color quality, 436–444
  environmental factors
    nonthermal processing techniques, 433–434
    pH effects, 421–428
    temperature, 418–421
    water relations, 428–433
  exogenous enzymes
    carbohydrates, 389–398
    enzymic pectin transformation, 401–404
    sugar transformation, 398–401
  flavor biogenesis
    hydroperoxide lyase, 448–450
    lipid-derived flavors, 450
    lipoxygenases, 444, 446–448
  lipid-transforming
    lipases, 413–416
    lipoxygenase, 416
    phospholipases, 417
  protein and nonprotein nature, 359–360
  pungent flavors, 451–455
  reaction kinetics
    graphical analysis, 377–381
    models, 374–375
    rate expressions, 375–376
  specificity and selectivity, 381–389
  textural quality
    carbohydrate polymers, 456
    protein degradation, 456–457
    sugar wall defect, 457–458
Epicatechin (EC), 871–872
Epicatechin-3-gallate (ECG), 871
Epigallocatechin (EGC), 871–872
Epigallocatechin-3-gallate (EGCG), 871–872
Epoxides, 826–827
Ergocalciferol, 566–567
Esterification, of proteins, 350
Estimated Average Requirement (EAR), 636–637
Ethylene
  biosynthesis, 1032–1034
  climacteric fruits, 1034–1037
  non-climacteric fruits, 1034–1037
  postharvest technologies
    adsorption, 1071
    avoidance, 1070–1071
    catalytic decay, 1071
    detrimental effects, 1069–1070
    inhibitors of, 1071–1072
    oxidation, 1071
    physiological/biochemical response, 1070
    production and sensitivity, 1069–1070
Ethylenediaminetetraacetic acid (EDTA), 633, 815–816
Ethylene glycol (CH_2OH-CH_2OH), 839
Ethylene oxide, 827, 852
Ethylmaltol, 769
Excitation–contraction coupling, 983–986
Exogenous enzymes
  carbohydrates, 389–398
  enzymic pectin transformation, 401–404
  flavor biogenesis
    hydroperoxide lyase, 448–450
    lipid-derived flavors, 450
    lipoxygenases, 444, 446–448
  sugar transformation, 398–401
Extracellular matrix (ECM)
  collagen, 967
    cross-linking reactions, 970–971
    groups, 967–968
    HP and LP, 970, 972
    polypeptides, 969–970
    primary structure, 969
    procollagen, 970
    telopeptide regions, 969
    tropocollagen, 968–969
    Type I, 969
    Type III, and V, 968
    Type IV, 968
  integrins, 967
  localization and interaction, 967
Extrusion texturization, 317–318

F

FAD, *see* Flavin adenine dinucleotide
Fast-scan mass spectrometry, 754
Fast-twitch glycolytic (FG) muscle, 985
Fast-twitch oxidative (FOG) muscle, 985
Fat mimetics, 229–230
  carbohydrate, 842
  protein, 842
  pseudo-moistness, 842
Fat replacers
  fat mimetics
    carbohydrate, 842
    protein, 842
    pseudo-moistness, 842
  fat substitutes, 841
  reduced-calorie synthetic triacylglycerol fat substitutes, 842–843
  reduced-fat foods, 841
  synthetic compounds
    polydextrose, 843–844
    sucrose polyesters, 844–845
    trialkoxycarballates, 843
Fat-soluble vitamins
  vitamin A
    analytical methods, 565
    bioavailability, 565
    degradation, stability and modes of, 561–566
    structure and general properties, 559–562
  vitamin D
    analytical methods, 567
    structure and general properties, 566–567
  vitamin E
    analytical methods, 572
    bioavailability, 570, 572
    oxidative degradation, 569–571
    singlet oxygen and α-tocopherol, reaction of, 570–571
    stability, 569
    structure and general properties, 567–570
  vitamin K
    analytical methods, 573
    structure and general properties, 572–573
Fatty acid-derived volatiles, 1054
Fenton reaction, 212
Ferments, 358
Ferrous sulfate, 662–663
Filamins, 981
Fischer numbering system, 690
Flavin adenine dinucleotide (FAD), 588–589
Flavin mononucleotide (FMN), 588–589
Flavonoids
  chemical and biological properties, 870
  classification, 869
  daily intake, 870
  edible plant tissues, 1052–1053
  function, 869
  genistein, 872–873
  green tea catechins, 870–872
  oolong teas, 870–871
  PMFs, 873–874
  quercetin, 872–873
  structures, 869
  theaflavins, 871
Flavor binding, of proteins, 308–309
  influencing factors, 310–311
  thermodynamics, 309–310
Flavors
  astringency, 771–772
  atmospheric pressure ionization mass spectrometry techniques, 755
  bitter taste substances
    amino acids, DG values for, 762–764
    caffeine, in coffee, tea, and cola nuts, 760
    humulone to isohumulone, thermal isomerization, 760–761
    limonin, 762–763
    molecular structure–receptor relationship, 759
    naringin, in grapefruit juices, 761–762
    peptides, 762–764
    phenylthiocarbamide, 763–765
    PROP, 764–765
    quinine, in soft drink beverages, 759–760
    theobromine in cocoa, 760
  chemistry and technology, 797–798
  cooling substances, 770–771
  electronic nose devices, 755
  fast-scan mass spectrometry, 754
  fats and oils
    hydrolysis, 785–786
    long-chain polyunsaturated fatty acid, 785–787
  gas chromatography, 754
  kokumi and flavor-modifying compounds, 767–769
  lactic acid–ethanol fermentations, 782–785
  muscle foods and milk
    fish and seafood flavors, 790–792
    nonruminant meats, 789–790
    ruminants, 787–788
  perception, molecular mechanisms of, 756–757
  process/reaction flavors
    carotenoids, oxidative cleavages of, 797
    thermally induced process flavors, 792–797
  pungent substances, 769–770
  quantitative information, 755
  salty taste substances, 765–766
  sensory assessments, 755–756
  sour taste substances, 766
  sweet taste substances, 757–759
  time-intensity release rate concept, 755
  umami taste substances, 766–767
  unmodified and modified food matrix compositions, 755
  vegetable and fruit flavors
    Allium sp., 772–773
    branched-chain amino acids, 777–778
    citrus flavors, 780–781
    Cruciferae, sulfur-containing volatiles, 774
    lipoxygenase-derived flavors, 776–777
    long-chain fatty acids, β-oxidation, 776–778
    methoxy alkyl pyrazines, 775–776
    Shiitake mushrooms, 775
    shikimic acid pathway, 779
    spices and culinary herbs, 782–784
    terpenes, 779–780
Flickering cluster model, 32
FMN, *see* Flavin mononucleotide
Foams
  and emulsions, quantitative differences, 527
  mechanical forces, 528–529

stability
coalescence, 532–533
drainage, 531–532
Ostwald ripening, 530–531
structure evolution, 529–530
supersaturation, 527–528
Folates
analytical methods, 609–610
bioavailability, 609
degradation, stability and modes of, 604–609
structure and general properties, 601–604
Folic acid, 546, 548
Food additives
acids
acetic acid, 806
cheeses, 805
chemical leavening systems (*see* Chemical leavening systems)
citric, 806
D-gluconolactone, 805
dissociation constants, 806–807
functions of, 805
gluconic acid formation, 805
lactic acid, 805–806
mineral acids, 807
pectin gels, 805
potassium acid tartrate, 806
short-chain free fatty acids, 806
anticaking agents, 851–852
antimicrobial agents
acetic acid, 821
antibiotics, 827–828
benzoic acid, 825–826
diethyl pyrocarbonate, 828–829
epoxides, 826–827
glyceryl esters, 824
lauric arginate, 824–825
medium-chain fatty acids, 821–823
natamycin, 825
nitrate salts, 820–821
nitrite, 820–821
p-hydroxybenzoate alkyl esters, 826
propionic acid, 821
sorbic acid, 821–823
sulfites, 818–820
sulfur dioxide, 818–820
antioxidants, 816–818
appearance control and clarifying agents, 846–849
bases, 811–812
bread improvers, 849–851
buffer systems and salts
pH control, 812–813
phosphates, in animal tissues, 814
processed dairy foods, 913–814
water binding, in animal tissues, 814
carbonation, 852–853
chelating agents, 814–816
fat replacers (*see* Fat replacers)
firming texturizers, 845–846
flour bleaching agents, 849–851
functional food ingredients, 804
functional purposes, 804
masticatory substances, 845
nonnutritive and low-calorie sweeteners
brazzein, 836
chlorosaccharides, 833–835
glycyrrhizin, 834
miraculin, 836
monellin, 836
neohesperidin dihydrochalcone, 836
peptide, 831–833
relative sweetness values, 829
stevioside, 836
sulfoamide, 830–831
talin, 836
thaumatin I and II, 836
oxygen, protection from, 852
polyols
applications, 837
comparative structures, 837
ethylene glycol, 839
functions, 837
glucose, hydrogenation of, 837–838
IM foods, 839–841
isomalt, 838–839
polyglycerol, 839
sorbitol, 839–840
sweetness and energy values, 837–838
propellants, 853
stabilizers, 841
summary, 854–861
thickeners, 841
use of, 804
Food adulteration, phases of, 3–4
Food-borne heavy metals, toxicology of, 653
cadmium, 656–657
lead, 654
mercury, 654–656
Food chemists
analytical approach, 5
vs. biological scientists, 1
societal role
involvement types, 14–15
reasons for, 13–14
1938 Food, Drug, and Cosmetic Act, 547
Food gels
caseinate gels, 508–509
gelatin, 508
globular proteins, 509–510
mixed gels, 510–511
polysaccharides, 506–508
Food gums, *see* Hydrocolloids
Food processing and handling
carbohydrates, 9
chemical interactions, 7, 11
food stability factors, 11–13
lipids, 10
proteins, 8
Food safety, 5
attributes and alterations, 6
chemical and biochemical reactions, 6
moisture absorption isotherm, 55
nanoparticles, 898
Food stability and molecular mobility
glass transition, 75–77
glassy state, 78–80
reaction rates, 77–78
state diagram, 80–82

Formaldehyde (HCHO) formation, 457–458
Fortification and enrichment, of mineral foods
  iodine, 664–665
  iron, 661–664
  zinc, 664
Free glycerol, 837
Free radicals, 217
Free-radical scavengers (FRSs), 217, 220, 223
Freeze-drying process, 23
Freezing point depression, 46
Fresh-cut processing, 1077–1079
Fructooligosaccharides, 162
D-Fructose, 95

G

γ-aminobutyric acid (GABA), 1050–1051
Garlic, 772–773
GAs, *see* Gibberellins
Gas chromatography, 754
Gelatin, 508, 848, 1004, 1067
Gelatinization, 136–137
Gelation, of proteins, 313–316
Gellan, 161
Gels, 495, 512–513
  caseinate gels, 508–509
  cold-set gels, 498
  consistency, 503
  diffusion, 505
  examples, 132
  gelatin, 508
  globular proteins, 509–510
  heat-set gels, 498–499
  mixed gels, 510–511
  modulus, 500–501
  particle gels, 497–498, 502–503
  permeability, 505
  physical stability, 503–504
  polymer gels, 497–498, 501
  polysaccharides, 506–508
  rheological and fracture parameters, 499–500
  swelling and syneresis, 505
  three-dimensional network structure, 131
  weak gels, 504–505
General Standard for Food Additives (GSFA), 735
Genetically modified organisms (GMOs), 1047–1075
Genetic engineering, 866
Genistein, 872–873
Gibberellins (GAs), 1032, 1037
Gibbs free energy of hydration, 36–37
Gibbs–Marangoni effect, 516
Ginger, 770
Gingerols, 876–877
Glass transition temperature
  determination, 83–84
  molecular weight dependency, 84–87
  water activity and moisture content relationships, 87–88
Gliadins, 320, 1067
Globular proteins gel, 509–510
Glucoamylase, 142
Gluconic acid, 805, 808
β-D-Glucopyranose, 98–99
D-Glucose, 93–94
Glucose syrup, 143
Glucosinolates, 879–880
Glutathione, 768
Glutathione peroxidase, 223
Glycans, 119
Glyceryl esters, 824
Glycine, 969
Glycolytic pathway, 1027–1029
Glycosides, 99–100
Glycyrrhizin, 834
GMOs, *see* Genetically modified organisms
Goiter, 650
Goitrin, 893, 896
Goitrogens, 651
Gordon–Taylor equation, 84
Gossypol, 817
Green staining, 698
Green tea catechins, 870–872
Guar gum, 151, 153
Guggenheim, Anderson, and De Boer (GAB) model, 64–65
Gum arabic, 160–161

H

Haber–Weiss reaction, 213
Hatch Act, 5
Haworth projection, 96–98, 641
HCN, *see* Hydrocyanic acid
Heat shock proteins (HSPs), 1073
Heme pigments, 648
  chemical structures, classification, 683–684
  cured meats, 683, 685, 688
  in fresh and cooked meat, 683, 685
  MAP techniques, 689
  meat color and pigment stability, 688–689
  myoglobin/hemoglobin
    chemical structure, 684, 686
    green discoloration, 688
    oxidation states, 684, 686–688
    tertiary structure, 684, 686
Hemicelluloses, 1040
Henderson–Hasselbalch equation, 242
High-molecular-weight (HMW) glutenins, 320–321
High-temperature short-time (HTST) processing, 700
Hofmeister series, 37
Homeostasis, 634
Humulone, 760–761
Hydrochloric acid, 850
Hydrocolloids, 121, 841
Hydrocyanic acid (HCN), 891, 893, 896–897
Hydrodyne®, 1000
Hydrogen, as food additives, 852
Hydrogenation, 101–102
Hydrogen bonds
  proteins, 262–263
  water
    electronegativity, 26–28
    molecular weight, 27
    potential energy, 28–29
    strength, 29
Hydrogen peroxide, 223
Hydrolysis products
  polysaccharides, 132
  starch, 141–143

Hydrophilic antioxidants, 224
Hydrophilic–lipophilic balance (HLB) values, 476
Hydrophobic effect, 40–43
Hydrophobic hydration, 41
Hydrophobic interactions, 42, 264–265
Hydrophobicity, of amino acids, 244–246
p-Hydroxybenzoate alkyl esters, 826
10-Hydroxychlorophylls, 698
Hydroxycinnamic acids, 890
10-Hydroxyethylflavin, 589
Hydroxyl group esters, 103
Hydroxyl group ethers, 103–105
Hydroxylysylpyridinoline (HP), 970, 972
Hydroxypropylation, 146
Hydroxypropylmethylcelluloses (HPMCs), 150–151
Hydroxypropylstarch, 146

I

IAA, *see* Indoleacetic acid
Ice
  density, 20, 22
  physical properties, 23
  proton defects, 31
  structure, 29–31
IM foods, *see* Intermediate-moisture foods
Indigoid-type dyes, 739
Individual minerals, in foods, 657
Indoleacetic acid (IAA), 1037
Indole-3-carbinol, 880–882
Indoles, 879–882
Inosine-5'-monophosphate (IMP), 766–767
Inositol hexakisphosphate (IP6), 642
Instant milk-gel puddings, 811
Instant starch, *see* Pregelatinized starch
Integrins, 967, 981–982
Interfacial tension, 473–474
Intermediate-moisture (IM) foods
  Maillard browning, 60–61
  polyhydric alcohols, 839–841
  technological challenges
    moisture migration, 70–73
    phase transition, 73
  water activity *vs.* food stability relationships, 55, 58–60
Inulin, 162
Invert sugar, 117
Iodine
  foods, fortification and enrichment of, 664–665
  nutritional aspects, 650–651
Iron
  foods, fortification and enrichment of, 661–664
  functional role, 670
  nutritional aspects, 647–649
Isinglass, 848
Isohumulone, 761
Isomalt, 838–839
Isomaltol, 107–108
Isothiocyanates, 879–882

J

Jasmonic acid (JA), 1039
Joint WHO/FAO Expert Committee on Food Additives
  (JEFCA), 734–735

K

Kaschin–Beck disease, 652
Kauzmann temperature, 76
Kelvin equation, 480
Keratin, 1067
Ketoses, 95
Kokumi taste substances, 767–768
Konjac glucomannan (KG), 161–162
Kosmotropes, 36–37

L

α-Lactalbumin, 921
Lactase, 115
Lactic acid, 805
Lactoferricin B, 942–943
Lactoferrin, 921
β-Lactoglobulin, 920–921
Lactose, 114–116
Land-Grant College Act, 4–5
Laplace pressure, 479–480
Lauric arginate, 824–825
Lead, 654
Lecithin enzymatic hydrolysis, water activity on, 59, 61
Lemon flavor, 781
Leucoanthocyanidins, *see* Proanthocyanidins
Lewis theory of acids and bases, 630–632
Ligands, 631
Limit dextrin, 142
Limonin, 762–763
Linamarin, 891, 895
Lipid hydroperoxides, 226
Lipids
  acylglycerols, 175–176
  crystallization, 189–199
  density, 186
  electrical properties, 188
  fatty acid compositions, 178–180
  fatty acid profiles, alteration of, 199–202
  fatty acids, 173–175
  food processing and handling, 10
  functionality
    appearance, 204
    flavor, 204–205
    texture, 204
  health benefits
    carotenoids, 229
    CLA, 229
    dietary lipids, 228
    low-calorie lipids, 229–230
    phytosterols, 229
    trans fatty acids, 228
    w-3 fatty acids, 228–229
  hydrolytic reactions, 205
  molecular properties, 181–183
  optical properties, 187–188
  oxidation reaction
    antioxidants, 216–224
    chemical pathway, 205–209
    decomposition products, 213–216
    measurement, 225–227
    oxidation rates, influencing factors of, 224
    prooxidants, 209–213

phospholipids, 176–177
refining, 180–181
rheological properties, 183–186
solid fat content, 188–189
sphingolipids, 177
sterols, 177–178
thermal properties, 186–187
waxes, 178
Lipid-transforming enzymes
lipases, 413–416
lipoxygenase, 416
phospholipases, 417
Lipophilic antioxidants, 224
Lipoxygenases (LOX), 211–212, 222
Liquid dispersions
aggregation, 493–494
changes in, 491
sedimentation, 491–493
Liquid water, structure of, 31–34
Lithium chloride (LiCl), 765
Locust bean gums (LBGs), 151, 153
Low-calorie lipids, 229–230
Low-density lipoprotein (LDL)-cholesterol levels, 228
Low-molecular-weight (LMW) glutenins, 321
Lutein, 868
Lycopene, 706, 867–869
Lysine, 945
Lysophosphatidylcholine, 141
Lysylpyridinoline (LP), 970, 972

M

MAE, *see* Microwave-assisted extraction
Maillard browning reaction, 58, 60, 105–110, 897–898, 1045
Malic acid, 1047–1048
Malonaldehyde (MDA), 227
Maltodextrins, 142
Maltol, 107–108, 769
Maltose, 114
Mangiferin, 725
D-Mannitol, 102
Marangoni effect, 483
1-MCP, *see* 1-Methycyclopropene
MDA, *see* Malonaldehyde
Meat, 956
conversion of muscle to
creatine phosphate, 987–988
delay phase, 987–988
glycogen, 987
muscle proteins, postmortem degradation of, 988–991
resolution phase, 988
rigor phase, 988
natural and induced postmortem biochemical changes
cold shortening, 994–996
DFD, 993–994
electrical stimulation, 996–997
pH, 992
PSE, 992–993
thaw rigor, 996
nutritive value
amino acid composition, 958
carbohydrates, 959
composition, 957
fatty acid composition, 958–959
lipid content, 957–958
mineral, 959–960
protein content, 958
water-soluble vitamins, 959–960
preservation, chemical changes in
chilling process, 997–998
freezing, 998–999
high-pressure treatment, 999–1000
irradiation, 1000–1001
refrigeration, 997–998
processed meats
categories, 1001
curing, 1001–1002
fat immobilization, 1006–1008
hydration, 1002–1004
protein gel matrix formation, 1004–1007
restructure, 1008–1009
stabilization, 1006–1008
surimi, 1009
water retention, 1002–1004
Meat factor, 640
Medium-chain triglycerides (MCTs), 842
Melanins, 1049
Melanoidins, 108
Mercury, 654–656
Methional, 793
Methionine, 793
Methoxy alkyl pyrazines, 775–776
10-Methoxychlorophyll, 698
10-Methoxylactone, 698
1-Methycyclopropene (1-MCP), 1071–1072
Methylcelluloses (MCs), 150–151
Metmyoglobin, 686
MFG, *see* Milk fat globules
MFGM, *see* Milk fat globule membrane proteins
Michael addition-type reaction, 216
Microbial myrosinase, 880
Microcrystalline cellulose (MCC), 149–150, 852
Microwave-assisted extraction (MAE), 889
Milk
biosynthesis, 912–913
casein micelles, 911
cheese, 908
commodity milk products (*see* Commodity milk products)
consumption, 908
cow's milk composition, 913
concentrations, 914
enzymes, 929–930
lactose, 929
lipids, 921–924
milk fat globule membrane (*see* Milk fat globule membrane)
primary structure and interactions, 915–918
protein (*see* Milk proteins)
salts and minerals, 915–918, 927–928
whey proteins, 920–921
facts and figures, 909–910
gross macronutrient composition, 910–911
nutritive value
contents, 943–944
daily values, 945, 947

digestibility, 944
fat, 943–944
food labels design, 945–946
lactose intolerance, 947–948
phylogenetic tree, 910
yogurt, 908
Milk fat globule membrane (MFGM) proteins
lipid composition, 925–926
protein content, 926–927
tripartite structure, 924–925, 927
Milk fat globules (MFG), 911, 913, 924
Milk proteins
adsorption, 939
allergic proteins, 940–941
applications, 939
casein
acid casein, 914
bioactive peptides, 941–942
caseinates, 938
γ-caseins, 915
κ-caseins, 916–917
coagulation, 935
coprecipitates of, 938
essential amino acids, 945
families, 914
lysine, 945
micelles, 914–918
phosphoserine, 916
rennet, 938
WPIs, 938–941
daily intake, 944–945
digestibility, 944
intermolecular interactions, 940
ion-exchange fractionation, 939
solubility, 939
surface activity, 939–940
whey protein, 940, 942–943
Mineral acids, 807
Minerals
acid/base chemistry
Bronsted theory of acids and bases, 629–630
Lewis theory of acids and bases, 630–632
chelate effect, 632–633
definition, 628
foods
animal foods, 660
ash, 657
fortification and enrichment, 660–665
individual minerals, 657
physical and chemical properties, 666–671
plant foods, 658–659
processing effects, 665–666
nutritional aspects
bioavailability, 637, 640–642
calcium, 644–646
chloride, 646–647
digestion and absorption process, 643
DRIs, 636–639
homeostasis, 634
iodine, 650–651
iron, 647–649
phosphorous, 646
potassium, 646–647
selenium, 651–653
sodium, 646–647
summary, 635–636
zinc, 649–650
solubility, 629
Minimally processed products, 1068, 1077–1079
Miraculin, 836
Mixed linkage β-glucans, 164
Modified atmosphere (MA), 1062–1065
Modified atmosphere packaging (MAP), 689
Modified food starches, 138
applications, 147–148
cross-linked starches, 144, 146–147
stabilized starches, 144–145
Moisture desorption isotherm, 67
Moisture resorption isotherm, 67
Moisture sorption isotherms (MSIs)
categories, 51–52
gluten, 63
hysteresis, 67–68, 70
interpretation, 52–55
Molecular mobility
glass transition, 75–77
glassy state, 78–80
reaction rates, 77–78
state diagram, 80–82
Monellin, 836
Monocalcium phosphate monohydrate [$Ca(H_2PO_4)_2 \cdot H_2O$], 810
Monolaurin, 824
Monosaccharides, 806
definition, 93
glycosides, 99–100
isomerization, 96
reactions
acrylamide formation, 110–113
aldonic acid oxidation, 100–101
caramelization, 109–110
carbonyl group reduction, 101–102
hydroxyl group esters, 103
hydroxyl group ethers, 103–105
nonenzymic browning, 105–109
uronic acids, 102–103
ring forms, 96–99
Monosodium L-glutamate (MSG), 766–767
Monostarch phosphates, 145
Montmorillonite, 848
Multidomain foods, moisture migration in, 70–73
Muscle contraction cycle, 984
Myofibers
ECM, 961
endomysium, 962, 987
myofilaments, 963
perimysium, 962, 987
sarcomere, 963–964
sarcoplasm, 962–963
SL, 962
sliding filament theory, 963–965
SR, 962–963
types, 985
Myofibrillar proteins, 966, 1004–1007
Myoglobin/hemoglobin, 683
chemical structure, 684, 686
green discoloration, 688

oxidation states
fresh and cured meats, reactions in, 686–687
vs. oxygenation, 684
oxygen partial pressure, influence of, 687–688
reduced ferrous (Fe^{2+})/oxidized ferric (Fe^{3+}) form, 684
tertiary structure, 684, 686
Myomesin, 981
Myosin, 966, 973–975, 985, 1005

N

NAD(P)H/FMN cofactor systems, 458
Naringin, 761–762
Natamycin, 825
National Nutrient Database for Standard Reference, 550
Natural colorants, 734–735
Nebulin, 978
Neohesperidin dihydrochalcone, 836
Neotame, 833
Niacin
analytical methods, 592
bioavailability, 592–593
structure and general properties, 591–592
Nickel, 670
Nicotinamide adenine dinucleotide (NAD), 591–592
Nicotinamide adenine dinucleotide phosphate (NADP), 591–592
Nicotinic acid, 591
Nisin, 828
Nitrate salts, 820–821
Nitric oxide (NO), 1038, 1074
Nitrite, 820–821, 1002
Nitrite burn, 688
Nitrogen tetroxide (N_2O_4), 850
Nitrosamines, 897–898
Nitrosylmyoglobin, 688
NO, *see* Nitric oxide
Nonanthocyanin (NA)-type flavonoids
absorption spectra, 721–722
anthoxanthin, 721
in foods, 721–722
proanthocyanidins
acid hydrolysis, 723
basic building block of, 722–723
tannins, 723–724
Nonenzymic browning, 7, 105–109
Non-freezable water, 120
Nonmineral elements, 628
Nordihydroguaiaretic acid, 817
Nutraceuticals
anticancer effects, 883
anti-inflammatory effects, 882
antioxidant protection, 882–883
apoptosis, 883
bioavailability of, 885–886
carotenoids, 866–869
combined dietary treatment, 884
flavonoids (*see* Flavonoids)
immune system, 884
indoles, 879–882
isothiocyanates, 879–882
organosulfur compounds, 878–879
phytoestrogens, 884
polyphenolic compounds, 875–878
polyphenols, 884–885
proanthocyanidins, 874–875
process-induced, 890–891
Nutritional aspects
of minerals
bioavailability, 637, 640–642
calcium, 644–646
chloride, 646–647
digestion and absorption process, 643
DRIs, 636–639
homeostasis, 634
iodine, 650–651
iron, 647–649
phosphorous, 646
potassium, 646–647
selenium, 651–653
sodium, 646–647
summary, 635–636
zinc, 649–650
proteins
biological methods, 328–329
chemical methods, 329–330
enzymatic and microbial methods, 330
Nutritional Labeling and Education Act of 1990, 732
Nutrition labeling, 550–551

O

Odor units (OUs), 756
Oleic acid, 923
Oligosaccharides
cyclodextrins, 117–119
lactose, 114–116
maltose, 114
sucrose, 116–117
trehalose, 117
Onion, 772–773
Optical properties, of amino acids, 246
Organic acids, 637, 1047–1048
Organic phosphates, 646
Organized biological macromolecular structures, 20
Organosulfur compounds, 878–879
Ostwald ripening, 481
emulsions, 520
foam, 530–531
Oxalates, 893
Oxidation intermediates, 222
Oxidation reaction, lipids
antioxidants, 216–224
ascorbic acid and thiols, 221
chemical mechanisms, 216
free radicals, 217
lipoxygenases, 222
metals, prooxidative activity of, 221–222
oxidation intermediates, 222
peroxides, 223
physical location, 224
plant phenolics, 220–221
prooxidants, 221
singlet oxygen, 222
superoxide anion, 222
synergistic activity, 223–224

synthetic phenolics, 220
tocopherols, 217–220
chemical pathway, 205–209
decomposition products
alkoxyl radicals, 216
β-scission reaction, 214–216
carbon–carbon crosslinks, 216
cholesterol oxidation, 216
reaction schemes, 213–214
measurement, 225–227
oxidation rates, influencing factors of, 224
prooxidants
autoxidation, 209–210
free radicals, 212
hydroperoxides, 210, 212
LOX, 211–212
singlet oxygen, 210–211
transition metals, 212–213
Oxidative pentose phosphate pathway, 1029–1030
Oxidative rancidity, 205, 212, 227
Oxidized starches, 147
Oxygenation
heme pigment, 687
lipoxygenases, 387
of myoglobin, 684
Oxygen concentration and lipid oxidation rates, 224
Oxytetracycline, 828

P

PAHs, *see* Polycyclic aromatic hydrocarbons
Pale, soft, exudative meat (PSE), 992–993
Pantothenic acid
analytical methods, 613
bioavailability, 613
degradation, stability and modes of, 612
structure and general properties, 612
Parabens, 826
Para-casein complex, 814
Pectin gels, 805, 846
Pectins, 158–160, 1040–1041, 1067
Pellagra, 547
Pepper, 770
Peptides, 831–833
Periodic table, 628
Peroxides, 223
Phenylthiocarbamide (PTC), 763–765
Pheophorbides, 696
Pheophytinase, 693
Phosphates, 666, 668–669
Phosphoric acid (H_3PO_4), 807, 816
Phosphorous, 646
Phosphorylation, of proteins, 349
Phosphoserine, 916
Physical denaturing agents, 272–279
Physically modified starches, 143
Phytic acid, 640–642
Phytochemicals, 1056
Phytoestrogens, 884
Phytosterols, 229
Pigments
anthocyanins
chemical structures, 711–712
color and stability, 711
copigmentation, 718–719
enzyme reactions, 719–720
light, 716–717
metals, 717–718
as natural food colorants, 720
oxygen and ascorbic acid, 716
structural transformations and pH levels, 712
structure, 708–711
sugars and degradation products, 717
sulfur dioxide, 718
temperature, 712–716
betalains
betacyanins, 726
betaxanthins, 726–727
chemical properties, 726–731
general structures and building blocks, 725–726
physical properties, 726–727
carotenoids
and apocarotenoids, structures and formulas of, 702–704
carotenoid-chlorophyll-protein complexes, 705
chemical properties, 706–707
commonly consumed foods, content in, 705
glycosides, 705
isoprene units, 704
methylerythritol 4-phosphate pathway, 704
occurrence and distribution, 705
in ocean algae population, 702
photoprotective role, 702
in photosynthetic organisms, 702
physical properties, extraction, and analysis, 706
stability, 708
vitamin A activity, 702
chemical structures, classification, 683–684
chlorophyll
allomerization, 698
color preservation, 699–702
enzyme activity, 692–693
heat and acid, 693–697
metallocomplex formation, 696–698
photodegradation, 698–699
physical characteristics and analysis, 691–692
structure and nomenclature, 690–691
thermal processing, color loss, 699
heme pigments
chemical structures, classification, 683–684
cured meats, 683, 685, 688
in fresh and cooked meat, 683, 685
MAP techniques, 689
meat color and pigment stability, 688–689
myoglobin/hemoglobin, 684–688
nonanthocyanin (NA)-type flavonoids
absorption spectra, 721–722
anthoxanthin, 721
in foods, 721–722
proanthocyanidins, 722–723
tannins, 723–724
quinones, 724–725
xanthone, 725
Pimaricin, 825
Plant foods, 658–659
Plant growth regulators (PGRs), 1032
Plant phenolics, 220–221
Plastein reaction, 351

Plasticizing water, 120
PMFs, *see* Polymethoxyflavones
Polar antioxidants, 224
Polyamines (PAs), 1038
Polycyclic aromatic hydrocarbons (PAHs), 893, 896
Polydextrose, 162–163, 843–844
Polyglycerol, 839
Polyhydric alcohols, *see* Polyols
Polyhydroxyflavone sulfonate, 717
Polymer gels, 497–498, 501
Polymers, 477–478
Polymethoxyflavones (PMFs), 873–874
Polyols
  applications, 837
  comparative structures, 837
  ethylene glycol, 839
  functions, 837
  glucose, hydrogenation of, 837–838
  IM foods, 839–841
  isomalt, 838–839
  polyglycerol, 839
  sorbitol, 839–840
  sweetness and energy values, 837–838
Polyphenols, 642, 884–886
Polysaccharides
  algins, 157–158
  carrageenans, agar, and furcellaran, 154–157
  cellulose, 149–151
  chemical structures and properties, 119–120
  crystallinity, solubility, and cryostabilization, 120–121
  fructooligosaccharides, 162
  gellan, 161
  gels, 131–132, 506–508
  guar gums, 151, 153
  gum arabic, 160–161
  hydrolysis, 132
  inulin, 162
  konjac glucomannan, 161–162
  locust bean gums, 151, 153
  pectins, 158–160
  polydextrose, 162–163
  solution viscosity and stability, 122–124, 131
  starch
    amylopectin, 134–135
    amylose, 133–134
    cold-water-swelling starch, 148
    commercial products, 133, 136
    complexes, 140–141
    cyclodextrins, 117–119
    granules, 133–137
    hydrolysis products, 141–143
    modified food starches, 143–148
    pregelatinized starch, 148
    retrogradation and staling, 140
    unmodified starches, 137–138
    vegetable tissues, 138–140
  xanthan, 153–154
Polyvinylpyrrolidone (PVP), 848–849
Porcine stress syndrome, 992
Postharvest technologies
  CA, 1062–1065
  edible coatings
    injurious anaerobic gas concentrations, 1068
    lipids, 1066
    materials, 1066
    minimally processed products, 1068
    nonfleshy products, 1066
    polysaccharides, 1066–1067
    protein, 1067–1068
    requirements, 1066
    ripening and senescence, inhibition of, 1068
    shellac and carnauba wax, 1066
    synthetic, 1066
  ethylene
    adsorption, 1071
    avoidance, 1070–1071
    catalytic decay, 1071
    detrimental effects, 1069–1070
    inhibitors of, 1071–1072
    oxidation, 1071
    physiological/biochemical response, 1070
    production and sensitivity, 1069–1070
  heat treatments, 1073
  ionizing radiation, 1073–1074
  MA, 1062–1065
  nitric oxide, 1074
  polyamines, 1074
  RH, 1062
  storage temperature
    chilling-sensitive, 1059–1061
    cooling methods, 1057–1058
    curing, 1057
    flavor and aroma compounds, 1058
    freezing injury, 1062
    low, 1057
    non-chilling-sensitive, 1059–1061
    respiration rate and relative storage life, 1058
Potassium, 646–647
Potassium acid tartrate, 806
Potassium bicarbonate ($KHCO_3$), 807
Potassium bromate, 850
Potassium carbonate, 811
Potato amylopectin, 134–135
Prebiotic fiber, 163–164
Pregelatinized starch, 148
Pressure-shift freezing, 999
Pressure–temperature phase diagram, 24
Pretzels, 811
Primary lipid oxidation products, 225
Proanthocyanidins, 874–875
  acid hydrolysis, 723
  basic building block of, 722–723
Prooxidants, 221
  autoxidation, 209–210
  free radicals, 212
  hydroperoxides, 210, 212
  LOX, 211–212
  singlet oxygen, 210–211
  transition metals, 212–213
Propionic acid (CH_3-CH_2-COOH), 821
Propylene glycol alginates (PGAs), 158
Propylene oxide, 827
6-*n*-Propyl-2-thio-uracil (PROP), 764–765
Proteasome, 989
Protein dispersibility index (PDI), 295
Protein hydration levels, 56–57
Proteins
  amino acid contents, 325–327

chemical modifications, 346–347
acylation, 348–349
alkylation, 347
esterification, 350
phosphorylation, 349
sulfitolysis, 349–350
coalescence, 522–523
conformational stability, 267–268
denaturation, 269–270
chemical agents, 279–284
physical agents, 272–279
thermodynamics, 270–272
digestibility
antinutritional factors, 327–328
processing, 328
protein conformation, 325, 327
dough formation, 318–321
emulsions, 299–304, 517–519
enzymatic modifications
cross-linking, 351
hydrolysis, 350–351
plastein reaction, 351
flavor binding, 308–309
influencing factors, 310–311
thermodynamics, 309–310
foams, 304–308
food processing and handling, 8
foods, sensory attributes of, 284–286
gelation, 313–316
hydration, 286–290
hydrolysates, 312–322
allergenicity, 324
bitter peptides, 324
functional properties, 322–324
interfacial properties, 295–299
emulsifying properties, 299–304
foaming properties, 304–308
nutritive value evaluation
biological methods, 328–329
chemical methods, 329–330
enzymatic and microbial methods, 330
primary structure, 251–253
quality, 325
quaternary structure, 260–261
secondary structure, 253–257
solubility, 290–292
ionic strength and, 293–294
organic solvents and, 295
pH and, 292–293
temperature and, 295
stability forces
disulfide bonds, 265–267
electrostatic interactions, 263–264
hydrogen bonds, 262–263
hydrophobic interactions, 264–265
steric strains, 261
van der Waals interactions, 261–262
surfactants, 477
tertiary structure, 257–260
texturization, 316–317
extrusion, 317–318
spun-fiber, 317
viscosity, 311–313
Protein solubility index (PSI), 295
PSE, *see* Pale, soft, exudative meat
PTC, *see* Phenylthiocarbamide
Pterostilbene, 876–877
Pulsed electric field (PEF)-assisted extraction, 889
Pungent substances
black and white pepper, 770
chili peppers, 769
ginger, 770
Pure Food and Drug Act, 5
PVP, *see* Polyvinylpyrrolidone
Pyrochlorophylls, 696–697
Pyropheophorbide-A, 893
Pyrrolidone carboxylic acid (PCA), 699
Pyrrolizidine alkaloids, 891, 895
Pyrroloquinoline quinone (PQQ), 617

Q

Quercetin, 872–873
Quinine, 759–760
Quinones, 724–725

R

Raffinose, 116
Reactive oxygen species (ROS), 867, 883, 1056
Recommended dietary allowances (RDAs), 550–552, 636–637
Red meats, 959
Reduced iron, 663
Reducing sugars, 101
Reference daily intakes (RDIs), 550–552
Regulatory proteins
tropomyosin, 976–977
troponin, 977–978
Reindeer milk, 909
Relative humidity (RH), 1062
Relative vapor pressure (RVP), 51
Resins, 1066
Resistant starch (RS), 165
Resveratrol, 876–877
Retinoids
structures of, 559–560
vitamin A activity, 560–562
Retrogradation, 137, 140
RH, *see* Relative humidity
Riboflavin
analytical methods, 591
bioavailability, 590–591
photochemical degradation, 589–590
stability, 589
structure and general properties
FMN and FAD, 588–589
human and cow's milk, distribution in, 589–590
oxidation–reduction behavior, 589
Ripening process, 1024–1025, 1068, 1076
Ripe olives, 811
Roquefort, 936
ROS, *see* Reactive oxygen species
Rosanoff structures, 94
Rosins, 1066

Rounding off phenomenon, 139
RVP, *see* Relative vapor pressure
Ryanodine receptor (RyR), 982–984, 993
RyR, *see* Ryanodine receptor

S

SA, *see* Salicylic acid
Saccharin, 831
Salatrim (XVI), 843
Salicylic acid (SA), 1039
Salty taste substances, 765–766
Sarcolemma (SL), 962, 982–983
Sarcoplasmic proteins, 966, 970, 972–973, 1004
Sarcoplasmic reticulum (SR), 962–963, 982–984
Saturated fatty acids (C_2-C_{12}), 822
Seafoods
  bromophenols, 791–792
  dimethyl sulfide, 791
  fresh fish aromas, 790
  saltwater fish species, 790–791
Secondary lipid oxidation products, 226
Selenium, 651–653
Selenocysteine, chemical structure of, 651
Selenomethionine, chemical structure of, 651
Senescence process, 1024–1025, 1068, 1076
Sensory analysis, of oxidized lipids, 225
Sequestrants, *see* Chelating agents
Serine, chemical structure of, 651
Serine proteases, 406
Sesamolin, 890–891
SFE, *see* Supercritical fluid extraction
Shiitake mushrooms, 775
Shikimic acid pathway, 779
6-Shogaol, 876–877
Singlet oxygen, 222
Single water molecule, schematic model of, 26
Skeletal muscle
  Ca^{2+} release and uptake mechanisms, 982–983
  excitation–contraction coupling, 985–986
  myofibers
    ECM, 961
    endomysium, 962, 987
    myofilaments, 963
    perimysium, 962, 987
    sarcomere, 963–964
    sarcoplasm, 962–963
    SL, 962
    sliding filament theory, 963–965
    SR, 962–963
    types, 985
SL, *see* Sarcolemma
Sliding filament theory, 963–965
Slow-twitch oxidative (SO) muscle, 985
Smoluchowski's equation, 78
Smooth muscles, 965–966
Sodium, 646–647
Sodium acid pyrophosphate, 810
Sodium acid sulfate, 807
Sodium aluminum sulfate, 808
Sodium bicarbonate ($NaHCO_3$), 807, 811
Sodium chloride (NaCl), 669–670, 765–766
Sodium tripolyphosphate ($Na_5P_3P_{10}$), 815
Soft solids
  biopolymers mixtures, phase separation, 495–496
    complex coacervation, 497
    thermodynamic incompatibility, 496–497
  cellular materials, 495
  closely packed systems, 495
  eating characteristics of foods, 510–513
  gels, 495, 512–513
    caseinate gels, 508–509
    cold-set gels, 498
    consistency, 503
    diffusion, 505
    gelatin, 508
    globular proteins, 509–510
    heat-set gels, 498–499
    mixed gels, 510–511
    modulus, 500–501
    particle gels, 497–498, 502–503
    permeability, 505
    physical stability, 503–504
    polymer gels, 497–498, 501
    polysaccharides, 506–508
    rheological and fracture parameters, 499–500
    swelling and syneresis, 505
    weak gels, 504–505
α-Solanine, 891, 895
Solid–liquid equilibrium line, 23–24
Solubility
  minerals, 629
  proteins, 290–292
    ionic strength and, 293–294
    organic solvents and, 295
    pH and, 292–293
    temperature and, 295
Soluble carbohydrates, 1043–1044
Sorbic acid
  activity of, 821
  antimicrobial properties, 822–823
  antimycotic action, 821
  lactic acid bacteria, 822–823
  and protein, 823
  sodium and potassium salts, 821
  sulfur dioxide, 822–823
Sorbitol, 838–840
Sour taste substances, 766
Soxhlet extraction, 888
Soy isoflavones, 866
Soy proteins, 811, 1067
Spans, 839
Spun-fiber texturization, 317
SR, *see* Sarcoplasmic reticulum
Stability constant, of metal complexes, 631–632
Stability forces, protein structure
  disulfide bonds, 265–267
  electrostatic interactions, 263–264
  hydrogen bonds, 262–263
  hydrophobic interactions, 264–265
  steric strains, 261
  van der Waals interactions, 261–262
Stabilized starches, 144–145
Stachyose, 116

Staling, 140
Starch, 132–133
  amylopectin, 134–135
  amylose, 133–134
  cold-water-swelling starch, 148
  commercial products, 133, 136
  complexes, 140–141
  cyclodextrins, 117–119
  granules
    crystalline framework, 135
    gelatinization and pasting, 136–137
    properties, 133–134
  hydrolysis, 1046
  hydrolysis products, 141–143
  modified food starches, 143–148
  pregelatinized starch, 148
  retrogradation and staling, 140
  unmodified starches, 137–138
  vegetable tissues, 138–140
State diagrams
  applicability, 83
  sucrose solution, 80–82
Stearoyl-2-lactylate emulsifiers, 850–851
Stereochemistry, of amino acids, 240
Steric strains, 261
Stevioside, 836
Stokes–Einstein equation, 78
Stokes sedimentation, 492
Stromal proteins, 966, 1004
Structural carbohydrates, 1040–1043
Structure
  amino acids, 238–240
  liquid water, 31–34
  proteins
    primary structure, 251–253
    quaternary structure, 260–261
    secondary structure, 253–257
    tertiary structure, 257–260
Structured low-calorie fats, 230
Sucralose, 833–835
Sucrose, 116–117, 1043
Sucrose diacetate hexaisobutyrate, 846
Sucrose fatty acid esters, 230
Sucrose polyesters, 844–845
Sugar wall defect, 457–458
Sulfites, 818–820
Sulfitolysis, of proteins, 349–350
Sulfoamide, 830–831
Sulforaphane, 880–881
Sulfur dioxide (SO_2), 718, 818–820
Supercritical fluid extraction
    (SFE), 888–889
Superoxide anion, 222
Superoxide dismutase (SOD), 222
Surface area and lipid oxidation rates, 224
Surface-dilational modulus, 481–482
Surface load, 474, 518
Surface phenomena
  adsorption, 474–475
  contact angles, 478–479
  curved interfaces, 479–481
  interfacial rheology, 481–482
  interfacial tension, 473–474
  surface tension gradients, 482–483
  surfactants
    amphiphiles, 475–477
    functions of, 483–484
    polymers, 477–478
Surface pressure, 475
Surface-shear viscosity, 481
Surfactants
  amphiphiles, 475–477
  functions of, 483–484
  polymers, 477–478
Sweet taste substances, 757–759
Synthetic dyes, 734–735
Synthetic phenolics, 220

T

Talin, 836
Tangential stress, 482
Tannins, 723–724, 848–849
Tartaric acid, 1047–1048
TBA, *see* Thiobarbituric acid
Temperature and lipid oxidation rates, 224
Terpenes, 779–780
Terpenoid volatiles, 1054
Tetrahydrofolic acid, 546
Tetrodotoxin, 893
Texturization
  food enzymes
    carbohydrate polymers, 456
    protein degradation, 456–457
    sugar wall defect, 457–458
  proteins, 316–317
    extrusion, 317–318
    spun-fiber, 317
Thaumatin I and II, 836
Theobromine, 760
Thiamin
  analytical methods, 588
  bioavailability, 587–588
  degradation, stability and modes of
    stability properties, 582, 584–587
    thermal degradation, rate and mechanism of, 586–587
  structure and general properties, 582–583
Thiamine, 792
Thiazoline, 794
Thiobarbituric acid (TBA), 227
Thiodipropionic acid, 817
Tilsiter, 935
Titin, 978
Tocopherols, 217–220
  structures of, 567–568
  in vegetable oils and foods, 568, 570
  vitamin E activity, 568–569
Tocotrienols
  structures of, 567–568
  in vegetable oils and foods, 568, 570
  vitamin E activity, 568–569
Tolerable Upper Intake Level (UL), 636–637
Trans fatty acids, 228
Transgenic plant products
  GMO, 1074–1075
  nutritionally enhanced food crops, 1075–1076
  ripening, modification of, 1076
  senescence processes, modification of, 1076

Transglutaminase catalyzed cross-linking, 351
Transition-state theory of enzyme catalysis, 362–364
Trehalose, 117
Triacylglycerols, 922
Trialkoxycarballates, 843
Tricarboxylic acid (TCA) cycle, 1027, 1029
Triphenylmethane dye, 739
Triple point, 23
Tropocollagen, 968–970
Tropomodulin, 981
Tropomyosin, 976–977, 984
Troponin, 977–978
Tweens, 839

U

UAE, *see* Ultrasound-assisted extraction
Ubiquinone, *see* Coenzyme Q_{10}
Ultrasound-assisted extraction (UAE), 889
Umami taste substances, 766–767
Unbranched glycans, 123
Universal Salt Iodization (USI), 664
Unmodified starches, 137–138
Uronic acids, 102–103
USDA Nutrient Database for Standard Reference, 657
U.S. Department of Agriculture, 5

V

van der Waals interactions, 261–262, 485
Vanillin, 769
Veri-Green process, 700
Vinyldithiin, 879
Viscosity, of proteins, 311–313
Vitamins
  analytical methods, 550, 553
  ascorbic acid
    analytical method, 581
    bioavailability, 581
    degradation, stability and modes of, 575–580
    functions, 580–581
    structure and general properties, 573–575
  betaine, 616
  bioavailability, 553
  biotin, 610–611
  carnitine, 616–617
  choline, 616
  coenzyme Q_{10}, 617–618
  dietary recommendations, 550–552
  folates
    analytical methods, 609–610
    bioavailability, 609
    degradation, stability and modes of, 604–609
    structure and general properties, 601–604
  functions, 545
  niacin, 591–593
  nutrients, addition of, 547–549
  pantothenic acid, 612–613
  pyrroloquinoline quinone, 617
  retention, optimization of
    losses, prediction of, 619
    packaging, effects of, 619
    thermal processing procedures, 618–619
  sources, 546–547
  stability, 545–546
  toxicity, 546
  variation/losses, causes
    blanching and thermal processing, 556–557
    chemicals and food ingredients, influence of, 558–559
    inherent variation, 554
    postharvest changes, 555
    postprocessing losses, 556–558
    preliminary treatments, 555–556
  vitamin A
    analytical methods, 565
    bioavailability, 565
    degradation, stability and modes of, 561–566
    structure and general properties, 559–562
  vitamin B1 (*see* Thiamin)
  vitamin B2 (*see* Riboflavin)
  vitamin B6
    bioavailability, 601
    degradation, stability and modes of, 596–601
    measurement of, 601
    structure and general properties, 592–596
  vitamin B12, 613–615
  vitamin C, 1055–1056
  vitamin D
    analytical methods, 567
    structure and general properties, 566–567
  vitamin E
    analytical methods, 572
    bioavailability, 570, 572
    oxidative degradation, 569–571
    singlet oxygen and α-tocopherol, reaction of, 570–571
    stability, 569
    structure and general properties, 567–570
  vitamin K
    analytical methods, 573
    structure and general properties, 572–573
VOCs, *see* Volatile organic compounds
Volatile lipid oxidation products, 226
Volatile organic compounds (VOCs), 1053–1054

W

Warmed-over flavor, 213
Water
  abnormal properties, 22
  anomalous properties, 21–22
  density, 20, 22
  hydrogen bonds
    electronegativity, 26–28
    molecular weight, 27
    potential energy, 28–29
    strength, 29
  phase relationship, 22–25
  physical properties, 23
  radial distribution function, 33
  temperature–density profile, 34
Water activity, 13
  BET monolayer determination, 62–65
  definition and measurement, 48–51
  food stability, 55

hysteresis, 67–70
lipid oxidation rates, 224
microorganisms growth, 61
MSIs
categories, 51–52
interpretation, 52–55
temperature and pressure dependence, 65–67
Water-soluble, nonstarch food polysaccharides, 125–130
Water-soluble vitamins
ascorbic acid
analytical method, 581
bioavailability, 581
degradation, stability and modes of, 575–580
functions, 580–581
structure and general properties, 573–575
biotin, 610–611
folates
analytical methods, 609–610
bioavailability, 609
degradation, stability and modes of, 604–609
structure and general properties, 601–604
niacin
analytical methods, 592
bioavailability, 592–593
structure and general properties, 591–592
pantothenic acid, 612–613
riboflavin
analytical methods, 591
bioavailability, 590–591
photochemical degradation, 589–590
stability, 589
structure and general properties, 588–590
thiamin
analytical methods, 588
bioavailability, 587–588
degradation, rate and mechanism of, 586–587
stability properties, 582, 584–587
structure and general properties, 582–583
vitamin B6
bioavailability, 601
degradation, stability and modes of, 596–601
measurement of, 601
structure and general properties, 592–596
vitamin B12, 613–615
Water–solute interactions, 34–35
Weber number, 521
ω-3 fatty acids, 228–229
Whey protein nitrogen index (WPN), 937
Whey proteins
edible plant tissues, 1068
immunoglobulins, 921
α-lactalbumin, 921
lactoferrin, 921
β-lactoglobulin, 920–921
lysine, 945
serum albumin, 921
Williams–Landel–Ferry (WLF) equation, 79–80, 82–83
World Wide Web, 15

X

Xanthan, 153–154
Xanthone, 725
Xylitol, 102

Y

Yogurt, 934
Young equation, 478–479

Z

Zeaxanthin, 868
Zinc
foods, fortification and enrichment of, 664
nutritional aspects, 649–650

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com