

Peter C.K. Cheung

Editor-in-Chief

Bhavbhuti M. Mehta

Editor

Handbook of Food Chemistry



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With 209 Figures and 179 Tables

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*This book is dedicated to my family members:
Carmen, Timothy, Rebekah, and Anthony.
Peter C.K. Cheung*

Preface

Food chemistry, unlike other branches of chemistry which focus on a particular class of compounds, is a very broad field that encompasses many different aspects. Food chemistry is involved not only in the determination of the chemical composition of the raw materials and food products but also deals with the desirable and undesirable changes which occur in foods during their production and storage. Since food is a very complex matrix by its nature, the chemical reactions of its constituents are very complicated and affect the nutritional value, toxicological and safety aspects, as well as the sensory quality of the food. Food chemistry is also closely related to the technological and economic aspects in the food manufacturing industry, catering, and health food business.

This Handbook is intended to be a comprehensive reference for the various chemical aspects of foods and food commodities. Apart from the traditional knowledge, this book will cover the most recent research and development of food chemistry in the areas of functional foods and nutraceuticals, organic and genetically modified foods, and nanotechnology.

This Handbook is divided into the following seven parts:

Part 1: Introduction encompasses the overview of food chemistry, which includes the general properties of major and minor food components; the major categories of food additives including preservatives, dietary ingredients, processing aids, flavors, sweeteners, colors, and texturizers as well as the applications of these food additives in general. This part aims at providing some basic information for those who may not have the background knowledge of food chemistry and can be read as an independent introductory section.

Part 2: Principles of Chemical Analysis of Food Components provides a very concise coverage of the basic chemical analysis of food components, which highlights some classical wet chemistry methods, sampling and sample preparation, and instrumental food analysis. The purpose of this part is to outline some of the essentials of food analysis which are used to determine the chemical composition of food and food commodities mentioned in Part 3.

Part 3: Chemical Composition of Food and Food Commodities is the major section in this Handbook, which consists of 12 categories including natural foods and food commodities from plant and animal origin as well as genetically modified

and organic ones. Some common processed food products are also included to reflect the diversity of food commodities. The information in this part should give the readers a comprehensive knowledge of the chemical composition of most of the major food groups listed in a food composition database.

Part 4: Chemical and Toxicological Aspects of Food Contamination is a special section which aims at covering the various types of toxins from plant and microbial origins as well as the different chemical toxicants that are likely to cause food contamination. Both the chemical nature and toxicological aspects of all these natural and man-made contaminants/toxicants are essential for evaluating the safety of our foods and food products.

Part 5: Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Processing focuses on the possible chemical changes of food components in regard to their nutritional and toxicological aspects during conventional thermal processing methods including heating, freezing, and drying as well as the unconventional pressure-assisted thermal processing. Emphasis in this part is on the chemical degradation of food components leading to the formation of harmful substances during processing and their implications to food safety and health risk.

Part 6: Chemistry of Bioactive Ingredients in Functional Foods and Nutraceuticals highlights the chemical properties of natural bioactive substances that are from plant, animal, and microbial origin as well as synthetic ones. This information will be useful for those who want to apply these substances as bioactive ingredients in functional foods and nutraceuticals for human consumption.

Part 7: Chemistry of Food Nanotechnology covers a few areas related to food nanotechnology including an introduction to food nanotechnology and its application in food safety via the development of biosensors as well as nanomaterials used for food processing. It reflects the very rapid and promising development in food nanotechnology that would attract great attention in the near future.

This book should be appropriate for undergraduates and postgraduates in the academics and professionals from the various disciplines and industries who are interested in applying knowledge of food chemistry in their respective fields.

Peter C.K. Cheung

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Part I

Introduction

Bhavbhuti M. Mehta and Peter C.K. Cheung

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Abstract

Among the different branches of food science, food chemistry focuses on the changes in the composition and chemical, physical, and functional properties of foods and food products during their different processing stages and storage periods. Food chemistry has rapid development in the past few decades based on modern chemistry and biochemistry. Its recent advances are mainly in the

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chemical aspects of food components and additives in the areas related to food quality and safety. The future development of food chemistry will be expanded into the field of functional foods and nutraceuticals.

Definition and Importance of Food Chemistry

Foods may be defined as natural products, fresh or processed, which are consumed by human beings for their nourishment. Foods are composed of various chemical constituents including mainly carbohydrates, lipids, proteins, vitamins, minerals, and enzymes. All these chemical constituents when acting alone or when interacting with others play an important role for the changes of the physical and chemical properties of foods and food products during their production, handling, processing, storage, and distribution.

The science of food therefore studies all these properties in foods. Food science is a discipline in which biology, physical sciences, and engineering are used to study the nature of foods, the causes of their deterioration, and the principles underlying food processing. Food science is thus a multidisciplinary subject which involves chemistry, microbiology, biology, engineering, as well as biotechnology, and all these different fields are interrelated.

Chemistry is the science of the composition of matter and the changes in composition that occur under changing conditions (Ira 1976). The developments in various branches of chemistry such as organic chemistry, analytical chemistry, and physical chemistry were considered as essential to the advances of food chemistry (Meyer 2004). Food chemistry is one of the major disciplines of food science which is mainly focusing on changes in the composition and chemical, physical, and functional properties of foods and food products during various stages from farm to fork. Such changes would ultimately affect the quality attributes and sensory characteristics of foods as well as the safety aspect. Therefore, the application of food chemistry mainly focuses on improving food quality and safety for the consumers.

Thorough knowledge of chemistry, biochemistry, botany, zoology, physiology, and molecular biology is the key criteria to understand the food chemistry. Food chemistry does not only concern with the composition of food raw materials and end products but also with the desirable and undesirable reactions which are controlled by a variety of physical and chemical parameters. There are extensive studies on the chemical composition of foods, micronutrients, contaminants, and additives as well as mechanisms of various reactions which affect the quality and safety of foods. Food chemistry can provide an in-depth understanding of the principles and mechanisms involved in the various reactions that happened during food preparation, processing, and storage. A thorough understanding of all these chemical reactions that occur in foods would allow us to maximize the production or preservation of health-promoting compounds while minimizing the formation of

harmful substances during food handling and preparation. Some typical examples of these chemical reactions include enzymatic and nonenzymatic browning (Maillard reaction), lipid oxidation, starch hydrolysis, formation of *trans*-fatty acids, cross-linking and denaturation of proteins, gel formation, starch retrogradation, toughening and softening of meat texture, degradation of vitamins, development of food flavor and off-flavors, formation of carcinogenic compounds during cooking, migration of various chemicals from packaging materials to food and their interactions, as well as those measures taken to reduce deterioration of foods during handling, processing, and storage.

Historical Development of Food Chemistry

At some point in the distant past, our ancestors who were hunter-gatherers had harnessed fire and learned to cook raw foods and started to experiment primitive methods to preserve excess foods by trial and error. These events gave birth to the earliest knowledge of food science in which the chemistry of food was the major area. Later, as foods became more abundant due to agriculture and farming, people were motivated to make their diets more interesting and palatable and had the luxury to enjoy the variety of food. Without knowing the principles behind, our ancestors started to explore different cooking methods and treatments to make foods more digestible, to remove toxins, to improve its taste, and to keep it longer. Hence, traditional cooking and food preparation methods were developed in different regions and handed down to later generations.

It is difficult to trace the origin of food chemistry, but it is closely related to the development of the different branches in modern chemistry and also biochemistry. One of the major areas in food chemistry is the study of food composition in terms of the different chemical components. It was not until in the eighteenth and nineteenth centuries that the quest for understanding of the chemical nature of our food by organic chemists had emerged. Firstly, the chemical structure and properties of macronutrients like carbohydrates, proteins, and lipids were characterized. This was followed by the discovery of various vitamins in the early twentieth century. Research on the biosynthesis of both macro- and micronutrients and their metabolism has also linked up food chemistry and food biochemistry together during the twentieth century. The chemical analysis of food (so-called wet chemistry) was developed in parallel with the study of food components at a similar time due to the demand to expose the malpractices of adulteration by food suppliers. With the advances in modern analytical instrumentation in the 1960s, food analysis has moved from the classical techniques of wet chemistry to sophisticated instrumental techniques using spectroscopy and spectrophotometry. The development of physical sciences in the field of texture and image analysis has allowed the sensory aspects of foods to be described in a more quantifiable manner and the physical characteristics of foods to be more well defined.

Chemical Aspects of Food Components and Additives in Quality and Safety

Water

Water is essential to life. Water (H₂O) contains strong covalent bonds that hold the two hydrogen atoms and one oxygen atom together. The bonds between oxygen and each hydrogen atom are polar bonds. The outer-shell electrons are unequally shared between the oxygen and hydrogen atoms, the oxygen atom attracting them more strongly than each hydrogen atom. As a result, each hydrogen atom is slightly positively charged and each oxygen atom is slightly negatively charged. Therefore they are able to form hydrogen bonds. The nature of hydrogen bonds allows water to bond with other water molecules as well as with proteins, pectin, sugar, and starches. Water is important as a solvent or dispersing medium, dissolving small molecules to form true solutions and dispersing larger molecules to form colloidal solutions depending on their particle size and solubility.

Water is an essential constituent of many foods. It may occur as an intracellular or extracellular component in vegetable and animal products, acting as a dispersing medium or solvent in a variety of products, as the dispersed phase in some emulsified products such as butter, and as a minor constituent in other foods (deMan 1999). Water is the major component of many foods, with its content varying from food to food. The water content of some food and food products is as follows: meats (50–82 %), fruits (80–95 %), vegetables (70–95 %), beer (90 %), milk (84–86 %), bread (30–35 %), butter (16 %), milk powder (4–5 %), and anhydrous milk fat (0.5 %). Water in the proper amount, location, and orientation profoundly influences the structure, appearance, and taste of foods and their susceptibility to spoilage (Fennema 1996; Reid and Fennema 2008). Water activity (a ratio of the vapor pressure of water in a solution to the vapor pressure of pure water) has a profound effect on the rate of many chemical reactions (e.g., hydrolytic reactions, nonenzymatic browning, lipid oxidation, color reaction) in foods and on the rate of microbial growth (Labuza 1980; deMan 1999).

Carbohydrates

Carbohydrates are the most abundant food component and the most important energy source (4 kcal/g) in our diet. Carbohydrates are defined as polyhydroxy aldehydes or ketones and their derivatives. Carbohydrates come in various shapes and sizes, from small sugar molecules to complex polymers containing thousands of simple sugar units. Important food carbohydrates include simple sugars, dextrans, starches, and nonstarch polysaccharides including celluloses, hemicelluloses, pectins, and gums. The latter ones are an essential source of fiber in the diet. Carbohydrates are important constituents of foods not only because of their nutritive values but also because of their functional properties. Carbohydrates, especially polysaccharides, can be used as sweeteners, thickeners, stabilizers, gelling agents,

and fat replacers. They are being used in a wide spectrum of convenience foods (Vaclavik and Christian 2008).

The intensity of sweetness of sugars varies depending on the specific sugar (e.g., fructose is the sweetest whereas lactose is the least sweet). The high water solubility of sugars can make syrups easily. However, sugars form various crystals as water gets evaporated or depending upon the saturation of the solution. Depending upon the type of product, sugar crystallization is desirable (e.g., candy making) or undesirable (e.g., sweetened condensed milk, ice cream). A high concentration of sugar reduces the water activity in certain food products like jams, jelly, and sweetened condensed milk which prevents the growth of spoilage microorganisms.

The addition of sugar makes food more viscous and provides body and mouth-feel to foods. Nowadays, consumers are more health conscious, but the replacement of sugar by nonnutritive sweeteners could not totally satisfy consumers who demand food body fullness. Hence, the addition of various gums or starches is necessary to get the desired body of the product. The most common energy source of microorganisms is carbohydrates which are metabolized into various components like lactic acid, acetic acid, pyruvic acid, and carbon dioxide. Sugar alcohols such as mannitol, sorbitol, and xylitol are obtained from the reduction of the carbonyl group to a hydroxyl group of the reducing sugar. These sugar alcohols are not readily fermented by microorganisms and are used in chewing gums because of their tooth decay prevention and can be used to replace sugar in noncaloric foods (Vaclavik and Christian 2008).

The reducing sugar, having a free carbonyl group, undergoes reaction with free amino acid groups of protein leading to the formation of various flavor and color compounds (e.g., melanoidins – a brown pigment). The interaction of the carbonyl group with amino acids is known as Maillard reaction which can impart a brown color to food products such as bakery products, UHT milk, and milk powder during their production and subsequent storage period. Maillard reaction may also lead to the formation of various toxic compounds and loss of the available lysine. On the other hand, at extremely high temperatures, sugars alone can be decomposed to produce brown color compounds, and the reaction is popularly known as caramelization, which is a nonamino acid type of browning. Moreover, lactose undergoes thermal degradation with the production of various organic acids such as formic acid, lactic acid, pyruvic acid, levulinic acid, and acetic acid which can lower the pH of milk during heat treatment leading to heat coagulation of milk and milk products.

Lipids

Food lipids are esters formed by fatty acids and glycerol and are commonly known as triglycerides or triacylglycerides. They are a heterogeneous group of naturally occurring substances which are sparingly soluble in water but soluble in organic solvents such as ether, chloroform, acetone, and benzene. Up to 99 % of the lipids in plant and animal materials are consisted of triglycerides known as fats and oils.

At room temperature, fats are solid while oils are liquid. The fatty acids in triglycerides can be saturated and unsaturated, depending on the number of carbon-carbon double bonds in the hydrocarbon chain.

Lipid is a principal dietary component of energy source and reserve which provides (9 kcal/g) energy. The lipid content of foods can range from very low to very high in vegetable and animal products such as haddock (0.1 %), cod (0.4 %), barley (1.9 %), milk (4–6 %), chicken (7 %), cheese (25–30 %), butter (80 %), and ghee/butter oil (99.5 %) (deMan 1999). Lipids also carry the fat-soluble vitamins A, D, E, and K as well as provide essential fatty acids for our body (Vaclavik and Christian 2008). Fatty acids such as monounsaturated fatty acid and conjugated linolenic acid (CLA) have beneficial effects to human health such as a decreased risk of coronary heart disease. Apart from its energy and nutritional values, lipid plays an important functional role in foods by providing mouthfeel, palatability, texture, and aroma. Lipids provide either tenderization (e.g., oil pie crusts) or flakiness (puff pastry) that imparts distinct characteristics to a food product. However, health-conscious consumers often demand food products that have reduced-fat, low-fat, or no-fat formulations produced by substituting lipids with a variety of fat replacers derived from carbohydrates, proteins, or fats.

The fatty acid profile of individual fats and oils is unique, and hence the measure of various parameters related to fatty acid composition (e.g., melting point, saponification number, iodine value, amount of short-chain fatty acids, Reichert Meissl and Polenske value, refractive index) is often used to check adulteration of lipids in the food products. The melting point of fat is usually not sharp but is within a certain range due to variation in low-, medium-, and high-melting triglycerides. Milk fat has a wide melting range (e.g., -40°C to $+40^{\circ}\text{C}$), whereas chocolate fat has a narrow melting range (close to body temperature) to allow its melting in our mouth. Fat has the ability to form different crystals upon cooling. Controlled crystallization of lipids can improve the functional properties in foods because small fat crystals can give a smooth texture to products like butter and anhydrous milk fat.

Food lipids are subjected to a number of chemical reactions that would affect their quality and applications. Lipid oils can be modified by hydrogenation process to reduce the number of double bonds to be used as solid fats in margarine. Interesterification of lipids can be used to produce more spreadable butter. Fats and oils undergo various rancidification reactions. There are different types of rancidity that can take place in food products having high fats and oils: hydrolytic (due to action of lipase/lipolysis), ketonic (due to growth of penicillin mold), and oxidative (due to chemical reaction with oxygen) rancidity. Limited lipolysis is essential in certain varieties of cheese for the production of typical flavors but can also be detrimental in anhydrous milk fat which leads to the production of short-chain fatty acids giving a butyric and offensive smell. The oxidative deterioration of fats/oils can lead to the formation of various off-flavors as well as generation of various toxic compounds. Certain fats/oils that have undergone repeated deep-frying processes can form harmful chemicals including various epoxides, free radicals, and other toxic compounds, causing deterioration in the quality of food products.

Proteins

Proteins play a central role in biological systems. They are utilized in the formation and regeneration of body muscle. Certain specific proteins serve as enzymes, while others serve to provide functions in metabolic regulations. The energy provided by proteins is 4 kcal/g. Proteins are polymers of different amino acids joined together by peptide bonds. Because of the various side chains that are linked to different amino acids, proteins have different chemical properties (deMan 1999). Proteins have four types of structure including primary, secondary, tertiary, and quaternary structure. These structures are stabilized by peptide bonds, hydrogen bonds, disulfide bonds, hydrophobic interactions, ionic interactions, and van der Waals interactions. Knowledge of protein conformation and stability is essential to understanding the effects of processing on food proteins. Food proteins may be defined as those that are easily digestible, nontoxic, nutritionally adequate, functionally useable in food products, and available in abundance. Traditionally, milk, meats (including fish and poultry), eggs, cereals, legumes, and oilseeds have been the major sources of food proteins (Damodaran 2008).

Several factors, such as content of essential amino acids and digestibility, contribute to the differences in the nutritive values of proteins. Cereal proteins from wheat, maize, rice, and barley are richer in methionine but are very low in lysine, while legume proteins are deficient in methionine but are higher in lysine content. 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. Food proteins of animal origin are more completely digested than those of plant origin. Proteins of animal origin, such as 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. Plant proteins (e.g., soy and other legume and oilseed proteins) are used to a limited extent in conventional foods.

Proteins have many useful functional properties in foods such as hydration, emulsification, gelling, and foaming. Therefore, they can be used as thickeners, binding and gelling agents, as well as emulsifiers or foaming agents (Vaclavik and Christian 2008). Proteins generally have a great influence on the sensory attributes such as texture, flavor, color, and appearance of foods. For example, the textural and curd-forming properties of yogurt are due to the unique colloidal structure of casein micelles; the sensory properties of bakery products are related to the dough-forming properties of wheat gluten; and the succulent characteristics of meat products are largely dependent on muscle proteins (Damodaran 2008).

Proteins are vulnerable to many chemical reactions that would affect their nutritional and functional properties. For example, proteins can undergo several chemical alterations involving lysyl residues when exposed to high temperatures and alkaline pH. Such alterations reduce their digestibility. The reaction of reducing sugars with ϵ -amino groups (e.g., Maillard browning) also decreases digestibility of lysine and produces certain toxic compounds (Damodaran 2008). Fermentation of proteins leads to the production of various bioactive peptides which have health

benefits. Moreover, excessive proteolysis in cheese is responsible for the production of peptides with bitter taste. Various processing treatments can lead to various chemical reactions involving protein-protein and protein-lipid interactions affecting both functional properties and nutritive values of food proteins (Parris and Barford 1991).

Minerals

Minerals usually refer to elements other than C, H, O, and N that are present in foods. The mineral material may be present as inorganic or organic salts or may be combined with organic material. Major minerals include calcium, phosphorus, magnesium, sodium, potassium, and chloride. Trace elements include iron, iodine, zinc, selenium, chromium, copper, fluorine, lead, and tin. Minerals play important roles in both living organisms and foods (deMan 1999; Miller 2008).

Minerals are chemically inert to heat, light, oxidizing agents, and extreme pH. Minerals can, however, be removed from foods by leaching or physical separation. The most important factor causing mineral loss in foods is milling of cereals and rice. Hence fortification is generally carried out in certain foods to compensate for the loss of iron. Calcium and phosphate are present in colloidal form with casein micelles, but they can be removed and transferred to the soluble aqueous phase during acidification. Loss of calcium from milk can occur when whey is drained during cheese making (Lucey and Fox 1993). Moreover, calcium plays a significant role in cheese making, and hence sometimes calcium chloride is added in milk to make a firmer body of cheese.

The presence of calcium, magnesium, phosphate, and citrate in milk is responsible for the heat stability of milk and milk products. Moreover, citrate plays a key role during fermentation of milk in the preparation of dahi and yogurt. Phosphate is a food additive having multifunctions including acidifying (soft drinks), buffering (various beverages), anticaking, leavening, stabilizing, emulsifying, water binding, and protecting against oxidation (Miller 2008).

Minerals have a tendency to interact with other food components which affect the physical and chemical properties of foods. For example, iron and copper are considered as prooxidants and are responsible for various oxidative deteriorations in high-fat food products. Iron serves as a color modifier in meat and has the ability to form blue, black, or green complexes with polyphenol compounds. For applications in the food industry, nickel is used in the hydrogenation of vegetable oil and copper can be used to produce heat-stable color pigment by replacing magnesium in chlorophyll.

Vitamins

Although vitamins are only a minor constituent in foods, they play an essential role in human nutrition. Some vitamins function as part of a coenzyme whereas others

occur in foods as provitamins. They fall into two groups: water-soluble and fat-soluble vitamins. Sources of vitamins are from both animal and plant products. Milk and dairy products contain riboflavin, pyridoxine, and vitamins B₁₂, A, D, E, and K; fish, poultry, and meats provide riboflavin, niacin, biotin, thiamin, vitamin B₁₂, and pyridoxine; fruits and vegetables are rich in vitamins A, K, and C, folate, and riboflavin; bread and cereals contain thiamin, folate, pantothenic acid, niacin, biotin, riboflavin, and pyridoxine, while fats and oils contain vitamins A, D, E, and K (Combs 1992).

Chemically, many vitamins are unstable during thermal processing and storage (deMan 1999). Vitamin A and carotenoids are relatively stable to heat in the absence of oxygen but quite susceptible to oxidation in the presence of light due to their unsaturation. Vitamin D is also susceptible to degradation by light. Certain milk products like UHT milk and milk powders are fortified with vitamins A and D to compensate the losses during heating. Vitamin E can act as antioxidants which provide stability of highly unsaturated vegetable oils. Vitamin K is quite stable to heat treatment but a certain fat substitute has been reported to impair vitamin K absorption (Jesse 2008). Vitamin C or ascorbic acid is widely distributed in nature, mostly in plant products such as citrus fruits, green vegetables, tomatoes, and berries. Ascorbic acid is commonly used as a food ingredient/additive because of its reducing and antioxidative properties. Ascorbic acid also prevents enzymatic browning, inhibits nitrosamine formation in cured meats, and contributes to the reduction of metal ions. Nevertheless, vitamin C is the least stable of all vitamins and is easily destroyed during thermal processing and storage. Water-soluble vitamins are easily leached out during processing treatments (deMan 1999).

Enzymes

Enzymes are proteins with catalytic properties. Although enzymes are only minor constituents of many foods, they play a major role in foods. Enzymes that are naturally present in foods can cause both desirable and undesirable changes in food composition. Since very often these changes are undesirable, the responsible enzymes must be deactivated (deMan 1999). Since enzymes are proteinaceous in nature, various chemical agents and physical factors such as heat, strong acids and bases, organic solvents can denature them and destroy their activity (Oort 2010).

Some examples of the chemical reactions involved by these enzymes include oxidation catalyzed by lipid peroxides, lipolysis catalyzed by lipases, and enzymatic browning catalyzed by polyphenol oxidases. Lipases and lipoxygenase are responsible for the formation of short-chain fatty acids and other off-flavor products that are responsible for rancidity in high-fat food products. Polyphenol oxidases are responsible for browning in cut fruits and vegetables after exposure to oxygen.

On the other hand, food enzymes can have many useful applications. For instance, amylases which are widely found in plants, animals, and some microorganisms are used in the brewing and baking industries. Proteolytic enzymes are used in meat tenderization and cheese production. Peroxidases are responsible for

the bleaching of flour during natural aging. Glycolytic enzymes are responsible for the development of rigor mortis in fish and seafood products.

Food Additives

Many chemical substances are incorporated into foods for functional purposes, and in many cases, these ingredients can also be found occurring naturally in some foods. However, when they are used in processed foods, these chemicals are known as food additives (Lindsay 2008). According to the FDA, food additives are substances added to foods for specific physical or technological effects. They may not be used to disguise poor quality but may aid in preservation and processing or improve the quality factors of appearance, flavor, nutritional value, and texture (Vaclavik and Christian 2008).

Based on their specific functions in the food products, food additives include anticaking/free-flow agents (e.g., calcium silicate), antimicrobials (e.g., benzoic acid), antioxidants (e.g., vitamin E), emulsifiers (e.g., lecithin), stabilizers (e.g., carboxymethylcellulose), humectants (e.g., glycerol monostearate), bleaching/maturing agents (e.g., benzoyl peroxide), bulking agents (e.g., sorbitol), firming agents (e.g., calcium chloride), flavoring agents (e.g., aldehydes)/flavor enhancers (e.g., monosodium glutamate), coloring agents (e.g., anthocyanins), curing agents (sodium nitrate), dough conditioners/improvers (e.g., ammonium chloride), leavening agents (e.g., ammonium bicarbonate), fat replacers (sucrose polyester), sweeteners (e.g., high-fructose corn syrup), low-calorie sweeteners (e.g., aspartame), and so on. Food additives can be used to maintain the nutritional quality (e.g., use of antioxidants), increase the shelf life and stability (e.g., use of antimicrobial agents), enhance the appearance (use of coloring, flavoring agents, stabilizers, bleaching agents), and act as processing aids (e.g., use of acids, buffers, sequestrants) in food production (Meyer 2004).

Conclusion and Future Directions

The ultimate goal of food scientists and technologists is to produce foods and food products that are fresh, natural, nutritious, safe, attractive, tasty, wholesome, convenient, readily available at all seasons, and affordable to the general public. Food chemistry has stood up to this great challenge. With the demand for more natural foods and minimum processed foods, the use of nonthermal and food additive-free preservation technologies is emerging. The chemical changes in the food components and the microbes induced by nonthermal treatments like high pressure processing and other emerging technologies like pulsed electric field and ohmic heating require extensive studies. The future application of food chemistry will also be shifted from the conventional food commodities to those that are organic and genetically modified in origin. The growing popularity of molecular gastronomy has opened up yet another interesting area in culinary science in which food chemistry is closely

associated with physical chemistry. The advancement in nanotechnology and its application in foods has initiated a new investigation of food chemistry with a main focus on polymer science. As the structure-function relationship of individual food components with human health and diseases is becoming more important among food scientists, nutritionists, and physicians, the future direction of food chemistry will be closely linked to life sciences and health sciences as well as the rapid development of functional foods and nutraceutical in the food industry. It is anticipated that food chemistry would evolve into an even more multidisciplinary science in the twenty-first century with higher impact to mankind.

Cross-References

- ▶ [Chemical Properties and Applications of Food Additives: Preservatives, Dietary Ingredients, and Processing Aids](#)
- ▶ [Chemical Properties and Applications of Food Additives: Flavor, Sweeteners, Food Colors, and Texturizers](#)
- ▶ [General Properties of Major Food Components](#)

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General Properties of Major Food Components

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Abstract

Food consists of major constituents or materials. Its composition will affect nutritional and sensory quality of food product, therefore elucidating the composition of food is very essential for food production chain. The major compositions of most foods are including water, lipid, protein, carbohydrate, and enzyme. Each component has its own physical and chemical characteristics which contribute to the final properties of food products. This chapter focuses on the structure, physicochemical properties, and functionality of water, lipid, protein, carbohydrate, and enzyme.

Introduction

Raw food products from plants and animals contains mostly water, lipid, protein, and carbohydrate as major components. Understanding the fundamental aspects such as the composition and physicochemical properties of raw food products during handling, processing, and storage will be very important.

Water is an essential constituent in which organisms' life processes occur. In food, water presents predominantly as an intracellular or extracellular component in plant and animal food products. Interaction of water with other food constituents results in controlling many chemical and physical reactions which significantly contributes to shelf life and quality values of food products (Luck 1981). For instance, removal of water from food by physical or chemical methods such as binding it with salt or sugar inhibits chemical and enzymatic reactions produced by microorganisms, resulting in shelf life improvement of food products (Lewin 1974; Luck 1981). On the other hand physical interaction of water with macromolecules may contribute to the physical appearance of food.

Food lipids are generally nonsoluble compounds 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. Food lipids play an important role in food sensory quality by contributing to attributes such as texture, flavor, nutrition, and caloric density.

Proteins play a central role in biological systems and food as well. All biologically derived proteins can be used as food proteins which must be easily digestible,

nontoxic, nutritionally adequate, functionally usable in food products, available in abundance, and sustainable agriculturally. The functional properties of proteins in foods are related to their structural and other physicochemical characteristics.

Carbohydrates are organic compounds that are mostly distributed and abundant on earth. They consist of carbon, hydrogen, and oxygen and can be simple or complex in structure of molecules. In food processing, a carbohydrate has functional role in its physical and chemical properties which act as sweeteners, thickeners, stabilizers, gelling agents, fat replacers, and precursors for aroma and coloring substances, especially in thermal processing. Carbohydrates are commonly divided into monosaccharides, oligosaccharides, and polysaccharides.

Enzymes are proteins involved in chemical reactions related to metabolism. Generally, food enzymes can be classified as endogenous and exogenous. Endogenous enzymes are enzymes that exist and are synthesized by biological cells in all organisms and thus enhance or deteriorate food quality, while exogenous enzymes are enzymes that are added to foods to cause a desirable change (Damodaran et al. 2008). Inactivation or controlling the rate of enzymes is important to result in desirable changes in food products.

In this chapter, general properties of water, lipid, protein, carbohydrate, and enzyme such as their chemical structure, physical and chemical properties, and functional properties in food are discussed in different sections.

Water

Physical Properties of Water and Ice

Physical properties of water are shown in Table 1; much of this information was obtained from De Man (1999). Table 2 shows physical properties of ice at various temperatures. Some of the physical properties of water and ice are exceptional; however, these exceptionally high values of the caloric properties of water would be important for food processing operations such as freezing and drying (Fennema 1973). In frozen food, density difference of water and ice may contribute in structural damage to foods. Structural change from solid to semisolid or vice versa as a result of fluctuating temperature will change also the density of ice, producing stresses and structural damage in frozen foods (Boutron et al. 1960; Vaclavik and Christian 2014).

Interactions of Water

Water-Solute Interaction

Water-solute interaction results in alteration of the properties of food systems. These changes result from molecular interactions between water and the nature of the solute, particularly from ions or charged groups which interact with water through electrostatic forces. These interactions alter the geometric structure of

Table 1 Physical properties of water

Water	Temperature (°C)					
	0	20	40	60	80	100
Vapor pressure (mm Hg)	4.58	17.53	55.32	149.4	355.2	760.0
Density (g/cm ³)	0.9998	0.9982	0.9922	0.9832	0.9718	0.9583
Specific heat (cal/g °C)	1.0074	0.9988	0.9980	0.9994	1.0023	1.0070
Heat of vaporization (cal/g)	597.2	586.0	574.7	563.3	551.3	538.9
Thermal conductivity (kcal/m ² h °C)	0.486	0.515	0.540	0.561	0.576	0.585
Surface tension (dynes/cm)	75.62	72.75	55.32	69.55	66.17	62.60
Viscosity (centipoises)	1.792	1.002	0.653	0.466	0.355	0.282
Refractive index	1.3338	1.3330	1.3306	1.3272	1.3230	1.3180
Dielectric constant	88.0	80.4	73.3	66.7	60.8	55.3
Coefficient of thermal expansion × 10 ⁻⁴	–	2.07	3.87	5.38	6.57	–

Source: De Man J M. (1999). Principle of Food Chemistry. Aspen Publisher. Gaithersburg, Maryland

Table 2 Physical properties of ice

Ice	Temperature (°C)						
	0	–5	–10	–15	–20	–25	–30
Vapor pressure (mm Hg)	4.58	3.01	1.95	1.24	0.77	0.47	0.28
Heat of fusion (cal/g)	79.8	–	–	–	–	–	–
Heat of sublimation (cal/g)	677.8	–	672.3	–	666.7	–	662.3
Density (g/cm ³)	0.9168	0.9171	0.9175	0.9178	0.9182	0.9185	0.9188
Specific heat (cal/g °C)	0.4873	–	0.4770	–	0.4647	–	0.4504
Coefficient of thermal expansion × 10 ⁻⁵	9.2	7.1	5.5	4.4	3.9	3.6	3.5
Heat capacity (joule/g)	2.06	–	–	–	1.94	–	–

Source: De Man J M. (1999). Principle of Food Chemistry. Aspen Publisher. Gaithersburg, Maryland

water molecules found in hydrophilic groups of solutes which result in their mobility retardation and reactivity (Brady and Romanov 1960). In contrast, the hydrophobic groups of solutes interact weakly with adjacent water, due to their preference to nonaqueous surrounding environment (Ashbaugh et al. 2002). However, this weak interaction may result in instability and structural consequences.

Types of Water

Most natural fresh foods contain water up to 70 % of their weight or greater; fruits and vegetables contain water up to 95 % or greater (Vaclavik and Christian 2014). Water presents in food in the form of free, bound, and entrapped water

(Fennema 1973; Schmidt 2004). Free water is water which can be removed easily from foods just by squeezing, grinding, or pressing, for instance, removing water from orange fruit just by squeezing it.

Bound water is water which cannot be extracted easily because it binds tightly onto water molecules (Fennema 1973). Thus, this type of water needs particular analysis methods to determine its value in the particular food. An example of bound water is the water present in cacti or pine tree needles; it cannot be squeezed or pressed out; even extreme desert heat or a winter freeze does not negatively affect bound water, and the vegetation remains alive.

Water may also present as entrapped water in foods such as water in pectin gel or in fruit or vegetable. Entrapped water is immobilized in matrixes, capillaries, or cells, but if released during cutting or damage, it flows freely. Entrapped water has properties of free water and no properties of bound water. Entrapped water in food is often associated with water holding capacity, a term that is frequently employed to describe the ability of a matrix of molecules, usually macromolecules present at low concentration, to physically entrap large amounts of water in a manner that inhibits exudation under the application of an external, often gravitational, force (Belitz et al. 2009; Damodaran et al. 2008; De Man 1999). Familiar food matrices that entrap water in this way include gels of pectin and starch and cells of tissues, both plant and animal.

Physically entrapped water does not readily flow from tissue foods, even when they are cut or minced. Nevertheless, this water behaves during processing with properties close to those of pure water. It is easily removed during drying, easily converted into ice during freezing, and readily available as a solvent. Thus, though its bulk flow is severely restricted, the movement of individual molecules is essentially similar to that of water molecules in a dilute salt solution (Fennema 1973, 1996).

Most of the water in tissues and gels can be categorized as physically entrapped, and impairment of this entrapment capability (water holding capacity) of foods has a profound effect on food quality. Examples of quality defects associated with impairment of water holding capacity are syneresis of gels, thaw exudation from previously frozen foods, and inferior performance of animal tissue in sausage resulting from the decline in muscle pH that accompanies normal physiological postmortem events. In all cases, the quality defect stems from the physical relocation of water molecules in space but does not necessarily reflect any significant change in the interactive properties of these molecules (De Man 1999; Schmidt 2004).

Water as Food Solvent and Dispersing Medium

Solutes in the form of food constituents can be either *dissolved*, *dispersed*, or *suspended* in water depending on their particle size and solubility. To form a solution, water should dissolve small molecules such as salts, sugars, or water-soluble vitamins which may be either ionic or molecular. An ionic solution is formed by dissolving substances that ionize in water, such as salts, acids, or bases. Sodium chloride contains sodium (Na⁺) and chloride (Cl⁻) ions held together

by ionic bonds; the added water molecules reduce the attractive forces between the oppositely charged ions, the ionic bonds are loosened, and thus the water molecules surround the individual hydrated ions. Each ion is usually surrounded by six water molecules; the ions move independently of each other.

The surrounding concept of water molecules occurs also in polar molecules to form molecular solutions, for instance, sugars. When a sugar crystal is dissolved in water, predominant hydrogen bonds between the polar hydroxyl groups are loosened and replaced by hydrogen bonds between water and the sugar molecules. Therefore, the hydration occurs gradually until each sugar molecule is fully surrounded by water molecules. Temperature also affects the hydrogen bond disruption between polar hydroxyl groups of sucrose molecules, then facilitating the formation of hydrogen bonds between water and sucrose and hydration of sucrose molecules. Therefore, sucrose has much better solubility in hot water than in cold water. Solute dissolution in water will increase the boiling point of water which is practically important to be understood for food process application like in candy or jelly making through determination of the sucrose concentration by measuring the boiling point of sucrose solution (Fennema 1996).

Big molecules with a size range 1–100 nm are difficult to dissolve fully to form true solutions, but they can be dispersed to form colloidal dispersion. Examples of such molecules include hydrocolloids, starches, and some food proteins. Colloidal dispersions which mostly apply in food are often unstable systems; stabilization is necessary to maintain water-solute binding, thus phase separation can be prevented. They are particularly unstable to factors such as heating, freezing, or pH change. In food application, changing the conditions in a stable dispersion can cause the system to be unstable such as precipitation or gelation; however, this is desirable in some cases, for instance, when making pectin jellies (Belitz et al. 2009). Colloidal dispersion is essential to be understood in food processing as many convenient or packaged foods have colloidal dimensions and their stability and sensitivity to certain types of reactions.

Particles that are too large, usually >100 nm, will form a *suspension* when mixed with water. Suspension is much more unstable, which separates out over a period compared with colloidal dispersion. An example of a suspension is when native starch grains are suspended in water.

Water and Food Stability

Since water is a predominant compound in food, it has a significant role to control food stability. Stability of food includes its physical, chemical, and microbiological aspect associated with quality of food; these parameters will affect and relate to each other. Food instability can lead to food perishability which degrades food quality. It has long been recognized that there is a relationship between water content and food perishability. In some foods, water is an undesired compound. Concentration and dehydration processes are performed primarily for the purpose

Table 3 Water activity of some foods

Food	aw
Leberwurst	0.96
Salami	0.82–0.85
Dried fruits	0.72–0.80
Marmalades	0.82–0.94
Honey	0.75

Source: Belitz HD, Grosch W, Schieberle P (2009). Food Chemistry. 4th revised and extended edition, Springer-Verlag Berlin Heidelberg

of decreasing the water content of a food, simultaneously increasing the concentration of solutes and thereby decreasing perishability (Scott 1957).

Interestingly, it has also been investigated that various types of food with the same water content have different significant perishability. Therefore, it is evident that the indicator of perishability is not only the water content. Since water can interact with solute, the intensity binding might expect that water engaged in strong associations to lead food quality degradation mainly by microorganism growth and hydrolytic chemical reactions. The term “water activity” (A_w) was introduced to reflect the intensity with which water associates with various solute constituents (Luck 1981; Scott 1957).

Although A_w is not a totally reliable factor in food stability and safety, A_w can give sufficient information regarding its correlation with rates of microbial growth and rates of many degradative reactions. A_w is considered high in living tissues of organisms because they require sufficient level of water to maintain cell metabolism. However, microorganisms such as bacteria, mold, and yeast multiply at high A_w (Labuza et al. 1972). Preservation techniques against spoilage because of these microorganisms take into account the controlling of water activity in the food. Less bacterial growth occurs if the water level is lowered to less than 0.85 according to FDA Model Food Code. Foods with a_w values between 0.6 and 0.9 (examples in Table 3) are known as “intermediate moisture foods” (IMF) (Vaclavik and Christian 2014).

A_w is not the only factor that contributes to bacterial growth; other factors such as pH and environment would contribute also to food stability and safety. These foods are necessary to be protected against microbial spoilage. In food application, an option for decreasing water activity and thus improving the shelf life of food is to use additives with high water binding capacities, such as sucrose, salt, sorbitol, and glycerol, which act as humectants (Fennema 1978). For instance, jams, jellies, and dried fruits are prepared using high concentrations of sugar and brines, whereas hams, salami, and sausages contain high concentrations of salt to prolong their shelf life.

The storage quality of food does depend on the water activity (A_w), not on the water content. The ratio of the moisture content of food and the relative humidity of the air surrounding it is known as the water activity. It is an important characteristic. Water activity (A_w) is determined by (Vaclavik and Christian 2014)

$$A_w = P/P_0 = ERH \quad (1)$$

P = partial vapor pressure of food moisture at temperature T_0
 P_0 = saturation vapor pressure of pure water at temperature T_0
 T_0 = equilibrium temperature of the system
ERH = equilibrium relative humidity

A relationship between A_w and ERH is noteworthy. First, A_w is an intrinsic property of the sample, whereas %ERH is a property of the atmosphere established in steady state with the sample. Second, equality exists in Eq. 1 only if equilibrium has been established between the product and its environment. Equilibrium condition of the samples is a time-consuming process and almost impossible for large samples, especially at temperatures below 20 °C (Damodaran et al. 2008).

Role of Molecular Mobility in Food Stability

Even though A_w has become an important tool for the food industry, this should not preclude consideration of other approaches that can support A_w as a tool for predicting and controlling food stability and processability. Compelling evidence has accumulated to indicate molecular mobility. In the molecular mobility approach, attention is paid to the mobilities of the constituent molecules. Molecular mobility M_m defined as rotational and translational mobilities is considered relevant. This consideration of mobilities implies that careful attention should be given to the diffusional aspects of many reactions, and in particular, the importance of diffusion-limited reactions to the quality in many foods such as glassy states, recrystallization, and collapse temperatures during freeze drying contributes to the properties of biological materials (Angibaud 1986).

Many of the basic concepts related to M_m in nonequilibrium systems consisting of synthetic amorphous polymers explained the important role of glassy and supersaturated states in food containing various sugars and suggested that the existence of these states had an important influence on the stability and processability of many foods (Ferry 1980; White and Cakebread 1966; White and Cakebread 1969; Williams et al. 1955). According to Duckworth et al. (1976) evidence of the relationship between M_m and food stability is important, since there is a relevance of M_m to rates of nonenzymatic browning and ascorbic acid oxidation.

The major concept regarding the relationship between molecular mobility and stability of foods is very simple. As a food is cooled, molecular mobilities decrease. The different molecular constituents of the food will give their own characteristic mobilities. Two possible mechanisms are proposed. First, as temperature is lowered, at some point the larger molecules' diffusion is highly restricted, and processes depending on their mobility will slow down as well. At some lower temperature, intermediate-size molecules also experience restricted motion that affects the properties of the system, and its reactions. This relationship exhibits temperature dependence, particularly in the temperature zone where restricted motion occurs (Boutron et al. 1986; Damodaran et al. 2008).

Second, as temperature is lowered, separation of new solid occurs. Ice solid phase would be more relevant to this mechanism. The principle is when ice separates out, concentration of solutes in the unfrozen aqueous phase increases; for this relationship it is known that molecular mobility is not solely a function of the temperature but also a function of the concentration, since at higher concentrations collisions and involvement become more likely. The combination of temperature and concentration prevail to a reduction in mobility with decreasing temperature, which is much more obvious than only driving force-temperature dependence. As previously, molecular size is a factor, with large molecules exhibiting severely restricted mobilities at higher temperatures than do smaller molecules (Damodaran et al. 2008; Duckworth et al. 1976; White and Cakebread 1966; White and Cakebread 1969).

In particular, Levine and Slade proposed the application of the Williams-Landel-Ferry (WLF) equation to food systems to describe interrelationships between WLF and molecular mobility. The WLF equation takes the form below (Damodaran et al. 2008):

$$\log \frac{\eta}{\eta_g} = \frac{-C_1(T - T_g)}{C_2 + (T - T_g)} \quad (2)$$

η = the viscosity at product temperature T (K)

η_g = the viscosity at product temperature T_g (K)

T_g (K) = product temperature (usually the glass transition temperature)

C_1, C_2 = constants

η can be replaced by $1/M_m$, the molecular mobility, or any other diffusion-limited relaxation process. This equation describes the dependence of system viscosity, and other diffusion-enabled processes, on the amorphous state behavior of a polymer. In polymer systems, universal values have been established for C_1 and C_2 . It is a topic of dispute as to whether these values can be usefully applied to systems of aqueous food glasses (Damodaran et al. 2008).

Diffusion-limited processes often play an important role in food stability, since the essential constituents considered in molecular mobility are water and the dominant solute or solutes; evidence implies that M_m is causatively related to diffusion-limited properties of foods which contain other substances besides water. Some types of these substances are substantial amounts of amorphous, primarily hydrophilic molecules, ranging in size from monomers to polymers, for example, starch-based foods, protein-based foods, boiled confections, intermediate-moisture foods, and dried, frozen, or freeze-dried foods (Slade and Levine 1995). When in a condition where M_m is greatly reduced, diffusion-limited properties become highly stable, changing very slowly or not at all with time. It is important to notice that while most processes of physical change are diffusion limited, not all processes of chemical change are so limited. Sometimes chemical reactivities are the dominant factor in food stability (Damodaran et al. 2008).

Food Lipids

Chemical Structure and Classification

Fats and oils are triglycerides, the major constituent of lipids. Overall, lipid is the umbrella term that includes the triglycerides, phospholipids, and sterols.

Glycerides

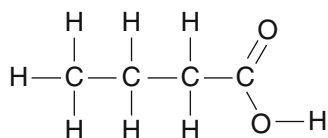
Glycerides include monoglycerides, diglycerides, and triglycerides. Glycerides have a glycerol molecule backbone joined to one or more fatty acid molecules (O'Keefe 2002). A monoglyceride contains glycerol esterified to one fatty acid molecule. Diglycerides are formed by esterification of two fatty acids to glycerol. If a triglyceride consists of three same fatty acids, it is called a simple triglyceride, otherwise if it contains two or three different fatty acids it is called a mixed triglyceride (O'Keefe 2002; Weiss 1983). The arrangement and specific type of fatty acids on the glycerol determine the chemical and physical properties of a fat.

In addition to glycerides and free fatty acids, lipids may contain small amounts of phospholipids, sterols, tocopherols, fat-soluble pigments, and vitamins (Nielsen 2003). Phospholipids are similar to triglycerides but consist of only two fatty acids esterified to glycerol. A polar group containing phosphoric acid and a nitrogen-containing group takes the place of the third fatty acid. Sterols contain a common steroid nucleus, an 8–10 carbon side chain, and an alcohol group (Giese 1996).

Fatty acids are long hydrocarbon chains with a methyl group (CH₃) at one end of the chain and a carboxylic acid group (COOH) at the other. Most natural fatty acids contain from 4 to 24 carbon atoms and an even number of carbon atoms in the chain; for instance, butyric acid is the smallest fatty acid, having four carbon atoms. Fatty acids may be saturated, in which case they contain only single carbon-to-carbon bonds and have the general formula CH₃(CH₂)_nCOOH (Fennema 1996). Fatty acids also can be divided into monounsaturated fatty acids which contain only one double bond (e.g., oleic acid) and polyunsaturated fatty acids which contain two or more double bonds (e.g., linoleic acid) (Weiss 1983). Generally, unsaturated fats have low melting points and liquid form at room temperature (Marangoni and Narine 2002) (Fig. 1).

The double bonds in fatty acids have different isomer structures which occur either in *cis* or *trans* configuration. In the *cis* form, the hydrogen atoms attached to the carbon atoms of the double bond are located on the same side of the double bond. In the *trans* configuration of the isomer, the hydrogen atoms are located on opposite sides of the double bond, across from one another. This configuration of the double bonds affects both melting point and shape of a fatty acid molecule.

Fig. 1 Example of saturated fatty acid (butyric acid)



The *trans* double bonds have a higher melting point than the *cis* configurations, and *trans* configurations do not significantly change the linear shape of the molecule, but a *cis* double bond forms coil structure in the chain (O'Keefe 2002).

Physical Properties

Crystal Formation

Fat molecules can bind each other to form crystals. When liquid fat is cooled, the molecular movement is reduced; the molecules will be attracted to each other by Van der Waals forces. These forces are weak and of minor significance in small molecules, but the effect is cumulative in large or long-chain molecules (O'Keefe 2002).

Crystal formation depends on symmetry of fat molecules and the similarity of fatty acid chain length (Marangoni and Narine 2002). Fats containing asymmetrical molecules and molecules containing coil structure of the double bonds align in more difficult manner, because they cannot pack together closely in space. Symmetrical fat molecules that align easily tend to form large crystals because they have high melting points resulting from less energy removed before they crystallize. On the other hand, asymmetrical molecules tend to form small crystals because more energy must be removed before they crystallize.

Polymorphism

Fat polymorphism is when fat can exist in different crystalline forms. A fat may crystallize in one of four different crystal forms, depending on the conditions during crystallization and on the composition of the fat. The smallest and least stable crystals are called α -crystals. They are formed when fats are chilled rapidly. Because of instability, they change readily to β' -crystals, which are small needle-like crystals approximately 1 μm long. Fats that can form stable β -crystals are good for use as shortenings, as they can be creamed easily and give a smooth texture. Unstable β' -crystals change to the intermediate crystal form, about 3–5 μm in size, and finally convert to coarse beta β -crystals, which can range from 25 to 100 μm in length (Timms 1991; Vaclavik and Christian 2014). These large crystals have the highest melting point.

Formation of small crystals is favored by rapid cooling with agitation. This allows formation of many small crystals, instead of slow growth of fewer large crystals (Akoh and Min 2002).

Formation of small or large crystals essentially depends on the cooling rate and homogeneity of fats (Marangoni and Narine 2002). Growth of large crystals occurs if cooling is slow and vice versa. The more heterogeneous the fat, the more likely that the molecules form small stable crystals. Large crystals are formed by homogeneous fats (e.g., lard), and small crystals are formed by heterogeneous fats (e.g., acetoglycerides). Increasing of the heterogeneity of the fatty acid composition prevents the formation of large crystals. Fats with small crystals are harder fats, have a smooth fine texture, and appear to be less oily because the oil is present

as a fine film surrounding the crystals, whereas the reverse is true of fats with large crystals. Smaller crystals are desirable if a fat contributes aeration to a food.

Melting Points

The melting point is an index of the force of attraction between molecules. A strong attractive force indicates a good degree of fit between the molecules. Molecules that do not fit together well do not have strong attractive forces holding them together, and so they have lower melting points (Marangoni and Narine 2002).

The melting point range of fat or oil is based on the component fatty acids (Akoh and Min 2002). The melting point of a fat or oil is actually a range, not a sharply defined temperature. Each fat or oil contains triglycerides that melt at different temperatures, depending on their component fatty acids. The more heterogeneous of fatty acids will be less easy to fit together, thus they will have lower melting points. Some fats have a wide melting range, whereas others, such as butter or chocolate, have a narrow melting range. Chocolate has a narrow melting range that is close to body temperature, and this accounts for its characteristic melt-in-your-mouth property.

The melting point is also determined by length of fatty acid chain, number of double bonds, and isomeric configuration. The first factor is length of fatty acid chain: long-chain fatty acids have a higher melting point than short-chain fatty acids, because there is more potential for attraction between long chains than there is between short chains; for instance, butyric acid (4:0) has a melting point of 18 F (−7), whereas stearic acid (18:0) has a higher melting point of 157 F (69 °C). The second factor is number of double bonds: higher the number of double bonds, lower the melting point. A third influence on melting point is isomeric configuration. The *cis* double-bond configuration introduces a number of coil structures which are more difficult to pack together into the molecule than the *trans* configuration, thus it has lower melting point (Weiss 1983).

Chemical Reaction

Hydrolytic Reaction

Off-flavors in fat-based products are caused by hydrolysis of fatty acids from triacylglycerols (Akoh and Min 2002). Free fatty acids may produce off-flavor, reduce oxidative stability, cause foaming, and reduce smoke point (the temperature at which an oil begins to smoke). In hydrolytic rancidity, free fatty acids are liberated from the glycerol backbone resulting in the development of off-flavors. Free fatty acids produce off-aromas, and long-chain fatty acids produce soapy tastes (Min and Boff 2002). Triacylglycerol hydrolysis also occurs in frying oils during high-temperature processing, the water from fried food inducing the hydrolysis of triacylglycerol. As the free fatty acid content of the frying oil increases, smoke point and oxidative stability decrease and the tendency for foaming increases. Triacylglycerol hydrolysis will also occur at extreme pH values. However, short-chain free fatty acids sometimes contribute to desirable flavor profiles, such as in yogurt or cheese products.

Enzyme lipases play a role in free fatty acid liberation. Enzyme lipases present in living tissues of raw food materials for foods, and the activity can occur naturally such as in olive oil processing. Oils from subsequent pressing and oil extracted from the pomace have higher free fatty acid concentrations as the cellular matrix is further disrupted and the lipases have time to hydrolyze triacylglycerols (Rousseau and Marangoni 2002).

Oxidative Rancidity

Oxidative rancidity is the predominant type of rancidity. In this process, the unsaturated fatty acids are subjected to autoxidation. The more double bonds there are, the greater the opportunity for addition of oxygen to double bonds, increasing the risk that the fat or oil will become rancid (Zhuang et al. 2002).

Autoxidation is an oxidative complex reaction and is promoted by heat, light, certain metals (iron and copper), and enzymes known as lipoxygenases (Min and Boff 2002). There are three stages in the autoxidation reaction: initiation, propagation, and termination. The initiation stage of the reaction involves formation of a free radical. The free radical results if a hydrogen on a carbon atom adjacent to one carrying a double bond is displaced (Buettner 1993). This reaction is propagated which displaces another hydrogen from another unsaturated fatty acid, forming another free radical. The reaction repeats, and this stage is called propagation stage that yields activated peroxide. The liberated hydrogen unites with the peroxide to form a hydroperoxide. Hydroperoxides are very unstable and decompose into compounds with shorter carbon chains, such as volatile fatty acids, aldehydes, and ketones (Decker and McClements 2001). These are responsible for the characteristic odor of rancid fats and oils. The termination stage of the reaction involves the reaction of free radicals to form nonradical products. Elimination of all free radicals is the only way to stop the oxidation reaction.

Oxidation can be prevented or delayed by avoiding situations that would serve as catalysts for the reaction (e.g., high temperature, presence of light, oxygen, and water vapor) and addition of antioxidant (e.g., EDTA, tocopherols, BHA (butylatedhydroxyanisole), BHT (butylatedhydroxytoluene), TBHQ (tertiary-butyl hydroquinone), and propyl gallate (Buettner 1993).

Functional Properties in Foods

The functionality of triacylglycerol plays important roles in determining physical and sensory properties of food products, such as texture, appearance, and flavor.

Texture

Functionality of triacylglycerols in food texture is determined by the physical state of the lipid in the food matrix. For partially crystalline fats, such as chocolate, baked products, shortenings, butter, and margarine, the texture is mainly determined by the concentration, morphology, and interactions of the fat crystals (McGrady 1994; Pszczola 2000). In particular, the melting profile of the fat crystals plays a major

role in determining properties such as texture, stability, spread ability, and mouthfeel.

The lipid concentration and behavior in the food matrix will also contribute to the overall texture; for example, oil-in-water (O/W) food emulsion is determined by the presence of fat droplets (e.g., creams, desserts, dressings, and mayonnaise). In these systems, the texture is represented by the viscosity of the overall system. In W/O food emulsions, the texture is represented by overall rheology of the system, which is largely determined by the rheology of the oil as dominant phase. The rheology of these products is determined by the morphology and interactions of the fat crystals present during crystallization and storage conditions. Formation of a three-dimensional network of aggregated fat crystals in the continuous phase provides fat product with spread ability (Moran 1994). On the other hand, interaction of lipids with other compounds in food (e.g., cookies, cakes, biscuits) impacts their texture by forming a network of interacting fat crystals that gives characteristics of firmness or snap (Marangoni and Narine 2002).

The W/O emulsion consists of water droplets embedded in a lipid phase that contains a network of aggregated crystals. The fat crystals formed are initially in the α polymorphic form but are converted to the more stable β' polymorphic form during the crystallization step. It is important to control the extent of this transformation during the manufacturing process since this determines the number and strength of the bonds formed between the fat crystals and therefore the rheology of the final product. On the other hand, it is also important to prevent the polymorphic transition from the β' form to the more stable β form during storage, since this leads to the formation of large crystals ($>30\ \mu\text{m}$) that are perceived as “grainy” or “gritty” in the mouth. This conversion can often be prevented by adding surfactants that interfere with the polymorphic transition, choosing lipids that will not form β crystals, or by appropriate blending of lipids to favor the β' -crystal habit (Lawler and Dimick 2002).

To obtain the desired functional characteristics in a particular product, it is important to choose a blend of fats and oils that gives the appropriate melting profile and polymorphic characteristics, then to process the fat using controlled cooling and shearing conditions to obtain the desired crystal type and structure (Ghotra et al. 2002). It is usually important that the lipid is partially crystalline at storage temperatures so that it maintains its structural integrity but melts during consumption to give a desirable mouthfeel.

Appearance

The characteristic appearance of many food products is represented by visual observation such as color and opacity. The color of bulk oils, such as cooking or salad oils, is mainly determined by the presence of pigment impurities that adsorb light, such as chlorophyll and carotenoids. The opacity of the fat depends on the concentration, size, and shape of the fat crystals present. Solid fats are usually optically opaque because of scattering of light by the fat crystals present, whereas liquid oils are usually optically clear. Food emulsions usually appear optically opaque because the light passing through them is scattered by the droplets (McClements 2002).

Instability problem associated with fat crystallization in lipid-based products such as chocolate can cause bloom. Bloom manifests itself as large grayish appearance on the surface of the product (Lonchampt and Hartel 2004). Fluctuating temperature during their storage causes the fat phase to melt and recrystallize (Erickson and Frey 1994). Bloom can often be retarded or prevented by using surfactants that limit the crystal transition or by carefully controlling the storage temperature to avoid fat polymorphic phase transitions (Erickson and Frey 1994; Lonchampt and Hartel 2004).

Flavor

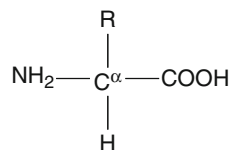
Edible fats and oils contribute to the flavor of food. Different fatty acid composition in fats and oils has distinctive flavor profiles. The flavor of many food products is indirectly influenced by the lipid phase because flavor compounds can partition between oil, water, and gaseous regions within the food matrix according to their polarities and volatilities (McClements 2004). For this reason, the perceived aroma and taste of foods are often strongly influenced by the type and concentration of lipids present. Physical states of lipids also influence the mouthfeel of many food products (Walstra 2003), for example, oily mouthfeel produced by liquid oils and “grainy” or “sandy” mouthfeel produced by fat crystals (Moran 1994). The melting of fat crystals in the mouth causes a cooling sensation, which is an important sensory attribute of many fatty foods (Walstra 2003).

Food Proteins

Proteins are present in all living things and have a key role in many biological processes. The nutritional energy value of proteins is 4 kcal/g. Amino acids are required building blocks for protein biosynthesis. Damodaran (2008) has defined food proteins as “those that are easily digestible, nontoxic, nutritionally adequate, functionally useable in food products, available in abundance, and sustainable agriculturally.” Proteins contribute to the flavor of food, precursors for aroma compounds and colors formed during various processing of food. Proteins have ability to build or stabilize gels, foams, emulsions, and fibrillar structures, which is essential in certain food products. The proteins can be obtained from milk, meat, grain, oilseeds, legumes, algae, yeasts, and bacteria (single-cell proteins).

Chemical Structure and Classification

There are 20 amino acids forming the building blocks of most proteins. Each amino acid contains a primary amine and carboxylic acid group, which is shown in Fig. 2. They link by peptide (amide) bonds formed between α -amino and α -carboxylic acid groups of neighboring amino acids in the polypeptide sequence. The amide linkage in proteins is a partial double bond.

Fig. 2 General structure of amino acid**Table 4** Common amino acids found in proteins

Type	Name	Abbreviation (three and one letter symbols)	
Simple	Glycine	Gly	G
Aliphatic	Alanine	Ala	A
	Valine	Val	V
	Leucine	Leu	L
	Isoleucine	Ile	I
Heterocyclic	Tryptophan	Trp	W
	Histidine	His	H
	Proline	Pro	P
Aromatic	Phenylalanine	Phe	F
	Tyrosine	Tyr	Y
Hydroxy	Serine	Ser	S
	Threonine	Thr	T
Acidic	Aspartic acid	Asp	D
	Glutamic acid	Glu	E
Amide	Asparagine	Asn	N
	Glutamine	Gln	Q
Basic	Lysine	Lys	K
	Arginine	Arg	R
Sulfur containing	Cysteine	Cys	C
	Methionine	Met	M

In the simplest case, $R = H$ (aminoacetic acid or glycine). In other amino acids, R is an aliphatic, aromatic, or heterocyclic residue and may incorporate other functional groups shown in Table 4.

Various side chains of the amino acids are involved in inter- and intramolecular interactions in proteins and classified into a number of ways, which is shown in Table 5.

Proteins are macromolecules whose name was derived from the Greek word *proteois*, which means of the first kind. Proteins contain 50–55 % carbon, 6–7 % hydrogen, 20–23 % oxygen, 12–19 % nitrogen, and 0.2–3.0 % sulfur on wt/wt basis. Protein synthesis occurs in ribosomes, and then subsequently some of them get modified by cytoplasmic enzymes (Damodaran 2008; Belitz et al. 2009). Proteins modified by enzyme or made complex with nonprotein components (prosthetic groups) are called conjugated (heteroproteins) proteins whereas modified proteins are known as homoproteins. Various proteins like glycoproteins, phosphoproteins, lipoproteins, metalloproteins, and nucleoproteins are some of the

Table 5 Base for classification of amino acids

Basis for classification	Amino acids
Charged side chains	Arginine, aspartic acid, glutamic acid, histidine, and lysine
Nonpolar, uncharged side chains	Methionine, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, and tryptophan
Uncharged, polar side chains	serine, threonine, cysteine, tyrosine, asparagine, and glutamine
Essential amino acids	Valine, leucine, isoleucine, phenylalanine, tryptophan, methionine, threonine, histidine, lysine, and arginine
Nonessential amino acids	Glycine, alanine, proline, serine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, and glutamic acid

examples of conjugated proteins. Moreover, based on structural organization, proteins can be classified as globular and fibrous proteins. Globular proteins exist in ellipsoidal or spherical shapes due to folding of a polypeptide chain on itself while fibrous proteins are rod-shaped molecules containing twisted linear polypeptide chains. Enzymes are globular proteins whereas collagen, keratin, and fibrin are fibrous proteins. Based on biological functions, proteins can further be classified as structural proteins, contractile proteins, transfer proteins, enzyme catalysts, storage proteins, protective proteins, hormones, and antibodies (Damodaran 2008).

Structure of Proteins

Amino acids are linked covalently through the α -carboxyl group of one amino acid and the α -amino group of another amino acid through an amide or peptide bond to form peptides, oligopeptides, polypeptides, and proteins (Ustunol 2015). Peptides are denoted by the number of amino acid residues as di-, tri-, tetrapeptides, etc., and the term “oligopeptides” is used for those with 10 or less amino acid residues. Higher molecular weight peptides are called polypeptides. The transition of “polypeptide” to “protein” is rather undefined, but the limit is commonly assumed to be at a molecular weight of about 10 kd; i.e., about 100 amino acid residues are needed in the chain for it to be called a protein. The amino acid residue with the free amino group is always placed on the left. The amino acids of the chain ends are denoted as N-terminal and C-terminal amino acid residues (Belitz et al. 2009) which are shown in Fig. 3.

Proteins are formed from amino acids through amide linkages. The structure of a protein is dependent on the amino acid sequence (the primary structure) which determines the molecular conformation (secondary and tertiary structures). Proteins sometimes occur as molecular aggregates which are arranged in an orderly geometric fashion (quaternary structure) (Belitz et al. 2009). Thus proteins have primary, secondary, tertiary, and quaternary structures, which are shown in Fig. 4.

Primary Structure

It refers to the linear sequence in which the constituent amino acids are covalently linked through amide bonds, also known as peptide bonds. The primary structure is determined by its genetic code and post-translational covalent modifications.

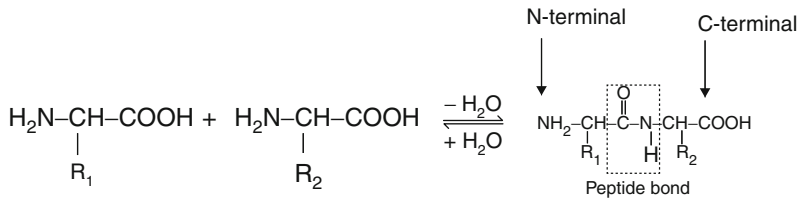


Fig. 3 Peptide bond with N- and C-terminal

The primary structure of a protein determines the physicochemical and the functional properties of food protein (Damodaran 2008; Ustunol 2015).

Secondary Structure

The primary structure gives the sequence of amino acids in a protein chain while the secondary structure reveals the arrangement of the chain in space. Secondary structure may result from aperiodic (random coil) or periodic structures. In aperiodic structures consecutive amino acid residues possess different sets of dihedral angles whereas *helical* and *extended* structures are periodic structures which result from consecutive amino acid residues in a segment having the same recurring set of φ and ψ angles (Damodaran 2008; Ustunol 2015). The α -, 3_{10} -, and β -helices are the helical structures, but the right-handed α -helix is the most common and is also the most stable in food proteins. Protein helical structures are formed when the φ and ψ angles of consecutive amino acid residues are twisted to a same set of values. Stability to α -helix is provided by the hydrogen bonding of the N-H and C=O groups of the fourth preceding residue. The hydrogen bonds are oriented parallel to the helix axis. The α -helical structure found in proteins is amphiphilic in nature. The cyclic imino acid, proline, cannot form α -helices. Proline is considered to be an α -helix breaker. It cannot form hydrogen bond due to the lack of hydrogen on the nitrogen atom. Proteins containing high levels of proline residues will take random aperiodic structures. A β -casein and α_1 -casein possess about 17 % and 8.5 % proline of the total amino acid respectively. Proline residues are uniformly distributed, and α -helices are not present in these proteins (Damodaran 2008; Ustunol 2015). These caseins have only primary (random) structures, and hence casein is known as naturally denatured proteins. The β -sheet is an extended structure where C=O and N-H groups are positioned perpendicular to the direction of the polypeptide chain, which then allows for hydrogen bonding between the two segments or β -strands. Two β -strands of the same molecule interact via hydrogen bonds, forming a sheet-like structure known as β -pleated sheet. Depending on the direction of the polypeptide strands, parallel β -sheet or antiparallel β -sheet are formed. The β -sheet structure is generally more stable than the α -helix (Damodaran 2008).

Tertiary Structure

It refers to the spatial arrangement attained when a linear protein chain with secondary structure segments folds further into a compact three-dimensional

LEVELS OF PROTEIN STRUCTURE

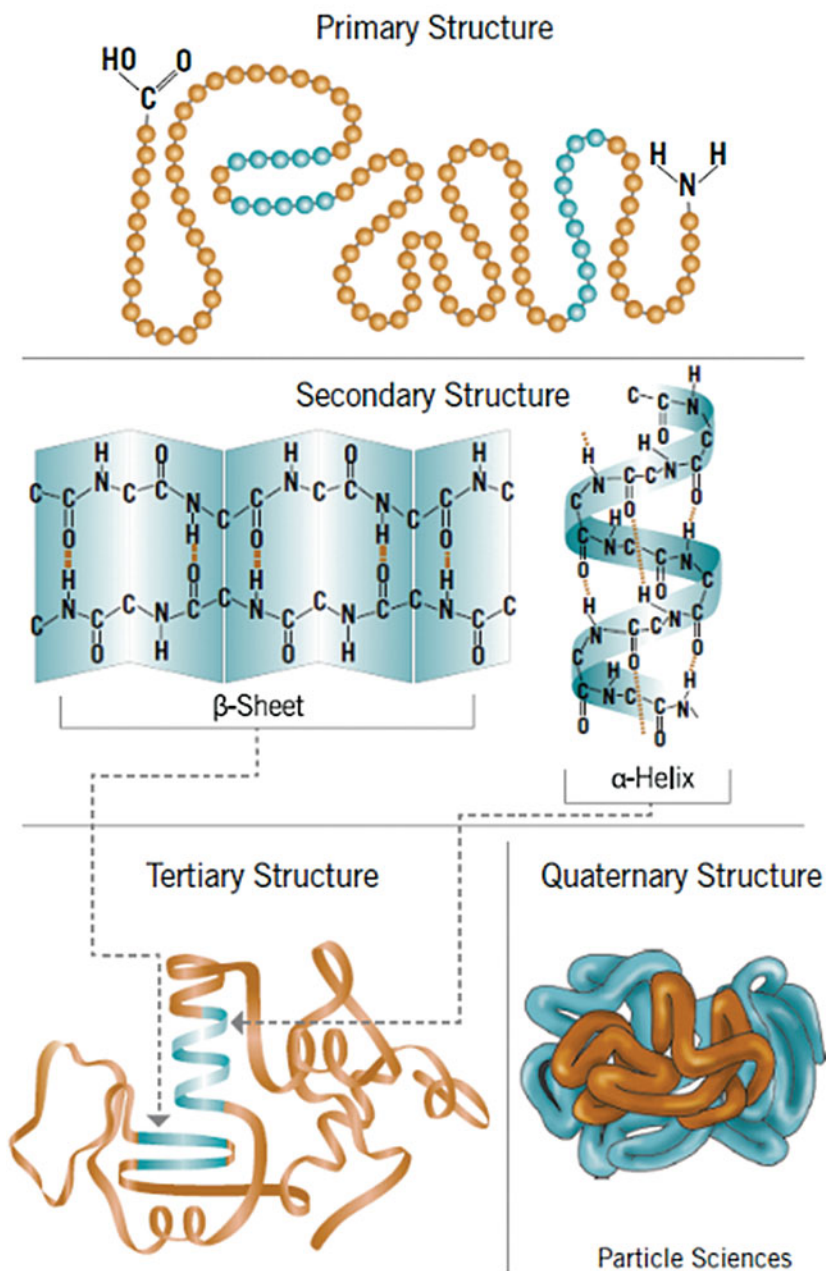


Fig. 4 Various levels of protein structures (Reprinted (adapted) with permission from Particle Sciences, Inc. Source: Protein Structure. Particle Sciences – Technical Brief: 2009; volume 8. Particle Sciences – Drug Development Services (www.particlesciences.com))

form. Formation of tertiary structure involves hydrophobic, electrostatic, van der Waals, and hydrogen bonding between various groups of proteins. The folding of the protein into a tertiary structure defines the size and shape of that protein. Folding of proteins brings hydrophobic residues at the interior as well as hydrophilic and charged residues on the surface of the molecule (Damodaran 2008; Ustunol 2015). Presence of hydrophilic and hydrophobic residues in the polypeptide determines the shape of proteins. Globular proteins are abundant in hydrophobic residues while elongated or rod-shaped proteins are abundant in uniformly distributed hydrophilic residues (Nelson and Cox 2013).

Quaternary Structure

It refers to the spatial arrangement of a protein when it contains more than one polypeptide chain, which are also referred to as subunits or oligomers. Formation of quaternary structure is primarily driven by the thermodynamic requirement to bury exposed hydrophobic surfaces of subunits. The quaternary structure is further stabilized by noncovalent interactions such as hydrogen bonding and hydrophobic and electrostatic interactions (Damodaran 2008; Ustunol 2015).

The folding of a random polypeptide chain into its three-dimensional structure is a complex process, and various forces are involved. These forces may be grouped into two categories, i.e., (i) intermolecular interactions result from the surrounding solvent (i.e., hydrogen bonding, electrostatic and hydrophobic interactions), and (ii) intramolecular interactions result from forces intrinsic to the protein molecule (i.e., steric, van der Waals) (Damodaran 2008; Ustunol 2015).

Physical Properties

Dissociation/Acid–Base Properties

In aqueous solution amino acids are present, depending on pH, as cations, zwitterions, or anions, which are shown in Fig. 5. In amino acids the acidity of the carboxyl group is higher and the basicity of the amino group lower. Amino acids behave both as acids and bases, i.e., they are *ampholytes*. At around neutral pH, both the α -amino and α -carboxyl groups are ionized, and the molecule is a dipolar or a zwitterion. The pH at which the dipolar ion is electrically neutral is called the isoelectric point (pI) (Damodaran 2008; Belitz et al. 2009).

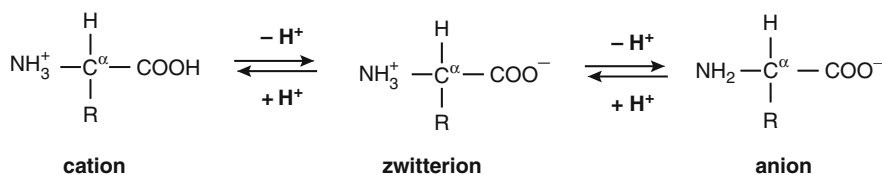
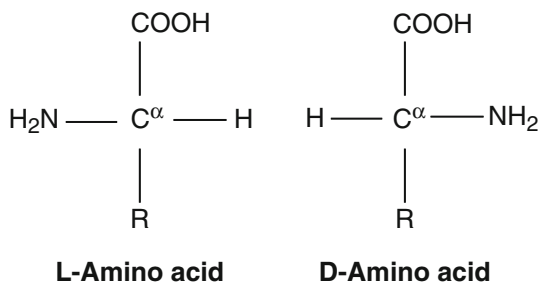


Fig. 5 Dissociation of amino acids

Fig. 6 L- and D- amino acids

Configuration and Optical Activity Properties

Amino acids, except for glycine, have at least one chiral center (α -carbon atom) and, hence, are optically active. Isoleucine, threonine, and 4-hydroxyproline have two asymmetric C-atoms, thus each has four isomers (Belitz et al. 2009). The L- and D- amino acids are shown in Fig. 6.

All proteins found in nature contain only L-amino acids. D-amino acids also occur in nature, for example, in a number of peptides of microbial origin. The specific rotation of amino acids in aqueous solution is strongly influenced by pH. It passes through a minimum in the neutral pH range and rises after addition of acids or bases. The optical activity of proteins is due not only to asymmetry of amino acids but also to the chirality resulting from the arrangement of the peptide chain (Belitz et al. 2009).

Solubility

The solubilities of amino acids in water are highly variable. Proline is extremely soluble; hydroxyproline, glycine, and alanine are quite soluble, whereas cystine and tyrosine have low solubilities in water. Addition of acids or bases improves the solubility through salt formation. The extent of solubility of amino acids in protein hydrolysates is different than that observed for the individual components. The solubility in organic solvents is not very good because of the polar characteristics of the amino acids. All amino acids are insoluble in ether. Protein solubility is variable and is influenced by the number of polar and apolar groups and their arrangement along the molecule (Belitz et al. 2009).

UV Absorption

Aromatic amino acids such as phenylalanine, tyrosine, and tryptophan absorb in the UV range of the spectrum with absorption maxima at 200–230 and 250–290 nm. Tyrosine and tryptophan also exhibit fluorescence in the ultraviolet region (250–300 nm). Histidine, cysteine, and methionine absorb between 200 and 210 nm. Absorption readings at 280 nm are used for the determination of proteins and peptides (Damodaran 2008; Belitz et al. 2009).

Sensory Properties

While the taste quality of amino acids does depend on configuration, peptides, except for the sweet dipeptide esters of aspartic acid, are neutral or bitter in taste with no relationship to configuration. The taste intensity is influenced by the hydrophobicity of the side chains. The taste intensity does not appear to be dependent on amino acid sequence. Bitter-tasting peptides can occur in food after proteolytic reactions (Belitz et al. 2009).

Chemical Reactivity of Amino Acids

Amino acids show the usual reactions of both carboxylic acids and amines. Reaction specificity is due to the presence of both carboxyl and amino groups and other reactive groups such as amino, carboxyl, sulfhydryl, phenolic, hydroxyl, thioether (Met), imidazole, and guanyl in free amino acids and proteins. Reactions occurring at 100–220 °C, such as in cooking, frying, and baking, are particularly relevant to food chemistry (Damodaran 2008; Belitz et al. 2009). Various type reactions which involve (i) amino groups (e.g., reductive alkylation, guanidation, arylation, acetylation, succinylation, thiolation, deamination), (ii) carboxyl groups (e.g., esterification, reduction, decarboxylation), (iii) sulfhydryl group (oxidation, blocking), (iv) serine and threonine (e.g., esterification), and (v) methionine (e.g., alkyl halides, β -propiolactone) take place. Some of the reactions can be used to alter the hydrophilic and hydrophobic properties and the functional properties of proteins and peptides as well as quantify amino acids and specific amino acid residues in proteins (Damodaran 2008; Belitz et al. 2009). For further details on various reactions, readers are advised to refer to some books like Damodaran (2008) and Belitz et al. (2009).

Denaturation of Proteins

Protein denaturation refers to changes in the secondary and tertiary structure of the protein. The primary structure (the amino acid sequence) of a protein remains unchanged. The denaturation is a reversible or irreversible change of native conformation without cleavage of covalent bonds. Denaturation does not involve any chemical changes in the protein. Any treatment that cleaves hydrogen bridges or ionic or hydrophobic bonds is responsible for denaturation. This can be accomplished by: changing the temperature, adjusting the pH, increasing the interface area, or adding organic solvents, urea, salts, guanidine hydrochloride, or detergents such as sodium dodecyl sulfate. Denaturation is generally reversible when the peptide chain is stabilized in its unfolded state by the denaturing agent and the native conformation can be reestablished after removal of the agent. Irreversible denaturation occurs when the unfolded peptide chain is stabilized by interaction with other chains (as occurs, for instance, with egg proteins during boiling). During unfolding reactive groups, such as thiol groups, that were buried or blocked may be

exposed. Their participation in the formation of disulfide bonds may also cause an irreversible denaturation (Belitz et al. 2009). Consequences of denaturation of a protein are that it loses its biological and enzymatic activity as well as solubility, gets aggregated, improves digestibility, inactivates antinutritional factors as well as deteriorative enzymes, and improves functional properties of food proteins that are used as ingredients in processed foods (Ustunol 2015).

Functional Properties in Foods

Proteins possess a range of dynamic functional properties and represent a most important class of functional ingredients. 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 (Damodaran 2008). Functional properties of protein depend on physicochemical properties like shape, size, composition and sequence of amino acid, distribution of charges, structural levels of proteins, hydrophobicity/hydrophilicity ratio, molecular flexibility, and ability to react/interact with other components (like lipids, sugars, polysaccharides, salts, and minor components). Various functional properties like organoleptic (flavor, odor, color), hydration (gelling, syneresis, swelling, thickening, solubility, wettability, water sorption, viscosity, gelation), kinesthetic (texture, grittiness, mouthfeel, smoothness), rheological/textural (aggregation, cohesiveness, chewiness, elasticity, adhesiveness, network formation, dough formation, texturizability, extrudability), and surface (foaming, emulsification, film formation) are commonly possessed by proteins. Proteins show versatility during processing. They can form networks and structures and provide essential amino acids. Moreover, they interact with other components and improve quality attributes of foods (Kinsella et al. 1994).

Solubility of Proteins

Protein solubility is variable. The solubility of proteins is considered as that proportion of nitrogen in a protein product which is in the soluble state under specific conditions. Proteins can be partly or completely soluble or completely insoluble in water. Protein solubility provides useful information on foams, emulsions, and gels. Protein solubility is influenced by sequence and composition of amino acid, molecular weight, and conformation and content of polar and nonpolar groups in amino acids. Water molecules bind to charged groups, backbone peptide groups, the amide groups of glutamine and asparagines, hydroxyl groups of threonine, serine and tyrosine residues, and nonpolar residues in the proteins. Ionic strength, type of solvent, pH, temperature, and processing conditions affect the solubility of proteins. Solubility is increased if electrostatic repulsion between the molecules is higher than hydrophobic interactions. At the isoelectric point (pI), proteins have a net zero charge, attractive forces predominate, and molecules tend to associate, resulting in insolubility. Above the pI, the net charge is negative and solubility is enhanced. Protein-water interactions increase at pH values higher or

lower than the pI because protein carries a positive or negative charge. At low ionic strengths, the solubility rises with increase in ionic strength. Neutral salts have a twofold effect on protein solubility. At low concentrations they increase the solubility (“salting in” effect) by suppressing the electrostatic protein-protein interaction (binding forces). Protein solubility is decreased (“salting out” effect) at higher salt concentrations due to the ion hydration tendency of the salts. Solubility of various proteins decreases variously with temperature and time of heating (Zayas 1997).

Water Holding Capacity of Proteins

Water hydration and holding, water retention, water binding, water imbibing, and water adsorption are some of the terms used interchangeably for interaction of proteins with water. The water holding capacity (WHC) of foods can be defined as the ability to hold its own and added water during the application of forces, pressing, centrifugation, or heating. WHC plays a major role in the formation of food texture, especially in comminuted meat products and baked doughs. Swelling is an important protein functional property as it is the first step in their solvation and can be defined as the spontaneous uptake of water by a protein matrix. Water retention is a critical factor in protein functionality because it affects the texture, color, and sensory properties of products. Water binding depends on the composition and conformation of the protein molecules. Water interacts with proteins in a number of ways, and significant amounts of water bounded by proteins are retained by hydrogen bonding. Interactions between molecules of water and hydrophilic groups of the protein side chains occur via hydrogen bonding. Structural water is held by hydrogen bonding between polypeptide groups of the proteins. Binding of water to proteins is related to the polar hydrophilic groups, such as imino, amino, carboxyl, hydroxyl, carbonyl, and sulfhydryl groups. The capacity of proteins to retain moisture is affected by the type and number of these polar groups in the protein polypeptide chain. The binding of water is due to the dipolar character of water. Proteins that contain numerous charged amino acids will tend to bind large amounts of water. Water binding of proteins can be predicted from their amino acid composition. The absorbed water is tightly bound to the protein molecules. Water binding of a protein is influenced by protein concentration; pH; ionic strength; temperature; presence of other components of foods such as hydrophilic polysaccharides, lipids, and salts; rate and length of heat treatment; and conditions of storage (Zayas 1997; Belitz et al. 2009).

Foam Formation and Foam Stabilization

Proteins function as foam-forming and foam-stabilizing components in many foods, e.g., baked goods, sweets, desserts, and beer. The most widely used protein foaming agents are egg white, gelatins, casein, other milk proteins, soy proteins, and gluten. Serum albumin foams very well, while egg albumin does not. Foams consist of an aqueous continuous phase and a gaseous (air) dispersed phase. Foam can be defined as a two-phase system consisting of air cells separated by a thin continuous liquid layer called the lamellar phase. Proteins stabilize by forming

flexible, cohesive films around the gas bubbles. Protein is adsorbed at the interface via hydrophobic areas followed by partial unfolding. Protein adsorption facilitates the formation of new interfaces by reducing surface tension. The more quickly a protein molecule diffuses into interfaces and the more easily it is denatured there, the more it is able to foam. Foam stability refers to the ability of protein to stabilize foam against gravitational and mechanical stresses. Foams collapse because large gas bubbles grow at the expense of smaller bubbles (disproportionation). The protein films counteract this disproportionation. That is why the stability of a foam depends on the strength of the protein film and its permeability for gases. Film strength depends on the adsorbed amount of protein and the ability of the adsorbed molecules to associate. An ideal foam-forming and foam-stabilizing protein is characterized by a low molecular weight, high surface hydrophobicity, good solubility, a small net charge in terms of the pH of the food, and easy denaturability (Zayas 1997; Belitz et al. 2009).

Gel Formation

A gel is an intermediate phase between a solid and a liquid. Gel is a substantially diluted system that exhibits no steady-state flow. Gelation is a basic process in the processing of various foods, milk gels, comminuted meat and fish products, other meat products, fruit jellies, bread doughs, pie and cake fillings, coagulated egg white, and others. Protein gels may be utilized to simulate the textural properties and mouthfeel of lipids. Gels are formed when partially unfolded proteins develop uncoiled polypeptide segments that interact at specific points to form a three-dimensional cross-linked network. Partial unfolding of proteins with slight changes in secondary structure is required for gelation (Zayas 1997; Belitz et al. 2009).

Emulsifying Effect

Emulsions are disperse systems of one or more immiscible liquids. They are stabilized by emulsifiers – compounds which form interface films and thus prevent the disperse phases from flowing together. The adsorption of a protein at the interface of an oil droplet is thermodynamically favored because the hydrophobic amino acid residues can then escape the hydrogen bridge network of the surrounding water molecules. In addition, contact of the protein with the oil droplet results in the displacement of water molecules from the hydrophobic regions of the oil–water boundary layer. Therefore, the suitability of a protein as an emulsifier depends on the rate at which it diffuses into the interface and on the deformability of its conformation under the influence of interfacial tension (surface denaturation). The diffusion rate depends on the temperature and the molecular weight, which in turn can be influenced by the pH and the ionic strength. A protein with ideal qualities as an emulsifier for an oil-in-water emulsion would have a relatively low molecular weight; a balanced amino acid composition in terms of charged, polar, and nonpolar residues; good water solubility; well-developed surface hydrophobicity; and a relatively stable conformation. The β -casein molecule meets these requirements because of less pronounced secondary structures and no cross-links due to the lack of SH groups (Belitz et al. 2009).

Food Carbohydrates

Chemical Structure and Classification

Carbohydrate in nature has 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 (Fennema 1996). However, the great majority of natural carbohydrate compounds produced by living organisms do not have this simple empirical formula (Vaclavik and Christian 2014). Rather, most natural carbohydrates are classified as simple sugars (mono- and disaccharides), oligomers (oligosaccharides), and polymers (polysaccharides). However, this chapter begins with a presentation of simple sugars and builds from there to larger and more complex structures.

Monosaccharides

Monosaccharides are simple carbohydrates containing three to eight carbon atoms, but only those with five or six carbon atoms are common. Two of the most important ones in foods are the six-carbon sugars, glucose and fructose, which have the general formula $C_6H_{12}O_6$ (Fennema 1996).

Glucose is categorized as an aldose sugar due to an aldehyde group (CHO) located on the first carbon atom of the chain. It is conventional to number the carbon atoms along the chain so that the carbon atom with the highest number is farthest away from the aldehyde (or functional) group. The aldehyde group therefore is located on carbon one in glucose (and in all other aldose sugars) (Belitz et al. 2009; Vaclavik and Christian 2014).

In nature, two isomers of glucose exist, which are mirror images of each other: D-glucose and L-glucose. D-glucose is the isomer that occurs naturally. In fact, there are two series of aldose sugars, known as the d-series and the l-series, in which each isomer is formed by adding CHO groups to build the carbon chain, starting from the smallest aldose sugar, which is D- or L-glyceraldehyde (Cho et al. 1999).

As shown in Fig. 7, each H-C-OH group within the chain is asymmetrical (since the H and OH groups are different). The highest-numbered asymmetric carbon atom of each d-series sugar has the same configuration as D-glyceraldehyde, rather than its l-isomer. In glucose, the highest-numbered asymmetric carbon atom is carbon-5, which is termed the reference carbon atom, due to structure configuration determination if the sugar is known as the d series or l series. The hydroxyl group attached to it is called the reference hydroxyl group, which is always on the right side in a d-series sugar (Stick and Williams 2009).

Oligosaccharides

The reducing group of one monosaccharide can connect to one of the hydroxyl groups on another through glycosidic bond, to form disaccharides. More connections of glycosidic bonds will give rise to trisaccharides, tetrasaccharides, etc., categorized as oligosaccharides and ultimately polysaccharides. The glycosidic bond is the result of the condensation of the hydroxyl of the hemiacetal group of

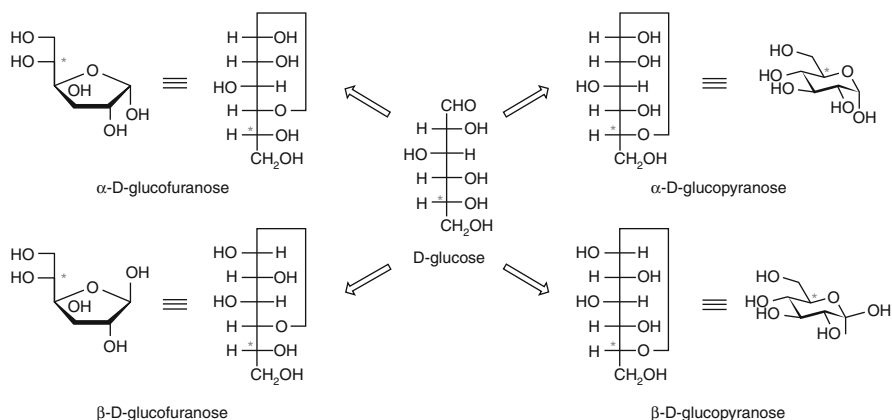


Fig. 7 The main isomers of D-glucose (Fischer projections) (Reprinted (adapted) with permission from Withers (2015)). Anomeric centre (alpha and beta) in CAZypedia, available at URL [http://www.cazypedia.org/index.php/Anomeric_centre_\(alpha_and_beta\)\)](http://www.cazypedia.org/index.php/Anomeric_centre_(alpha_and_beta)))

C-1 with the hydroxyl of an alcohol. When a glycosidic linkage is established only between the lactol groups of two monosaccharides, then a nonreducing disaccharide is formed, and when one lactol group and one alcoholic HO group are involved, a reducing disaccharide results (Belitz et al. 2009).

Polysaccharides

When oligosaccharides have more than ten monosaccharides the unit is known as polysaccharide. Polysaccharides consist of monosaccharide units bound to each other by glycosidic linkages. Their complete acidic hydrolysis yields monosaccharides. Partial chemical and enzymatic hydrolysis giving rise to them is important for structural elucidation, the analysis of which elucidates monosaccharide sequences and the positions and types of linkages (Stephen 1995; Whistler and BeMiller 1993).

Polysaccharides (glycans) can have only a type of sugar structural unit (homoglycans) or several types of sugar units (heteroglycans). Polysaccharides may have a linear pattern (as in cellulose and amylose) or a branched fashion (amylopectin, glycogen, guaran) of monosaccharide unit linkage. The frequency of branching sites and the length of side chains can vary greatly (glycogen, guaran). The monosaccharide residue sequence may be periodic, one period containing one or several alternating structural units (cellulose, amylose, or hyaluronic acid), the sequence may contain shorter or longer segments with periodically arranged residues separated by nonperiodic segments (alginate, carrageenans, pectin), or the sequence may be nonperiodic all along the chain (as in the case of carbohydrate components in glycoproteins). The monosaccharide structural unit conformation and the positions and types of linkages in the chain determine the chain conformation of a polysaccharide (Fennema 1996; Stephen 1995).

Physical Properties

Hygroscopicity of sugar in crystallized form is the ability of moisture uptake by sugars. The hygroscopicity varies and depends on the sugar structure, isomers present, and sugar purity (Belitz et al. 2009).

Monosaccharides tend to be soluble in polar solvent; they are also soluble to a small extent in ethanol but not soluble in organic solvents such as benzene, chloroform, and ether. When the crystal sugars cake together, the solubility will decrease. Sugar solution in high concentrations, e.g, glucose syrup, is used in the baking industry to retain food moisture. The solubility of mono- and oligosaccharides in water is good. However, anomers may differ substantially in their solubility, as exemplified by α - and β - lactose (De Man 1999). The physical and sensory properties of oligosaccharides are covered by monosaccharides since oligosaccharides consist of not more than 10 sugar molecules.

While sugars form true solutions, polysaccharides form colloidal solutions and are, therefore, difficult to purify. The polysaccharides are tasteless and amorphous. Polysaccharides can be classified as (a) homoglycans: single monosaccharides linked together – starch, cellulose, and glycogen; (b) heteroglycans: two or more different constituents – hemicelluloses, mucilages, pectins, and resins; and (c) conjugated compounds made up of saccharides, proteins, or lipids (Stephen 1995; Whistler and BeMiller 1993).

Isolated polysaccharides are utilized to a great extent in food processing, either in native or modified form, such as thickening or gel-setting agents (starch, alginate, pectin, guaran gum), stabilizers for emulsions and dispersions, film-forming, coating substances to protect sensitive food from undesired change, and inert fillers to increase the proportion of indigestible substances in a diet.

Chemical Reactions

Carbohydrates can perform wide chemical reactions with other compounds because besides having hydroxyl groups, some monosaccharides and sugars also have carbonyl groups available for reaction. This section will focus on two most common reactions of carbohydrates in food processing, Maillard and caramelization.

Maillard Reaction

Maillard is categorized as nonenzymatic browning and plays an important role in improving appearance and taste of cooked food, particularly roasting, toasting, and baking food. The compounds that contribute to Maillard reaction were sugars and amino acids in water, which was first reported in 1912 by Louis-Camille Maillard. The chemistry underlying the Maillard reaction is very complex. It encompasses not one reaction pathway but a whole network of various reactions. The original comprehensive reaction scheme of Hodge (Fig. 8) has been developed and elaborated by food technologists ever since, so the understanding of the reaction is advancing steadily.

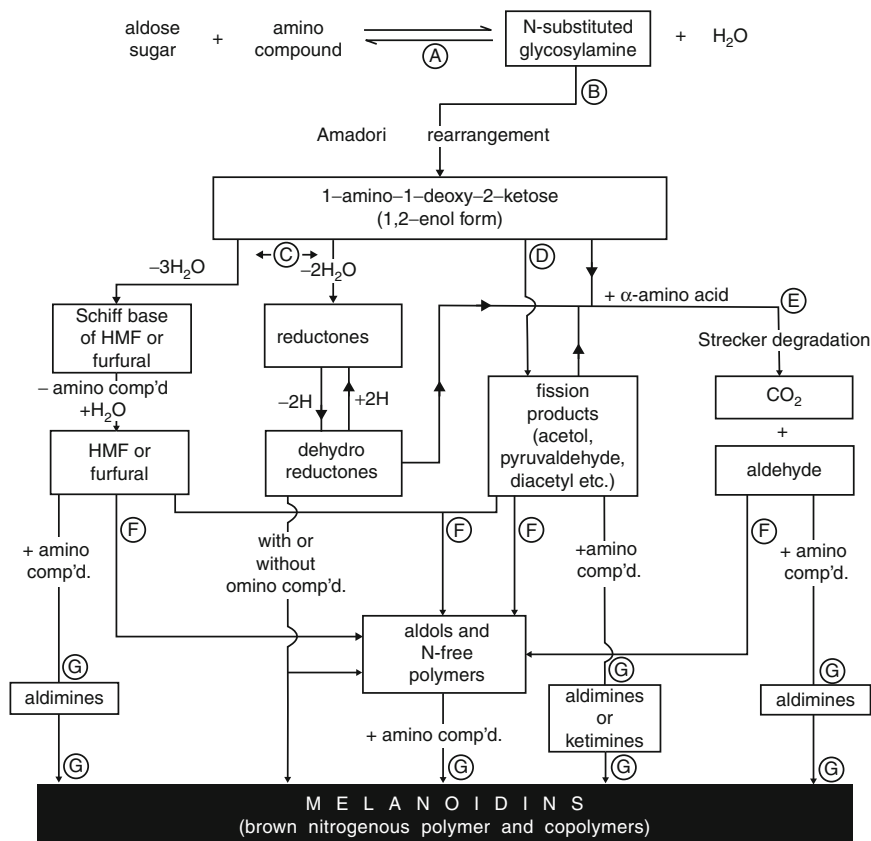


Fig. 8 Maillard reaction (Hodge) scheme (Reprinted (adapted) with permission from Hodge (1953). *Chemistry of Browning Reactions in Model Systems*. *J Agric Food Chem* 1: 928–943. Copyright (1953) American Chemical Society)

In the early stage of Maillard, a reducing sugar, like glucose, reacts with a compound possessing a free amino group (of an amino acid or in proteins mainly the ε-amino group of lysine but also the α-amino groups of terminal amino acids) resulting in a condensation product N-substituted glycosylamine. This product will lead to form the Amadori rearrangement product (ARP). The subsequent degradation of the Amadori product is dependent on the pH of the system. At pH 7 or below, it undergoes mainly 1,2-enolization with the formation of furfural when pentoses are entangled or hydroxymethylfurfural (HMF) when hexoses are entangled. At pH >7 the degradation of the Amadori compound is thought to involve mainly 2,3-enolization and formation of reductones, such as 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one (HMF^{one}), and a variety of fission products, including acetol, pyruvaldehyde, and diacetyl as well. All these compounds are highly reactive and take part in further reactions. Carbonyl groups will condense with free amino groups, which results in the incorporation of nitrogen into the reaction

products. Dicarbonyl compounds will react with amino acids with the formation of aldehydes and α -aminoketones known as the Strecker degradation. Subsequently, in an advanced stage, a range of reactions takes place, including cyclizations, dehydrations, retroaldolizations, rearrangements, isomerizations, and further condensation. In the final stage, melanoidins were formed, which are brown nitrogenous polymers and copolymers (Martins et al. 2001).

Caramelization

Although it produces the same brown color, caramelization is different from Maillard. In caramelization, sugar decomposition occurs at high temperature and produces brown color; this reaction does not involve proteins and produces different variety of compounds than Maillard, including organic acids, aldehydes, and ketones. Reaction is facilitated by the presence of an acid, base, or salt. Heating causes dehydration of the sugar molecule with introduction of double bonds or formation of anhydro rings. As in Maillard browning, intermediates such as 3-deoxyosones and furans are formed. The unsaturated rings may condense to form useful, conjugated double-bond-containing, brown-colored polymers. Catalysts increase the reaction rate and are used to direct the reaction to specific types of caramel colors, solubilities, and acidities. Caramel served as colorant and flavor in cola, acidic beverages, baked goods, syrups, candies, and dry seasoning (Damodaran et al. 2008).

Functional Properties in Foods

In food, simple forms of carbohydrates have main role as sweeteners in candies and many other food products. The most obvious sensory property of sugars such as glucose, fructose, and sucrose is their sweetness, which varies depending on the specific sugar. Lactose, which is commonly found as dominant sugars in milk, is the least sweet, whereas fructose is the sweetest sugar. In addition, sugars can form solution because they are soluble in water and form syrups when the concentration in water is high. In syrup production, water is evaporated to lead to crystal formation, thus the molecular solutions are formed because of hydrogen-bond interchange. When sugar is placed in water, the water molecules immediately form hydrogen bonds with the sugar molecules, thus hydrating them and removing them from hydrogen bonds between the sugar crystals. At high concentrations, sugars act as a food preservative which prevents food spoilage by growth of microorganisms. This mechanism is related to reducing the water activity in food to a level below which bacterial growth cannot be supported. Examples of foods preserved in this way include jams and candied fruit (Lee 1983; Vaclavik and Christian 2014).

Mono- and polysaccharides in appropriate concentration or proportion contribute to the viscosity of foods, thus giving mouthfeel to foods. This functionality related the binding capacity of the molecules particularly with water; if they are replaced by a chemically sweetening additive such as aspartame or saccharin, it

Table 6 Application of polysaccharides in food

Area of application/food	Suitable polysaccharides
Stabilization of emulsions/suspensions in condensed milk and chocolate milk	Carrageenan, algin, pectin, carboxymethylcellulose
Stabilization of emulsions in coffee whiteners, low-fat margarines	Carrageenan
Stabilization of ice cream against ice crystal formation, melting, phase separation; improvement of consistency (smoothness)	Algin, carrageenan, agar, gum arabic, gum tragacanth, xanthan gum, guaran gum, locust bean flour, modified starches, carboxymethylcellulose, methylcellulose
Water binding, improvement of consistency, yield increase of soft cheese, cream cheese, cheese preparations	Carrageenan, agar, gum tragacanth, karaya gum, guaran gum, locust bean flour, algin, carboxymethylcellulose
Thickening and gelation of milk in puddings made with and without heating, creams; improvement of consistency	Pectin, algin, carrageenan, guaran gum, locust bean flour, carboxymethylcellulose, modified starches
Water binding, stabilization of emulsions in meat products (corned beef, sausage)	Agar, karaya gum, guaran gum, locust bean flour
Jellies for meat, fish, and vegetable products	Algin, carrageenan, agar
Stabilization and thickening, prevention of syneresis, freeze-thaw stability of soups, sauces, salad dressing, mayonnaise, ketchup; obtaining “body” in low-fat and low-starch products	Gum tragacanth, algin, karaya gum, xanthan gum, guaran gum, locust bean flour, carboxymethylcellulose, propylene glycol alginate, modified starches
Stabilization of protein foam in beer, whipped cream, meringues, chocolate marshmallows	Algin, carrageenan, agar, gum arabic, karaya gum, xanthan gum
Prevention of starch retrogradation in bread and cakes, water binding in dough	Agar, guaran gum, locust bean flour, carrageenan, xanthan gum
Thickening and gelation of fruit pulp (confiture, jams, jellies, fruit pulp for ice cream and yoghurt)	Pectin, algin
Gelation of jelly candies, jelly beans, glaze, icing, water-dessert jellies	Pectin, algin, carrageenan, agar, gum arabic, modified starches
Sediment stabilization in fruit juices, obtaining “body” in beverage powders	Algin, pectin, propylene glycol alginate, gum arabic, xanthan gum, guaran gum, methylcellulose
Stabilization of powdery aroma emulsions, encapsulation of aroma substances	

Source: Belitz HD, Grosch W, Schieberle P (2009). Food Chemistry. 4th revised and extended edition, Springer-Verlag Berlin Heidelberg

doesn't affect the consistency of the food. Another substance will be added to give the expected body or mouthfeel to the food, for example, hydrocolloids to the desired consistency without addition of natural sugar (Belitz et al. 2009; Fennema 1996). Table 6 shows the application of polysaccharides in food industry.

The outlined functions of polysaccharides depend on their highly variable properties. They may vary from insoluble (cellulose) to those with good swelling power and solubility at different temperatures of solvent (starch, guaran gum).

The solutions may have low viscosities even at very high concentrations (gum arabic) or may have exceptionally high viscosities even at low concentrations (guaran gum). Some polysaccharides, even at a low concentration, set into a thermoreversible gel (alginates, pectin). While most of the gels melt at elevated temperatures, some cellulose derivatives set into a gel.

Food Enzymes

Classification

The systematic classification and designation of enzymes based on reaction specificity are based on the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB)–adopted rules last amended in 1992. All enzymes can be classified into six major classes (Belitz et al. 2009):

1. Oxidoreductases: catalyzing oxidoreduction reactions
2. Transferases: transferring a group from one compound to another
3. Hydrolases: catalyzing the hydrolytic cleavage of C-O, C-N, C-O, and some other bonds
4. Lyases: clearing C-C, C-O, C-N, and other bonds by elimination
5. Isomerases: catalyzing geometric or structural changes within one molecule
6. Ligases or synthetases: catalyzing the joining together of two molecules coupled with the hydrolysis of a pyrophosphate bond in ATP or a similar triphosphate

Some other enzymes without the suffix “-ase” terminology are trypsin, rennin, and ficin.

Each enzyme has a systematic name and number derived from the six classes above. Each class can be subdivided into subclasses which more specifically denote the type of reaction, e.g., by naming the electron donor of an oxidation-reduction reaction or by naming the functional group carried over by a transferase or cleaved by a hydrolase enzyme. Moreover, each subclass is further divided into sub-subclasses. For example, sub-subclasses of oxidoreductases are denoted by naming the acceptor which accepts the electron from its respective donor (Belitz et al. 2009). An example is the enzyme ascorbic acid oxidase. Its systematic name is L-ascorbate: oxygen oxidoreductase, and its systematic number is E.C. 1.1.10.3.3. It is essential to mention the origin of the enzymes, particularly the type of organism and subcellular fraction of origin (cytoplasmic, mitochondrial, or peroxisomal), for example, ascorbate oxidase (E.C. 1.1.10.3.3) from cucumber.

E.C.	1.	10.	3.	3.
Enzyme commission	Oxidoreductase	Subclass of oxidoreductase (donor naming diphenols or ascorbic acid)	Sub-subclass (naming the acceptor: oxygen)	Serial number of the enzyme within the sub-subclass

Catalytic Actions

Catalytic actions of enzymes depend on their properties such as enzyme kinetics and specificity. Enzyme kinetics are affected by enzyme concentration and enzyme inhibition. The concentration of an enzyme in an enzyme-catalyzed reaction is directly proportional to the rate of the reaction (Cornish-Bowden 1995).

Michaelis and Menten (1913) were the first to introduce mathematical analysis of the velocity of enzyme-catalyzed reactions as affected by the substrate concentration (Fig. 9). It is assumed that an intermediate enzyme-substrate complex is formed, which is the important feature of this theory. In addition, the assumption is made that the conversion rate of the enzyme-substrate complex to products of the reaction and the enzyme determines the conversion rate of the substrate to the reaction products. This equation is expressed as follows:

$$v = \frac{V [S]}{K_m + [S]} \quad (3)$$

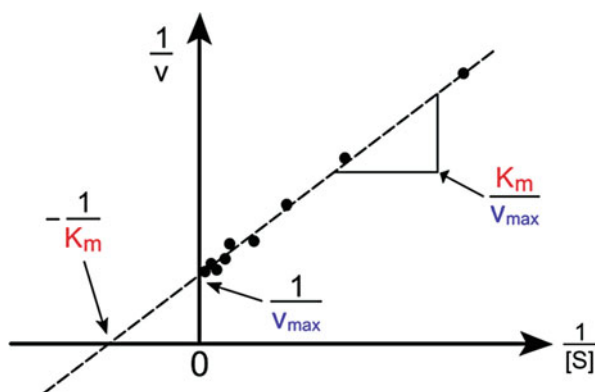
where v = initial velocity, V = maximal velocity, $[S]$ = total concentration of substrate, K_m = Michaelis-Menten constant.

$$\frac{1}{v} = \frac{K_m}{V} \frac{1}{[S]} + \frac{1}{V} \quad (4)$$

If one plots $1/v$ versus $1/[S]$, K_m/V is the slope, and intercept on $1/v$ axis is $1/V$. One gets a straight line rather than a sigmoid curve as in Michaelis-Menten. It is possible to calculate K_m (Fig. 9).

The Michaelis-Menten equation can be analyzed by the effects of enzyme inhibitor on the reaction kinetics. There are three classes of enzyme inhibition: competitive, noncompetitive, and uncompetitive. All of these are reversible. In competitive inhibition, another substance related to the substrate competes with the substrate to bind at the active site of the enzyme. This can inhibit the activity of the

Fig. 9 Linear plots of the Michaelis–Menten equation (Source: Dibberri (<http://commons.wikimedia.org/wiki/User:Dibberri>))



enzyme in the formation of the complex with the normal substrate. In noncompetitive inhibition, the inhibitors do not bind at the active site of the enzyme but at another site that frequently alters the enzyme as a result. In uncompetitive inhibition, an inactive enzyme-substrate-inhibitor complex is formed by the combination with the enzyme-substrate complex, which is usually formed. This inhibitor itself does not react with the enzyme to form a complex. In irreversible inhibition, some agents are able to alter permanently and covalently a functional group of certain enzymes that is necessary for catalysis, resulting in irreversible inactivation of the enzyme (Copeland 2000; Cornish-Bowden 1995).

As particular enzyme only fits with very specific substrate, lock-and-key theory has been suggested by Fischer (1894) to describe the relationship. However, the lock needs some adjustment, thus allowing the key to combine with the lock with varying degrees of specificity, from highly specific to only moderately so. Urease, highly specific, will act on urea only and on no other known compound. Some enzymes act on a definite stereoisomeric structure. For example, racemic compounds have been separated by means of enzymes because a specific enzyme was found to act only on the dextro form, leaving the levo form undisturbed. Some enzymes are intermediate in specificity, for instance, invertase, which hydrolyzes sucrose and other β -fructosides. Lipases are enzymes of low specificity, which hydrolyze not only triacylglycerols to fatty acids and glycerol but also simpler esters to alcohols and acids (Pleiss et al. 1998).

Enzymes act only in the specific area due to the molecular size of enzymes, known as the active site. In order for this reaction to proceed asymmetrically, the enzyme and substrate must have a specific spatial relationship. The enzyme-cofactor complex that is catalytically active is known as the holoenzyme. The protein remaining after the removal of the cofactor is known as the apoenzyme, which is, by itself, catalytically inactive. However, the holoenzyme has the ability to combine with the substrate, yielding the final products (Gutfreund 1972).

Rate of Reaction

The rate of enzymatic reaction is referred to as the number of molecules of a substrate decomposed by a molecule of a given enzyme per minute. As an example, catalase decomposes 5,000,000 molecules of H_2O_2 in a minute. If the molecular weight of the enzyme is not known or if the material employed is not pure, it is internationally accepted that one unit of the enzyme material used will catalyze the reaction 1 μ mol of substrate per minute under controlled conditions. Frank A. Lee (1983) describes some factors that considerably affect the rate of an enzyme reaction, as the following:

1. Temperature

Enzymes are denatured at high temperatures. Temperature can increase the rate of the reaction when the optimum temperature is selected, whereas if the temperature reaches the denaturation point of enzyme, it can decrease the rate of reaction. The bulk of enzymes are most active around 30^o-40 °C. Denaturation begins around 45 °C, and the activity of the enzyme starts to decline. Some

enzymes are more heat stable. The temperature coefficient, Q_{10} , is used as an expression of the change in rate of reaction for a 10 °C change in temperature. The value of Q_{10} for many enzyme-mediated reactions is about 2. The Q_{10} value is determined by dividing the reaction rate at a given temperature plus 10 °C by the reaction rate at that given temperature.

2. Regeneration (Renaturation)

It is reported that the enzyme trypsin can recover its enzyme activity when it is cooled following denaturation by heat (Northrop 1932). Also, it is well known that peroxidase in vegetables which has been inactivated by blanching can recover at least part of its enzyme activity during frozen storage.

3. pH

pH can be used to control the rate of enzymatic reaction since the hydrogen ion concentration of the medium affects significantly the activity of the enzyme. The pH is controlled either to inhibit the enzyme activity or to increase the maximum enzyme activity. The pH can be lowered in fruit products by the addition of such compounds as phosphoric or citric acids.

4. Activators

Enzymes such as pyruvate kinase and enolase require either Mn^{2+} or Mg^{2+} to be activated. Bisulfite ion or cysteine can prevent inactivation of papain, bromelain, and ficin, plant proteolytic enzymes, when exposed to oxygen. These reducing compounds maintain the sulfhydryl groups in the enzyme protein.

5. Active Sites of Enzymes

Great catalytic efficiency depends on precise location of the substrate at the catalytic group of the active site of the enzyme. Of the enzymes that contain only amino acids it must be noted that not all are involved as part of the active site. In certain enzymes large parts of the protein can be removed with no change in the activity of the enzyme.

From the classification scheme given earlier, it is stated that enzymes are classified by the kinds of reactions they catalyze. Those that bring about hydrolysis and those active in oxidation and reduction are of particular interest in dealing with foods. Various types of enzyme with their specific catalytic actions are given below (Belitz et al. 2009; Lee 1983):

1. Esterases, among others, are able to bring about hydrolysis, or reversibly, synthesis, of esters.
2. Lipase hydrolyzes fats and oils to glycerol and fatty acids. Lipase is also able to hydrolyze the ethyl esters of long-chain fatty acids.
3. Glycosidases, the enzymes effective in the synthesis and breakdown of carbohydrates, belong to the general group of hydrolases.
4. Pectinesterases hydrolyze the methyl ester groups naturally occurring in pectin. They are used in the production of low-ester pectins which, in turn, are used for the manufacture of jellies and jams of low sugar content for which products the ordinary pectin will not produce a gel.

5. Amylases have the ability to hydrolyze starch and glycogen. Three types of amylases are considered: α -amylases, β -amylases, and amyloglucosidases.
6. Phosphatases are able to split phosphoric acid from some types of organic phosphates.
7. Proteinases, otherwise known as proteolytic enzymes, include papain, ficin, trypsin, pepsin, and others and have the ability to cleave the CO-NH (peptide) bond.
8. Peroxidases catalyze reactions in which hydrogen emulsifying the fatty material increases the speed at which this reaction takes place.

Applications in Foods

In vegetable processing, the main purpose of enzyme inactivation is to prevent the quality deterioration of vegetables. Blanching or steaming vegetables before low-temperature storage can prevent their deterioration. Lee (1958) describes the liberation of fatty acids as a result of peroxidases found in unblanched peas during storage at -18°C , whereas the compounds were not formed in the blanched peas. On the other hand, blanching vegetables before drying process can eliminate the development of haylike flavor and aroma. While low water content protects food from spoilage by microbial action, it does not stop deterioration as a result of enzyme activity. An increase in the moisture content can result in an increase in enzyme activity.

In beverage industry, pectic enzymes are used to increase the yields of juice and to extract completely the pigments from fruits. In alcoholic beverages, microorganisms produce some enzymes that convert sugars to alcohol (Whitehurst and Law 2002).

Proteolytic enzymes are used to make meat tender. Wrapping meat with papaya leaves has been applied to tenderize meat since long time ago. These leaves contain proteolytic enzymes. In modern practice, allowing the carcass to hang at a low temperature (4°C) for several weeks improves the activity of tenderness enzyme. Another method of tenderizing is achieved by injecting small amounts of proteolytic enzymes into the animal just prior to slaughter. In dairy processing, cheese production is achieved by curdling milk with rennet, an enzyme produced by calf stomachs (Nagodawithana and Reed 1993; Whitehurst and Law 2002).

Enzymes are frequently isolated from parts of organisms and microorganisms. In enzyme isolation, the richest available source should be used in order to reduce the amount of work necessary to prepare the enzyme. The extraction should not be done in extreme temperature, pH, and ionic strength, which causes enzyme instability and inactivity. The isolation can be done by chromatography with some media such as Sephadex, cross-linked polyacrylamide, and ion-exchange, or another recent technique involves the use of isoelectric focusing (Nagodawithana and Reed 1993).

Enzymes can be advantageous because of the following characteristics. (a) They are efficient catalysts. (b) The reaction can be stopped by applying heat sufficient to destroy the enzymes. (c) Temperature, pH, and time can be used to control the

reactions. (d) Enzyme activity can be standardized. (e) They are nontoxic and can be left in the product unless it is necessary to destroy them (Whitehurst and Law 2002).

Conclusion and Future Directions

Water molecule structure accounts for its special behavior which determines its interaction with other compounds. Since water can determine the shelf life of food because of its interaction with food components, water activity is important. Water activity is the ratio of the moisture content of a food and the relative humidity of the air surrounding. Besides, physical and chemical properties of water affect and control food stability. Stability of food is associated with quality of food. Food instability can lead to food perishability which degrades food quality. A combination between rapid techniques to detect water content in food and novel methods to control physicochemical properties of water will be a focus to improve food quality in the future. Some novel techniques both for entrapping and eliminating water from the system will be interesting research to be developed in the future, such as eliminating water with minimized effect on the physical and chemical changes of food or entrapping water in the gel structure using food-grade biopolymer.

Lipids in food play an important role in food sensory attributes such as texture, flavor, nutrition, and caloric density. Lipids enhance and modify flavor, aerate doughs, contribute flakiness and tenderness, emulsify, transfer heat, and provide satiety. They are composed of a glycerol molecule with one, two, or three fatty acids attached creating mono-, di-, or triglycerides, respectively. Since lipids produce much calories, food scientists and manufacturers will need to modify the physical and chemical properties of food lipids in order to produce healthy foods with high consumer acceptability. In other words, the content of unhealthy lipids (e.g., saturated fats and *trans* fatty acids) will be reduced or replaced in food without changing much food texture or flavor.

Proteins have a key role in many biological processes as well as foods. Amino acids are required building blocks for protein biosynthesis. A protein containing all of the essential amino acids is considered a complete protein and will have a high biological value. Major animal proteins generally have high biological value as compared to plant proteins due to deficiency in some of the essential amino acids in plant foods. Food proteins are responsible for flavor, color, and texture as well as gel and foam formations. The functional properties of proteins in foods are related to their structural and other physicochemical characteristics. Food proteins can be powerful allergens for some people and have adverse effects in the diet. Food processing can alter the nutritional value and functional properties of proteins.

Food carbohydrates are differentiated by their sugar molecules, chemical structures, and physicochemical properties. Each carbohydrate has its own characteristics for various specific applications in food products. Microstructure manipulation of polymeric assembly of carbohydrates can have great potential in developing

novel food. This polymer can be used as food texturing, encapsulant, emulsifier, and gelation agent.

Enzymes are classed as proteins, and the classification of enzymes is based on the reactions they catalyze. Enzymes act by combining with the substrate at a small area of activity which is known as the active site. The efficiency of an enzyme is known as the molecular activity. This is the number of molecules of a substrate which are decomposed by a molecule of a given enzyme in specific time. Enzymes are important in a number of industrial processes involving foods. In future food application, increasing food product quality through enzyme inactivation, isolation, and optimization will still become a main concern for food research and development.

Cross-References

- ▶ [Chemical Composition of Fat and Oil Products](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Heating and Cooking](#)

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General Properties of Minor Food Components

3

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Abstract

Vitamins and minerals are minor components of foods that play an essential role in human nutrition. The vitamins are generally divided into two main groups, the water-soluble and the fat-soluble vitamins. The occurrence of the vitamins in the various food groups is related to their water or fat solubility. Minerals may be present as inorganic or organic salts or may be combined with organic material. Many vitamins and minerals are unstable under certain conditions of processing and storage; therefore, their content in processed foods may be considerably reduced. Some vitamins and minerals function as part of an enzyme cofactor; without them, the enzyme would be ineffective as a biocatalyst. This chapter focuses on vitamins and mineral classification, their chemical properties and stability, and their functional properties in food.

Introduction

Vitamins and minerals are quantitatively minor constituents in food that play an important part in the nutritional value of foods. They are required to maintain health and, in younger animals and adults, growth. Only small amounts of them are necessary for these purposes.

The vitamins comprise a diverse group of organic compounds that are nutritionally essential micronutrients. They function as enzyme precursors, components of the antioxidative defense system, factors involved in genetic regulation. Vitamins have a variety of chemical structures and they mainly can be categorized as water- and fat-soluble vitamins. In food, several of the vitamins influence the chemical nature of food, by functioning as reducing agents, radical scavengers, reactants in browning reactions, and as flavor precursors. Stability and properties of vitamins in complex food systems are affected by 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) (Belitz et al. 2009).

Mineral is assigned to the trace elements. Its involvement in a biochemical function in a vital tissue or organ is possible to be detected. Main and trace elements have very varied physiological functions, such as electrolytes, enzyme constituents, and building materials, e.g., in bones and teeth. Mineral contents in the same food raw material may be different, it can fluctuate greatly depending on genetic and climatic factors, agricultural procedures, composition of the soil, and ripeness of the harvested crops, among other factors (Lee 1983). Mineral materials contribute to structure and physiological value (Damodaran et al. 2008). Changes in the mineral content usually occur as well in the processing of raw materials, e.g., in thermal processes and material separations (Belitz et al. 2009).

The major objective of this chapter is to discuss the chemistry of the individual vitamins and minerals including the chemical structure and properties that influence their stability and functional properties in foods.

Food Vitamins

Vitamin Classification

Vitamins can be mainly categorized into two general classes: the fat-soluble vitamins, such as A, D, E and K, and the water-soluble vitamins, B₁ (thiamin), B₂ (riboflavin), B₃ (niacin), B₅ (pantothenic acid), B₆ (pyridoxine), B₇ (biotin), B₉ (folic acid), and B₁₂ (cyanocobalamin).

Vitamins are essential for human growth and development, either because they are not produced in the body or because they are produced in insufficient amounts (e.g., niacin, choline). Each of these vitamins regulates many important functions in the body by acting as antioxidants, coenzymes, cofactors, and hormone regulators (Combs 2008). Some of the health benefits derived from these vitamins include: improved vision and immunity boost (vitamin A); enhanced energy levels, mood regulation, and immune boost (vitamin B-complex); immune boost and promoting healthy skin, gums, tendons, and ligaments (vitamin C); healthy bones and teeth and prevention of cancer and heart diseases (vitamin D); skin and brain vitality and protection against bladder and prostate cancers (vitamin E); and potential role in bone metabolism, nerve signaling, and prevention of atherosclerosis and kidney stones (vitamin K) (Fairfield and Fletcher 2002; Ryan-Harshman and Aldoori 2005).

Structure and General Properties

Vitamin A

Vitamin A refers to a group of unsaturated hydrocarbons, including retinol and related compounds and certain carotenoids. Vitamin A is insoluble in water but soluble in ethanol and freely soluble in organic solvents including fats and oils (de Man 1999).

Carotenoids provide significant vitamin A activity to foods of both plant and animal origin. Of approximately 600 known carotenoids, about 50 exhibit some provitamin A activity (i.e., are partially converted to vitamin A in vivo). In animal tissues, vitamin A activity is predominantly in the form of retinol or its esters, retinal, and, to a lesser extent, retinoic acid. The term retinoids refers to the class of compounds including retinol and its chemical derivatives having four isoprenoid units. For a compound to have vitamin A or provitamin A activity, it must exhibit certain structural similarities to retinol, such as at least one intact nonoxygenated β -ionone ring and an isoprenoid side chain terminating in an alcohol, aldehyde, or carboxyl function (Fennema 1996) (Fig. 1).

Vitamin D

All provitamins D found in nature are chemically sterols, carbon 3, which has a hydroxyl group attached and also contains the conjugated 5,7-diene (Lee 1983). Vitamin D activity in foods is associated with several lipid-soluble sterol analogs including cholecalciferol (vitamin D₃) from animal sources and ergocalciferol

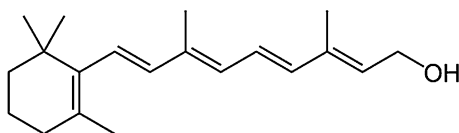
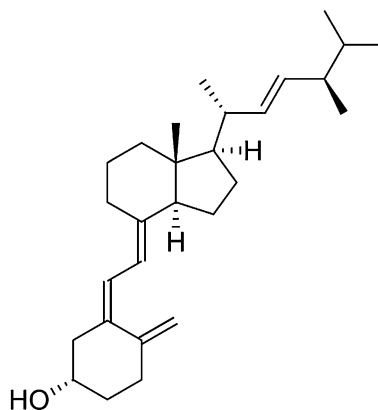


Fig. 1 Chemical structure of retinol, one of the major forms of vitamin A (Source: <http://commons.wikimedia.org/wiki/User:NEUROtiker>)

Fig. 2 Chemical structure of ergocalciferol (vitamin D₂) (Source: <http://commons.wikimedia.org/wiki/User:Calvero>)



(vitamin D₂) produced synthetically (Fennema 1996). Vitamins D₂ and D₃ are white to yellowish crystalline compounds. They dissolve readily in organic solvents such as hexane, ether, acetone, and ethanol, are less in vegetable oils, and are insoluble in water. Both of these compounds are used in synthetic form for food fortification.

Vitamin D is synthesized *in vivo* from dietary vitamin D with the help of sunlight exposure. 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 (Lee 1983). On the other hand, ergocalciferol is an exclusively synthetic form of vitamin D that is formed by commercial irradiation of phytosterol (a plant sterol) with ultraviolet (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 (Damodaran et al. 2008) (Fig. 2).

Vitamin E

Vitamin E is the generic term for tocopherols and tocotrienols that exhibit vitamin activity similar to that of α -tocopherol (Fig. 3). Tocopherols are 2-methyl-2-(4,8,12-trimethyltridecyl)chroman-6-ols, while tocotrienols are identical except for the

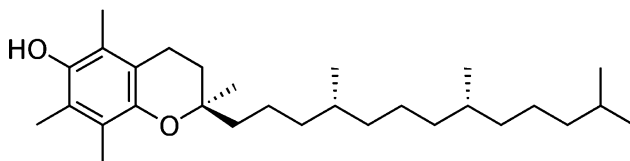


Fig. 3 Chemical structure of α -tocopherol (Source: <http://commons.wikimedia.org/wiki/User:Calvero>)

presence of double bonds at positions 3', 7', and 11' of the side chain. 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) (de Man 1999).

The α , β , γ , and δ forms of tocopherol and tocotrienol differ according to the number and position of the methyl groups and thus distinguish significantly in vitamin E activity (Fennema 1996). In a new system of reporting vitamin E activity (Institute of Medicine 2000), 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. The stereochemical configuration at these positions affects the vitamin E activity of the compound. The naturally occurring configuration of α -tocopherol shows the greatest vitamin E activity.

α -Tocopherol is a pale, yellow, viscous oil which melts at 2.5–3.5 °C and boils under very reduced pressure at 350 °C with decomposition. It is insoluble in water, but soluble in alcohol and ether (de Man 1999).

Vitamin K

Vitamin K consists of a group of naphthoquinones that exist with or without a terpenoid side chain in the 3-position. 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 (Belitz et al. 2009).

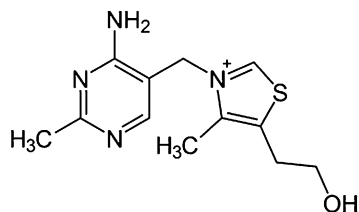
Phylloquinone (vitamin K₁) is a product of plant origin, while menaquinones (vitamin K₂) of varying chain length are products of bacterial synthesis, mainly by intestinal microflora. Vitamin K₁ is a yellow viscous oil which has a melting point of –20 °C. It is insoluble in water, but soluble in alcohol and ether. Vitamin K₂ is a crystalline substance with a melting point between 35 °C and 60 °C (Damodaran et al. 2008).

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 menaquinones are absorbed from the lower (Belitz et al. 2009).

Vitamin B₁ (Thiamin)

Thiamin is widely distributed in plant and animal tissues. Most naturally occurring thiamin exists as thiamin pyrophosphate, with lesser amounts of nonphosphorylated

Fig. 4 Chemical structure of α -thiamine (Source: <http://commons.wikimedia.org/wiki/User:NEUROtiker>)



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 (de Man 1999). Thiamin is commercially available as the hydrochloride and mononitrate salts, and these forms are widely used for food fortification and as nutritional supplements. It is soluble in water, methanol, and glycerol but practically insoluble in acetone, ether, chloroform, and benzene (Shils et al. 1999) (Fig. 4).

Vitamin B₂ (Riboflavin)

Riboflavin, formerly known as vitamin B₂, is the generic term for the group of compounds that exhibit the biological activity of riboflavin. Riboflavin is soluble in water, giving rise to a bright yellow-colored solution. The parent compound of the riboflavin family is 7,8-dimethyl-10(1'-ribityl)isalloxazine, and all derivatives of riboflavin are given the generic name flavins. Riboflavin is involved in several dehydrogenase reactions such as interconverts NAD⁺/NAD(H) and NADP⁺/NADPH couples (Belitz et al. 2009).

Vitamin B₃ (Niacin)

Niacin is also known as nicotinic acid and nicotinamide. This is otherwise known as the anti-pellagra factor. Niacin is 3-pyridinecarboxylic acid, and nicotinamide is the corresponding form of acid amide of this compound. Niacin crystallizes in the form of needles from water or alcohol, with a melting point of 236–237 °C, and sublimes without decomposition (Harris 1971). It is slightly soluble in water and alcohol, but soluble in hot solvents, but nicotinamide is very soluble in water and alcohol. It appears as a white powder but forms needles from benzene, melts at 129–131 °C, and boils under reduced pressure (Harris 1971).

Nicotinamide is involved in enzyme activity of electron transport reactions. It is a part of two acceptor pyridine protein enzymes which are known as nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). They have also been known as diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) (Fennema 1996).

Vitamin B₅ (Pantothenic Acid)

Pantothenic acid, or d-N-(2,4-dihydroxy-3,3-dimethyl-butyryl)- β -alanine), is a water-soluble vitamin (Fennema 1996). Pantothenic acid oxidizes coenzyme A in the synthesis/oxidation of fatty acid. It is 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 (Damodaran et al. 2008). Pantothenic acid is essential for all living things and is distributed widely among meats, cereal grains, eggs, milk, and many fresh vegetables.

Vitamin B₆ (Pyridoxine)

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. It crystallizes in needles from acetone and has a melting point of 160 °C. It sublimes and is very soluble in water and soluble in alcohol. Pyridoxine hydrochloride crystallizes in plates from alcohol and acetone. It melts at 206–208 °C, sublimes, and is very soluble in water and slightly soluble in alcohol (Lee 1983).

Glycosylated forms of vitamin B₆ are present in most fruits, vegetables, and cereal grains, generally as pyridoxine-5-β-D-glucoside (Gregory and Ink 1987). 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 (Belitz et al. 2009). Several other glycosylated forms of vitamin B₆ are also found in certain plant products.

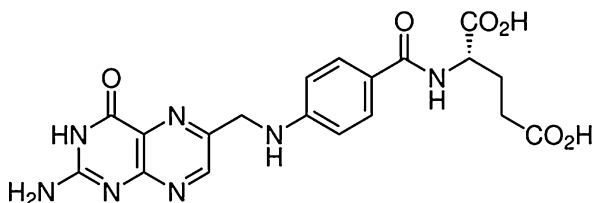
Vitamin B₇ (Biotin)

Biotin naturally forms free D-biotin and biocytin (biotinyl-L-lysine). It is a water-soluble vitamin that functions coenzymatically in carboxylation and transcarboxylation reactions (Lee 1983). Biocytin functions as the coenzyme form and actually consists of a biotinylated lysyl residue, formed by post-translational biotinylation, that is, covalently incorporated in a protein chain of various carboxylases including a role in fatty acid synthesis (de Man 1999). 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 (bis-norbiotin 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 (Belitz et al. 2009).

Vitamin B₉ (Folic Acid)

Folic acid (pteroyl-L-glutamic acid) exists naturally only in trace quantities. Folic acid consists of L-glutamic acid that is coupled with its α-amino group to the carboxyl group of para-aminobenzoic acid that, in turn, is linked to a 2-amino-4-hydroxypteridine (Fig. 5). In folic acid, the pteridine moiety exists in oxidized form (Lee 1983). Vitamin B₉ is essential for numerous bodily functions; it acts as coenzyme in single-carbon metabolism (Belitz et al. 2009).

Fig. 5 Chemical structure of folic acid (Source: <http://commons.wikimedia.org/wiki/User:Calvero>)



Vitamin B₁₂ (Cyanocobalamin)

Vitamin B₁₂ is the generic term for the group of compounds (cobalamins). Cyanocobalamin is crystallized into red, odorless, and tasteless needles. It has the least water solubility (12.5 mg/ml) among all the water-soluble vitamins (Fennema 1996). These compounds are corrinoids, tetrapyrrole structures in which a cobalt ion (Co) is coordinately covalently bonded to the four pyrrole nitrogens. The fifth coordinate covalent bond to Co is with a nitrogen 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 (Damodaran et al. 2008). It acts as coenzyme for methylmalonyl-CoA mutase and homocysteine methyltransferase (Belitz et al. 2009). Cyanocobalamin is commonly available in a synthetic form used in food fortification and nutrient supplements.

Vitamin C (Ascorbic Acid)

Ascorbic acid is a naturally occurring organic compound. It is a white solid, but impure samples can appear yellowish. It dissolves well in water to give mildly acidic solutions. Ascorbic acid is one form of vitamin C. This compound is highly polar; thus, it is readily soluble in aqueous solution and insoluble in less nonpolar solvents. Ascorbic acid 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 ($pK_{a2} = 11.4$) (Lee 1983). It contains two optically active centers at the C₄ and C₅ positions. Ascorbic acid is 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 it has no nutritional value. Because of the high solubility of ascorbic acid aqueous solutions, the potential exists for significant losses by leaching from freshly cut or bruised surfaces of fruits and vegetables.

Stability and Degradation

Almost every food preparation process reduces the amount of micronutrients in food, including vitamins. Vitamins are very unstable to high levels of heat, light, and oxygen which can cause the greatest loss. Vitamins can also be “washed out” from foods by fluids that are introduced during a cooking process. For example, boiling a potato can cause much of the potato’s B and C vitamins to migrate to the

boiling water. Similar losses also occur in different processes, such as broiling, roasting, or frying in oil, and then draining off the drippings.

On the other hand, stability issues of vitamin related to the formulation and delivery challenges for functional purpose are also an important concern. Both water-soluble and oil-soluble vitamins suffer from stability issues concerning mainly their oxidative stability in the presence of heat, ultraviolet light, and metal ions (Ball 2004). It is quite straightforward to imagine that the formulation of oil-soluble vitamins in food beverages or aqueous-based products is severely limited due to their insolubility in water. More specific details related to the stability of vitamins are given as follows.

Vitamin A: Vitamin A and provitamin A carotenoids are very sensitive to oxygen in air, especially in the presence of light and heat. The sensitivity to oxidation is enhanced in the presence of trace metal ions especially Fe^{++} and Cu^{++} . Food processing and storage can lead to 5–40 % destruction of vitamin A and carotenoids (Belitz et al. 2009). In the absence of oxygen and at higher temperatures (e.g., cooking or food sterilization), vitamin A can be degraded by reaction of isomerization and fragmentation. In the presence of oxygen, oxidative degradation leads to a series of products, some of which are volatile (Fennema 1996).

Vitamin D: Vitamins D_2 and D_3 are white to yellowish crystalline compounds. Both vitamins are rapidly dissociated by light, oxygen, and acid, particularly in solution or in finely powdered form. They are stable in alkaline solutions but tend to isomerize in oily solutions. Vitamin D degradation may occur in glass-packaged milk during retail storage. Approximately 50 % of cholecalciferol added to skim milk is lost during 12 days of continual exposure to fluorescent light at 40 °C (Belitz et al. 2009). Like other unsaturated fat-soluble components of foods, vitamin D compounds are susceptible to oxidative degradation.

Vitamin E: Vitamin E content in food can be lowered by several detrimental factors such as light, oxygen, and heat. In some foods it may decrease by 50 % during two weeks of storage at room temperature. However, vitamin E compounds show 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. Oxidative degradation of vitamin E is strongly influenced by the same factors that influence oxidation of unsaturated lipids. On the other hand, frying causes large losses of vitamin E in vegetable oils. Esters of α -tocopherol (α -tocopheryl acetate and α -tocopheryl succinate) are used for supplements because they are more resistant to oxidation during storage (Friedrich 1988; Jenness et al. 1988).

Vitamin K: Vitamin K compounds are moderately stable to heat and reducing agents, but are sensitive to acid, alkali, light, and oxidizing agents (Jenness et al. 1988). They are relatively stable to atmospheric oxygen and exposure to heat. Vitamin K_1 is a light-yellow oily liquid that is decomposed by light and alkaline substances. Vitamin K_2 is a crystalline substance with a melting point between 35 °C and 60 °C (Lee 1983).

Vitamin B₁: Vitamin B is stable in acidic conditions but highly unstable at alkaline pH (Shils et al. 1999). In fact, thiamine is considered one of the most

unstable B vitamins; it is unstable to heat and when exposed to UV and γ -irradiations. Moreover, because of its chemistry, thiamine is also known to react strongly in Maillard-type reactions (Mahan and Escott-Stump 2000). It has been reported that processes such as baking, pasteurization, or boiling of foods fortified with thiamine can reduce its content by up to 50 % (Lee 1983).

The following losses of thiamine can be estimated between 15 % and 25 % in canned fruit or vegetables stored for more than a year; 0–60 % in meat cooked under household conditions, depending on temperature and preparation method; 20 % in salt brine pickling of meat and in baking of white bread; 15 % in blanching of cabbage without sulfite; and 40 % with sulfite. Losses caused by sulfite are pH dependent. Practically no thiamine degradation occurs in a stronger acidic medium (e.g., lemon juice) (de Man 1999).

Vitamin B₂: Riboflavin is quiet stable to heat and other processing operations but degrades easily in the presence of light (Zhuge and Klopfenstein 1986). The wavelengths of light responsible for the riboflavin destruction are in the visible spectrum below 500–520 nm, while UV light has no destructive effect on riboflavin. This photolytic reaction cleaves ribitol from the vitamin, converting it to lumiflavin. Riboflavin gives yellow color in solution (Damodaran et al. 2008). Moreover, one can also assume that due to its bright color-imparting nature, the aesthetics of the fortified products may be altered. Riboflavin is relatively stable in normal food handling processes.

Vitamin B₃: Niacin exhibits an excellent stability in the presence of heat, light, air, and alkali. However, in the presence of other micronutrients such as minerals especially coupled with high temperatures, the stability of niacin is altered (Lee 1983).

Vitamin B₅: Pantothenic acid is yellow, viscous, and hygroscopic oil. It is stable to heat in slightly acid to neutral conditions, but decomposes rapidly in alkaline conditions (Gutzeit et al. 2007). It is stable in solution in the pH range of 5–7 and less stable outside this range. Due to its low stability in the gastrointestinal conditions, pantothenic acid supplements are sold as calcium and sodium salts. In solution, pantothenic acid is most stable at pH 5–7 (de Man 1999).

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 %. 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 (Belitz et al. 2009).

Degradation of pantothenic acid during thermal processing conforms to first-order kinetics (Hamm and Lund 1978). 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.

Vitamin B₆: Pyridoxine is relatively stable to heat, but pyridoxal and pyridoxamine are not. Vitamin B₆ is decomposed by oxidation and ultraviolet light and in an alkaline environment (Henry and Chapman 2002).

Vitamin B₇: Vitamin B₇ is also known as biotin. Biotin is quite stable to heat, light, and oxygen. Heat treatment results in relatively small losses. The vitamin is also stable to air at neutral (de Man 1999). Extremes of high or low pH can cause degradation, possibly because they promote hydrolysis of the $-NH-C=O$ (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. Losses of biotin during food processing and storage have been documented and summarized (Hoppner and Lampi 1993). Such losses may occur by chemical degradation processes as mentioned above and by leaching of free biotin. Little degradation of biotin occurs during low moisture storage of fortified cereal products.

Vitamin B₉: Folic acid loses its activity in the presence of light, oxidizing or reducing agents, and acidic and alkaline environments. However, it is relatively stable to heat and humidity (de Man 1999). There is no destruction of folic acid during blanching of vegetables, while cooking of meat gives only small losses. Its losses in milk are apparently due to an oxidative process and parallel those of ascorbic acid. Ascorbic acid added to food can preserve folic acid from its degradation (Lee 1983).

Vitamin B₁₂: Cyanocobalamin is crystallized into red, odorless, and tasteless needles. Vitamin B₁₂ is stable to heat, but slowly loses its activity when exposed to light, oxygen, and acid or alkali-containing environments (Henry and Chapman 2002). The stability of vitamin B₁₂ is very dependent on a number of conditions. It is fairly stable at pH 4–6, even at high temperatures. In alkaline media or in the presence of reducing agents, such as ascorbic acid or SO₂, the vitamin is destroyed to a greater extent.

Vitamin C: Ascorbic acid is easily destroyed during processing and storage through the action of metals, such as copper and iron. It is oxidized in the presence of air under neutral and alkaline conditions. Vitamin C is also sensitive to heat and light. The rate of destruction is increased by the action of metals, especially copper and iron, and by the action of enzymes (especially enzymes containing copper or iron, such as ascorbic acid oxidase, phenolase, cytochrome oxidase, and peroxidase) (de Ritter 1976).

Functional Properties in Food Processing

In addition to their role as essential micronutrients, vitamins may serve as food ingredients for their varied functional properties, such as antioxidant and colorant.

Several vitamins have been extensively used in food processing, such as vitamin C, vitamin E, and vitamin A. Vitamin C and vitamin E have been used as antioxidants in food. Provitamin A carotenoids such as β -carotene and β -apo-8-carotenal are used as colorants in fat-based as well as water-based foods.

In lipid systems, vitamin E may be used as an antioxidant in fats. Vitamin C or ascorbic acid in the form of its palmitic acid ester, ascorbyl palmitate, is an effective antioxidant in lipid systems. Ascorbyl palmitate prevents the formation of lipid free radicals and thereby delays the initiation of the chain reaction that leads to the deterioration of the fat (Liao and Seib 1987). In vegetable oils, ascorbyl palmitate acts synergistically with naturally occurring tocopherols.

Ascorbic acid reduces other food components including nitrous acid (HO-NO) and oxidized phenolic compounds. The reduction of nitrous acid with high concentrations of ascorbic acid (>1000 ppm) to nitric oxide occurs during curing of meat and prevents the formation of *N*-nitrosamine, considered to be mutagenic. Ascorbic acid is also widely used to prevent enzymatic browning in fruit products. The reduction of quinones by ascorbic acid to regenerate phenolic compounds is important in preventing the enzymatic browning reactions and in restoring the antioxidant activity of polyphenolic compounds by a synergistic mechanism (Liao and Seib 1987). Other functions of ascorbic acid are inhibition of can corrosion in canned soft drinks, protection of flavor and color of wine, prevention of black spot formation in shrimp, stabilization of cured meat color, and dough improvement in baked goods (Institute of Food Technologists 1987).

Food Minerals

Mineral Classification

Minerals are the constituents which play an important part, particularly in nutritional value of foods. They are usually present in small amounts, frequently in traces, but their importance is well recognized. They are divided into main elements, trace elements, and ultratrace elements. The major elements include Na, K, Ca, Mg, Cl, and P which are essential for human beings in amounts >50 mg/day. Trace elements (Fe, I, F, Zn, Se, Cu, Mn, Cr, Mo, Co, Ni) are essential in concentrations of <50 mg/day (Belitz et al. 2009). The role of selenium in human is not completely established. On the other hand, ultratrace elements (Al, As, Ba, Bi, B, Br, Cd, Cs, Ge, Hg, Li, Pb, Rb, Sb, Si, Sm, Sn, Sr, Tl, Ti, W) are elements that have been tested in animal experiments over several generations and deficiency symptoms have been found under these extreme conditions. Some of those minerals categorized as toxic elements include arsenic, cadmium, mercury, lead, and antimony (Lee 1983). Food materials are the important source of the major and minor elements. Table 1 shows some of the minerals found in a variety of foods.

Table 1 Minerals in representative foods^a

Food	Ash (g)	Calcium (mg)	Magnesium (mg)	Phosphorus (mg)	Iron (mg)	Sodium (mg)	Potassium (mg)
Almonds, dried	3.0	234	270	504	4.7	4	733
Apricots, raw	0.7	17	12	23	0.5	1	281
Bananas, raw common	0.9	8	33	26	0.7		533
Beans, lima immature seeds boiled and drained	1.0	47	62	121	2.5	1	422
Beef, sirloin broiled	1.1	10	21	191	2.9		
Cabbage, raw	0.7	49	13	29	0.4	20	233
Carrots, raw	0.8	37	23	36	0.7	47	341
Chicken, fryer, flesh only, fried	1.2	13		257	1.6	78	381
Lemon juice, raw	0.3	7	8	10	0.2	1	141
Lettuce, iceberg raw	0.6	20	11	22	0.5	9	175
Macaroni, dry enriched	0.7	27	48	162	2.9	2	197
Ocean perch, Atlantic, fried	1.9	33		226	1.3	153	284
Oranges, peeled raw	0.6	41	11	20	0.4	1	200
Peanuts, roasted with skins	2.7	72	175	407	2.2	5	701
Peas, green raw, immature	0.9	26	35	116	1.9	2	316
Potatoes, raw	0.9	76	34	53	0.6	3	407

Source: Watt and Merrill (1963)

^aIn 100 g, edible portions

Chemical Properties and Role of Minerals

Mineral elements are present in foods in many different chemical forms. These forms are commonly referred to as species and include compounds, complexes, chelates, and free ions and undergo complex changes during processing, storage, and digestion of foods (van Dokum 1989). The large diversity of chemical properties among the mineral elements and the complexity of their presence in food, make them difficult to be isolated and characterized. Therefore, the knowledge of the exact chemical forms of minerals in foods remains limited. However, guiding predictions about the behavior of mineral elements in foods have been established according to principles and concepts of inorganic, organic, physical, and biochemistry, such as its solubility in aqueous systems, acid/base chemistry, and chelating properties (Damodaran et al. 2008) (Table 2).

As a part of essential nutrients, the availabilities and reactivities of minerals depend on their solubility in water. The species (forms) of elements present in food vary considerably depending on the chemical properties of the element. Predominant free ionic species (Na^+ , K^+ , Cl^- , and F^-) are highly water soluble and have low affinities for most ligands, and thus, they present primarily as free ions in aqueous systems. Most other minerals are present as weak coordinate complexes, chelates, or oxygen-containing anions. The solubilities of mineral complexes and chelates may be very different from solubilities of inorganic salts. For example, if ferric chloride is dissolved in water, the iron will soon precipitate as ferric hydroxide. However, ferric iron chelated with citrate is quite soluble. Conversely, calcium as calcium chloride is very soluble, while calcium chelated with oxalate ion is insoluble (Damodaran et al. 2008).

Applying the concepts of acid/base chemistry can help to understand the chemistry of the mineral elements. Moreover, acids and bases may profoundly influence functional properties and stabilities of other food components by altering the pH of the food. A key to understanding the chemistry of minerals in foods is the Lewis acid/base concept because metal cations are Lewis acids and they bind to Lewis bases. The complexes produced by reactions between metal cations and food molecules range from metal hydrates, to metal-containing pigments such as hemoglobin and chlorophyll, to metalloenzymes. The electron-donating species in these complexes are commonly referred to as ligands. Many food molecules including proteins, carbohydrates, phospholipids, and organic acids are ligands for mineral. The principal electron-donating atoms in ligands are oxygen, nitrogen, and sulfur (Damodaran et al. 2008).

The term chelate is derived from *chela*, the Greek word for claw that 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. Since the structure is very stable because a chelating ligand (also called a chelating agent) must contain at least two functional groups capable of donating electrons, chelates have greater thermodynamic

Table 2 Trace minerals in the human body and their daily intake^a

		Content ^b	Adequate intake ^c (/day)	Note
Main	Na	1–1.5 g/kg	2.5–3.3 g	Present mostly as an extracellular constituent and maintains the osmotic pressure of the extracellular fluid
	K	2–2.5	2–5.9 g	The most common cation in the intracellular fluid
	Mg	0.4–0.5	300–500 mg	As a constituent and activator of many enzymes
	Ca	10–20	1 g	Involved in the structure of the muscular system and controls essential processes
	Cl	1–1.2	A molar basis to the sodium requirement	Serves as a counterion for sodium in extracellular fluid and for hydrogen ions in gastric juice
	P	6–12	0.8–1.2 g	Present as a phosphate, free or bound as an ester, or present as an anhydride, plays an important role in metabolism
Trace	Fe	60 mg/kg	15 mg	Most of it is present in the hemoglobin (blood) and myoglobin (muscle tissue) pigments
	F	37	2.9–3.8	Its deficiency in animal study resulted in growth and reproduction disorders
	Zn	33	10–15	Its deficiency in animals causes serious disorders, while high zinc intake by humans is toxic
	Cu	1.0	1.0–1.5	It is a component of a number of oxidoreductase enzymes
	Se	0.2	0.03–0.07	Selenium is an antioxidant and can enhance tocopherol activity
	Mn	0.2	2–5	The metal activator for pyruvate carboxylase, and, like some other divalent metal ions, it activates various enzymes
	I	0.2	0.2–0.26	Its deficiency results in enlargement of the thyroid gland (iodine deficiency-induced goiter)
	Ni	0.1	0.025–0.03	An activator of a number of enzymes
	Mo	0.1	0.05–0.1	A component of aldehyde oxidase and xanthine oxidase
	Cr	0.1	0.003–0.1	It is important in the utilization of glucose
	Co	0.02	0.002–0.1	Vitamin B ₁₂ contains cobalt as its central atom
Ultra trace	Sn			A growth-promoting effect was detected in rats, it is disputed
	Al	50–150 mg	2–10 mg	Proven nontoxic in animal studies but the accumulation in human can damage the nervous cells

(continued)

Table 2 (continued)

	Content ^b	Adequate intake ^c (/day)	Note
B	28 mg/kg	1–2 mg	Promotes bone formation by interaction with calcium, magnesium, and vitamin D
Si	1 g	21–46 mg/day	The toxicity of silicic acid is apparent only at concentrations ≥ 100 mg/kg
As	-	12–25 μ g/day	Its metabolic role is not yet understood

Source: With kind permission from Springer Science+Business Media: Food chemistry. 4th revised and extended edition, 2009, Belitz HD, Grosch W, Schieberle P

^aAverage values

^bBody weight

^cEstimated for adults

stabilities than similar complexes that are not chelates, a phenomenon referred to as the “chelate effect” (Damodaran et al. 2008). Several factors that interact to affect the stability of a chelate are ring size, number of rings, Lewis base strength, charge of ligand, chemical environment of the donating atom, resonance in chelate ring, and steric hindrance (Kratzer and Vohra 1986). Most complexes resulting from interactions between metal ions and food molecules are chelates. Chelates are very important in foods and in all biological systems; for instance, iron or copper can be added to food to prevent them from acting as prooxidants. Furthermore, preformed chelates, such as ferric sodium EDTA, may be added to foods as fortificants (Bothwell and MacPhail 2004).

Since minerals are essential to physiological function and metabolism, 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 bio-availabilities in the food supply are low, fortification has been used to help assure adequate intake by all segments of the population. Fortification of minerals such as iron and iodine has dramatically reduced deficiency of particular diseases associated with these nutrients in both developed and developing countries.

Functional Properties 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 their interactions with other food components. Overviews of mineral-food interactions as well as their roles are summarized in Table 3.

Minerals also contribute to the nutritive/physiological value and the physical state of food. For example, minerals may dramatically alter the color, texture, flavor, and stability of foods. Discolorations of fruit and vegetable products or metal-catalyzed reactions are responsible for losses of some essential nutrients, for example, ascorbic acid oxidation. Also, they are responsible for taste defects or

Table 3 Functional roles of minerals and their salts/complexes in foods

Mineral		Functional role	References
Main	Na	NaCl is used as preservative. Na ₂ CO ₃ is used as leavening agents	(Belitz et al. 2009)
	K	KCl is used as salt substitute	(Damodaran et al. 2008)
	Mg	Removal of Mg changes color in green vegetables from green to olive brown	(Damodaran et al. 2008)
Trace	Ca	It forms gel when it combines with negatively charged macromolecules such as anionic polysaccharides. Maintaining the texture in canned vegetables product	(de Man 1999)
	P	Present as phosphates. Phosphates aid emulsification in cheese. H ₃ PO ₄ is used as acidulent in soft drinks	(Damodaran et al. 2008)
	Fe	Fe ²⁺ and Fe ³⁺ catalyze lipid peroxidation in foods. It can modify color of fresh meat	(de Man 1999)
	Zn	It affects water-binding capacity in meat products	(de Man 1999)
	Cu	It may cause black discoloration in canned, cured meats. It catalyzes nonenzymatic browning	(de Man 1999)
	Mn	It acts as cofactor of enzymes (pyruvate carboxylase)	(de Man 1999)
	I	KIO ₃ improves baking quality of wheat flour	(Damodaran et al. 2008)
	Ni	It is used as catalyst in hydrogenation of vegetable oils	(de Man 1999)
	Se	It acts as cofactor of enzymes	(de Man 1999)
	S	SO ₂ acts as browning inhibitor in dried fruits and antimicrobial in wine production	(Belitz et al. 2009)
	Br	KBrO ₃ is used as bread improver, because wheat flour contains bromine	(de Man 1999)
Al	Na ₂ SO ₄ ·Al ₂ (SO ₄) ₃ is used as leavening Na ₃ Al(PO ₄) ₂ is used as emulsifying agent in processed cheese	(Damodaran et al. 2008)	

off-flavors, for example, as a consequence of fat oxidation. Therefore, the removal of many interfering metal ions by chelating agents or by other means is important to the food processing. 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 (Damodaran et al. 2008). In contrast, minerals may be added to achieve a particular functional effect, such as manipulating rheology in food colloid, and stabilize food emulsion.

Mineral elements, unlike vitamins and amino acids, are indestructible and resistant to heat, light, oxidizing agents, extreme pH, or other factors that affect organic nutrients. However, minerals may be removed from foods intentionally or unintentionally by leaching or physical separation. Grain milling is the major cause of mineral loss because minerals tend to be concentrated in the bran layers and the germ. Similar losses occur during milling of rice and other cereals. Another example of mineral loss in cheese occurs when the pH of cheese manufacturing is too low. 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; thus, soluble Ca losses when the whey is drained (Lucey and Fox 1993). Moreover, since many minerals have significant solubility in water, it is reasonable to expect that cooking vegetables in water would result in some losses of minerals compared to steaming (Lachance and Fisher 1988). Complexes formation of minerals with protein can retain the loss of minerals during food processing. The loss of iron during pasta cooking is relatively low due to iron is bound to protein molecules.

Conclusion and Future Directions

Vitamins and minerals are trace compounds of varying composition which are essential for the maintenance of health. Vitamins exist in two groups, water-soluble and fat-soluble vitamins. Those vitamins that are also not synthesized by the organism must be supplied in the diet. The mineral in food is at low concentrations, but it plays important role not only in physiological function but also in food processing, such as altering the color, texture, flavor, and stability of foods.

In food processing, both vitamins and minerals suffer from stability issues concerning food processing conditions. Almost all food processing use high temperature to process raw food material (e.g., drying, cooking, heating, pasteurizing). Besides, other food processing treatments such as pH adjustment, exposure to oxygen and light, and the presence of metal ion can lead to a reduction of vitamin and mineral content in food. To date, challenges on protection of essential vitamins and minerals from the loss during food processing are important issues that need to be addressed.

Vitamin and mineral encapsulation is an approach to protect them from their degradation. Encapsulation techniques can offer various advantages for minor food components (e.g., vitamins and minerals), including improving their solubility, improving their chemical stability, arresting and slowing down their chemical degradation rates, enhancing their bioavailability with dissolution and absorption limitation, and controlling their release for their biological performance enhancement. The type of encapsulation techniques and materials corresponded to the physical and chemical characteristics of micronutrients, economics of the process, and the objective of application (using the micronutrients as food fortificants in food formulation).

Nowadays, with the recent interest in the development of functional foods and nutraceuticals (partly due to the increased lifestyle-based disorders and partly due to the increased awareness among consumers), research on encapsulation technology for overcoming poor stability of micronutrients in food processing has been receiving a lot of attention. Moreover, the use of encapsulated micronutrients in food formulation is also a prominent challenge and very relevant to the development of new value-added products with enhanced health benefits.

Cross-References

- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Drying](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Heating and Cooking](#)

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Chemical Properties and Applications of Food Additives: Preservatives, Dietary Ingredients, and Processing Aids

4

Pak Nam Albert Chan

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Abstract

Food additives are used in processing, storage, and packaging to maintain the original form or enhance the quality of the food. In this chapter, the various preservatives, dietary ingredients, and processing aids that are added to foods are discussed, including descriptions of their chemical properties and some of their conventional applications. Updates from relevant recent studies are included. Other additives such as flavorings, sweeteners, acidulants, food colorants, starches, emulsifiers, and hydrocolloids are discussed in ► [Chap. 5, “Chemical Properties and Applications of Food Additives: Flavor, Sweeteners, Food Colors, and Texturizers.”](#)

Introduction: Foods and Food Additives

People eat to stay alive. Basically, foods are what can be eaten to maintain life and to grow, so foods must be edible and nutritious. Therefore, foods must contain water, carbohydrates, protein, fats, vitamins, minerals, and many bioactive compounds. These contents are all derived from plants and are called phytochemicals. Dietary fibers are also needed. Although they are not absorbed by the body, fibers play important roles in regulating the digestive system so it absorbs nutrients properly.

The role of food is not simply to provide nutrition. Food also provides pleasure, and meals function as the focal point for gatherings and meetings. Food is judged based on whether it tastes good or bad, in addition to its nutritional value.

The most primitive forms of food are plants and animals. Various parts of cereals, vegetables, legumes, and fruits are used for food. Animals and marine life are used for their meat, meat parts, milk, and eggs. The more popular animals that are raised for consumption are cattle, pigs, sheep, and chicken, and seafood include fish, crustaceans, and shellfish. Primitive foods are processed into various forms such as beverages, dairy food, confectioneries, baked goods, snacks, pasta, processed meats, and canned foods, so that people can have a great variety of food to enjoy.

Most food consumers cannot eat foods immediately after they are produced. Many foods need to be stored and transported. To preserve a food's quality, different kinds of food additives are used in processing. Other food additives are incorporated to replace the nutrients lost during processing or to enhance their nutritional value. Foods are sold in markets so some additives are added to enhance the appearance of the food to influence the consumer.

Bakers in ancient times had already discovered that yeast could make bread rise. They knew how to add yeast into flour when preparing their staple foodstuffs. Food additives are defined differently by different countries and different ethnic groups. The 1958 Amendment to the Federal Food, Drug and Cosmetic Act (FFDCA) of the United States stated: “The term ‘food additive’ means any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food . . . if such substance is not generally recognized among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use. . .” (Burdock and Carabin 2004). Food additives may be described as substances that are added to a food or during food processing in small amounts to maintain or modify its characteristics. They are used for a variety of reasons: to maintain freshness during storage or transportation, to add nutrients or increase the nutritional level, to have a more appealing appearance or taste, or to make processing easier. Food additives are mostly synthetic chemicals but many are natural. They usually are incorporated into foods in very small quantities, for example, 0.1–0.5 % dosage, although some are added at a higher level, for instance, starch may be added at a level of 1–5 %.

At the beginning of the twentieth century, the U.S. government began to take notice of food additives present in various foods. The authorities passed regulations to control the types and extent of the additives used. In 1906, the Pure Food and Drug Act was signed by President Theodore Roosevelt (Burdock and Carabin 2004). In 1938, the Federal Food, Drug and Cosmetic Act (FFDCA) was passed, which was amended in 1958. The act became the primary federal law regulating the use of food additives in the U.S. The 1958 amendment included the important Delaney anticancer clause that forbids the addition of carcinogenic materials to foods, as referred to FFDCA 409(C)(3)(A). Since 1950, the Food and Drug Administration (FDA) has exercised authority over the use of food additives in almost all kinds of food produced and marketed in U.S. The U.S. Department of Agriculture (USDA), which regulates meat processing and products, has adopted the FDA’s definitions of food additives in the areas under its control. From 1958 to 1962, after proper study or inspection of the relevant safety of food additives, the FDA compiled a list of Generally Recognized As Safe (GRAS) food additives and incorporated it into the Code of Federal Regulation (CFR). A GRAS substance must comply with the applicable specifications, must have an appropriate function in food, and must be present at a level no greater than the minimum amount needed to accomplish the intended effect (Kracov 2002). The GRAS list is revised periodically. The up-to-date list can be found in 21 CFR 170.3(n).

The Codex Alimentarius Commission of the United Nations (UN) was established under the auspices of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). The commission compiled a set of

standards for foods that are traded internationally, called the Codex Alimentarius. The Committee of Food Additives endorses or establishes the maximum levels of individual food additives allowed in specific food items (Wodicka 1980).

Food additives can be categorized into many groups, including preservatives, antioxidants, and dietary ingredients, including vitamins, minerals, fibers, phytochemicals, bioactive extracts from animals, and pre/probiotics, and processing aids, including sequestrants or chelating agents, enzymes, humectants, and coating agents, all of which are discussed in this chapter. The next chapter discusses flavorings, including flavors and flavoring materials, proteinoous and Maillard reaction materials, herbs, spices, essential oils and oleoresins, flavor enhancers, acidulants, natural and synthetic sweeteners, artificial and natural food coloring, starches, emulsifiers, and hydrocolloids. The classification of an additive is based on its function in food. It is not uncommon for an additive to have several functions, so it is mentioned in more than one category. For example, ascorbic acid is an antioxidant and a vitamin; gum acacia is a hydrocolloid, an emulsifier, and a soluble fiber. There are many additives that are used in different parts of the world so it is impossible to list them all. Therefore, only the commonly used ones are discussed in this chapter.

Antimicrobial Preservatives

When food is not consumed immediately after being processed, it may undergo many changes, resulting in changes in form or quality. Most of the time, the quality deteriorates mostly as a result of microorganisms, oxygen, or internal enzymatic development. To retain the form and quality of foods for a certain period of time for storage and transportation, foods can be preserved by heating, cooling, refrigeration, freezing, air-proof packaging, drying, and fermentation. However, sometimes these treatments are not suitable and it is necessary to add chemicals to preserve the food. In many instances, chemicals are added to supplement the preservation that results from a particular treatment. Preservatives are used to prevent spoilage caused by bacteria, molds, fungi, yeasts, and other microorganisms. Artificial preservatives are commonly used but they may be carcinogenic; thus, they are gradually being replaced by their natural counterparts.

People in ancient times knew that adding salt and sugar can preserve their foodstuffs. They act as preservatives because they kill bacteria and many other organisms by reducing the amount water in the food; without enough available water, microorganisms cannot survive. However, for continuous preservation, salt or sugar must be present in the food in large quantities. Use of large amounts of salt or sugar changes the food into another form known as preserved or salted food. Thus, salt and sugar are regarded as major ingredients of this food form and are not considered preservatives. Another preservative is wood smoke. Besides fuming, the smoke deposits a variety of chemicals on the foodstuff which act as preservatives. It would be more appropriate to describe fuming as food processing rather than call wood smoke a preservative.

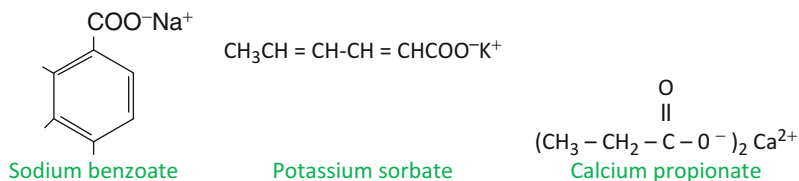


Fig. 1 Chemical structure of conventional preservatives

Foods are nutritious materials that attract a great variety of microorganisms that invade and then feed on the food. A specific chemical in a preservative may work on a limited spectrum of food invaders. However, it is impossible to find just one chemical with the ability to kill all microorganisms or to stop them from feeding on the food. An antimicrobial in a preservative is chosen for its gross effect depending on various factors such as its solubility in water, the most effective pH range, ease of application, and cost. There are several types discussed below.

Conventional Preservatives

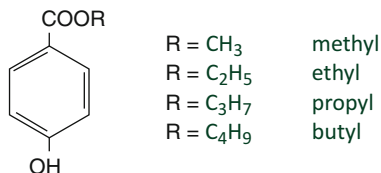
Conventional preservatives include sodium benzoate, potassium sorbate, and calcium propionate. These are the most commonly used low-cost preservatives. Together with their acid forms, all of them have GRAS status (see Fig. 1).

Sodium benzoate has been used as a preservative for more than 100 years. It occurs in nature, especially in cranberries, prunes, plums, and cinnamon, at ~0.05 %. Sodium benzoate is a white granule, potassium sorbate is a colorless to pale yellow scale-like crystal, and calcium propionate is a white powder. All three do not act as preservatives when dissolved in water; however, when in an acidic medium, their undissociated acid forms become effective preservatives:



Since the acid forms are barely soluble in water, their use as preservatives is not convenient. The salt forms are readily soluble in water; therefore, commercially they are more readily available. In water, 1 g sodium benzoate is soluble in 2 ml, 1 g potassium sorbate in 1.7 ml, and 1 g calcium propionate in 3 ml. Sodium propionate is also available and has higher solubility. The maximum effectiveness of preservation is achieved when there is a maximum degree of undissociated acids from the

Fig. 2 Structure of the parabens



salts, which depends on the pH of aqueous solvent: for sodium benzoate, it is $\text{pH} < 4$; for potassium sorbate, $\text{pH} < 6.5$; and for calcium propionate, $\text{pH} < 5.5$. Their acid forms dissolve the cell walls of the microorganism causing the cell to rupture and then lyse, thus suppressing growth. Since these preservatives have practically no effect on spores, they are not bactericidal. Benzoic acid controls molds and yeast, sorbic acid controls bacteria, molds, and yeasts, and propionic acid is extremely effective against molds but not yeast.

The cost of sodium benzoate is usually lower (sometimes a third of the cost) than that of potassium sorbate. Both preservatives are used in foods at 0.1 % or less. The former is widely used in foods of high pH. The preservatives are used largely in beverages, including fruit juices and carbonated drinks, followed by sauces (including soya and oyster sauces), pickles, ciders, vinegar, fruit jams and jellies, pie fillings, and salad dressings. Potassium sorbate is used extensively in nearly all types of foods with a lower acidic pH, especially noncarbonated beverages, and certain bakery products like cakes. Since calcium propionate has no effect on yeast, it is widely used in all yeast-raised flour goods, especially breads. It also works on flour that contains spores of *Bacillus mesentericus* that produce a rope-like texture; a dose of 0.2 % is an effective rope inhibitor.

Parabens

Paraben is the commercial name of a family of chemicals known as para-hydroxybenzoic acid esters, which are closely related to benzoic acid. They are made by the esterification of the carbonyl group of benzoic acid (see Fig. 2 for the structure of paraben). There are four commercially available parabens: methyl, ethyl, propyl, and butyl.

The parabens are barely soluble in water. Each paraben behaves differently. With an increase in the carbon chain length of the alkyl group (R), there is a decrease in solubility in water but an increase in solubility in a nonaqueous solution. The toxicity decreases but the antimicrobial effect increases. Although benzoic acid exhibits its effect in solution with $\text{pH} < 4$, parabens are effective at pH 7 or higher, which no conventional chemicals can achieve. Originally, parabens were used in cosmetics and pharmaceutical products, but it was found that their preservation effect extended into the acidity range of benzoic acid in food products. The four parabens have different antimicrobial effects, with the methyl paraben better for molds and propyl paraben for yeast. Therefore, the parabens are mixed so the

preservative mixture covers a wider spectrum of microorganisms. Since only methyl and propyl parabens have GRAS status, the mixture is usually 3:1 methyl:propyl. However, in many instances, the individual paraben or a mixture of parabens is blended with benzoic acid to reinforce the antimicrobial effect on foods in the extended pH range 4–8.

Parabens work against molds and yeasts, but less so against bacteria, especially gram-negative bacteria. They are usually used at the 0.1 % level; however, since they emit an odor when heated, a level of <0.05 % is recommended. The parabens are first dissolved in NaOH solution, acetic acid, or ethyl alcohol before they are applied to food formulation.

The parabens are used in all kinds of foods with neutral or slightly alkaline pH, i.e., pH 6.5–8, such as baked goods, sports drinks, cheese, creams, jams and jellies with artificial sweeteners, olives, pickles, syrup, dairy products, processed meats, and marine products.

Sulfur Dioxide and Sulfites

Now we discuss sulfur dioxide gas and its associated compounds, including sodium and potassium sulfites, bisulfites, metabisulfites, and hydrosulfites. Sulfur dioxide gas forms by burning sulfur. It can be liquidized at -10°C (14°F). Sulfurous acid forms when the gas is in contact with water. When sulfur dioxide reacts with sodium or potassium cations, the above-mentioned chemicals are formed and extracted. The metabisulfites are more stable than the bisulfites, followed by the sulfites, and all have GRAS status. Sulfur dioxide is a very old preservative; its use was recorded as early as the Egyptian and Roman periods. People smoked their foodstuffs to preserve them so they could be stored for a long time. At that time, people regarded sulfur dioxide as the best spoilage inhibitor for dried fruits, various types of fruit juices, and wines made in the household.

Sulfur dioxide and related chemical compounds function not only as antimicrobials, but also as bleaching agents or strong antioxidants. They transform into sulfate by oxidizing food in which the colors are usually bleached away. To avoid an extreme reaction, caution must be taken when the gas or the chemicals are applied in solution. For certain products like fruit juices, how much preservative to add must be calculated carefully so that preservation is achieved but the foods do not lose their color. However, slightly higher doses can also be applied to bleach away certain unwanted color in foods. The addition of sulfur dioxide to foods is referred to as sulfuring or sulfurization. Many people are allergic to these preservatives.

Sulfur dioxide and its associated compounds are very effective at inhibiting yeasts, molds, and bacteria, especially at lower pH. They also inhibit plant food browning due to the natural occurrence of polyphenol oxidase (PPO). In one study, sodium metabisulfite was shown to completely inhibit the PPO of yam flour (elubo) and meal (amala) during processing (Evans et al. 2013). In many instances, sulfur dioxide and its compounds can be used in conjunction with

other preservatives such as sodium benzoate. These preservatives are used extensively in fruit and vegetable products:

1. In fruit purees, juices, syrups, concentrates, and cordials, 350–600 ppm can be used for preservation. In the bottles in which these foods are contained, the sugar levels are so high that molds are attracted to the food. The added salt form decomposes to liberate the sulfur dioxide gas into the air head space, which will also be sterilized.
2. Dehydrated fruits and vegetables are fumed with sulfur dioxide at 1,500–2,000 ppm to preserve colors.
3. In wine making, sulfur dioxide is used in different processing stages to sanitize equipment, to treat raw grape juice extract so it eliminates the natural microbial flora, to act as an antioxidant and clarifier (50–100 ppm) during fermentation, and to preserve the finished products during shelf storage.
4. Sulfur dioxide is also used to condition bakery dough, and in corn foods, gelatin, molasses, and fresh shrimp to prevent enzymatic browning.

Nitrites and Nitrates

Nitrates and nitrites are found naturally in many foods such as cheese. In food processing, they are applied as a mixture of sodium salts dissolved in brines to cure meats. The nitrates slowly turn into nitrites which fix the pink color of meats. Because carcinogenic nitrosamines are formed by the reaction of nitrites with the amino groups of meat proteins, the dosages are usually regulated and limited by various state authorities to ~100–1,000 ppm of the mixture.

Acidulants as Preservatives

Acidulants are added to foods to adjust the pH; this has a preservative effect. In the presence of other preservatives, acidity shortens the sterilization time. In the pH range of 4.5–5, acids prevent the germination of microorganism spores, which is believed to be due to the lethal effect of their hydrogen anion working with other anions. Citric acid, lactic acid, acetic acid, tartaric acid, and many other acids are used extensively as preservatives in beverages such as carbonated drinks and fruit juices. Additional acids are added to juices and drinks when the natural acid content of the fruits is not enough. Acids are also used in other foods such as canned artichokes and figs, whipping cream, frankfurters, and salad dressings for their bactericidal properties. Use of acetic or citric acid in commercial sesame paste significantly minimizes the risk of contamination from the pathogen *Salmonella typhimurium* (Al-Nabulsi et al. 2014). A mixture of citric and lactic acids in ice is used for on-board chilled storage of two fish species, European hake and megrim (Garcia-Soto et al. 2014). Sodium lactate is used to preserve cooked beef. In many applications, the dosages of these acids are limited by the pH factor.

Natural Preservatives

There is a wide range of natural plant extracts being used to preserve foods. These extracts are used in fresh fruits to control microorganism growth or to reduce enzymatic respiration so as to extend their shelf life. The aromatic components from essential oils and oleoresins of cinnamon, Chinese Cassia, oregano, and red thyme were evaluated and showed antimicrobial activity against some food pathogens (Dussault et al. 2014). This activity is enhanced with the addition of hydroxypropyl- β -cyclodextrins (Liang et al. 2012). After dissolving in acidic pH, chitosan, derived from chitin of insects or crustacean shells, forms a protective coating for fruits and is regarded as a preservative. Finally, some microorganisms or their attackers are effective preservatives, namely, lactic acid bacteria (LAB), bacteriophage, and lysozyme. LAB belongs to the genera *Lactobacillus* and is isolated from dairy products; the *Lactobacillus brevis* strain shows the best inhibitory effect (Rushdy and Gomaa 2013) by producing antimicrobial peptides such as bacteriocins. Bacteriocins, including natamycin, nisin, and tylosin, are polypeptides produced by microorganisms; some can be obtained from cheese whey, a low-cost milk by-product (Schirru et al. 2014).

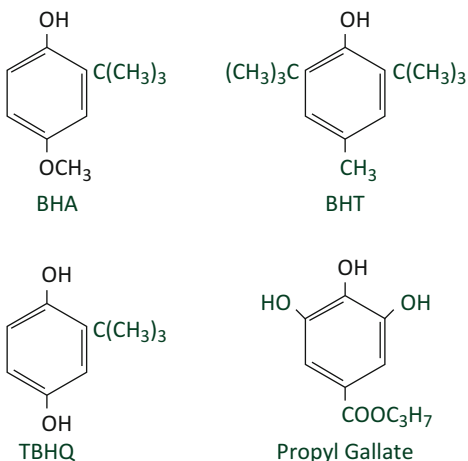
Antioxidants

It is a natural phenomenon that oily food materials become rancid when exposed to air for a long time. All natural fats, oils, vitamins, essential oils, and waxes that contain unsaturated bonded chemicals are subject to oxidation, upon which they become rancid. Oxidation depends on many factors: the material itself, the storage conditions such as temperature and humidity, the degree of exposure to air and light, and the presence of catalysts such as enzymes and metals, especially iron and copper. Oxidation begins with the accumulation of peroxide in the presence of air so that a foul odor is formed; then the peroxide attacks the unsaturated bonds via a catalytic reaction resulting in the strong unpleasant rancid smell. Antioxidants are used to slow down or even prevent oxidation. Good antioxidants must not impart any odor, taste, or color to foods and must be safe to use. There are several types of antioxidant in common use.

Phenoxy Radical Type

Phenoxy radical-containing antioxidants are used extensively in edible oils. They include butyrate hydroxyanisole (BHA), butyrate hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), and propyl gallate. The phenoxy radicals help brake the catalytic reaction to stop autoxidation; a dose as low as 0.025–0.1 % is sufficient. The chemical structures of BHA, BHT, and TBHQ are similar (see Fig. 3). They have excellent solubility in fats and oils. The former two are heat-stable, so they are used in baking. BHT and propyl gallate have been in use the longest. Propyl gallate

Fig. 3 Chemical structure of BHA, BHT, TBHQ, and propyl gallate



is not heat-stable at temperatures over 165 °C; therefore, it is usually mixed with any one of the other phenoxy radicals. In many foods that contain water or are moist, these radicals are used together with citric acid. BHT synergizes α -tocopherol in a nonpolar solvent such as toluene by regenerating tocopherol from the tocopheryl radical (Marteau et al. 2014).

Acids and Their Salts

Citric acid, ascorbic acid, and erythorbic acid are antioxidants as are their sodium salts. They are all water-soluble. Erythorbic acid, also known as isoascorbic acid, is the isomer form of ascorbic acid. Since it is less expensive, it is preferred where the food does not need active vitamins. Citric acid chelates trace metals which stops their catalytic autoxidation degradation. Ascorbic and erythorbic acids scavenge oxygen. These acids and salts are used in beer, wine, dairy products, fruits, vegetables, fresh meats, and seafood to prevent color change or malodor development due to oxidation or formation of trimethylamine. The nitrite meat-curing process is hastened with the use of these acids. Ascorbic acid is also applied to shrimp to stop blackening. Ascorbyl palmitate is oil-soluble and used in vitamin-rich items.

Tocopherols

The E vitamins consist of eight different isomers and are one of the fastest growing antioxidants. They are natural products extracted from edible oils, usually soya and palm oils, but are not heat-stable. In an oil-in-water (o/w) emulsion system of both canola and soybean oils, the α and δ forms of tocopherols are synergistic in their

antioxidant interaction at low concentration (Winkler-Moser et al. 2014). They are used mainly in snacks, candies, cereals, nuts, whipped toppings, citrus drinks, and baby foods. Tocopherols are dissolved in the fat or oil part of the food to help the antioxidant effect.

Rosemary and Tea Extracts

Rosemary contains carnosol, rosmanol, carnosilic acid, and ursolic acid. Green tea contains tea catechins, particularly epigallo catechins gallate (EGCG). In vivo, they are powerful antioxidants that provide various benefits to the body's metabolism. It has been discovered that their antioxidant properties can also be utilized in preserving foodstuffs, comparable to BHA or BHT. The dosage used is 0.05–0.1 % or lower. In one study, rosemary extract and β -carotene were used as antioxidants at 0.02 % to improve stability against oxidation in sunflower oil, extra virgin sunflower oil, lard, and soybean oil. The peroxide values decreased at room temperature (Georgescu et al. 2013). Both rosemary and green tea extracts were tested for their ability to stabilize the color of a dry-fermented beef sucuk sausage. Both had a significant positive impact, but the green tea caused unacceptable sensory quality (Savanovic et al. 2014).

Other Natural Antioxidants

A study by Uchida et al. (2001) showed that phytic acid (inositol hexaphosphate) was more efficient at protecting skipjack tuna lipids against oxidation than α -tocopherol. Chen et al. (2014b) evaluated the antioxidant activities of cumin oil and its main components and found that δ -terpinene was the most efficient antioxidant. In another study, Yu et al. (2005) found that while cold-pressed black caraway and cranberry seed oil extracts were able to considerably suppress lipid peroxidation in human low-density lipids (LDL), their methanolic extracts and those from carrot and hemp seed oil may be used as natural additives for food quality, stability, and human health.

Dietary Ingredients

Carbohydrates, proteins, and fats are the major essential nutrients needed for survival; these are called macronutrients. In addition to these, there are many more nutrients that also are needed but in small quantities; these are called micronutrients. Their shortage in diets results in various health defects. For example, vitamin deficiency was one of the most serious diseases that afflicted sailors in the eighteenth century. Scurvy was common due to a lack of ascorbic acid or vitamin C. Not until the twentieth century was the etiology of such diseases recognized. In addition to vitamins, minerals are also essential to the diet. Recently,

dietary fibers, phytochemicals, polyunsaturated lipids, and pre- and probiotics have been found to be essential for good health.

These micronutrients have many functions: they have a role in bone formation or repair, they are constituents in hormones and enzymes, and act as antioxidants and bactericides. Based on these functions, micronutrients are osteoprotectants, coenzymes, and cofactors in catalysis of the essential biochemical metabolism. They protect against hypotension and hypocholesterolemia and are antiaggregants, anti-inflammatories, and anticarcinogenic. These nutrients are consumed either separately as supplements and nutraceuticals or as dietary ingredients added to foods. Foods with extra nutrients added are known as reinforced or functional foods. Addition of vitamins or minerals to foods, especially drinks, has been common for a long time. Nowadays, foods are not only nutritious, they are also therapeutic. It is not surprising to see many more foods in the markets in which micronutrients, including proteins and amino acids, are incorporated for better body function and healing. These dietary materials are bioactive ingredients.

Proteins and Amino Acids

Proteins are macronutrients that are fundamental building blocks for body tissues and they have a role in the body's functions while behaving like enzymes. They are composed of amino acids. While proteins come from daily staple foods, protein supplements are taken to make up some of the amino acid deficiency in those foods and to enhance a specific function. For example, whey protein concentrate (WPC) is consumed by those who do heavy exercise to obtain extra leucine, an amino acid, in order to build muscle. In addition to WPC, soy and pea proteins are common nutritional additives. Soy milk contains isoflavone but it is in a form that converts to aglycones during storage. An emerging technology, Ultra High Pressure Homogenization (UHPH), produced a product with a lower amount of aglycones (Toro-Funes et al. 2014). A study of cumin seed protein isolate revealed the potential source of a bioactive protein, or the ingredient of functional and health-promoting foods (Siow and Gan 2014). Furthermore, an analysis of the amino acid content of rice dreg protein showed that rice dreg met the suggested nutritional requirements for children according to FAO/WHO. Compared with soy protein isolate (SPI), rice dreg protein is easier to digest so it could replace soy protein (Zhao et al. 2014).

More than 100 amino acids occur naturally, but only 20 of them are needed by the human body and 8 of them are essential. Amino acids synthesize the body's proteins, structurally and enzymatically. Essential amino acids are absorbed from foods. The amino acids that are at their lowest level in food are the limiting amino acids, which determine the utilization of other essential acids. In such a case, the diet must be supplemented to increase those limiting amino acids. For example, it is recommended that methionine be taken to supplement leguminous foods, and rice protein, which contains threonine, and milk protein, rich in leucine, are added to the

diet for same purpose. Other amino acids such as arginine and lysine are added to foods for specific health effects. A low glutathione concentration and low synthesis rate in erythrocytes are associated with cysteine shortage in edematous children with acute malnutrition. Methionine supplementation increases cysteine flux since cysteine is made from methionine (Green et al. 2014).

Vitamins

In the past, vitamin deficiencies were the major cause of diseases like pellagra, beriberi, and scurvy, which usually led to death. Vitamins are essential for life and well-being. They are organic compounds that must be supplied by the diet; however, a few can be synthesized in the body, e.g., vitamin D. Some vitamins are water-soluble, such as vitamin C and the B vitamins, and others are fat-soluble, such as vitamins A, D, E, and K. From 2007 to 2010, the total intake of vitamins A, C, D, and E and the minerals calcium and magnesium in a large portion of population in the U.S. was below the average requirements. Government authorities in many countries issue Dietary Vitamin Allowances for their citizens.

Vitamin A occurs in nature as retinal, retinol, retinoic acid, and retinyl palmitate. Carotenoids are abundant natural botanical substances. The α and β forms such as α - and β -carotenes and β -cryptoxanthin are provitamin A; they can be converted to vitamin A after being absorbed by the body. Vitamin A palmitate and acetate are two substances that are added to food such as margarine for fortification.

Vitamin B complex comprises the more essential B₁ (thiamine), B₂ (riboflavin), niacin, pantothenic acid, B₆ (pyridoxine), folic acid, B₁₂ (cyanocobalamin), and choline. Biotin is included in vitamin B complex, although it is often denoted as vitamin H. Other B vitamins are B₁₃ (orotic acid), B₁₅ (pangamic acid), and B₁₇ (laetrile). The B vitamins usually are consumed as a mixture. Yeasts are the most abundant source of B vitamins in nature. Synthesized forms of individual vitamins are also available. B₁, B₂, and niacin are added, along with iron, to bread or flour for enrichment.

Vitamin C ascorbic acid, is required for collagen formation. It prevents scurvy due to its enediol structure, which reduces superoxide, hydroxyl, and many reactive oxygen species (Howard 2001). An investigation in Korea showed that dietary intake of vitamin C was associated with better hearing in the elderly (Kang et al. 2014).

Vitamin D is unique in that it can be ingested or synthesized by the body via sun exposure. There are two inactive precursor forms, cholecalciferol (D₃) and ergocalciferol (D₂). They are transformed in the liver and kidney into 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, which prevent rickets and other musculoskeletal diseases. Vitamin D plays a role in a wide variety of health issues, including osteoporosis, arthritis, cardiovascular disease, and cancer. The vitamin-D-binding protein is the primary carrier of vitamin D in the body and it regulates the bioavailability of 25-hydroxyvitamin D (Moy et al. 2014). It is added to milk and milk formula to prevent rickets in children.

Vitamin E is a mixture of tocopherols and tocotrienols, each one with α , β , γ , and δ forms. This vitamin is extracted from crude soy and palm oils. Tocopherols are polyisoprenoid derivatives that function as scavengers of reactive oxygen species (ROS) such as peroxy and superoxide radicals for lipid peroxidation. Their strong antioxidant property is due to their ability to donate their hydrogen ions to lipid free radicals.

Vitamin K also has two forms: phylloquinone K_1 is in almost all green leafy vegetables, and menaquinones K_2 is synthesized in the gut by microflora. They reduce bone loss and improve bone strength.

Minerals

Minerals serve a wide range of physiological functions. For example, phosphorus (P) is needed in bone and teeth. It is also involved in respiration and energy metabolism. Phosphorus deficiency causes hypophosphatemia. Other essential minerals are needed in abundance and some minerals are merely trace elements. Sodium (Na) and chlorine (Cl), essential constituents of the daily diet and consumed in large quantities, are usually not considered as additives. The macroelements are required in the daily diet at levels >100 mg and include calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), and sulfur (S). The microelements are required in the diet at levels <100 mg and include iron (Fe), zinc (Zn), copper (Cu), iodine (I), manganese (Mn), chromium (Cr), and cobalt (Co) (Nabrzyski 1997). Trace minerals are nickel (Ni), selenium (Se), vanadium (Vd) strontium (St), and silicon (Si). These minerals cannot be absorbed in elemental form; they are usually absorbed as organic substances or inorganic salts, like calcium acetate, potassium iodate, and potassium iodide. Many are added to foods to fortify them. Minerals are vital to life so many countries and the World Health Organization (WHO) established recommended daily allowances (RDA) for consumers.

Dietary Fiber

Dietary fiber is the edible part of plants and is resistant to digestion and absorption in the intestines. Lack of fiber in the diet is associated with gastrointestinal diseases, including constipation, colon cancer, and hemorrhoids; cardiovascular diseases, including hypercholesterolemia, stroke, and ischemic heart; and metabolic diseases, including obesity and diabetes. Hypercholesterolemia is an important risk factor for cardiovascular diseases (CVD). A study on the combined effect of dietary fiber and plant sterols showed that they could be used to treat CVD (Castellanos-Jankiewicz et al. 2014). Another study showed that high dietary fiber intake is inversely and independently associated with the incidence and risk of stroke (Casiglia et al. 2013). High-fiber and low-carbohydrate diets may enhance satiety and promote weight loss, which was verified by a study on a high-fiber bean-rich diet to fight obesity (Tonstad et al. 2014).

Many plant materials are valuable sources of dietary fibers. The cell wall of the fruiting body of mushroom comprises nondigestible macromolecules and is made up of four parts: the outer part is made of polysaccharide, a cold alkali-soluble part of hetero-polysaccharides, a hot alkali-soluble part of hyperbranched glucans, and an alkali-insoluble part of glucan-chitin complex. The composite of all the parts is a rich source of fiber that is beneficial to human health (Chen and Cheung 2014). Further details can be obtained by referring to the chapter “Preparation and Characterization of Polysaccharides from Mushrooms” in Zhu et al. 2015. Other valuable sources of food fiber are food residue, which is often thrown away, e.g., soybean hulls and lime rinds, and resistant starch, which is a small fraction of starch that is resistant to digestion and can be fermented in the large intestine by microbiota (Homayouni et al. 2014).

Fibers are insoluble or soluble. Insoluble fibers include cellulose, hemicellulose, and lignin, of which cellulose is the most consumed. Cellulose is a polysaccharide composed of basic glucose units, similar to starch. However, starch is soluble since the units are mostly $\alpha(1 \rightarrow 4)$ bonds and hydrolyzable by amylase or α -glucosidase, but unit bonds in cellulose are $\beta(1 \rightarrow 4)$ links and not digestible by enzymes in the intestine. Commercially, most cellulose comes in powdered form and is derived from wheat, rice, soybean, citrus fruits, and bamboo. In food preparation, insoluble fibers absorb and retain a small amount of water. In making bread, this results in an increase in volume, softer texture, improved mouthfeel, and longer shelf life. Insoluble fiber intake reduces the risk of coronary heart and cardiovascular diseases (Threapleton et al. 2013), as well as prostate cancer (Deschasaux et al. 2014). The functional properties of insoluble dietary fiber can be improved by microfluidization which effectively reduces the particle sizes to submicron scale (Chen et al. 2013).

Soluble fibers are represented by hydrocolloids, mucilages, resistant starches, and oligosaccharides. Hydrocolloids are high-molecule polysaccharides. Because they are soluble in water, they bind a portion of fluid present in the digestive tract, especially the small intestine. This delays the absorption of digested glucose and hinders the recirculation of bile acids composed of cholesterol. As a result, the glycemic index (GI) of foods containing these fibers is greatly decreased, thus reducing blood cholesterol. In turn, a reduced GI and cholesterol level will decrease the incidence of diabetes, obesity, and other diseases. Natural pectin and guar gum and synthetic sodium carboxymethyl cellulose (CMC) were marketed as antiobesity supplements. In one study, patients with type 2 diabetes who were given partially hydrolyzed guar gum showed improved cardiovascular and metabolic profiles because of their reduced waist circumference, glycated H6 (H6A1c), 24-h urinary albumin excretion, and serum *trans*-fatty acids (Dall’Alba et al. 2013). Another study showed that dietary supplements containing soluble fiber improved the GI of those with type 2 diabetes (Silva et al. 2013). Consumption of soluble fiber is associated with a variety of physiological responses, one of which is the modulation of postprandial glycemia due to the increased viscosity of fiber caused by the digestive process. Xanthan retains its viscosity significantly (Fabek et al. 2014). Recently, konjac gum has become more popular because of

its relatively high aqueous viscosity. These hydrocolloids must be hydrated *in vivo* so they can achieve their maximum effect. Further detail can be obtained by referring to the chapter “Pharmaceutical Applications of Various Natural Gums” in Deshmukh et al. 2015.

Phytochemicals

Phytochemicals are micronutrients present in almost all parts of plants that are consumed as food. They were discovered after vitamins and are so important for human health that some nutritionists regard them as vitamins. There have been continuous attempts to improve health by replacing the intake of pharmaceuticals with natural compounds derived from plants (Lavecchia et al. 2013). Many people are under stress and have chronic disorders such as diabetes, arthritis, allergies, cardiovascular disease, fatigue, and cancer. The intake of green leafy vegetables lowers the risk of chronic diseases (Rahal et al. 2014). Fruits and other plants provide abundant bioactive substances as well. Enhanced release of these bioactives from plant cells by cell disruption and extraction through the cell wall can be optimized using enzyme preparation (Puri et al. 2012). Another way to obtain phytochemicals is by metabolic engineering of microorganisms to synthesize natural products. DNA sequencing and recombinant DNA technology have elucidated the biosynthetic pathways that create these products (Marienhagen and Bott 2013). Nosocomial infections caused by fungi have increased greatly due to the increasing number of immunocompromised patients. Plants produce a variety of medicinal components that can inhibit pathogen growth; many are secondary metabolites, phenolic compounds, essential oils, and extracts (Negri et al. 2014). Aging is an inevitable process influenced by genetics, lifestyle, and the environment. Increasing evidence shows that aging is slightly associated with a chronic increase in reactive oxygen species (ROS). Recent studies show that phytochemicals are effective in extending the lifespan of various animal species by reducing ROS (Si and Liu 2014). Scientists are developing personalized supplements comprising specific phytochemicals for cancer prevention and other therapies (Vallinas et al. 2013). Some phytochemicals with anticancer effects have already been identified: resveratrol (red grapes), allicin (garlic), lycopene (tomato), indole-3-carbinol (cruciferous vegetables), [6]-gingerol (ginger), emodin (aloe), sulforaphane (mustard), ellagic acid (pomegranate), myricetin (cranberry), carnosol (rosemary), and eugenol (cloves) (Khuda-Bukhsh et al. 2014). Phytochemicals can be classified by their various chemical structures: (1) isoprenoids, including carotenoids (lycopene, β -carotene, lutein, zeaxanthin, astaxanthin), saponins, and terpenes; (2) phenolic compounds, including anthocyanins, isoflavones (daidzein and genestein from soybean), flavonones (hesperetin and naringenin from citrus), flavonols (myricetin, rutin, quercetin), flavanols (catechin, epicatechin, epigallocatechin gallate), and resveratrol; and (3) protein or amino acid-based, including capsaicinoids, isothiocyanates, indole-3-carbinol, allyl sulfur compounds, and folate. The unique structure of individual phytochemicals renders a specific

pathway for certain therapeutic treatments. Naringin protects the nigrostriatal dopaminergic projection in a neurotoxin model of Parkinson's disease by induction of glia-derived neurotrophic factor (GDNF) (Leem et al. 2014). Resveratrol is frequently used as an activator of sirtuin 1 to inhibit cell apoptosis induced by radiation that results in the accumulation of ROS which contributes to neurodegenerative changes (Li et al. 2014). Further information can be obtained from the chapter "Flavonoids" in Azimova and Vinogradova 2013.

Materials of Animal Sources and Unsaturated Fatty Acids

There are several dietary nutrients that are derived from animal sources:

- (i) Conjugated linoleic acid (CLA), a geometrical and positional isomer of linoleic acid (LA), is extracted from grazing animals for anticarcinogenic purposes, but is used mostly for weight management by helping to reduce body fat and maintain lean muscle mass. This ingredient is produced by bacteria in the rumen of animals from ingested linoleic acid and distributed to the flesh and milk. It can modulate dendritic cell cytokine production to suppress initiating inflammatory diseases by directing T-helper cell differentiation (Draper et al. 2014).
- (ii) Polyunsaturated fatty acids (PUFA) include the omega-3's, which are important to health, especially the brain (cognition) and the heart. One study showed that telomere shortening in the elderly with mild cognitive impairment may be attenuated with omega-3 fatty acid supplements (O'Callaghan et al. 2014). This positive and encouraging result has been echoed by many other studies on the metabolics and physiology of the human aging process. The important PUFAs are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are present in deep water fish like salmon and crustaceans. The fatty acids are produced by the algae and phytoplankton in the sea by converting the precursor linolenic acid; the fish or crustaceans consume the algae and phytoplanktons and the fatty acids are deposited as lipids in their bodies. The PUFAs are extracted from the animals or the algae. Microalgae from *Isochrysis*, *Nannochloropsis*, *Pavlova*, and *Thalassiosira* contain enough omega-3 PUFAs for extraction (Ryckebosch et al. 2014). Flax seed oil can be enriched with omega-3 fatty acids by adding alkaline lipase from *Aspergillus fumigatus* (Rajan et al. 2014).
- (iii) Carnitine is found in animal meats, especially the red meats. It is an antiobesity ingredient that transports body fat to the mitochondria which then burns it away to produce energy. Presently, the material is available by synthesis.
- (iv) Chitosan is another weight management agent, chemically converted from chitin. Chitin is one of the main components of the shell or exoskeleton of insects and crustaceans, with the chemical structure of the β -1,4 homopolymer of *N*-acetylglycosamine, very similar to pectin. Insoluble chitin is deacetylated to become soluble in dilute acetic acid.

Pro- and Prebiotics

A subset of foods that are good for health and affect the live microorganisms that inhabit the intestines, particularly the colon, are known as probiotics, which are commonly ingested as yogurt. A probiotic is a microorganism that improves the beneficial properties of the indigenous microbiota in the digestive tract, which produce plenty of B vitamins and short-chain fatty acids (SCFA): acetic, propionic, and butyric. These nutrients are absorbed into the body and enhance calcium. The colony of microorganisms usually occupies the area inhabited by toxin-producing bacteria such as *E. coli*, coliform, and *Clostridium* by inhibiting their growth. Because the destination of probiotics in foods is the intestines via the digestive tract, they must endure the very acidic and enzymatic conditions of the stomach and the intestines. Probiotics are able to colonize there and they are safe to consume. The main probiotics are *Lactobacillus* and *Bifidobacterium*, each consisting of several species. Other probiotics are *Streptococcus salvarius*, *Lactococcus lactis*, and *Saccharomyces boulardii*, a yeast. Age-related alterations in the composition of the gut microbiota have a direct impact on the immune system; modulation of the microbiota with pre- and probiotics could improve immune system responses to infections in the elderly (Yaqoob 2014). Many studies have proven that manipulation of microbiota through the diet prevents metabolic disorders such as obesity (Chen et al. 2014a). Dietary probiotics affect low-density lipoprotein cholesterol (LDL-C); thus, they could be used in a lifestyle change to prevent cardiovascular disease (DiRienzo 2014).

Another subset of foods, known as prebiotics, is actually nutrients for the probiotics. These are mainly oligosaccharides that occur in many plant foods. Common prebiotics are soy oligosaccharides (SOS), galacto-oligosaccharides (GOS), xyloligosaccharides (XOS), raffinose, stachyose, and inulin. Some disaccharides such as lactulose and palatinose, are prebiotics. GOS forms during enzymatic hydrolysis of lactose which is part of the production of a prebiotics-enriched milk (Colinas et al. 2014).

Processing Aids

Processing aids are another variety of additives that are used in small amounts in food formulations.

Anticaking Agents

Anticaking agents are normally used in powdered foods that are highly hygroscopic, e.g., foods rich in protein. They are usually minute mesh-sized particles. The particles are fluffy, with air space channels between them. They are able to absorb moisture from the atmosphere, thus keeping foods dry and free flowing for

an extended period. Deliquescent, highly soluble crystalline ingredients are prone to caking and dissolution when they are stored in places with relative humidity above a certain level. Anticaking agents are added to improve the flowability of powders and to prevent or reduce caking (Lipasek et al. 2012). While many powdered starches and fiber can be used as anticaking agents, the commonly or conventionally used agents are (1) the silicates: sodium aluminum silicate, calcium silicate, calcium aluminum silicate, talc (also known as talcum powder, which is magnesium hydrogen metasilicate), and magnesium silicate; (2) the stearates: calcium stearate and magnesium stearate; (3) silica gel, which is silicon dioxide; (4) calcium phosphate and tricalcium phosphate (TCP); and (5) microcrystalline cellulose (MCC), magnesium carbonate, and magnesium oxide. Silicon dioxide and calcium stearate were the only anticaking agents shown to improve the physical stability of powdered sodium ascorbate (vitamin C) (Lipasek et al. 2011). Varying amounts of TCP, maltodextrin, and glycerol monostearate were applied to mango powders produced by vacuum drying so that optimum feed mix composition was obtained (Jaya and Das 2004).

Enzymes

Enzymes are present in all living organisms to catalyze specific reactions. Industrially, they are cultured from fungi and carried in diluents like water, salt, sugar, and maltodextrin, and properly preserved and stabilized. Since their activities are to be preserved before application, enzymes must be handled with care. Formulated in foods in very low dosages, each enzyme has a specific function and action:

- Amylase and glucosidase degrade starch to glucose molecules; it is used in baking bread to improve volume and softness;
- Cellulase and protease are used in wine and beer production for clarification;
- Proteases are used for meat tenderizing; papain, bromelain, and ficin are used extensively; and new proteases are actinidin and zingibain (Bekhit et al. 2014);
- Pectinase is used in juice concentration to destroy pectin so as to lower the viscosity of the juice;
- Transglutaminase is used in the meat industry to make the meat pieces stick together. The enzyme is able to modify soybean proteins and improve the functional properties and quality of various low-fat and salted meat products.

Humectants

Humectants are the hygroscopic materials that are able to bind and retain water molecules to prevent drying out; they are used in very small quantities (a few percentage points). Common humectants are glycerol, sorbitol and

propylene glycol. Glycerol prevents crystallization of syrup and is a plasticizer in sausage casing. Sorbitol is used in confectioneries, chewing gum, baked goods, and meats to help retain softness; in addition, its soft-tasting effect reduces the bitter aftertaste of some sweeteners like saccharin. These humectants in any combination are valuable to the production of many processed meat items; for instance, cured jerkies made from pork or marine food are kept tender with humectants.

Baker's Dough Conditioners and Leavening Agents

Natural milled wheat flour is yellow, too soft, and cannot form baker's dough which needs to be strong, stable, and elastic. Oxidization is required to condition the flour (1) to prevent proteinase action in the flour, (2) to oxidize the -SH group so that the protein matrix can be formed, and (3) to bleach it for a white appearance. Benzoyl peroxide is a conventional conditioner; ascorbic acid and iodates are also used in conjunction with enzymes. With some wheat bran bread, the action of ascorbic acid together with vital wheat gluten compensates for the diluted gluten proteins, thus improving the quality of the bread with respect to loaf volume yield, grain structure, softness, and moisture content (Dizlek et al. 2013). Wheat germ enzyme lipoxygenase in bread increases the volume and decreases the firmness (Bahal et al. 2013).

For making bread and many bakery items, leavening or increasing the product volume is the result of carbon dioxide generated by decomposing carbohydrate. Yeast is the natural leavening agent used in bread. The amount of yeast used has an effect on the rheological properties and microstructure of bread dough, which are related to the size and distribution of the generated bubbles (Upadhyay et al. 2012). For other baked goods, other materials that liberate carbon dioxide upon heating are baking soda (sodium bicarbonate), cream of tartar (tartaric acid and potassium salt), sodium aluminum sulfate, and fumaric, propionic, and glycono-delta-lactone acids, used alone or in combination.

Additives to baker's dough affect the structure, properties, and aging of the loaves. Supplementation of gluten-free breads with nongluten proteins (albumin, collagen, pea, lupin, and soya) affects volume, hardness, chewiness, and sensory acceptance to varying degrees (Ziobro et al. 2013). Whey protein isolate (WPC) and buttermilk powder affect dough and bread quality in a similar manner (Madenci and Bilgili 2014). Different enzymes are used for dough conditioning to control the bread's texture. Xylanase, transglutaminase, protease, lipase, and amylase are often used in gluten-free breads.

Sesquestrants and Chelating Agents

In solution, sesquestrants and chelating agents suppress the activities of other materials, usually metals, by forming coordinate bonds. The common agents are ethylene

diamine tetra-acetic acid and its salts (EDTA) and sodium hexametaphosphate (SHMP). EDTA chelates copper and iron to stop the precipitation of metallic salts in liquid foods such as salad dressings and canned foods. SHMP is a long-chain polyphosphate salt with the formula $(\text{NaPO}_4)_6$. In solution, the tetrahedra nature of potassium compounds results in a geometrical arrangement such that three phosphate groups are in close proximity to one metal ion to form a complex. The main use for SHMP is in dairy products, e.g., SHMP solubilizes calcium oxalate precipitants in milk. Its complexation reaction with calcium cations results in the disappearance of the precipitant. One study compared the chelating capacities of ethylenediaminetetraacetic acid (EDTA) and sodium triphosphate (STPP) to prevent lipid oxidation due to the catalysis of fish hemoglobin with iron with that of other acidic chelators. The study showed that STPP was the most efficient, followed by EDTA, whereas citric acid and adenosine-5-triphosphate (ATP) were ineffective (Maestre et al. 2009). EDTA and cysteine were identified as effective chelators in eliminating mercury from mackerel fillet (Hajeb and Jinap 2012). Many acids and their salts also act as sequestrants. Malic, citric, tartaric, and lactic acids were investigated for their ability to prevent precipitation in sand lance fish sauce; citric acid was found most effective (Moon et al. 2008). The effects of six potential chelating agents (citric, lactic, malic, and ascorbic acids, glucose, and xylitol) were tested to maximize the solubility of mineral elements (Ca, Mg, Fe, Mn, Zn, K) in oat flakes; it was concluded that citric acid was the most efficient (Ekholm et al. 2000).

Conclusion and Future Directions

One of the important roles of food is to provide nutrition. It is the job of food formulators to produce better foods; better foods feed more people. Researchers at various institutions are dedicated to upgrading the quality of various kinds of food, for which they have earned respect. However, while people in developed countries enjoy the fruits of their studies and research, it should not be forgotten that there are many areas in the world, such as sub-Saharan Africa, where the people are still suffering from starvation; they simply do not have enough food to eat. People there are suffering from serious poverty. Statistically, our earth is able to grow enough food for everyone living on it. There are many reasons for these tragic situations. Besides political causes, there is uneven distribution of food and improper processing so that foods do not keep from spoiling while being transported long distances and spending a long time in storage, which are necessary when food must be delivered far from where it is produced. Food includes processed items and agricultural produce. To reduce regional poverty, food scientists must improve production methods to preserve the nutrition of food, especially the micronutrients of food products so surplus foods in the developed countries can be transported to the areas where it is needed. Proper use of food additives is required so as to increase not only the nutritional value but also the density of the nutrients. Another mission is to add value to the existing local produce, such as grown crops, fruits, and meats from farming or stock culturing. On the one hand, food scientists must

work with agriculturalists to increase the harvest. On the other hand, research should be done to upgrade the quality of various foods containing micronutrients so that the food can help people avoid diseases by having a better immune system.

Hippocrates (460–370 BC), the Father of Medicine, said, “Let Food Be Thy Medicine.” For the last several decades, the percentage of the population in developed countries suffering from various chronic diseases such as obesity, diabetes, cancer, and cardiovascular diseases has increased dramatically. The national expenditure on health care and people’s individual medical bills are increasing every year due to these chronic diseases. Recently, food scientists have identified a wide range of dietary vitamins and minerals to be used as health supplements in foods. The focus for improving health has moved to dietary fibers, prebiotics, probiotics, and phytochemicals. Many studies have shown that extracts of these food ingredients do have therapeutic value, especially when given to those with chronic diseases.

The human body is a miniature universe; nature can correct abnormal phenomena, including human health disorder. Treating chronic diseases with pharmaceuticals is not an ideal solution because there are side effects and drugs are somewhat toxic. Fortunately, studies have shown many dietary ingredients with respective therapeutic properties and able to reduce or even eradicate disease symptoms. It is expected that in the near future, food or dietary ingredients will not only help the body fight off disease, it will also be effective medicine to cure disease completely. Medicine or pharmaceuticals are mostly synthetic materials; their prescriptions will decrease and finally be abandoned someday. The public is looking to the entire discipline of food science for the breakthrough.

Cross-References

- ▶ [General Properties of Major Food Components](#)
- ▶ [General Properties of Minor Food Components](#)

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Chemical Properties and Applications of Food Additives: Flavor, Sweeteners, Food Colors, and Texturizers

5

Pak Nam Albert Chan

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Abstract

Foods are nutrients. Besides nutrition, there are other major parameters that affect a person's like of or preference for various foods, such as appearance, odor, and taste. The environment, including noise made when biting food and background sound such as music, also has an effect. The addition of flavorings, sweeteners, and acids, either natural or synthetic, to processed foods is meant to make up the loss of odor and taste that occurs during processing, or to enhance the quality of the food. Similarly, natural or synthetic colorants are used to improve the appearance of food. Starches, emulsifiers, and hydrocolloids are food texturizers with different chemical properties that affect taste via their respective characteristics. Recently, there have been many studies on the production of foodstuffs with specific and unique characteristics in which additives were used.

Introduction: Foods and Their Taste Attributes

Foods are nutrients for people. Before being eaten, foods are first seen and smelled, so they must be attractive to people in appearance and odor. It is essential that foods have good coloring and a pleasant smell to attract potential customers. Zellner et al. (2014) studied how food presentation affected whether a person liked the taste of the food. Subjects liked a food more when it was presented in a more attractive manner. Color and taste, natural characteristics of foodstuffs, reflect the quality of the food. The sensation of the taste of food in the mouth is a complex composite of odor, taste, and texture that is affected by the appearance of the food and the sound made when biting the food and by the movement of muscles in the mouth, especially the tongue. A preference for certain flavors is a complicated combination of genetics and eating behavior traits. Adventurous people are less afraid of new foods, a trait linked to the *TAS1R1* and *PKDK3* genes (Törnwall et al. 2014). The taste buds are minute depressions located mostly on the tongue. They comprise many very small sensory receptors that detect the five basic electrical messages and send them to the brain: sweet, sour, salty, bitter, and umami. In addition, there are many volatile molecules in the flavoring materials that stimulate the nose's sensory cells. Environmental factors also affect the reception and sensation of food. A study

of the type of background music played while dining showed that food is accepted differently depending on not only the type of food served but also the kind of music played and the performer (Fiegel et al. 2014). However, this topic is beyond the scope of this chapter.

Aroma-active compounds, together with sugars, fat, and nucleotides, are the main chemical species that determine the characteristic aroma and taste of food (Lin et al. 2014). The food processing industry usually adds colors and flavors to improve the quality of the food. Adding flavors to certain types of foods is necessary to restore some of the original flavors that are partly or totally lost during the manufacturing process. Also, flavors are added to enhance the food when cooked in a microwave. Extra flavoring is necessary for many so-called health foods, along with replacing some portion of sugar or fat, so they are acceptable to consumers. Acidulants and sugar are taste integers. Currently, multiple sweeteners are used extensively in foods to replace sugar for health reasons. In this chapter, flavors, acidulants, and sweeteners added for taste are discussed. In addition, since texture contributes to the feeling of food in the mouth, starches, emulsifiers, and hydrocolloids are discussed.

Flavors

Flavors, known as flavoring materials, are composed of volatile or aromatic substances of very low molecular weight, most below 300. There are about 2,500 flavoring materials used in the food industry. Because of their strength, flavors are used in extremely small quantities, mostly on a parts per million (ppm) scale or smaller. Cavemen knew that smoking meats with apple wood could impart a pleasant barbecue flavor, which was due to the presence of volatile flavoring materials in the smoke. During the Middle Ages in Europe, natural flavor spices were used to keep meats tastier while in long-term storage. Alchemists at that time started to distill spices and herbs to obtain the concentrates, the early forms of natural flavors. Flavors are classified as natural or synthetic.

Natural Flavors

Natural flavors are extracted from volatile compounds found in different parts of plants, mostly herbs. Natural flavors come in various forms: oleoresins, which are crude extractions and 5–20 times more condensed; essential oils, which are processed by distillation, cold pressing, or solvent extraction and 80–100-fold more concentrated; and fruit juice concentrates or purees, which are added to foods.

Popular oleoresins are usually from spices and herbs such as anise, basil, cardamom, cinnamon, clove, coriander, mace, cumin, nutmeg, rosemary, sage, thyme, and vanilla. Their naturally occurring wax and oils are used as essential flavoring materials in processing culinary seasonings, condiments, and sauces. Oleoresins are oil dispersible so their application is limited. An emulsifier such as

lecithin is blended with an oleoresin to convert it into a water-dispersible aquaresin, which can have many applications.

Essential oils are used across the entire food industry. In addition to the oleoresins that are processed into this form, there are also the essential oils of orange, lemon, lime, almond, garlic, ginger, onion, peppermint, spearmint, and wintergreen. Besides their use in cooking, essential oils are used in candies, chewing gums, bakery items, pickling, beverages, frozen desserts, and snacks. Essential oils are oil-soluble flavors and are easily used by adding directly to food or to the oily portion of foods at a dose of ~0.5 to 1 %. In addition to contributing flavor and taste to foods, essential oils are therapeutics because of the specific therapeutic properties of some active bioingredients in the aromatic compounds. One study showed that L-carvone from spearmint induced apoptosis of breast cancer cells (Patel and Thakkar 2014). Diallyl sulfide, a natural organosulfuric aromatic compound in garlic, inhibits tumor necrosis factor- α (TNF- α) and histamine-induced proinflammatory responses in the aortic smooth muscle cells of rats (Ho et al. 2014).

Terpenes are major constituents of almost all essential oils. Orange, lemon, and lime oils are citrus oils that have the same constituent terpenes like D-limonene. Terpenes are hydrocarbons that are not very soluble in water, easily oxidizable in air due to the presence of double bonds, and weak in flavor. Since citrus oils are important flavors that need to be stable and soluble in water, folded oils were developed by removing the unwanted L-limonene. Terpene makes up more than 98 % of orange oil. Removal of 80 % by weight of terpene results in a fivefold oil. Similarly, 10-, 25-, and 50-fold oils are formed by removing 90 %, 96 %, and 98 % of terpenes. Terpeneless oils are also available. Folded oils have stronger flavors and are more stable and water-soluble; thus, they are used in beverages where water is the solvent. All essential oils, including the folded oils, are natural flavors used in foods at the 0.1–0.5 % level, or they are used as components in synthetic flavors.

The third type of natural flavor is obtained from the concentration of the aromatic components of fruity extracts. These volatile organic and aromatic components are flavoring materials. Gas chromatography (GC) and mass spectrometry (MS) are used by flavorists to identify the aromatic components in the extracts. Their flavor and value are highly influenced by their aroma, as dictated by volatile flavoring materials. A simple and robust method of sampling these compounds on polydimethylsiloxane has been developed (Allwood et al. 2014). In addition, during the manufacture of some fruity foods, the volatile fraction is captured and added to reinforce the extracts. Some natural flavors can be blended in small percentages with extracts from other fruits to form the “natural flavor WONF,” where WONF stands for “with other natural flavors” that one sees on an ingredients label.

Flavorists must understand the type and nature of flavoring materials in natural flavors before creating synthetic flavors. Both aliphatic and aromatic flavoring materials are esters, aldehydes, alcohols, ketones, and hydrocarbons. The characteristics of the dominant flavoring materials of some individual flavors are listed in Table 1.

Because of the complicated interaction of flavoring materials with some natural foods, the analysis of their flavor spectrum is very challenging for flavorists.

Table 1 Flavors and their characteristic aromatic chemicals

Flavor	Characteristic aromatic chemical	Flavor	Characteristic aromatic chemical
Almond	Benzaldehyde	Anise	Anethole
Apple butter	Benzyl isovalerate diacetyl	Banana	Isoamyl acetate
Coconut	γ -Nonalactone	Cherry	Benzyl acetoacetate
Garlic	Allicin	Coffee	Methyl furoate
Grape	Phenylethyl anthranilate	Ginger	Zingiberone
Lemon	Citral	Honey	Benzyl cinnamate
Onion	Diallyl disulfide	Melon	Octyl butyrate
Plum	Citronellyl butyrate	Peach	γ -Undecalactone
Strawberry	3-Methyl-3-phenylglycidic acid ester	Raspberry	Geranyl formate
		Vanilla	Vanillin

For example, the analyses of the extremely delicate flavors of mango, grape, and many tropical fruits are tedious. Recently, there has been some success with the analysis of mango where its characteristic aromatic component has been identified as 4-hydroxy-2,5-dimethyl-3(2H)-furanone; 54 flavoring materials were revealed of which 16 were reported for the first time (Munafa et al. 2014). Alkyl-methoxypyrazines were shown to be important flavoring materials in the cultivars of grapes and their wines, in which a new flavoring material, 2,5-dimethyl-3-methoxypyrazine, was identified as possibly an important and common odorant in red wines (Botzatu et al. 2014).

Synthetic Flavors

In the food industry, the use of natural flavors has several disadvantages. First, their flavor profile is not consistent. It is difficult for all aromatic ingredients from raw materials to be consistent in content because of variation of the batches in harvesting and processing. Second, the strength of the flavor is usually not great enough because of the presence of many other natural components. Third, natural flavors cost more to use because of their limited quantity in nature. Synthetic flavors are an alternative to natural flavors. They are composed of volatile flavoring materials that are mixed together using the same formula as that for natural flavors. All ingredients are dissolved in a liquid carrier to form a synthetic flavor. The solvents selected for use as carriers depend on the application, i.e., ethyl alcohol is used for beverages, vegetable oil is used for any food with a lipid phase, and propylene glycol or Triacetin is used for confectioneries and baked goods. Water is used on rare occasions. Several flavoring materials may react with each other in the flavor solution to yield a stronger flavor; this is known as synergism. Flavorists create synthetic flavors that reference their natural counterparts. A good flavorist knows how to utilize synergism to obtain strong flavors with a minimal amount of

materials to keep the cost down. The dosage in foods is generally 0.1–0.2 % in baked goods or hard-boiled candies when high-temperature heating is required for processing and 0.05–0.1 % in other food products.

All flavoring materials must be generally recognized as safe (GRAS). In addition to those found in nature, there are plenty of flavoring materials that have been synthesized and have high flavoring strength. To be accepted for use as food flavoring, adequate toxicity tests must be performed on flavoring materials to verify their safety and their GRAS status must be determined. For example, vanillin is a dominant ingredient in the vanilla flavor; ethyl vanillin, a modified form of vanillin not found in nature, is more heat stable, about three times stronger, and is an important component in many flavors used by bakers.

For some convenience foods like powdered or instant formulas, synthetic flavors are preferred in powdered form. Instead of substrating the array of flavoring materials in liquid form, they are spray-dried in the presence of a solid-phase carrier such as gum acacia or glucose to form powdered flavors. One study showed that rosemary essential oil was encapsulated by spray-drying the oil with whey protein and insulin blends as the carrier (Fernandes et al. 2014). However, due to the heat involved in the spray-drying process, it is more difficult to maintain the genuine original flavor profile and the strength of the original flavor.

Flavor Enhancers

Flavor enhancers do not have their own specific flavor but enhance the effect of other flavors, producing a taste sensation known as umami. Umami can be measured by electronic tongue and near-infrared spectroscopy (Bagnasco et al. 2014). A typical flavor enhancer is monosodium glutamate (MSG). It was discovered in Japan in 1908 as an extract from seaweed that enhanced the taste of food when added during cooking. It was produced commercially the following year, and today it is made by fermentation. Other flavor enhancers are nucleotides originally extracted from bonito tuna – disodium 5'-inosinic monophosphate (IMP) and disodium 5'-guanylic monophosphate (GMP) – which have a much stronger enhancing effect than MSG. The naturally occurring mixture of 5'-IMP and 5'-GMP, known as “I + G,” has an excellent enhancing effect. Any one of these materials can be used with MSG to optimize flavor enhancement. Other popular flavor enhancers are maltol and the synthetic ethyl maltol, used in foods at 250 and 150 ppm, respectively. They are common flavoring materials used in many confectionery flavors.

Meaty Flavors

While meats are cooking, natural characteristic flavors and attractive odors are generated. In principle, meaty flavors come from two sources: the small amino acids and peptides from protein hydrolysis and the volatile molecules resulting

from the Maillard reaction. The Maillard reaction is the chemical reaction between amino acids and reducing sugars that produces sulfur- or nitrogen-containing molecules from the fragmentation of amino acids and peptides. The characteristic flavor of a particular meat is due to the type of meat and the cooking or hydrolysis process used. For example, the main active compounds that were found to contribute to the aroma of beef extract are 2,3,5-trimethylpyrazine, 1-octen-3-ol, 3-methylbutanoic acid, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (Takakura et al. 2014). The strong smell of cooked ham is the result of 2-methyl-3-furanthiol, 2-methyl-3(methyledithio) furan, and bis(2-methyl-3-furyl) disulfide (Thomas et al. 2014). Enzymatic hydrolysis has replaced chemical reaction so that less unwanted salt and impure by-products are produced. A study found that the enzyme bromelain hydrolyzes a seaweed protein and the end-product hydrolysate was characterized as the precursor of a thermally processed seafood flavor (Laohakunjit et al. 2014). The final flavor profile is purer and much more refined. Natural meaty flavors can be produced by hydrolysis of plant proteins chemically or enzymatically. Plant or vegetable protein is broken down into tasty peptides and amino acids commonly known as hydrolyzed vegetable protein (HVP). Soy protein is usually the raw material from which HVP is made. The protein slurry is hydrolyzed at high temperature by the addition of hydrochloric acid or sodium alkaline; it is then neutralized accordingly. To obtain yeast extract, yeast undergoes autolysis, i.e., the protein inside the rigid cell wall compartment is digested or hydrolyzed by its own enzyme, to yield the much smaller volatile molecules that give yeast extract that characteristic meaty taste. Important aromatic compounds are furan derivatives, pyrazines, and sulfur-containing aldehydes, acids, and ketones, similar to typical natural meat flavors but with different component ratios. HVP and yeast extract are synergistic and used in typical meaty flavorings.

A specific flavor profile is determined by the content of the Maillard reaction materials, many constituents of which are available by chemical synthesis. To produce synthetic flavors, the product of the protein hydrolysis part of the Maillard reaction can be replaced by HVP or yeast extract. The Maillard reaction product is obtained by the finely controlled reaction between selected amino acids and reducing sugars at high temperature in a reactor. The addition of glycerol to the reacting vessel as a flavor precursor when processing a roasted meat flavor was shown to contribute to the formation of specific proline materials such as 2-propionyl-1(3),4,5,6-tetrahydropuridines, which are known for their roasted aroma (Smarrito-Menozi et al. 2013). Meaty flavorings are added at 0.5–2.0 % concentration to any type of food that needs a characteristic meaty taste, such as soups, sauces, processed meats and seafoods, imitation meat analogs, snacks, and packaged meals.

Acidulants

Acids are naturally present in almost every kind of fruit, contributing to their acidity and tartness. Acids stimulate the appetite because they affect the olfactory nerve, creating a desire for more food. Therefore, selecting the proper acid and the right

amount when formulating a food product to obtain the desired physiological and psychological reactions is a subtle skill. Citric, malic, lactic, tartaric, and phosphoric acids are those most commonly used in the food industry. Citric acid is compatible with all citrus fruit items; malic acid is usually used as a flavor enhancer; tartaric acid is added for a grape taste; and phosphoric acid is used for colas. Addition of acids to a wide variety of foods, including beverages, jams, jellies, preserves, bakery items, confectioneries, chewing gums, and dairy products, is necessary to strengthen the function of the natural acid components or to adjust the pH for taste optimization. The acidic pH can be buffered by adding a corresponding salt, usually sodium.

Citric Acid

Citric acid is present in the human body, plants, and animals and functions as an intermediary in respiration and energy metabolism. It has been used in food processing for over 100 years. It is present in about 60 % of the world market. Citric acid is tribasic and it is used mainly in citrus-type beverages, especially carbonated orange, lemon, and lime drinks, as well as in fruit juices, concentrates, syrups, and cordials. For confectioneries, chewing gums, desserts, ice creams, jams, and jellies, citric acid is an absolute must. It is also valuable in meat curing, mayonnaise, cheese, and cottage cheese. In many cases, sodium citrate is used with citric acid to buffer the pH. Citric acid is produced mainly from the fermentation of corn sugars or molasses. It is also obtained from raw glycerol by fermentation of the yeast *Yarrowia lipolytica* (Morgunov et al. 2013).

Malic Acid

Malic acid is found in many vegetables and fruits, especially apple. Its acidulating properties resemble those of citric acid, but the characteristic tartness occurs over time. Malic acid is usually paired with citric acid to reduce the sharp note of food so that the taste is more smooth and natural.

Lactic Acid

Lactic acid is widely found in nature, especially in milk and fermentation products, and has been used since the early days of human history. Since it is a viscous liquid, the acid is present in both D- and L-stereoisomers, but in general the food industry uses the DL racemic mixture. Lactic acid is mildly tart and is used in dairy products, wine, frozen desserts, and especially added to packing of Spanish olives.

Tartaric Acid

Tartaric acid occurs naturally in grapes, limes, currants, gooseberries, raspberries, and many other fruits, and contributes to the superior flavor profile of fruit drinks, jellies, preserves, sherbet, and cakes, especially when combined with citric acid. Its potassium salt, monopotassium bitartrate, commercially known as cream of tartar, is a common leavening agent in the bakery industry. Most tartaric acid is produced from the pressed cake of unfermented grape juice produced during wine manufacturing.

Phosphoric Acid

Phosphoric acid is the only inorganic acid used in the food industry, and perhaps the least costly acidulant that can achieve the required acidity in foods. Its principal use is in carbonated beverages, particularly colas, root beer, and sarsaparilla, at a usage level of a few hundred parts per million. It is also used in some dairy products such as cheese. The acid is produced by treating phosphorus-containing rock with sulfuric acid, reducing the product to elemental phosphorus, burning it to form phosphorus pentoxide which is then hydrated to yield 75–85 % phosphoric acid, and finally purifying the acid to a commercial grade of 75 %, 80 %, or 85 %.

Other Acids

Other acids include acetic acid, adipic acid, fumaric acid, tannic acid, and glucono delta-lactone (GDL). Acetic acid is not directly used in foods, but vinegar, the major ingredient of which is acetic acid, is a common additive. Adipic acid is not common but sometimes is used in bakeries instead of tartaric acid to sustain the flavor notes longer. Fumaric acid is a structural isomer of maleic acid, is more “tart” than citric acid, and can be a substitute for tartaric acid. GDL, produced from the oxidation of glucose, is the inner ester of gluconic acid and is used in the preparation of some cheeses and bean curd (tofu).

Sweeteners

People of all ages, cultures, and races desire sweet-tasting foods. Throughout evolution, sweetness played a role in human nutrition by orienting humans to ingest food for energy and essential nutrients (Drewnowski et al. 2012). Sugar or sucrose is a universal sweetener present in almost every type of food. After absorption in the gut, sucrose is hydrolyzed to glucose, which is the energy source for life. Unfortunately, overconsumption of sugar or glucose can cause serious

chronic diseases such as diabetes mellitus and obesity. *Streptococcus mutans* is considered the primary etiologic agent of dental caries and contributes significantly to the virulence of dental plaque, especially in the presence of sucrose. Sugar substitutes interfere with the formation of *Streptococcus mutans* biofilms (Durso et al. 2014). Thus, replacing sugar with alternative sweeteners in foods is an important issue in food production. In food formulation, sugar is important not only for sweetening, but also for bulking. Other parameters in food preparation, such as viscosity and humidity control, are also significant. As replacements for sugar, both the polyols and artificial or natural intense sweeteners have their specific roles. First, a suitable bulking material to replace the volume of sugar should be selected; polyols are good choice. Then, since polyols are not as sweet as sugar, an artificial or natural intense sweetener is chosen to provide the required sweetness. In addition, a small amount of hydrocolloids is recommended to adjust moisture and viscosity.

Polyols

Polyols are sugar alcohols, a group of low-calorie digestible carbohydrates similar to sugar. Polyols contain hydroxyl groups that are substitutes for aldehydes. They occur naturally in fruits and vegetables and are produced by certain bacteria, fungi, yeasts, and algae (Ortiz et al. 2013). They are produced in the food industry from catalytic hydrogenation of natural sugars, but biotechnological production by lactic acid bacteria (LAB) has been investigated as an alternative (Ortiz et al. 2013).

The first generation of polyols is derived from hydrogenation of monosaccharides: sorbitol from glucose, xylitol from xylose, and mannitol from mannose, and their sweetness is reminiscent of that of glucose. Many polyols can be produced by various microorganisms. Xylitol can be produced from xylose and corn cob hydrolysate by assimilation of the tropical mangrove yeast *Cyberlindnera saturnus* (Kamat et al. 2013). Microalga *Chlamydomonas reinhardtii* can be genetically engineered to produce xylitol at low cost (Pourmir et al. 2013). Mannitol, the first crystalline polyol discovered, is present in many plant exudates, seaweeds, and grasses. By promoting the effective use of raw glycerol, mannitol is produced by the activity of the yeast *Candida azyma* (Yoshikawa et al. 2014).

The second generation of polyols is from disaccharides: isomalt from isomaltulose, lactitol from lactose, and maltitol from maltose. Isomaltulose is a derivative of sucrose and is a mixture of two disaccharide alcohols: gluco-mannitol and gluco-sorbitol.

Erythritol is a sugar alcohol that is present in a wide variety of fruits and fermentation products. It is about 85 % as sweet as and with a profile similar to that of sucrose. It used to be produced only by fermentation on glucose media. An alternative method is production of erythritol by the yeast *Yarrowia lipolytica* on pure and crude glycerol (Mironczuk et al. 2014). A study by Heikel et al. (2012)

established the optimal synergism of erythritol with either rebaudioside A or sucralose. It was concluded that the binary sweeteners achieved sucrose-like flavor and texture profiles at an adjusted sweetness of 1.0 for both combinations (Heikel et al. 2012).

Artificial Intense Sweeteners

There are a limited number of intense artificial sweeteners that the US FDA has approved for industrial use as sucrose substitutes: acesulfame potassium, aspartame, neotame, saccharin, and sucralose. The isosweetness concentration of these sweeteners compared to sucrose, or relative sweetness (RS), depends on the type of food in which the sweetener is incorporated. The isosweetness of neotame and sucralose, together with natural rebaudioside (from Stevia), were analyzed in a chocolate formulation and found to be 8,600, 570, and 200, respectively, which is a similar profile to that of other foods (Palazzo et al. 2011). Descriptions of the artificial sweeteners and their relative sweetness values are given below:

- (i) Acesulfame potassium, also known as acesulfame-k, has an RS of 200, a slightly bitter aftertaste, and is suggested for use in beverage mixes, gelatin confectioneries, and chewing gums.
- (ii) Aspartame, a dipeptide ester with the amino acids aspartic acid and phenylalanine, has an RS of 200, a taste in the sharp direction, and is suggested for use in desserts and beverages. Its sweetness is synergistic with acesulfame-k. It may be metabolized to phenylalanine, which can cause sensitivity for individuals with phenylketonuria.
- (iii) Neotame is a derivative of the dipeptide composed of aspartic acid and phenylalanine, has an RS in the range of 7,000–13,000, and is not metabolized to phenylalanine.
- (iv) Saccharin is the oldest artificial sweetener, having been in use since before 1900, has an RS of 300, is very stable to heat and acid, and has a slightly bitter aftertaste. There was controversy about its possible carcinogenicity, but there is no strong evidence.
- (v) Sucralose, manufactured from sugar, has an RS of 600, a profile close to that of sugar, a good clean taste, and is very stable.

The Natural Sweeteners

Because they are natural products, the compounds derived from the South American plant *Stevia rebaudiana* and monk fruit are used more and more. The leaves of the stevia plant contain a range of diterpene glycoside steviolosides, with rebaudioside A the sweetener of industrial interest. The RS of rebaudioside A ranges from 30 to 45. It has a clean taste, although there is some bitter aftertaste.

The triterpene glycoside mogrosin extracted from the monk fruit *Siraitia grosvenorii* is also gaining in popularity (Pawar et al. 2013). Monk fruit is also called *luo han guo*, a common Chinese herb. It has an RS of 250.

Food Colors

Like flavor, the appearance of food is an essential sensory characteristic. A food's appearance is the first thing that a consumer uses for a rapid judgment of quality. Food manufacturers utilize brilliant colors to attract customers in their products. In ancient Greece and Rome, dyes from inorganic heavy metal salts were added to wines. However, the inorganic coloring materials have slowly been replaced by their organic counterparts. In 1886, the US began regulating dyes by allowing organic dyes to be added to butter. In 1900, USDA established guidelines for the use of colors together with preservatives. Today, the US FDA is involved in use of food colorants to help protect the safety and health of consumers.

There are two types of food colorants approved for industrial use, certified and uncertified. Certified food colorants are synthetic dyes for which testing and approval must be processed by the FDA for every manufactured lot, whereas uncertified food colorants are natural colors that do not need batch approval by the FDA. All approved synthetic colors are assigned a Food, Drug and Cosmetic (FD&C) number. Table 2 gives the major commercial synthetic dyes.

The amount of each color that needs to be added in processed food is small, with about 10–100 ppm for red and yellow and from 3 to 10–20 ppm for blue. These additives are generally stable in acidic pH. The dyes are usually dissolved in ethyl alcohol before being added to foods in the manufacturing process. In addition to their own characteristic shades, other shades can be produced by mixing two or more dyes. Because of the insolubility of food colorants in fats or oils, lakes are used instead. Lakes are derivatives of food colorants and are made by depositing individual dyes on a substrate of aluminum or calcium salts. With a dye content of 10–40 %, the lakes are dispersed in lipid medium when added to food. The usage level of food colorants is 0.1–0.3 %.

In nature, plants and animals have a wide variety of colorful appearances. The natural color dyes are extracted from a large variety of plants. These are not certified dyes; in many cases, when they are added to foods, food processors do not have to label them as colors because they have other functions. However,

Table 2 Synthetic dyes and their FD&C numbers

Synthetic dye	Color shade	FD&C No.
Erythrosine	Bluish pink	Red #3
Allura red	Yellowish red	Red #40
Tartrazine	Yellow	Yellow #5
Sunset yellow	Reddish yellow (orange)	Yellow #6
Brilliant blue	Greenish blue	Blue #1
Indigotine	Deep blue	Blue #2

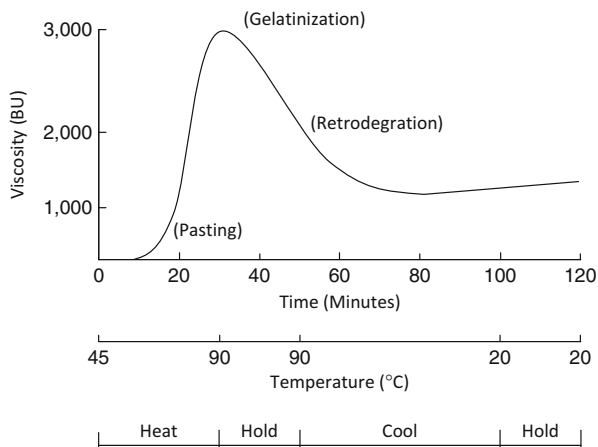
sophisticated techniques must be used and much care must be taken when using natural colors because most are soluble in oil but not in water, and they are not concentrated so that large quantities are required for use in food. In addition, they are not quite pH stable, so some natural color extracts need extra protection to safeguard them from the effects of high temperatures. One study evaluated the red cochineal extract, which showed changes in its visual color in association with high thermal stability (Fernandez-Lopez et al. 2013). Microencapsulation techniques were used to increase stability. In the extrusion of rice flour, microencapsulated lycopene yielded better color retention in the extrudates than free lycopene (Choudhari et al. 2012); the freeze-drying process was favorable for microencapsulation of curcumin by maltodextrin (Sousdaleff et al. 2013); and the encapsulation approach was a promising way to enhance the stability and dispersibility of carotenoids using sodium caseinate as the carrier (Zhang and Zhong 2013).

At present, natural dyes are increasing in popularity. The majority come from plants, e.g., Amaranthus red, annatto extract of bixin and norbixin, betanin from beets, β -carotene from carrots, curcumin from turmeric, gardenia blue, konjac rice red, lutein, paprika red, paprika orange, riboflavin yellow, spirulina blue, and tomato red. Recently, anthocyanins from blueberry and eggplant, orange color from avocado, and betanin were studied in more depth. The extraction of bixin from annatto seeds has increased because it is nontoxic (Barrozo et al. 2013). Paprika and tomato were studied for use as natural pigments in cured meat products to compensate for nitrite-reduced meat batters (Bazan-Lugo et al. 2012). In addition to extracts from plant sources, there are other natural colors: carmine cochineal comes from an insect; caramel is manufactured from the polymerization of phenolic molecules that result from high-temperature treatment of sugar; carbon black is made from minute purified charcoal particles, with one of the pure forms processed from rice husk by hydrolysis, carbonization, and pyrolysis (Wang et al. 2011); and titanium oxide is a mineral used for opaque white with a bright shiny look. A new method for extracting carminic acid from the dried bodies of cochineal insects is by pressurized liquid and supercritical fluid extraction techniques (Borges et al. 2012). Caramel-colored solution can undergo ultrafiltration to remove as much of the toxic chemical 5-hydroxymethyl-2-furaldehyde as possible to increase the intensity of browning (Guan et al. 2011).

Texturizers: Starches and Their Modified Forms

Starches are the most widely used food texturizers and are well known worldwide, especially to chefs and bakers. Since they are readily available almost everywhere, they are inexpensive. Starches are obtained from cereal grains such as wheat, corn, and rice, as well as from tubers like the potato and cassava root (tapioca). As a major constituent of the human diet, starches are a primary energy source for the human body. In addition to being a source of nutrition, starches and their modified forms can be added to many kinds of foods for needed thickening, gelling, moisture retention, and texturization. Many chefs and bakers are experts in making use of

Fig. 1 Brabender viscosity curve of a typical starch



starches in their foods and bakery items. In the food industry, the above-mentioned properties have been fully utilized in food processing to attain various texturization functionalities.

Native starches are contained in granules extracted directly from various sources. Chemically, starches are polysaccharides, which are polymers consisting of *d*-glucopyranose or anhydroglucose units (AGU) linked together by α -1,4 and α -1,6 glycosidic bonds. The polymers that are linked together by α -1,4 bonds forming straight chains are amylose, and those molecules with a number of units linked by α -1,6 bonds are known as amylopectin. Amylose is a linear structure and is smaller than amylopectin. Amylopectin is branched and much bigger, with a molecular weight about 300 times that of amylose; therefore, it is more thermally stable and has a higher viscosity in water. The difference in the properties and functionalities of native starches from different sources is due largely to their ratio of amylose to amylopectin. Waxy corn has an exceptionally high ratio of amylopectin of almost 100 %; potato, tapioca, and rice starches also have a high amylopectin content compared to that of wheat and dent corn starches. The behavior of starch also depends on small quantities of phosphorus, protein, and lipids.

The different sizes and shapes of starch granules also contribute to the diverse properties of starches. Different starches have their own unique characteristics and behave differently, which is reflected by the change in viscosity when the starch grain is dispersed in water, heated, and then cooled. These viscosity changes are recorded by viscoamylography. The Brabender viscosity curve shows the change in viscosity in a standardized heating environment where the starch is heated from 45 to 90 °C from 0 to 30 min, held at 90 °C from 30 to 50 min, cooled to 20 °C from 50 to 100 min, and then held at 20 °C from 100 to 120 min (Fig. 1).

When a slurry of starch grains is heated, the amylose and amylopectin gradually become hydrated so that the swelling of the granules causes an increase in the viscosity of the water solvent. The pasting temperature is where the viscosity begins to increase. The granules pass into a paste form; this is known as the cooking or gelatinization

process. After the viscosity reaches its peak, the amylose chains begin to reassociate in a parallel array structure causing a drop in viscosity; this is called retrogradation. Letting the starch stand leads to dispersal by loosening the hydration and rigid gel formation. Different starches have their own specific viscosity and Brabender curve shape. Different treatments also affect the properties of starches, e.g., heating or freezing the starch suspension. According to one study, repasting the potato starch produced by preheating preparation increased the viscosity by 50 % compared to that of its native form; the strength of the produced gel was also greater from preheating (Gryszkin et al. 2014). Waxy maize starch prepared in nanocrystal form could be used as a particle emulsifier to stabilize oil in water emulsion because of its ability to absorb both phases at the oil-water interface, resulting in stabilization (Li et al. 2014). The addition of syrups, amino acids, and acidulants greatly affects certain parameters of starches such as pasting temperature swelling power, aqueous solubility, gel strength, and light transmittance. The addition of hydrocolloids has significant effects on starches, which is particularly evident when they are added to foods.

To create starches with specific properties, parameters, stability, and applications, they are modified chemically or physically. There are various modified starches on the market.

Cross-Linking

One way to increase the stability of starch is by cross-linking. It is the most common chemical modification technique and is the result of the reaction between the slurry of granular starch containing 30–45 % solid and an agent in the alkaline medium. Covalent bonds form between the agent and the hydroxy groups of two strains of starch polymers with the same or different molecules. Cross-linking affects the swelling of starch granules so that they can resist high temperature, high acid, and stress conditions. Common agents used for cross-linking are sodium trimetaphosphate (STMP) and phosphorus oxychloride. A cross-linked tapioca starch prepared with 0.25–6 % concentration of STMP and sodium tripolyphosphate (STPP) at a ratio of 99:1 w/w was tested for its physiochemical properties. The paste clarity decreased with higher STMP/STPP concentration, and variations in swelling power, solubility, pasting, gelatinization, and rheological properties were observed such that cross-linking exhibited the strongest gel characteristic and greater shear resistance at 1.0 % (Wongsagonsup et al. 2014). Another study indicated that cross-linked wheat starch was more resistant to acids than its native counterpart; the effect of lactic acid was greater than that of acetic acid on the degradation of starch properties (Majzooobi and Beparva 2014).

Substitution

Introduction of a monofunctional agent into the starch amylose molecule blocks it and delays amylose reassociation, i.e., retrogradation. Starches with this

substitution are called starch esters. Starch acetate is formed by the agent acetic anhydride, which is used in food products such as refrigerated and frozen foods to increase stability. Starch with a substitution by 1-octenyl succinic anhydride (OSA) or succinic anhydride results in starch octenyl succinate, which is an effective emulsifier for carbonated beverages and an alternative to gum acacia when there was a shortage. There is also starch ether, a hydroxypropylated starch that results when the starch slurry reacts with propylene oxide under highly alkaline conditions. Starch ether paste is more viscous but clearer, with better freeze-thaw stability and less syneresis. Thus, it is used extensively in gravies, sauces, puddings, and pie fillings.

Acid Hydrolysis and Dextrinization

Acid-converted starches are hydrolyzed products produced by acidifying starches in slurry under stringent control; they are called thin-boiling starches. The lower aqueous viscosity of an acid-converted starch makes it readily dispersible in water without excessive unrequired thickening. A specific use for these starches is in candies with soft and jellylike yet firm texture. When starches are heated dry under agitation, many glycosidic bonds are broken to form dextrans, which are used in the coating of foods. Controlled enzymatic hydrolysis with amylase results in a range of products such as maltodextrin, corn syrup, and dextrose (glucose), depending on the degree of conversion. A study on maltodextrin obtained from tapioca starch after enzymatic esterification showed that it had a higher viscosity than native maltodextrin. It could be used as an emulsifier to make *n*-hexadecane oil-in-water (o/w) emulsions that are characterized according to their oil droplet behavior (Udomrati and Gohtani 2014).

Oxidation

Oxidation is used to bleach starches to remove colored impurities like carotene, xanthophyll, and related pigments. The solution has a slightly lower viscosity but becomes very transparent, suitable for use in batters or breadings to coat many types of meats and vegetables. A study on ozonation of cassava starch found that pH 3.5 reduced the peak viscosity, breakdown, setback, and final viscosity (Klein et al. 2014).

Pregelatinization

Pregelatinization is when starches are gelatinized and then dehydrated to dry powder form. When they are placed in water, hydration occurs immediately to form gel. Commercially, pregelatinized starches are cold-water swelling (CWS) starches or pregels that are used as thickeners in foods that require a minimal amount of heating, e.g., instant soups and sauces. The concentration of pregelatinized waxy maize starch has a significant impact on the stability of the emulsion with respect to creaming and

the capacity to hold fat, a worthwhile trait to exploit for the development of low-fat health-oriented food emulsions (Bortnowska et al. 2014).

Additional information on starches is available in the chapter “Starch and Nanoparticle” in Dufresne 2015.

Emulsifiers

Water and oil cannot be mixed together; they automatically separate into two distinct phases. However, an emulsifier can cause them to mix, forming an emulsion. An emulsion is a two-phase system, where one phase presents as finite globules in the other continuous phase. In most cases, the two phases are aqueous and lipid. Emulsifiers play important roles in humans, with the bile salts, cholesterol, and saponins just a few examples. There are many emulsion systems in foods, e.g., citrus oil-containing beverages, milk, dressings, shortening, coffee creamer, meat products, margarine, and peanut butter. To function properly, an emulsifier must have hydrophilic and lipophilic groups. It must be soluble in either or both phases. The groups align themselves on the phase boundary in one direction and in one layer forming micelles to separate the phases. By reducing the surface tension at the phase boundary in this manner, the system is stabilized. There are two systems: oil-in-water (o/w) and water-in-oil (w/o). The o/w system is more stable after emulsification with an emulsifier with a stronger hydrophilic group, whereas with the w/o system, the reverse is true. The suitability of the use of a specific emulsifier is determined by its hydrophilic and lipophilic balance (HLB) value that fits the particular emulsion system. The HLB value ranges from 2 to 18 and is based on the tendency of the emulsifier to dissolve in oil or water: a low HLB (2–8) indicates that it is more oil-soluble and a high HLB (14–18) indicates that it is more water-soluble. Table 3 lists the HLB values of some of the more commonly used emulsifiers (O’Brien 1998).

There are many emulsifiers that are used in a wide range of foods. Only the more common ones are discussed below.

Fatty Acid Esters

The most commonly used emulsifier is glycerol monostearate (GMS) of the mono- and diglyceride series. GMS was the first emulsifier to be added to a food (margarine) and has about a 70 % market share of all emulsifiers, with its major use in bakeries. GMS is produced by adding glycerol to fat or oil which results in a mixture of monoglyceride and diglyceride. One emulsifier has a minimum monoglyceride content of 40 % and another has a minimum monoglyceride content of 52 %. A GMS with a monoglyceride content of over 90 %, which resulted from distillation, is valuable in the production of w/o emulsion. GMSs have various forms: liquid, semiliquid, soft plastic, flakes, beads, and powder. The main use for GMS is to delay flour or starch retrogradation in yeast-raised baked goods.

Table 3 Emulsifiers and their HLB values

Emulsifier	HLB value
Mono- and diglycerides	
40 % mono (min)	2.8
52 % mono (min)	3.5
Distilled monoglycerides	4.3
Propylene glycol ester (PGME)	3.4
Sorbitan ester monostearate (Span 60)	4.7
Sorbitan ester tristearate (Span 65)	2.1
Polyoxyethylene sorbitan ester	
Tween 60	14.9
Tween 65	10.5
Tween 80	15.0
Lactated ester SSL	2.6

Retrogradation occurs when the baked goods are left standing for several days and the amylose of the flour retrogrades forming a parallel array of polymers and the amylopectin crystallizes slowly, resulting in a loss of crispness and flavor of the bread crumbs. The added emulsifier at a concentration of 0.25–0.5 % attaches to the amylose to form a helical complex that retards the retrogradation of the starch. In another application of GMS, the addition of 1.5 % GMS significantly influenced the rheological properties of pistachio spread, such as the consistency, thixotropy, and yield stress, because the monoglycerides as lipophilic emulsifiers prevented oil separation (Shakerardekani et al. 2013).

By reacting with acids, the chemical structure of mono- and diglycerides can be modified to that of ester derivatives that are soluble in water. Diacetyl tartaric acid ester of monoglycerides (DATEM) and lactic acid ester are used extensively in baking, e.g., the aeration of cakes, icings, and toppings. A study on frozen bread dough showed that the addition of DATEM (0.75 %) with hydrocolloids to both hard and soft wheat flours yielded the best results with respect to baking quality, including loaf volume and prevention of staling (Sungur and Ercan 2013).

Other emulsifiers include the propylene glycol monoesters (PGME); the so-called Span range, i.e., the sorbitol-derived sorbitan esters; and the Tween or polysorbate range, i.e., the polysorbate fatty acid esters produced by the reaction of sorbitan esters with ethylene oxide. Span 60 and 65 are sorbitan monostearate and tristearate, respectively; Tween 60, 65, and 80 are polyoxyethylene sorbitan monostearate, tristearate, and mono-oleate, respectively. Span 60 was added to whipping cream to study its effect on particle size distribution, microstructure, apparent viscosity, partial coalescence of fat, and overrun of this emulsion. The study showed improvement in overrun and organoleptic properties (Zhao et al. 2013). With the need for confectionery fat blends that are heat-stable, the addition of Span 60 could be the answer (Peyronel and Marangoni 2014). Tween 80, in combination with a hydrocolloid (e.g., guar, xanthan, carrageenan, or alginate), improved the stability of conventional meat sausages during cooking and storage (Ramos et al. 2004).

Another popular emulsifier range includes the lactic acid esters or lactated esters; the most common ones are sodium stearoyl lactylate (SSL) and calcium stearoyl lactylate (CSL). When wheat flour dough is baked, the added SSL affects the extent and rate of gluten polymerization by causing less gliadin to be incorporated into the polymer gluten network and by interacting with gluten (Steertegem et al. 2013). Many of these types of emulsifier are used in combinations of two or more, and preferably with GMS. The HLB values of the individual emulsifiers create a synergism that improves the flour grain to produce softer dough. Improvement in quality is attained with the use of emulsifiers, yielding better product structure with denser but finer air cells, larger volume, anti-staling characteristics, and longer shelf life.

In addition to baked goods, these emulsifiers are valuable additives in other foods that have both aqueous and lipid phases. They are used in ice cream, shortenings, salad dressings, and mayonnaise, as well as for defoaming in pudding production, lubrication of extruded goods, oil stabilization in peanut and sesame butters, and starch complexing for pasta.

Lecithin

Lecithin was the first emulsifier discovered, in egg yolk, in 1846. It is present naturally in almost all living cells. Today, most lecithin comes from soybean oil. Lecithin is not a single product but a group of four phosphatidyl (PP) lipids: PP-choline, PP-ethanolamide, PP-inositol, and phosphatic acids. Rich in polyunsaturated fats and with no cholesterol, most lecithin is in the form of flakes, while the de-oiled version is granular. It is an emulsifier with a wide HLB range of 3.5–6.5 and used extensively in both w/o and o/w emulsions.

Hydrocolloids

Gum acacia (or gum arabic) and propylene glycol alginate (PGA) are excellent emulsifiers. Gum acacia contains arabinogalactan protein complex (AGP), which comprises hydrophilic and lipophilic groups. It emulsifies citrus oil in sugar solution to form stable citric emulsified flavors for carbonated beverages. PGA is manufactured by the reaction of propylene oxide with alginic acid. In foods rich in oil, it improves acid stability and resists precipitation by calcium and other polyvalent metal ions. It is used in salad dressings and mayonnaise.

Protein Concentrates

Both soy protein isolate (SPI) and whey protein isolate (WPI) are more than 90 % protein and are used to emulsify fat in sausage-making; they prevent fat from leaking from the meat emulsion filling. One study used WPI hydrolysate to produce

o/w nanoemulsions with good stability and better storage properties. This demonstrated the value of tailor-made nanoemulsions for use in a wide range of food applications and they should be studied further (Adjonu et al. 2014). Sodium caseinate is another excellent emulsifier for use in high-fat-content beverages. In coconut drinks, it distributes the coconut oil evenly thus preventing an oily layer to form on top of the beverage.

Hydrocolloids: The Dominant Texturizers

Like starches, hydrocolloids are food-texturizing agents. However, they are used in much smaller quantities and their behavior and function are different. Texture is important for food palatability and safety, and hydrocolloids play an important role in controlling food texture (Funami 2011). In fact, hydrocolloids and starches are synergistic when added together, although the amylose content of the starch is a greater determinant of pasting, paste, and gel properties than the added hydrocolloid (Kim et al. 2013). Hydrocolloids are also known as gums. They are dispersible in water and have a strong water-binding characteristic. A grain of gum dropped into water will immediately modify the rheology by either increasing viscosity or gelling. In food, a high aqueous viscosity increases water or moisture retention, particle suspension, emulsion, and foam stabilization; improves volume; and prevents ice crystal formation. The formed gel controls movement of water molecules and stabilizes the freeze-thaw cycle. Hydrocolloids, like starches, are polysaccharides, but unlike starch in AGU, various gums have a different chemical composition of complex monosaccharides such as glucose, mannose, galactose, arabinose, and rhamnose. Some monosaccharides are attached with positive or negative charges. Each monosaccharide has unique properties that play a role in its application and performance in food texturization.

Hydrocolloids are extracted from various parts of plants such as tree exudates, seeds, seaweeds, fruits, tubers, and microorganisms, and some come from chemical modification of wood pulp. Hydrocolloids are used in almost all kinds of foods that contain water; they cannot be used in chocolate, chewing gum, and edible oil due to the absence of enough water to function. Because of their strong water-binding capacity, only small amounts of hydrocolloid are added to food, usually in the range of 0.05–0.2 %. Hydrocolloids are commonly used in bakery products, including dough conditioner, glazes, and pie filling; beverages such as fruit juices, concentrates, and soft and hard drinks; dairy products such as ice cream, yogurt, cheese, whipping cream, and pudding; desserts like water gel and smoothies; frozen foods; sauces and dressings; snacks; and meat products and their analogs.

Gum Acacia

Gum acacia is also known as gum arabic. It is extracted from the true exudates of the plant *Acacia senegal*, which grows in Sudan. The gum was traded by Arabs in

Table 4 Seed gums and their galactose:mannose ratio

Seed gum	Galactose:mannose
Fenugreek gum	1:1
Guar gum	1:2
Tara gum	1:3
Locust bean gum	1:4

the Middle Ages, which is how the name gum arabic came about. Gum acacia is a mixture of glycoproteins and the polysaccharides arabinose, galactose, rhamnose, and glucuronic acid. Its exceptionally low aqueous viscosity makes it usable in high-protein drinks, and its great adhesive qualities make it useful in gum drop-type candies. As an emulsifier, gum acacia is used in citrus beverages that contain citrus oil.

Seed Gums

Seed gums are a family of galactomannans. Commercially, they include fenugreek gum, guar gum, tara gum, and locust bean gum (LBG), which are produced in Canada, South Asia (India and Pakistan), South America, and Mediterranean countries, respectively. All gums have polymannose as the backbone formed by α -1,4 bonds, with a galactopyranose side chain of α -1,6 bonding to mannose in different ratios, as shown in Table 4.

The properties of seed gums vary. Fenugreek and tara gums are cold water-soluble and LBG dissolves in hot water. Fenugreek and guar gums cannot form a gel, while tara gum and LBG react with xanthan, carrageenan, and konjac to form gels of different strengths. Guar gum is used to increase viscosity in foods like sauces and gravies. The viscosities of guar and xanthan synergize such that they are used in gluten-free flour to strengthen the air cells in the flour matrix. The antisyneresis property of LBG makes it useful in jellies either alone or in combination with xanthan. Tara gum can be used to replace both guar gum and LBG when they are in short supply. Fenugreek gum contains amino acids that increase sensitivity to insulin; thus, it is used mainly in herbal drinks.

Marine Gums

There are three marine gums: agar, alginate, and the carrageenans.

- (i) Agar is extracted mostly from the genera *Gracilaria* and *Gelidium*; its sugar units are galactose-linking sulfate acid ester groups that form agarose and agarpectin. It dissolves in near-boiling water, forms a strong, brittle, and hysteretic gel at 40–50 °C, and then melts at 80–85 °C. Agar is synergistic with LBG. Agarose is the jellifying agent in stabilizing the structure of foam and is used extensively in jelly sweets and aerated products like marshmallow.

- (ii) Algin, or alginic acid, is extracted from the brown algae *Phaeophyceae*. The backbone polysaccharide is composed of mannuronic acid and guluronic acid. Since algin is insoluble, it is chemically converted to sodium alginates of various viscosities. Sodium alginate reacts with a calcium cation to form the thermoirreversible gel calcium alginate; gel formation is usually controlled by using phosphate or polyphosphate as the sequestrant. The characteristics of algin have made it useful in many gelation products such as ice cream and imitation fruit purees. In molecular gastronomy, sodium alginate is the “magic” material used in making caviar.
- (iii) Carrageenan comes from the red seaweeds *Rhodophyceae*, *Chondrus crispus*, and *Gigartina*, with structures of mixed galactan and half ester sulfates. The sulfate is at position 1 or 3 in about every two monosaccharide units, forming κ (kappa) carrageenan and λ (lambda) carrageenan, respectively. In addition, the structure of the red seaweed *Eucheuma spinosum* has two sulfates in two monosaccharides, forming the ι (iota) carrageenan. Since sulfate groups affect the stereostructure of the backbone chain, the κ -carrageenan reacts with potassium ions to form a rigid but elastic gel and the ι -carrageenan reacts with calcium ions to form a softer gel; the λ -type carrageenan does not gel. The carrageenans react with milk protein to prevent whey-off; therefore, they are used in dairy products like ice cream, milk puddings, flan, pie fillings, and chocolate milk. κ -Carrageenan is also used in water gel, either alone with potassium salt or synergistically with konjac gum or locust bean gum. κ -Carrageenan was analyzed for any therapeutic uses upon enzymatic hydrolysis to oligosaccharides. A study of the mixture of κ -neocarrabiose-sulfate, κ -neocarrahexaose-sulfate, and κ -neocarraoctaose-sulfate showed antitumor and antiangiogenic activities in vivo and in vitro (Yao et al. 2014).

Pectins

Pectin is extracted from the peel albedo and lamella of citrus fruits, mostly lemon and lime, as well as from apple pomace. This hydrocolloid is composed of partial methyl esters of polygalacturonic acids, with side chains comprising arabinose, galactose, and xylose; its molecular weight is in the range of 20,000–40,000. Upon extraction, it is 70–75 % esterified and is regarded as high-methoxyl (HM) pectin. De-esterification occurs upon acid hydrolysis. When the percentage of esterification goes below 50 %, it becomes low-methoxyl (LM) pectin. Alkaline can be used in low-temperature hydrolysis. If ammonia is involved, some ester groups are converted to acid amide groups and the pectin is regarded as amidated (AM). HM pectins are soluble in hot water and at high temperature form smooth solid gels with sugar of 65° brix. They are used in fruity jams, jellies, and marmalades. HM pectins in foods give a sense of fullness with respect to mouthfeel; people are sensitive to a pectin concentration of just a few parts per million. It is also used in yogurt, whereas the positive charges in the pectin keep milk protein from coagulating when the acidity of the lactic acid bacterial culture decreases to its isoelectric point.

Commercial pectins are classified as rapid set (RS), medium set (MS), and slow set (SS), depending on the degree of methylation: the higher the degree, the more rapid the setting. The LM and AM types of pectin behave very differently. They are soluble in cold water and form thixotropic, brittle, and irreversible gel with calcium ions, very similar to alginate gels. The thixotropy characteristic is valuable in making center-filling gels for cakes, breads, and biscuits.

Tuber Gum

Konjac gum is extracted from the tubers of *Amorphophallus konjac*. It is composed of polyglucomanan of β -1,4 linkages and has a molecular weight of 200,000–2,000,000 Da; its nonionic structure is composed of glucose and mannose in a 1:1.6 ratio. There are acetyl groups along the backbone in every 9–19 sugar units. Because of its high molecular weight, it exhibits very high viscosity in water with a pH of 4–7. Konjac is soluble in cold water and reacts with xanthan, carrageenan, locust bean gum, and tara gum to form thermoreversible gels. However, when treated with alkaline, an irreversible gel is formed via alkaline cleavage of the acetyl groups. Konjac also amplifies the pasting and gelling effects of starches. Konjac gum is used extensively in a large variety of foods such as konjac jello, which is a gelation of konjac and carrageenan. It is also used to make “konjac noodles” by spray-extruding slurries of concentrated konjac solution into the alkaline calcium hydroxide bath. By esterification with octenyl succinic anhydride using the microwave method, a new polymeric surfactant, konjac glucomannan octenyl succinate, was created, with good hydrophilic and lipophilic characteristics (Meng et al. 2014).

Biogums

The biogums are represented by xanthan and gellan.

- (i) Xanthan is extracted from a culture of *Xanthomonas campestris* grown on glucose or starch. It is a polysaccharide with its main backbone chain composed of α -1,4-linked *D*-glucopyranose units, and the trisaccharide side chains of mannose and glucuronic acid are attached to every other backbone sugar. The solution is stable within a wide pH range of 2–12 and is thermally stable, and its viscosity behaves in pseudoplastic fashion. Xanthan synergizes with guar resulting in an increase in viscosity, and it forms gels of various characteristics with locust bean gum, tara, carrageenan, and konjac. It is a universal hydrocolloid with many applications. In many starchy food systems, the addition of as little as 0.05–0.5 % of xanthan reduces the amount of starch needed while improving the rheology and stability. The effect of modified tapioca starches and xanthan on the viscoelasticity and texture of dough was investigated after starch sheets for Chinese shrimp dumplings were stored at 4 °C, and hydroxypropylated starch and hydroxypropylated-cross-linked

starches were substituted for natural starch. The viscoelasticity of the dough made with the hydroxypropylated starch was much softer and the dough made with the cross-linked counterpart was stiffer and more strain-resistant. Both formulations gave the gel sheet the least change in texture, which is potentially beneficial for frozen/chilled dumpling wrappers (Seetapan et al. 2013). Xanthan was evaluated as a carrier of a preservative solution in an edible coating applied to a freshly cut apple. The solution, which contained 1 % glycerol, 0.5 % xanthan, 1 % calcium chloride, 1 % ascorbic acid, and 0.25 % citric acid, reduced mass loss and oxidative browning of the apple, increased its firmness, and decreased the growth of psychotropic microorganisms, molds, and yeasts. Total and thermotolerant coliforms *Escherichia coli* and *Salmonella* sp. were also absent with the use of the preservative solution containing xanthan (Freitas et al. 2013).

- (ii) Gellan is a relatively new gum that is extracted from the culture of *Sphingomonas elodea*. There are two types of gellan: high acyl and low acyl. Both form very transparent gels when mixed with calcium ions at very low dosage in water. The gel of high-acyl gellan is soft, elastic, and nonbrittle, while that of low-acyl gellan is harder, less elastic, and more brittle. Combining the two gels using different ratios yields gels of various structures. Some food manufacturers use gellan in the colored beads that float in fancy beverages. The addition of hydrocolloids improves the quality of drinks; for example, for reduced-calorie carrot juice, adding 0.3 % gellan gum greatly enhances the stability of the juice cloud during storage (Sinchaipanit et al. 2013). A study concluded that a mixture of 0.2 % gellan and 8 % modified starch was the best replacement for gelatin in puddings in terms of gel strength, water absorption range, and elasticity (Wu et al. 2013).

Cellulose Derivatives

Cellulose is fiber that is present in all plants. Similar to starch, it is composed of a long chain of anhydroglucose units (AGU) that are bonded with β -1,4 linkages, which cannot be hydrolyzed by digestive enzymes like amylase. In the food industry, cellulose is converted to different kinds of gums.

- (i) Microcrystalline cellulose (MCC) is conventionally used for anticaking in cheese. To produce MCC, which is also known as cellulose gel, a caustic soda is used to swell the selected soft wood pulp, which is then spray-dried to obtain fine microsized crystals. Although insoluble in water, these crystals are porous and are able to absorb and attach an ample amount of contact water after vigorous shearing. Therefore, they can be used as a carrier of oil or water to transform some foods from paste to solid form. After taking up water, the MCC crystals become gel-like and can be used in a wide variety of health or low-fat foods because they mimic fat. Commercially, MCC at 85–90 % is co-compressed with 10–15 % of other gums like guar, carboxymethyl cellulose, or maltodextrin so that it can take up water faster.

- (ii) Sodium carboxymethyl cellulose (CMC) is the sodium salt of the polycarboxymethyl ester of cellulose, the gum most widely used by food manufacturers because it is inexpensive. CMC is obtained by treating the hydroxyl groups of softwood cellulose with caustic soda, followed by reaction with sodium chloroacetate. Commercial CMC, also known as cellulose gum, has many viscosity grades. It does not form a gel, but its low cost and wide range of viscosities enable manufacturers to use it in many kinds of foods that need increased viscosity, including bakery dough, yogurt, ice cream, beverages, soups, sauces, and dressings. Unlike other hydrocolloids which are natural, CMC is considered an artificial gum.
- (iii) Methyl cellulose (MC) and hydroxypropylmethyl cellulose (HPMC) are also artificial gums. MC is produced by the reaction of methyl chloride with caustic-treated wood cellulose. To prepare HPMC, there is an additional reaction with propylene oxide and then the by-product methoxy groups are removed as impurities. Aqueous solutions of these two hydrocolloids do not form a gel at low temperature, but gelation occurs upon heating; the HPMC solution has a higher viscosity. MC and HPMC are used to stabilize foods, in particular, batter coating. Recently, studies have compared the effect of HPMC when other hydrocolloids were added to various foodstuffs. In developing an onion powder-containing pasta, the inclusion of HPMC was shown to yield better product quality and sensory characteristics by increasing the shear value of the pasta dough (Rajeswari et al. 2013). To prepare sweet potato starch noodles with a high content of broccoli powder, several hydrocolloids with distinct water-binding capacity were tested to control rheology and structure. HPMC and xanthan were the most effective because they were more efficient in controlling the degree of swelling of the vegetable particles and starch granules in the starch noodles (Silva et al. 2013). The addition of hydrocolloids is essential for gluten-free dough and bread because as polymeric substances they mimic the viscoelasticity of gluten and increase the gas-retaining ability of the dough. A study showed that adding selected hydrocolloids at 1–1.5 % to dough led to increased loaf volume, superior color, and prolonged shelf life; bread containing 1.5 % HPMC was preferred by a trained panel (Sabanis and Tzia 2011). Hydrocolloids are effective in reducing oil uptake in fried goods. Among the hydrocolloids tested, HPMC demonstrated the best moisture retention and oil uptake in French fries, which were prepared from potatoes after pretreatment with water, calcium chloride, and citric acid, and then coated with different hydrocolloids (Pahade and Sakhale 2012).

Conclusion and Future Directions

Foods are meant to be eaten. They come into contact with a large number of sensory cells in the mouth before being swallowed into the body via the digestive tract. Foods are for living, and quality foods are for quality living. Quality foods must be

nutritious and palatable so that people can enjoy them, especially at that initial contact in the month.

Foods provide energy for the activities and metabolism of the body. The source of all kinds of energy on earth is the sun, and all kinds of foods, whether produced from plants, animals, microorganisms, or minerals, originate from the surface of the earth. The earth transforms the energy of the sun into food form for people to enjoy. All native crops and livestock can be considered a primary form that resulted from the intimate interaction between the sun and earth. They are later transformed into many varieties of food for human beings to enjoy. The colorful, nutritious, and tasty foods are works of art created by nature, our father sun and mother earth. All creatures, including human beings, depend on food to survive. Today, food processors transform native foods into other foods for modern man's enjoyment. Unlike our ancestors who lived in the wilderness, we dwell in communities where the area where primary foods are produced is far away. Processed foods are necessary.

Processors break down primary foods into different portions of their components and then recombine the selected components to structure them into secondary foods, which go to the markets and eventually consumers' plates and palates. People want to live quality life. In addition to the necessities to sustain life, people need enjoyment, and that includes eating. Eating has now become an art; therefore, food processing is also an art, and food processors are actually artists. These artists use various parts of primary foods as building blocks and combine them with colorants, acidulants, sweeteners, flavoring materials, emulsifiers, starches, and hydrocolloids, which are for combining with nutrients, preservatives and processing aids, to satisfy palatal desires and nutritional needs.

Quality foods are created by combining the best available components. Today, people are enjoying delicious foods at home and in restaurants. They should appreciate the artists who create the wonderful secondary foods. In addition, these artists are expected to create much more, so we can continue to lead quality lives.

From all perspectives, nature's art is the most beautiful and perfect. What will our secondary artists do then? Learn and discover more from nature. Reflect nature's beauty in developing our food. Yes, there have been many wonders in natural foodstuffs, particularly the ingredients that have been discovered by scientists. However, there is much more to be discovered and used, like natural colors, natural stabilizers, and natural flavoring substances. It is the mission of all food scientists to create the most wonderful foods, in harmony with nature.

Cross-References

- ▶ [Chemical Composition of Bakery Products](#)
- ▶ [Chemical Composition of Beverages and Drinks](#)
- ▶ [Chemical Composition of Sugar and Confectionery Products](#)

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Part II

Principles of Chemical Analysis of Food Components

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Abstract

Analysis of foods is requesting the development of more durable, influential, fragile, and cost-effective analytical methodologies. Also food analysis guarantees the safety, quality, and traceability of foods in coherence with law and consumers' demands. The old methods used at the beginning of the twentieth century called as "wet chemistry" have improved the current powerful instrumental techniques used in food laboratories. In addition to modern analytical instrumentation, wet chemical analyses are offered. Often, modern instrumentation cannot determine results which many specific wet chemical tests provide. Wet chemistry includes basic experimentation techniques such as measuring, mixing, and weighing chemicals,

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conductivity, density, pH, specific gravity, temperature, viscosity, and other aspects of liquids. Wet chemistry is usually qualitative. Qualitative means to determine the presence of a specific chemical rather than the exact amount. Some quantitative techniques are used in wet chemistry, and they occur as gravimetric (weighing) and volumetric analysis (measuring).

Introduction

Chemistry is the study of matter, including its composition and structure, its physical properties, and its reactivity. Chemistry is divided into five fields: organic chemistry, inorganic chemistry, biochemistry, physical chemistry, and analytical chemistry.

Analytical chemistry is the study of the separation, identification, and quantification of the chemical components. Analytical chemistry is the science of obtaining, processing, and communicating information about the composition and structure of matter and is used for determining the chemical composition of samples of matter. Analytical chemistry is responsible for characterizing the composition of matter, both qualitatively and quantitatively.

Analytical methods can be classified as classical and instrumental. Classical methods are also known as wet chemistry methods and are a term used to refer to chemistry generally done in the liquid phase, which is also called as bench chemistry. However, bench chemistry and wet chemistry terms differ in two primary ways: first term can involve dry chemicals, while second term always involves at least one substance in the liquid phase. Moreover, wet chemistry involves high-tech equipment, while bench chemistry only includes techniques that use simple devices. Both types of chemistry use many of the same techniques and equipment.

Wet chemistry is sometimes considered as an old-fashion chemical science and includes precipitation, extraction, distillation, and qualitative analysis by color, melting point, etc. Traditionally, laboratory glasswares (beakers, flasks, etc.) are used, and it excludes quantitative chemical analysis using instrumentation. Wet chemistry methods are achieved by measurement of weight or volume such as gravimetry and titrimetry. Besides, wet chemistry techniques can be used for qualitative measurements. Some uses for wet chemistry include tests for pH, moisture, fat characterization (refractive index, melting point, etc.), protein analysis, fat analysis (Goldfish, Soxhlet, etc.), hardness of water, etc.

Compositional Analysis of Foods

Moisture Analysis

Water occurs as the main constituent of some raw products. The determination of the water (moisture analysis) is an important step of many industrial and scientific disciplines.

The term of total water content of food refers to “free” and “bound” water, equilibrium moisture content, moisture adsorption, moisture desorption, etc. The most important term is bound water. Bound water was defined by many researchers as the water which remains in an unchanged form when the food is subjected to a particular treatment (Karel 1975).

Various methods for moisture analysis are described according to their principles, procedures, applications, cautions, advantages, and disadvantages. The methods applied can be classified into two groups: direct or indirect methods. In direct methods, the moisture is normally removed from the material by oven-drying, desiccation, distillation, extraction, and other physicochemical techniques. Also, in indirect methods, the moisture is not removed from material. Oven-drying methods, distillation methods, chemical methods, and physical methods are used for determination of the amount of moisture in the sample as wet chemistry methods.

In oven-drying methods, the loss of weight is used to calculate the moisture content of the sample. The methods are simple. The time required may be from a few minutes to over 24 h. These methods are approved for determining the amount of moisture in many food products as air oven, vacuum oven, microwave drying, infrared drying, rapid moisture analyzer, etc. (Bradley 2010).

Air oven-drying method: Approximately 2–10 g, depending on solid content, is weighed into a metallic flat, previously dried at 90–100°, and weighed. Sample is dried in an air oven, with vents open, for 2–3 h at 98–100°. Water is removed and cooled in a desiccator; it is weighed after it reaches room temperature and returned to oven and redried for 1 h and weighed. Process is repeated until change in initial and final weight difference is 2 mg. The loss of weight is calculated as percent moisture (Hart and Fisher 1971). Lipid oxidation and a resulting sample weight gain can occur at high temperatures in an air draft oven. If samples have the amount of high carbohydrates, they should be dried in a vacuum oven at a temperature no higher than 70 °C. Reduced pressure (typically 25–100 mmHg) is used in vacuum oven. Their dried mass is determined within a 3–6-h drying time (Marwaha 2010).

In microwave drying, a sample is placed between the two pads, and then pads are centered on the pedestal, and weighed against the tare weight. Time for the drying operation is set by the operator, and “start” is activated. The microprocessor controls the drying procedure, with percentage moisture indicated in the controller window (Bradley 2010).

Distillation methods involve measuring the amount of water removed from a food sample by evaporation. Distillation methods cause less thermal decomposition of some foods. Distillation methods are best illustrated by the Dean and Stark method. A known weight of food is placed in a flask with an organic solvent such as xylene or toluene. The flask is attached to a condenser and the mixture is heated. The water in the sample moves up into the condenser and converts back into liquid water and then gathers in the graduated tube. Distillation is stopped when the amount of collected water is stable, and the volume of water is read (Marwaha 2010).

The Karl Fischer titration is a chemical method, which is quite rapid and accurate. This method is based on the fundamental reaction involving the reduction

of iodine by SO_2 in the presence of water (Bradley 2010). The Karl Fischer method is preferable for products with very low levels of moisture. The volume used in the titration allows for the calculation of moisture content. The method uses no heat and is rapid and sensitive but is subject to error from interfering food constituents (Nielsen 2007).

Density, electrical conductivity, or refractive index is used with physical methods to determine the moisture content of foods. The electrical properties of water are determined by the dielectric method and measured according to the change in capacitance or resistance. Hydrometry is used for measuring specific gravity or density. Pycnometers are also commonly used for routine testing of moisture. The moisture of beverages, salt brines, and sugar solutions are measured by pycnometers. Moisture in liquid sugar products and condensed milks can be determined using a refractometer. When water is added to a food product, many of the physical constants are altered. Some properties such as vapor pressure, freezing point, boiling point, and osmotic pressure of solutions depend on the number of solute particles as ions or molecules present. Measurement of any of these properties can be used to determine the concentration of solutes in a solution (Bradley 2010).

The amount of water alone is not a reliable indicator of food stability. A relationship exists between the water content of food and its perishability. Various types of food with the same water content differ significantly in perishability. The term “water activity” (A_w) was developed to account for the intensity with which water associates with various nonaqueous constituents. There are various techniques to measure A_w . A commonly used approach relies on measuring the amount of moisture in the equilibrated headspace above a sample of the food product, which correlates directly with sample a_w (Fennema 1996).

Ash Analysis

Ash means the inorganic residue that is remained after either ignition or complete oxidation of organic matter in a foodstuff. There are two major types of ashing: dry ashing and wet ashing.

Dry ashing refers to the use of a muffle furnace. Temperatures of 500–600 °C are used. Therefore, water and volatiles are vaporized, and organic substances are burned, and most minerals are converted to oxides, sulfates, phosphates, chlorides, and silicates. Some (Fe, Se, Pb, and Hg) of elements may partially volatilize with this procedure. If ashing is a preliminary analysis, other methods can be used. Acids and oxidizing agents or their combinations are used for wet ashing. Minerals are solubilized without volatilization. Wet ashing often is preferable to dry ashing.

Plant material with 15 % or less moisture may be ashed without prior drying. Animal products, syrups, and spices require treatments prior to ashing because of high fat, moisture (spattering, swelling), or high sugar content (foaming). So, they need to be evaporated to dryness or added one or two drops of olive oil (Marshall 2010).

AOAC 923.03

Ash of Flour

Weigh well-mixed sample into shallow, relatively broad ashing dish that has been ignited, cooled in desiccator, and weighed soon after reaching room temperature. Ignite in furnace at ca. 550 °C until light gray ash results or to constant weight. Cool in desiccator and weigh soon after reaching room temperature AOAC (1990f).

AOAC 935.13

Calcium in Animal Feed

Wet Ash Method

Weigh sample into Kjeldahl flask. Add a little HNO₃ and boil gently for a couple of minutes to oxidize all easily oxidizable matter. Cool solution somewhat and add 70–72 % HClO₄. Boil until solution is colorless, so dense white fumes appear. Use particular care not to boil to dryness at any time. Cool slightly, add H₂O, and boil to drive out any remaining NO₂ fumes. Cool, dilute, filter into volume, flask, dilute to volume, and mix thoroughly. Some suitable aliquot may be taken and titrated with 0.1 N KMnO₄ AOAC (1990g).

Total ash and acid-insoluble ash contents are important indices to illustrate the quality and purity of sample. Total ash includes “physiological ash,” which is derived from the plant tissue itself, and “nonphysiological ash,” which is often from environmental contaminations such as sand and soil. Total ash content alone is not sufficient to reflect the quality of sample, since the plant materials often contain considerable levels of physiological ash, calcium oxalate in particular (Rao and Xiang 2009).

Fat Analysis

Fat determination is one of the key analyses used for food labeling and quality control. Lipids are insoluble in water and soluble in organic solvents such as hexane, diethyl ether, or chloroform. Lipids are determined as fatty acids and their derivatives. Sterols, tocopherols, and carotenoids are also common components of lipid extracts (Moreau and Winkler-Moser 2010; O’Keefe 2008; Shahidi and Wanasundara, 2008).

The terms lipids, fats, and oils are often used interchangeably. The term “lipid” commonly refers to the broad, total collection of food molecules, whereas fats generally are determined as those lipids that are solid at room temperature and oils are also determined as those lipids that are liquid at room temperature (Van Camp and Dierckx 2004).

Foods have different types of the lipid compounds (e.g., simple lipids (fats, waxes, etc.), compound lipids (phospholipids, cerebrosides, sphingolipids), and derived lipids) (Verkade 1985).

The total lipid content of foods is commonly determined by organic solvent extraction methods, which can be classified as continuous (e.g., Goldfish), semicontinuous (e.g., Soxhlet), discontinuous (e.g., Mojonnier, Folch), and nonsolvent wet extraction methods (e.g., Babcock or Gerber). These methods are changeable for certain types of food products.

Continuous solvent extraction (Goldfish) is designed according to the methods prescribed in the Association of Official Analytical Chemists (AOAC) or other technical methods involved with the use of a continuous extractor. In this method, solvent from a boiling flask continuously flows over the sample held in a ceramic thimble. Fat content is measured by weight loss of the sample or by weight of the fat removed. The continuous methods give faster and more efficient extraction than semicontinuous extraction methods (Lai and Varriano-Marston 1980).

The Soxhlet method is an example of the semicontinuous extraction method. In this method, the solvent builds up in the extraction chamber and completely surrounds the sample and then siphons back to the boiling flask. Fat content is measured by weight loss of the sample or by weight of the fat removed. However, this method requires more time than the continuous method (Kumoroa et al. 2009).

Discontinuous solvent extraction methods are known as Mojonnier method and chloroform–methanol procedure. In Mojonnier method, fat is extracted with a mixture of ethyl ether and petroleum ether in a Mojonnier flask, and the extracted fat is dried to a constant weight and expressed as percent fat by weight. This method does not require removal of moisture from the sample. It can be applied to both liquid and solid samples (Bligh and Dyer 1959). Chloroform–methanol procedure is called as Folch extraction. The chloroform–methanol extraction procedure is rapid and well suited to low-fat samples and can be used to generate lipid samples for subsequent fatty acid compositional analysis. The procedure has been more applied to basic commodities, rather than to finished product samples (Folch et al. 1957).

Nonsolvent wet extraction methods are known as Babcock method for milk fat and Gerber method for milk fat. In the Babcock method, the fat is measured volumetrically, but the result is expressed as percent fat by weight. Gerber method is similar to that of the Babcock method. The sulfuric acid digests proteins and carbohydrates, releases fat, and maintains the fat in a liquid state by generating heat (Bligh and Dyer 1959).

There is no single standard method for the determination of fats in different foods. To measure total fat, various methods have been used (Table 1).

AOAC 963.15

Fat in Cacao Products

Soxhlet Extraction Method

Chill sweet or bitter chocolate until hard, and grate or shave to fine granular condition. Mix thoroughly and preserve in tightly stopped bottle in cool place. Accurately weigh chocolate liquor into beaker. Add slowly, while stirring, boiling H₂O to give homogenous suspension. Add HCl and few defatted SiC chips or other

Table 1 Various methods for measuring total fat

Description	Solvent	Apparatus	Method
Fat in raw milk	None, acid hydrolysis	Babcock milk test bottle	AOAC 989.04 (1990k)
Fat in baked products and pet foods	Acid hydrolysis and ethyl ether extraction	Mojonnier	AOAC 954.02 (1990i)
Crude fat in food	Anhydrous ethyl ether	Soxhlet	AOAC 920.39 (1990h)

antibumping agent, and stir. Cover with watch glass, bring slowly to boil, and boil gently for 15 min. Rinse watch glass with H₂O. Continue washing until last portion of filtrate is Cl⁻-free as determined by addition of AgNO₃. Transfer wet paper and sample to defatted extraction thimble, and dry for 6–18 h in small beaker at 100 °C. Place glass wool plug over paper.

Add few defatted antibumping chips to Erlenmeyer, and dry for 1 h at 100 °C. Cool to room temperature in desiccator and weigh. Place thimble concentrating dried sample in Soxhlet, supporting it with spiral or glass beads. Rinse digestion beaker, drying beaker, and watch glass with three petroleum ethers and add washing to thimble. Reflux digested sample for 4 h, adjusting heat so that extractor siphons ≥ 30 times. Remove flask, and evaporate solvent on steam bath. Dry flask at 100–101 °C to constant weight AOAC (1990j).

Protein Analysis

Proteins are an abundant component in animal and human cells. Proteins are naturally constituted by 20 amino acids, so protein quality strongly depends on its amino acid content. They are composed of elements including hydrogen, carbon, nitrogen, oxygen, and sulfur (Aristoy and Toldr 2004).

Numerous methods have been developed to measure protein content. The basic principles of these methods include the determinations of nitrogen, peptide bonds, aromatic amino acids, dye-binding capacity, ultraviolet absorptivity of proteins, and light-scattering properties.

In the Kjeldahl procedure, chemical and instrumental methods for determination of nitrogen and protein are reviewed for their mode of action and utility in analysis of proteins and products. Despite the fact that the Kjeldahl method is satisfactory for determining total nitrogen, it is imprecise for determining total protein content. Presence of variable amounts of nonprotein nitrogenous components produces error if the formula ($N \times 6.25$) is used to calculate crude protein (Benedict 1987). Proteins and other organic food components in a sample are digested with sulfuric acid in the presence of catalysts (AOAC 920.152) AOAC (1990r). The total organic nitrogen is converted to ammonium sulfate. The digest is neutralized with alkali and distilled into a boric acid solution. The borate anions formed are titrated with standardized acid, which is converted to nitrogen in the sample. The result of the analysis represents the crude protein content of the food since nitrogen also comes from nonprotein components (Chang 2010).

In Dumas method, samples are combusted at high temperatures (800–1,000 °C) with a flow of pure oxygen. All carbon in the sample is converted to carbon dioxide during the flash combustion. Nitrogen-containing components produced include N₂ and nitrogen oxides. The nitrogen oxides are reduced to nitrogen in a copper reduction column at a high temperature (600 °C). The Dumas method requires less than 5 min per sample, can be semiautomated, avoids the use of corrosive and hazardous chemicals, and is a relatively safe procedure (Jung et al. 2003). Ultrahigh-purity acetanilide and EDTA (ethylenediamine tetraacetate) may be used as the standards for the calibration of the nitrogen analyzer. The nitrogen determined is converted to protein content in the sample using a protein conversion factor (AOAC 968.06) AOAC (1990q).

Carbohydrate Analysis

Total carbohydrate content is of interest for nutrition-labeling purposes as well as carbohydrates are important in foods as a major source of energy. Digestible carbohydrates, which are converted into monosaccharides, provide metabolic energy. Nondigestible polysaccharides comprise the major portion of dietary fiber. Carbohydrates also contribute other attributes, including bulk, body, viscosity, stability to emulsions and foams, water-holding capacity, freeze–thaw stability, browning, flavors, aromas, and a range of desirable textures (from crispness to smooth, soft gels). They also provide satiety.

The “**total carbohydrate**” content of a food must be calculated by subtraction of the sums of the weights of crude protein, total fat, moisture, and ash from the total weight of the food in a serving (Nielsen 2007).

Carbohydrates are classified as mono- and oligosaccharides, starch, and total dietary fiber. At least 90 % of the carbohydrate is in the form of polysaccharides. All other polysaccharides except starch are nondigestible. Nondigestible polysaccharides can be divided into soluble and insoluble classes. Along with lignin and other nondigestible polysaccharides occur dietary fiber (Köksel 2005).

The Trowell definition (Trowell 1974; Trowell et al. 1976) became the basis definition for the first official AOAC International method issued in 1985 as AOAC 985.29 AOAC (1990u).

Mono- and oligosaccharides consist of glucose, fructose, sucrose, lactose, maltose, raffinose, or stachyose. Samples may require extraction before analysis. Extraction is used to remove interfering substances from samples prior to analysis for mono- and oligosaccharides (Nielsen 2007).

AOAC 896.02

Sucrose in Sugars and Syrup

Weigh sample and dilute. Also weigh sample and dilute this second solution. Filter both solutions, and obtain direct polariscopic readings. Invert each solution and obtain invert readings.

True direct polarization of sample four times direct polarization of diluted solution minus direct polarization of undiluted solution AOAC (1990d).

AOAC 923.09

Invert Sugar in Sugars and Syrup

Lane–Eynon Method

This method is called as Lane–Eynon method and used for measuring invert sugar. This is often the most accurate method for the estimation of reducing sugars. It is required to reduce completely a known volume of alkaline copper reagent. The end point is indicated by the use of an internal indicator, methylene blue AOAC (1990n).

AOAC 955.36

Invert Sugar in Sugars and Syrup

Berlin Institute Method

Select amount of sample containing invert sugar. Pipet Müller solution and sugar solution into flask and cover. Mix and heat exactly 10 min in H₂O bath so vigorously that immersion of flask does not interrupt boiling. Place flask. After heating period, cool flask rapidly without agitation. Add acetic acid to cooled solution, mix, and immediately excess 0.0333 N I solution from buret. After all Cu₂O precipitate dissolves, titrate excess I with 0.0333 N Na₂S₂O₃ AOAC (1990b).

AOAC 968.28

Total Sugars in Molasses as Invert Sugar

Lane–Eynon Constant Volume Volumetric Method

Fill buret with working standard solution containing 5 mg invert sugar/ml.

Accurately pipet each Soxhlet solution and mix and add 30 ml H₂O. Add from buret almost all standard working solution necessary to reduce the Cu. Add few boiling chips. Place cold mixture on heater, regulate heat so that boiling will begin in approximately 3 min, and maintain at moderate boil exactly 2 min, reducing heat, if necessary, to prevent bumping. Without removing flask from heater, add 1 % aqueous methylene blue solution and complete titration. Maintain continuous evolution of steam to prevent reoxidation by air. Repeat standardization several times. Factor F is average number ml standard sugar solution required to completely reduce 20 ml. use average of ≥ 3 titrations.

Pipet filtrate and add 5 ml HCl. Let it stand for 24 h at 20–25 °C or 10 h at >25 °C. Add few drops phenolphthalein and neutralize with 20 % NaOH solution. Add few drops 0.5 N HCl until red disappears. Dilute to volume with H₂O and mix well.

Determine approximate sugar content of sample as follows: Accurately pipet each Soxhlet solution and mix and add aliquot inverted sample solution. Add 40 ml H₂O so that volume H₂O plus volume sample solution is 50 ml. Mix without heating by swirling. Add few boiling chips. Place flask on heater, regulate heat so that boiling begins in approximately 3 min. After liquid boils 10–15 s, observe change in color of solution. If blue color persists, add working standard sugar solution 0.5–1.0 ml at time, with few second actual boiling after each addition until unsafe to add more without risk of passing end point. Add drops methylene blue solution and continue adding sugar solution, approximately 1 ml at time, at intervals of approximately 10 s, until indicator is completely decolorized. Calculation approximates percent invert sugar in sample.

Pipet each of Soxhlet solution mix, add aliquot inverted sample solution as indicated. Add ml H₂O specified in table so that volume H₂O plus volume solution is 50 ml, and mix without heating by swirling. Add few boiling chips. Place flask on heater, regulate heat so that boiling begins in approximately 3 min, and during boiling, rapidly add working standard invert sugar solution from buret, so that 0.5–1.0 ml is required to complete titration AOAC (1990v).

The starch content of a food is best determined by a combination of enzymes. Firstly, starch is converted to D-glucose and then D-glucose is measured as enzymatically. Dietary fiber constituents are cellulose, lignin, hemicellulose, pectins, and hydrocolloids (Nielsen 2007).

AOAC 985.29

Total Dietary Fiber in Food

Enzymatic–Gravimetric Method

Total dietary fiber should be determined on dried, low-fat, or fat-free sample. Homogenize sample and dry overnight in 70 °C vacuum oven. Cool in desiccator, reweigh, and record weight loss due to drying. Dry-mill portion of dried sample mesh. If sample cannot be heated, freeze-dry before milling. If high fat content (>10 %) prevents proper milling, defatting with petroleum ether three times with 25 ml portions (per g of sample) can be applied before milling. When analyzing mixed diets, always extract fat before determining total dietary fiber. Record weight loss due to fat. Correct final percent dietary fiber determination for both moisture and fat removed. Store dry-milled sample in capped jar in desiccator until analysis is run.

Run blank through entire procedure along with samples to measure any contribution from reagents to residue. Weighing duplicate sample should differ by less than 20 mg from each other. Add 50 ml phosphate buffer (pH 6.0) to each beaker and check pH with pH meter. Adjust if pH does not equal 6.0 ± 0.1 . Add heat-stable α -amylase solution. Cover beaker with aluminum foil and place in boiling water bath for 15 min. Cool solutions to room temperature. Adjust to $\text{pH } 7.5 \pm 0.1$. Check pH with pH meter. Add protease solution. Cover beaker with aluminum foil and incubate

at 60 °C with continuous agitation for 30 min. Cool and adjust pH to 4.5 ± 0.2 . Check pH with pH meter. Add amyloglucosidase, cover with aluminum foil, and incubate for 30 min at 60 °C with continuous agitation. Add 95 % EtOH (ethanol) preheated to 60 °C. Let precipitate form at room temperature for 60 min. Weigh crucible containing Celite, then wet by using stream of 78 % EtOH from wash bottle. Apply suction to draw Celite onto fritted glass as even mat. Wash residue successively with three 78 % EtOH, two 95 % EtOH, and two acetone. Dry crucible containing residue overnight in 70 °C vacuum oven or 105 °C air oven. Cool in desiccator. Subtract crucible and Celite weights to determine weight of residue. Analyze residue from one sample of set of duplicates for protein by using N x 6.25 as conversion factor. Incinerate second residue sample of duplicate for 5 h at 525 °C. Cool in desiccator and weigh to determine ash AOAC (1990u).

This method is based on the enzymatic removal of starch and protein of the samples by amylase and protease at 90 °C and 60 °C, respectively. Then, insoluble dietary fibers (IDF) are separated by filtration and followed soluble dietary fibers precipitated by 78 % ethanol and collected by filtration. These fiber fractions are dried and weighed. The sum of them gives the total dietary fiber content of the sample. For chemically defined dietary fibers (fructans, galactooligosaccharides, pectin, resistant starch, resistant maltodextrins), special methods were devised for measurement in different matrices. However, the major problems of these methods are digestion of starch and protein at nonphysiological temperatures, partial hydrolysis of resistant starch, and lack of detection of low molecular weight dietary fiber (Hollmann et al. 2013). These problems were resolved by AOAC method 2009.01. In this method, at the beginning, the sample is incubated with α -amylase at 37 °C, then protein is digested at 60 °C by protease, and, as the third step, insoluble and high-molecular-weight soluble dietary fibers are precipitated at 78 % ethanol and finally determined gravimetrically. Nondigestible oligosaccharides (NDO) are measured in the ethanol filtrate by HPLC. The amount of NDO is calculated from the area under the curve. These nondigestible oligosaccharides which have presumably positive health effects are not measured by AOAC method 985.29 (Mc Cleary et al. 2012). In the AOAC2011.25, in addition to IDF, SDF, and TDF, resistant starch (RS), alcohol-H₂O-soluble nondigestible oligosaccharides, and polysaccharides of DP > 3 are also determined (Yang et al. 2014).

Vitamin Analysis

Vitamins are organic compounds, and they have been in the food in trace amounts. The vitamins contain vitamin D, vitamin E, vitamin A, and vitamin K which are fat-soluble vitamins and thiamin (vitamin B₁), riboflavin (vitamin B₂), pantothenic acid, folate (folic acid), vitamin B₁₂ (cyanocobalamin), biotin, vitamin B₆, niacin, and vitamin C (ascorbic acid) which are water-soluble vitamins (Colakoglu and Ötleş 1985).

Vitamins are generally defined by using spectrophotometric methods and high-performance liquid chromatography (HPLC). Only vitamin C is determined by titrimetric method.

AOAC Method 967.21

2,6-Dichloroindophenol Titrimetric Method

In the presence of significant amounts of ferrous (Fe), cuprous (Cu), and stannous (Sn) ions in the biological matrix to be analyzed, it is advisable to include a chelating agent such as ethylenediaminetetraacetic acid (EDTA) with the extraction to avoid overestimation of the ascorbic acid content. The light but distinct rose-pink end point should last more than 5 s to be valid AOAC (1990e).

Mineral Analysis

Most minerals are combinations of elements, while some minerals are elements by themselves. The mineral compounds in food are K, Mg, Ca, Cu, Zn, Fe, Na, Mn, etc. This chapter describes traditional methods for analysis of minerals involving titrimetric procedures.

AOAC 973.52

Hardness of Water

Water hardness is determined by EDTA complexometric titration of the total of calcium and magnesium. Dilute sample with H₂O in porcelain casserole, add some drop buffer solution, NaCN, and indicator powder, and titrate with EDTA standard solution slowly, with continuous stirring, until last reddish tinge disappears, adding last few drops at 3–5 s. Color at end point is blue in daylight and under daylight fluorescent lamp. Complete titration within 5 min from time of buffer addition AOAC (1990i).

AOAC 920.195

Silica in Water

Sample is used in order to determine approximately amount of Ca and Mg present.

Amount of sample equivalent evaporate to CaO or Mg₂P₂O₇. Acidify sample with HCl and evaporate on steam bath to dryness in dish. Drying continues approximately for 1 h. Add enough H₂O to bring solvent salts into solution. Heat on steam bath until salts dissolve. Filter to remove most of SiO₂ and wash with hot H₂O. Filtrate is evaporated to dryness and treated residue with 5 ml HCl and enough H₂O to dissolve solution salt, as before. Heat, filter, and wash with hot H₂O. The two residues to crucible are transferred, ignited, heated over blast lamp, and weighed. Contents of crucible with few drops H₂O are moistened, few drops H₂SO₄ are added and evaporated and volatilized. Dry carefully on hot plate,

ignite, heat over blast lamp, and weigh. Difference between the two weights is the weight of SiO_2 . Add weight residue to that of Al_2O_3 and Fe_2O_3 obtained AOAC (1990t).

AOAC Method 960.29

Salt in Butter

Salt in foods may be estimated by titrating the chloride ion with silver. The orange end point in this reaction occurs only when all chloride ion is complexed, resulting in an excess of silver to form the colored silver chromate. The end point of this reaction is therefore at the first hint of an orange color. When preparing reagents for this assay, use boiled water to avoid interferences from carbonates in the water.

Chemical Properties and Characteristics of Foods

pH and Titratable Acidity

There are two methods in food analysis for dealing with acidity: pH and titratable acidity. Titratable acidity is determined as measurement of the total acid concentration within a food. pH is also called active acidity. pH is measurement of H-ion activity and indicates acidity. It may be measured by determining electric potential between glass and reference electrodes, using commercially apparatus standardized against NIST primary standard pH buffers (AOAC 981.12) AOAC (1990p).

AOAC 975.124

Acidity Cheese

Titrimetric Method

To prepared sample, add H_2O at 40°C to volume of 105 ml, shake vigorously, and filter. Titrate 25 ml portion filtrate, representing 2.5 g sample, with standard NaOH, preferably 0.1 N, by using phenolphthalein. Express result as lactic acid. 1 ml 0.1 N NaOH = 0.0090 g lactic acid. Results may also be expressed as ml 0.1 N NaOH/100 g AOAC (1990a).

Fat Characterization

Numerous wet chemistry methods exist for the characterization of lipids, fats, and oils. First method is called as the refractive index (RI) method that is defined as the ratio of the speed of light in air (technically, a vacuum) to the speed of light in the oil (Cemeroğlu 2010). Second method is determined as melting point of fats. Melting points of fats are measured as relation to their physical properties (hardness and thermal behavior) (Nassu and Gonçalves 1999). Melting point is usually

defined as the point at which a material changes from a solid to a liquid, but natural fats do not have a true melting point. Pure compounds have sharp and well-defined melting points, but fats and oils are complex mixtures of compounds. For a melting point, one point within the melting range must be selected with a defined method. Only with rigid and specific definition of the conditions of the fat pretreatment and the test procedure can a melting point be determined. Finally, iodine value is measured for fat characterization. The iodine value is a chemical constant for a fat or oil. It is a valuable characteristic in fat analysis that measures unsaturation but does not define the specific fatty acids (O'Brien 2009).

AOAC 921.08

Index of Refraction of Oils and Fats

RI is related to the amount of saturation in a lipid. RI is also influenced by free fatty acid content, oxidation, and heating of the fat or oil. Samples are measured with a refractometer at 20 °C for oils and at specified higher temperatures for fats AOAC (1990m).

AOAC 920.156

Melting Point of Fats and Fatty Acids

Let melted and filtered fat fall 15–20 cm from dropping tube upon piece of ice or upon surface of cold Hg. Disks thus formed should be 1–1.5 cm in diameter and weighed. Remove disks when solid, and let them stand for 2–3 h to obtain normal melting point.

Alternatively, disks may be prepared using apparatus consisting of Al plate approximately 3 mm thick and 100 mm square with perforations approximately 10 mm in diameter and steel plate approximately 10 mm thick and 150 mm square. Chill steel plate in refrigerator and place Al plate on top. Pour melted and filtered fat into holes of Al plate, and let it stand in refrigerator for ≥ 2 h. Remove fat above surface of Al plate and remove disks.

Place 30×3.5 – 3.8 cm test tube, containing alcohol–H₂O mixture, in a tall 35×10 cm beaker containing ice and H₂O, and leave until mixture is cold. Drop disk of fat into tube. It will sink immediately to point where density of alcohol–H₂O mixture is exactly equivalent to its own. Lower accurate thermometer that can be read to 0.1 into test tube until bulb is just above disk. To secure even temperature in all parts of alcohol–H₂O mixture around disk, stir gently with thermometer. Slowly heat H₂O in beaker, constantly stirring with air steam or other suitable device.

When temperature of alcohol–H₂O mixture rises to approximately 6 °C below melting point of fat, disk of fat begins to shrivel and gradually rolls up into irregular mass. Lower thermometer until fat particle is even with center of bulb. Rotate thermometer bulb gently and so regulate heat that approximately 10 min is required for last 2 °C increase in temperature. As soon as fat mass becomes spherical, read thermometer. This is Wiley melting point. At this point, temperature of bath must

be ≤ 1.5 °C above melting point of sample. Conduct 2 additional determinations exactly as above. Second and third results should agree closely.

If edge of disk touches side of tube, make new determinations AOAC (1990o).

AOAC 920.158

Iodine Absorption Number of Oils and Fats

Hanus Method

Weigh fat or oil and dissolve in CHCl_3 . With pipet, add 25 ml Hanus solution, draining pipet in a definite time, and let it stand for 30 min in the dark, shaking occasionally.

Add 15 % KI solution, shake, and add freshly boiled and cooled H_2O , wash down any free on stopper. Titrate with standard 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$, adding it gradually with constant shaking, until yellow solution turns almost colorless. Add few drops starch indicator, and continue titration until blue entirely disappears. Toward end of titration, stopper bottle and shake violently, so that any remaining in solution in CHCl_3 may be taken up by KI solution AOAC (1990c).

Conclusion and Future Direction

Wet chemistry is sometimes considered as an old-fashion chemical science; however, the methods used for wet chemistry are preferred nowadays. Instruments are not necessary and determinations are made as both quantitative and qualitative. Wet chemical preparation is still the major time factor in an instrumental analytical procedure. However, wet chemistry steps have also been shortened by instrumentation.

Cross-References

► [Instrumental Food Analysis](#)

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Abstract

Sampling and sample preparation include the treatment of the theory, the methodology of sampling in all physical phases, and the theory of sample preparation for all major extraction techniques. The analytes in food sample are randomly distributed and variability. The analytical results depend on the type of sample and on the sample preparation techniques (handling, filtering of water, sieving of soils/sediments, drying, homogenizing). So, the highest-quality data are obtained as well as analytical techniques and methods offer excellent performance in achieving high sensitivity, selectivity, and identification capabilities for a wide range of contaminants.

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List of Abbreviations

AOAC	Association of Official Analytical Chemistry
ASE	Accelerated solvent extraction
FFF	Field-flow fractionation
GPC	Gel permeation extraction
IUPAC	The International Union of Pure and Applied Chemistry
LC-MS	Liquid chromatography-mass spectrometry
LLE	Liquid-liquid extraction
MAE	Microwave-assisted extraction
M-SPE	Miniaturized SPE
NMR	Nuclear magnetic resonance
PCBs	Polychlorinated biphenyls
PCDD/Fs	Polychlorinated dibenzo-dioxins/furans
SBSE	Stir bar sorptive extraction
SFE	Supercritical fluid extraction
SHE	Static headspace extraction
SM-SPE	Semimicro SPE
SPE	Solid phase extraction
SPLE	Combined extraction and cleanup in selective pressurized liquid extraction
SPME	Solid phase microextraction

Introduction

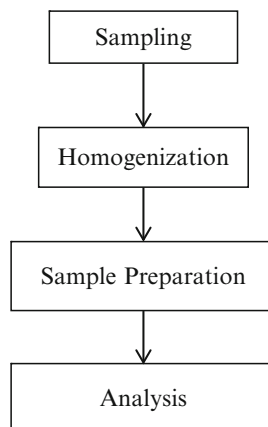
Food is a complex heterogeneous mixture of chemical substances. The isolation and measurement of individual chemical compounds in food is usually difficult task. Procedures for preparation of the sample should be developed, evaluated, and published as an integral part of any analytical method (Lichon 2004).

If the sample is not prepared properly for analysis, or if the components become altered during preparation, the results will be inaccurate regardless of the effort, the precision of the apparatus, and the techniques used in the analysis. The aim of sample preparation is to mix a large sample in the laboratory. Moreover, representative piece of material is extracted from a larger amount (Pomeranz and Meloan 1994).

There are three steps in sample preparation for chemical analysis of foods: sampling, homogenization, and sample preparation (Fig. 1). The aim of each of the three steps is to increase the accuracy and precision of the analysis. At the same time, each step introduces inherent errors (Lichon 2004).

Sample preparation includes some steps. These steps depend on the sample, the matrix, and the concentration level at which the analysis needs to be carried out. After the sample preparation is complete, the analysis can be carried out.

Fig. 1 Steps in a measurement process



Sampling

There is a great attention over the validity of analytical methods. The precision and accuracy of methods include measures such as international collaborative trials. However, the issue of sampling is not examined (Lichon 2004).

The International Union of Pure and Applied Chemistry (IUPAC) defines a sampling plan as: “A predetermined procedure for the selection, withdrawal, preservation, transportation, and preparation of the portions to be removed from a lot as samples” (IUPAC 1997). Sampling purposes vary widely among different food industries; however, the most important categories include nutritional labeling; detection of contaminants and foreign matter; statistical process control (quality assurance); acceptance of raw materials, ingredients, or products; release of lots of finished product; detection of adulterations; microbiological safety; authenticity of food ingredients; etc. (Morawicki 2010). Sampling can often be the greatest source of error in chemical analysis. When the samples cannot provide the required information, they are less worth the time and expense of analysis. Correct planning for sampling must be an integral part of any study (Lichon 2004).

Factors affecting the choice of sampling plans are classified as the purpose of the inspection, the nature of the product, the test method, and the nature of the population (Puri et al. 1979). Sampling plans are designed for examination of either attributes or variables. While, in attribute sampling, sampling is performed to decide on the acceptability of a population based on whether the sample possesses a certain characteristic or not, in variable sampling, it is applied to estimate quantitatively the amount of a substance or a characteristic on a continuous scale (Horwitz 1988).

The sample selected for analysis must be truly representative of the entire lot of food to be analyzed because the amount of substance actually used in quantitative

analysis is comparatively small. The composition of natural foods is variable. The sugar, acid, and water content of fruits and vegetables are varied from a wide range, depending on variety, the amount of sunlight during the growing period, the soil and climatic conditions under which they are grown, the stage of maturity, and the length and conditions of storage. Meats vary depending fat content. Fish vary according to season. The carbohydrate, fat, and protein content of foods of vegetable origin is more constant than the content of the essential mineral elements. Although the total ash content may be fairly constant, the elements which compose this ash such as the calcium, iron, phosphorus, and sulfur may vary between wide limits. Moreover, the composition of food changes between various parts of food. For example, the essential oils of citrus fruit occur only in specialized cells embedded in the flavedo layer, and anthocyanin pigments of certain grapes are located only in the epidermal cells. Others may be distributed throughout the plant but are present in markedly different concentrations at various portions (Joslyn 1950).

The difficulties in obtaining good representative sampling depend on the nature of the population. Discrete populations are relatively easy to sample. For example, a shipment of pallets of canned food may be mathematically modeled and sampled. However, sampling of continuous populations is by far more common. A three-dimensional (3D) bulk population is the most difficult sampling problems, and direct sampling is rarely possible. So, it is converted the bulk into a one-dimensional (1D) flowing stream. In a factory situation, the best sampling points are often conveyor belts. Another method for treatment of a 3D bulk is to convert it into a flattened two-dimensional (2D) configuration. The 2D bulk may be fashioned into a long narrow pile and cross-sampled in space as a 1D bulk (Lichon 2004).

Production sampling may purpose to determine a market price based on nutritional content (such as oil in seeds) or to select on the basis of contaminant content (aflatoxins, pesticides, etc.) (Lichon 2004).

Details for the sampling of specific food products are described in the *Official Methods of Analysis* of AOAC International. Such examples for specific foods follow.

The AOAC method 922.01 describes the method for sampling plants (AOAC 1990e). When more than one plant is sampled, include enough plants in sample to ensure that it adequately represents average composition of entire lot of plants sampled. Other sampling method is AOAC method 939.14 for eggs and egg products (AOAC 1990d). Samples are taken from representative number of containers in lot. Sampling tube or dipper, auger, spoon, and hatcher are sterilized by wiping with alcohol-soaked cotton and flaming over alcohol lamp or other burner. Between sampling, instruments are washed, dried, and reesterilized. Open and sample all containers under as nearly aseptic conditions as possible. For example, liquid eggs are mixed in the container with sterile sample tube or dipper, and transfer to sterile sample container. Keep samples at $<5^{\circ}\text{C}$, but avoid freezing. Odor of each container sampled is observed and recorded as normal, abnormal, reject, or musty.

Homogenization

The complex structure and composition of food substrates necessitate homogenization prior to most chromatographic analysis (Joslyn 1950). The homogenization of a sample for analysis generally involves reduction in amount and in particle size and mixing of the product. Therefore, the portion used represents the average composition of the entire mixture (Joslyn 1950). There are two functions of homogenization, reduction of particle size and mixing (Lichon 2004). Particle size can be reduced by cutting, shattering and shearing. Mixing can occur in very different ways and depends on the matrix. Within these steps, often two or more techniques are sequentially used to guarantee final homogeneity (Lacorte et al. 2012). In the analysis of liquids, homogeneous mixtures are easily obtained by stirring or mixing, but solid mixtures whose constituents differ in hardness, specific gravity, and particle size and are nonuniformly distributed must be ground and mixed to yield accurate samples (Joslyn 1950).

Particle Size Reduction

To formulate a meal without including particulate foods is difficult. The presence of the particles occurred in the character, texture, and flavor of food (Robins 2000). Reduction of particle size involves cutting, shattering, and shearing. The various homogenization devices achieve these in different ways to differing degrees (Lichon 2004). The two most direct methods for particle size analysis, microscopy and sieving, are discussed initially followed by three classes of optical technique, comparatively simple forward or back scattering, angular-dependent light scattering, and dynamic light scattering. The next technique to be addressed is the electrical sensing zone originally developed. Sedimentation techniques follow gravitational sedimentation, centrifugal sedimentation, and field-flow fractionation (FFF). The next two techniques are both acoustic in operation, either direct ultrasonic attenuation or electroacoustics. Finally, the novel applications based on nuclear magnetic resonance (NMR) are presented (Robins 2000).

The importance of particle size reduction is intuitive; the smaller the particles, the greater number of particles will be included in a given test portion, therefore greater probability of sampling of more parts of the original bulk. Particle size may also influence the analytical procedure such as surface area and/or particle radius are critical to reagent exposure and penetration. Point of view, the degree of particle size reduction may affect the accuracy as well as the precision of the analysis (Lichon 2004).

AOAC International provides details on the preparation of specific food samples for analysis. AOAC 983.18 method includes preparation of meat and meat products (AOAC 1990b). Ground meat samples should be stored in glass or similar containers, with air and water tight lids. Fresh, dried, cured, and smoked meats are to be bone free and cut no more than 3 mm wide. The sample then should be mixed and analyzed.

Mixing

While particle size reduction helps to improve sampling of the test portion by increasing the number of particles sampled, the sample must be well mixed to achieve the gain in accuracy and precision (Lichon 2004).

Most sampling and homogenization processes for foods lead to powdered samples defined as free flowing and cohesive. Free-flowing powders known as rice or coarse sugar exhibit a smooth flow. They have an attractive nondusty appearance and adhere little to container walls. However, the flowable properties lead to mixtures unmixing as easily as mixing. Cohesive powders such as flour have also an erratic stick-slip flow. They are often dusty and stick to container walls. Cohesive powders lack mobility as light individual particles are held in a structure and cannot easily move independently (Lichon 2004).

Mixing procedures need attention to two areas such as adequate shearing and the inclusion of all the samples. Adequate shearing is necessary to overcome cohesive forces and to break up and disperse aggregates into the mixture. The inclusion of all the samples requiring attention is to ensure the inclusion of the whole sample in the mixing process. The processes that prevented adequate mixing of foods are classification, agglomeration, and phase separation. Differentiated particle sizes and shapes, surface adhesivity, electrostatic charging, disruption of stable structures maintaining surface tension, destruction of encapsulating structures cause these processes.

Sample Preparation

Sample preparation is one of the important steps in flavor and food analysis, which has remove interferences and analyte preconcentration and converts the analytes to suitable form for separation and detection (Moein et al. 2014). It is necessary in order to minimize the required sample preparation time (Silva et al. 2013). This includes all steps from design and implementation of sampling in addition to particle size reduction and mixing such as drying, weighing, dilution, and any number of physical or chemical manipulations prior to analysis. These may also include apportionment, storage, digestion, extraction, cleanup, separation, and derivatization (Linchon 2000).

Several workers have also emphasized that for nutritional evaluation of food it is desirable to prepare the sample in the same way as it is commonly consumed prior to analysis.

Drying

AOAC 922.02 method is used for preparation of sample in plants (AOAC 1990c). For mineral constituents, all foreign matter from material is removed. Air or oven-dry as rapidly as possible to prevent decomposition or weight loss by respiration,

grind, and store in tightly stoppered bottles. If results are to be expressed on fresh weight basis, record sample weights before and after drying. When Cu, Mn, Zn, Fe, Al, etc. are to be determined, avoid contaminating sample by dust during drying and from grinding and sieving machinery.

Weighing

A practically universal step in the manipulation of the test portion is the weighing step. A judicious choice of balance can maintain this precision (Linchon 2000).

Apportionment

Some research is required to decide what parts of the sample are to be analyzed. For example, is fresh produce to be washed prior to pesticide analysis? The aim of this separation is removal of bones and trimming of excess fat from meat; and exclusion of brines from canned vegetables and inclusion of liquid from canned fruits.

Storage and Preservation

Storage and identification of samples require meticulous attention and control. Foods are generally frozen in sealed plastic after preliminary homogenization or freeze-dried before and after further homogenization treatments and stored in desiccators (Linchon 2000; Lichon 2004).

Extraction

The chemical properties of the analyte are important to an extraction. These properties are fundamental to understanding extraction theory: vapor pressure, solubility, molecular weight, hydrophobicity, and acid dissociation (Wells 2003).

Techniques used for extraction are liquid–liquid extraction (LLE), liquid–solid extraction (LSEs), solid phase extraction (SPE), and solid phase microextraction (SPME), stir bar sorptive extraction (SBSE), soxhlet extraction, automated soxhlet extraction, ultrasonic extraction, supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), microwave-assisted extraction (MAE), static headspace extraction (SHE), dynamic headspace extraction (purge and trap), and membrane extraction.

In liquid–liquid extraction (LLE), phases A and B are both liquids. The two liquid phases must be immiscible. For that reason, LLE has also been named as immiscible solvent extraction. In practice, one phase is usually aqueous, while the other phase is an organic solvent. The LLE process can be completed successfully by shaking both two phases together in a separatory funnel. Then, the layers are

allowed to separate. The high-density phases are separated from the bottom of the separatory funnel by a glass or Teflon stopcock. Commonly, separatory funnels are globe, pear, or cylindrically shaped. They may be shaken mechanically but are often shaken manually (Koltho et al. 1969). The main disadvantages of LLE are suffered from large amount of sample and large volume of toxic organic solvent (Cai et al. 2014).

The liquid–solid extractions (LSEs) are used to concentrate semivolatile organic compounds from liquids into the solid phase. The liquid phase contacts with bulk solid extracting phase. An equilibrium is allowed to occur between the two phases and then physical separation of solid and liquid phases follows it. During the past quarter century, different approaches to solid phase extractions have appeared: solid phase extraction (SPE), solid phase microextraction (SPME), and stir bar sorptive extraction (SBSE) (Wells 2003).

Solid phase extraction (SPE) is a particularly attractive technique for isolation and preconcentration of analytes. In SPE, an aqueous sample is passed through a small tube filled with porous solid particles such as poly or silica C₁₈. Moreover, a membrane disk containing sorbent particles may be used. The organic analytes are extracted. After a brief wash, the analytes are eluted by a small volume of an organic solvent. A portion of the eluate can then be analyzed by gas or liquid chromatography or by capillary electrophoresis (Fritz and Macka 2000; Huck and Bonn 2000). *SPE* related to LLE are reduced analysis time, cost, labor, organic solvent consumption and disposal and supplied potential for formation of emulsions. SPE provides higher concentration factors than LLE and can be used to store analytes in a sorbed state or as a vehicle for chemical derivatization. SPE is a multistaged separation technique providing greater opportunity for selective isolation than LLE, such as fractionation of the sample into different compounds or groups of compounds (Fritz and Macka 2000; Huck and Bonn 2000).

Solid phase extraction procedures are used both to extract traces of organic compound from environmental samples and to remove the interfering components of the complex matrices in order to obtain a cleaner extract containing the analytes of interest (Żwir-Ferenc and Biziuk 2006). Table 1 shows the application of solid phase extraction technique in analysis of different compounds in food sample.

By *solid phase microextraction* (SPME), samples are analyzed after equilibrium is reached or at a specified time prior to achieving equilibrium. Microextraction methods are usually based on using an amount of extraction phase much smaller than that of sample. The most popular microextraction method is SPME (Musteata 2013). SPME is distinctly different from SPE because SPE techniques, including semimicro SPE (SM-SPE) and miniaturized SPE (M-SPE), are exhaustive extraction procedures (Fritz 1999). The most important advantage of SPME is a solventless sample preparation procedure. SPME is a relatively simple involving only sorption and desorption (Beltran et al. 2000). SPME is compatible with chromatographic analytical systems. SPME sampling devices are portable. SPME has the advantages of high concentrating ability and selectivity (Wells 2003).

Table 1 Application of SPE as extraction and purification of extract technique

Sample	Analytes	References
Water	Pesticides	Stajbaher and Zupancic-Kraljl (2003)
Drinking water	Pesticides	Focant and Pauwe (2002)
Wheat, maize	Pesticides organophosphorus	Manirakiza et al. (2000)
Fruits	Pesticides	Ramos et al. (1999)
Fruits, vegetables (apples, peas, green beans, oranges, raspberries, clementines, carrots, wheat)	Pesticides	Esilssons and Björklund (2000)
Fruit, vegetables	Pesticides	Dabrowska et al. (2003)
Fruits, vegetables	Pesticides	Wilgfield et al. (1996)
Vegetables	Pesticides	Junker-Buchheit and Witznbacher (1996)
Oranges, sweet potatoes, green beans	Pesticides	Lawrence et al. (1996)
Tomatoes	Pesticides	Kim et al. (2001)
Fatty food (chickens, pork meats)	PCDD/Fs	Doong and Lee (1999)
Fish meal, lard fat	PCBs	Beneyp et al. (1996)

Stir bar sorptive extraction (SBSE) is used as a solventless sample preparation method for the extraction and enrichment of organic compounds from aqueous matrices. In this method, the solutes are extracted into a polymer coating on a magnetic stirring rod. The extraction is controlled by the partitioning coefficient of the solutes between the polymer coating and the sample matrix and by the phase ratio between the polymer coating and the sample volume (David and Sandra 2007).

Soxhlet extraction system has three components. The first part is a solvent vapor reflux condenser. The second part occurs in a thimble holder with a siphon device and a side tube. The thimble holder connects to a round-bottomed flask at third part. The sample is loaded into a porous cellulosic sample thimble and placed into the thimble holder. Consequently, the sample is always extracted with fresh solvents in each cycle. Automated Soxhlet extraction offers a faster alternative to Soxhlet, with comparable extraction efficiency and lower solvent consumption (Kou and Mitra 2003).

Ultrasonic extraction, also known as sonication, uses ultrasonic vibration to ensure intimate contact between the sample and the solvent. This extraction is fast, but the extraction efficiency is not higher than the other techniques. Also, ultrasonic irradiation may cause the decomposition of some organophosphorus compounds (Kotronarou et al. 1992).

Supercritical fluid extraction (SFE) can be operated online or offline. While in the online configuration, SFE is coupled directly to an analytical instrument, such as a gas chromatograph, high-performance liquid chromatography or supercritical fluid chromatography (SFC), etc., in the offline SFE, it is a stand-alone extraction

method independent of the analytical technique to be used. A supercritical fluid is a substance above its critical temperature and pressure. The density of supercritical fluids is close to that of liquid and their viscosities are gas-like. In SFE, the sample is loaded into an extraction cell and placed into the heating oven. The temperature, pressure, flow rate, and the extraction time are adjusted, and then the extraction is started. The extract is collected either by a sorbent trap or by a collection vial containing a solvent (Mitra and Wilson 1991). SFE is fast and uses a little amount of solvents per sample. CO₂ is nontoxic, nonflammable, and environmentally friendly.

Accelerated solvent extraction (ASE) is referred as pressurized fluid extraction (PFE) or pressurized liquid extraction (PLE). ASE is developed because SFE is matrix dependent and often requires the addition of organic modifiers. After the sample is loaded into the extraction cell, the solvent is pumped in and the cell is heated to the desired temperature and pressure. This is referred to as the prefill method (Richter et al. 1996). ASE uses a little amount of solvent. ASE is very fast and fully automated. It is very easy to use. Filtration is applied in ASE, so there is no need for additional filtration. Moreover, ASE has some opportunity such as flexibility of changing solvents without affecting the extraction temperature and pressure. However, initial equipment cost is very higher (Kou and Mitra 2003).

Microwave-assisted extraction (MAE) is more advanced than the traditional solvent extraction method (Li et al. 2011). MAE is an efficient extraction technique because of its low usage of extraction solvents and shorter extraction time (Teo et al. 2013). The matrix internally and externally without a thermal gradient is heated and so, functional compounds can be extracted efficiently and protectively. Moisture inside the cells is heated due to the microwave effect and evaporates. MAE has many advantages such as including shorter extraction times and dramatically reduced solvent volumes (Li et al. 2011).

Among these extraction techniques, *static headspace extraction* is a simple and fast technique to implement because no sample preparation or solvent is needed. SHE is used in food flavor analysis for the extraction of volatile compounds (Cavalli et al. 2003). This extraction method is combined with gas chromatography. In SHE-GC extraction, the vapor phase directly above and in contact with a liquid or solid sample in a sealed container is sampled, and an aliquot is transferred to a gas chromatograph for separation on a column, detection, and quantitation (Snow and Bullock 2010).

The dynamic headspace technique separates volatile sample constituents from the matrix by a continuous flow of an inert gas either above a solid or liquid sample or by bubbling through a sintered glass of high pore density through a liquid sample, preferably an aqueous one. This technique is called as purge and trap (P&T) (Kolb and Ettre 2006).

Membrane-based extractions offer some alternatives to classical sample preparation techniques. It has some advantages such as the possibility of tuning the selectivity by chemical means and decreasing solvent consumption and automatically. They allow high selectivity, high enrichment factors, and giving good possibilities for automation (Jönsson and Mathiasson 1999).

Digestion, Cleanup, Filtration, and Derivatization

Digestion, cleanup, filtration, and derivatization should all be quantitative (Linchon 2000).

Most of the solid–liquid techniques used for the extraction of organic compounds are not selective, and, thus, a cleanup step is usually necessary. The major cleanup treatments are sulfur elimination, solid phase extraction (SPE), gel permeation extraction (GPC), combined extraction and cleanup in selective pressurized liquid extraction (SPLE), and other cleanup approaches. The cleanup methods decrease the matrix effect and produce relatively high enrichment factors (Zuloaga et al. 2012).

AOAC 920.202 method can be exemplified preparation of sample for determination of manganese, iodine, bromine, and arsenic and boric acid in water (AOAC 1990a). 0.5–2 L sample is evaporated to dryness after addition of small amounts of solid Na_2CO_3 . Residue is boiled thus transferred to filter and washed with hot H_2O . Residue remaining on filter for determination of Mn is used.

Derivatization aims to modify the structure of the target compounds and, thus, the chemical and physical properties. Derivatization is integrated with some chromatographic methods such as LC-MS. The advantages of that include improvement of selectivity and separation, enhancement of ionization efficiency, improvement of structural elucidation, removal of endogenous interference, and facilitation of isomer separation (Qi et al. 2014).

Conclusion and Future Directions

Sampling and sample preparation are the mean steps for the good analytical results because the results of the experiments depend on the quality of the starting material. The sample needs to be both representative, homogeneous, and with an even surface in order to eliminate factors that can influence the results. Analytical errors usually cost money such as wrong decisions based on erroneous results, lost business, false security, and litigation. The analyst must be careful for accuracy and precision in each of the four steps of analysis. While the analysis step is often taken as routine in laboratories, the other steps are often less so, despite their frequent domination of experimental error. The greatest effort should be expended to reduce contributions in the error-dominating steps. In future, sampling and sample preparation may aid the analyst for solving the problem being investigated.

Cross-References

- ▶ [Classical Wet Chemistry Methods](#)

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Abstract

The instrumental analysis of foods is an important step in food processing and manufacturing companies because of the presence and interactions of various compounds in foods during storage and processing. While traditional methods are still used, most analysis involves the use of different instruments.

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This chapter is structured to provide a description of the information each technique can provide, a simple explanation of how it works and examples of its application, and facilitates comparison of techniques. The focus of this chapter is on spectroscopic, chromatographic and electrophoretic methods, including specific examples of instruments such as capillary electrophoresis, high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), atomic absorption, emission and inductively coupled plasma, fluorescence spectroscopies, gel electrophoresis, etc., among many others.

Introduction

Modern analytical chemistry is “the qualitative and quantitative characterization of matter”, which generally requires a small sample using different instruments. Qualitative analysis is the identification of one or more chemical species present in a material, whereas quantitative analysis is the determination of the exact amount of a chemical species present in a sample. Separations can be achieved with selective qualitative and quantitative methods. Instrumental methods are carried out with specially designed electronic instruments controlled by computers and are used for the interaction of electromagnetic radiation and matter, the determination of some physical properties of matter, and characterization of the sample being analyzed. These instruments have automated sample introduction, automated data processing, and even automated sample preparation. The most suitable instrumental technique depends on the physico-chemical characteristics of the analytes, the detection limit, and the chromatographic resolution required.

Accurate determination of components, residues, and contaminants in food is important to ensure both the quality and safety of products for consumers. Methods of analysis have to be robust and accurate; however, the time spent on sample preparation must be decreased and more environmentally friendly techniques must be used, such as using smaller volumes of organic solvents. Food products are complex mixtures of vitamins, sugars, proteins and lipids, fibers, aromas, pigments, antioxidants, and other organic and mineral compounds. Before such substances can be analyzed, they have to be extracted from the food matrix using different methods.

This chapter covers the fundamentals and application of instrumentation, which have been classified into the following areas: spectroscopic methods, chromatographic methods, and the electrophoretic method. Spectroscopic techniques involve the visible region, ultraviolet (UV) region, the near-infrared (NIR) region, mid-infrared (MIR), Fourier transform infrared (FT-IR), atomic spectroscopy, etc. Chromatographic methods are commonly defined as gas, liquid, supercritical fluid, paper, thin-layer chromatography (TLC), etc. Hyphenated chromatographic methods have also now been developed. The final instrumental method is electrophoretic analysis, classified as conventional and capillary electrophoresis (CE).

To summarize, in this chapter the basic knowledge and terminology required for understanding instrumental methods and their applications in food are introduced at an elementary level.

Spectroscopy

The range of wavelengths from 350 to 700 nm is known as the visible region of the spectrum. While UV radiation is defined from 200 to 350 nm, the NIR region starts immediately at 750 nm and ranges up to 2,500 nm. The classical infrared (IR) region extends from 2,500 to 50,000 nm. The energies of IR radiation range are not sufficient to cause electron transitions, but they do cause vibrational changes within molecules. The principles involved in these spectroscopic techniques are discussed in this chapter.

Absorption Spectroscopy (Ultraviolet/Visible)

UV/visible (UV/VIS) absorption spectroscopy has been used in food sampling for many years. Although much of the routine work is performed using high analysis systems, absorption spectroscopy still has an important place in most food samples.

UV and visible radiation interacts with matter, causing electronic transitions. Vibrational and rotational energy levels cause broad absorption. While the UV region falls in the range between 190 and 380 nm, the visible region falls between 380 and 750 nm. When light passes through a sample, some of the light may be absorbed and the remainder transmitted through the sample. This molecular absorption influences the chemical called a chromophore. A chromophore involves a multiple bond (such as C = C, C = O, or C \equiv N) and may be conjugated with other groups to form complex chromophores (Siouffi 2004).

UV/VIS spectroscopy is the study of how a sample responds to light. When a beam of light passes through a substance or a solution, some of the light may be absorbed and the remainder transmitted through the sample. The laws of light absorption are defined by two rules: Lambert's Law and Beer's Law.

In Lambert's Law, the proportion of incident light absorbed by a transparent medium (I) is the intensity of the light (I_0). This proportion is called transmittance (T).

$$\frac{I}{I_0} = T \quad (1)$$

The second rule, Beer's Law states that the absorption of light is directly proportional to both the concentration of the absorbing medium and the thickness of the medium in the light path.

A combination of the two laws is known as the Lambert–Beer Law and defines the relationship between absorbance (A) and transmittance (T). ϵ is known molar absorptivity ($\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$), c is molar concentration (mol dm^{-3}), and b is path length (cm). The Lambert–Beer law uses only monochromatic light.

$$A = \log \frac{I_0}{I} = \log \frac{100}{T} = \epsilon cb \quad (2)$$

A spectrophotometer needs a source of radiation, a monochromator, and a detector. The source of radiation is used to produce appropriate wavelengths. UV light derived from a deuterium arc provides an emission of high intensity and adequate continuity in the 190–380 nm range. A quartz or silica envelope is necessary. Visible light is normally supplied by a tungsten lamp in the 320–380 nm range. A glass or quartz envelope is very useful in this region. Spectrophotometers have either a single-beam or double-beam design. A monochromator is used to isolate light of a single wavelength and transmit it to the sample compartment. A detector helps to detect and measure the light intensity (Yetim and Cam 2012).

A UV/VIS spectrophotometer can be used to determine very different food compounds such as monosaccharides, disaccharides, fatty acids, sterols, HMF (hydroxymethylfurfural), protein carbonyl, antioxidant compounds, vitamins, etc. This spectrophotometer is available for raw and processed foods, cereals, grains, nuts, dairy products, fruits, vegetables, juices, jam, beverages, honey, oils, fats, meat products, fish, etc. Gao et al. (2015) determined the uronic acid content of polysaccharide from *Lilium lancifolium* Thunb by means of spectrophotometry with *m*-hydroxybiphenyl at 525 nm using D-galacturonic acid as the standard. Chen et al. (2015) measured the total phenolic content in natural products via the Folin–Ciocalteu method using a spectrophotometer at 730 nm. Pal et al. (2014) developed an experimental oil refining unit and tested sunflower oil using it. The quality and composition of crude and refined oil were analyzed and compared with a spectrophotometer at 460 nm. Javeria et al. (2013) found β -carotene in different vegetables such as carrots, corn, paprika, and pumpkin and quantified its concentration using a spectrophotometer at 452 nm.

Fluorometry

When UV light is shone onto certain paints or certain minerals in the dark, they spit visible light. These paints and minerals are said to fluoresce. For fluorescence to occur, a molecule must absorb a photon and be raised from its ground state to an excited vibrational state in a higher electronic state. Fluorescence and phosphorescence are both types of luminescence. Specifically, they are types of photoluminescence, meaning that the excitation is achieved by absorption of light. There are also other types of luminescence. If the excitation of a molecule and emission of light occurs as a result of chemical energy from a chemical reaction, the luminescence is called chemiluminescence (CL). The light emitted by a firefly is an example of bioluminescence (Robinson et al. 2005). A typical fluorometer includes radiation sources, monochromators, a sample cell and detector such as a UV/VIS spectrophotometer. Cui and Lv (2014) investigated the effect of turbidity on the determination of chlorophyll a using laboratory fluorometry RF5301-spectrofluorophotometer. They analyzed the turbidity effect on fluorimetric determination results of chlorophyll a. Utrera and Estévez (2012) assessed tryptophan depletion by quantifying the loss of natural tryptophan fluorescence during

metal-catalyzed oxidation using fluorescence spectroscopy. Villaverde and Estévez (2013) analyzed advanced glycation endproducts using a fluorescence spectrometer in myofibrillar proteins.

Infrared Spectroscopy

The IR absorption spectrum can be used to identify the molecule as a ‘fingerprint’ of a molecule with covalent bonds. Qualitative and quantitative identification of organic and inorganic compounds is a primary use of IR spectroscopy. The IR region begins after the visible region at 700 nm. From 700 to 2,500 nm is called the NIR. The classical IR region is from 2,500 to 50,000 nm. The energies of IR radiation range from 48 kJ mol^{-1} at 2,500 nm to 2.4 kJ mol^{-1} at 50,000 nm. Electron transitions cannot be due to these low energies, only vibrational changes within molecules occur. IR spectra differ from the typical UV or visible spectrum with many relatively sharp peaks. Because of this, IR spectroscopy is ideal for qualitative analysis of organic compounds. As do all spectrometers, IR has basic units: a source of electromagnetic radiation, a dispersion device, a sample holder, optical devices for collimating and focusing, a detection device, and a data readout or storage system (Jespersen 2006).

FT-IR spectrometry is the preferred method of IR spectroscopy because it meets the criteria of efficient analysis, i.e., easy to use, fast, and inexpensive (Bunaciu et al. 2011). Changes in the position and intensity of bands in the FT-IR spectra would be associated with the changes in the chemical composition of a sample (Sun et al. 2010). The advantages of FT-IR application are that it is effective, specific, rapid, and non-separative (Gad et al. 2013). The FT-IR spectrometry process consists of a source from which IR energy is emitted from a glowing black-body, an interferometer where the ‘spectral encoding’ takes place, a sample compartment where the beam is transmitted through or reflected off the surface of the sample, a detector used to measure the spectral interferogram signal, and a computer where the measured signal is digitized and sent (Ismail et al. 1997). NIR spectroscopy has found extensive use in food analysis and is concerned with NIR region of spectrum (between the wavelengths of ~ 750 and ~ 2500 nm in wavelength or ~ 13500 to $\sim 4000 \text{ cm}^{-1}$) where as MIR spectroscopy is a well-established technique for identification and structural analysis of chemical compounds in the MIR region of spectrum (between ~ 2500 and ~ 23500 nm or ~ 4000 to $\sim 450 \text{ cm}^{-1}$) (Cai et al. 2010).

IR spectroscopy is an appealing technology for the food industry due to simple, rapid, and non-destructive measurements of chemical and physical components. IR combined with multivariate data analysis methods make this technology ideal for large volumes, rapid screening, and characterization of minor food components down to parts per billion (ppb) levels. The food industry is already familiar with the technology and it has potential to expand to monitoring for food adulteration. The FT-IR is based on a Michelson interferometer and consists of four optical arms, usually at right angles to each other, with a beam splitter at their point of intersection. Radiation passes down the first arm and is separated by the beam splitter into

two perpendicular beams of equal intensity. These beams pass down into other arms of the spectrometer. At the ends of these arms, the two beams are reflected by mirrors back to the beam splitter, where they re-combine and are reflected together onto the detector. One of the mirrors is fixed in position; the other mirror can move toward or away from the beam splitter, changing the path length of that arm. Anjos et al. (2015) calculated the sugar content in honey samples using a FT-IR spectroscopic method. The FT-IR was shown to be a good methodology to quantify the main sugar content in honey and can easily be adapted to routine analysis. Jaiswal et al. (2015) used FT-IR spectroscopy to determine whether milk-like products from vegetable sources (soy milk) were added to milk without declaration. FT-IR spectroscopy has demonstrated potential as a rapid, quality monitoring method. Mossoba et al. (2014) evaluated the total concentration of *trans* fatty acids in the fat extracted from 19 representative fast foods using a portable FT-IR analyzer. These results indicated that the portable FT-IR analyzer was suitable for the rapid and routine quantification of total *trans* fat measured from fats extracted from fast foods. Bhat and Yahya (2014) evaluated functional groups of Belinjau (*Gnetum gnemon* L.) seed using FT-IR spectral-analyzed amines, amides, amino acids, polysaccharides, carboxylic acids, esters, and lipids.

The NIR region is used for quantitative analysis of solid and liquid samples containing OH, NH, and CH bonds, such as water and proteins. Ferreira et al. (2014) applied NIR and MIR spectroscopy techniques for several agricultural products to develop and compare these two spectroscopic techniques and determine the parameters of quality, such as moisture, protein, lipid, and ash content, in varieties of soybean. Both IR techniques have predictive abilities.

Atomic Spectroscopy

Atomic spectroscopy (AS) measures the concentration of chemical elements in a sample. When elements are transformed into atomic vapor at high temperatures, emission or absorption of light may occur and this can be accurately measured at a unique resonant wavelength. Modern AS can be divided into three related techniques. Whereas atomic absorption spectrometry (AAS) measures the quantity of light absorbed by atoms of the analyte, atomic emission spectrometry (AES) and atomic fluorescence spectrometry (AFS) measure the quantity of radiation emitted by analyte atoms (Evans et al. 2014).

The basis of AAS is the absorption of discrete wavelengths of light by ground-state, gas-phase free atoms. Free atoms in the gas phase are formed from the sample by an 'atomizer' at high temperature. AAS is an elemental analysis technique and used to determine ppb and parts per million (ppm) concentrations of most metal elements. AAS supplies the accurate results in rapid analysis times. However, AAS gives no information on the chemical form of the analyte, which makes AAS of very limited use for qualitative analysis. Since AAS is used for quantitative analysis of elements, the use of the term 'spectrometry' is preferred rather than 'spectroscopy.' AAS can measure down to ppb of a gram ($\mu\text{g dm}^{-3}$) in a sample. AAS

corresponds to the energy needed to promote electrons from one energy level to a higher energy level (Parsons 2000). AES is the study of the radiation emitted by excited atoms and monatomic ions. Excited atoms and ions relax to the ground state. The emitted atom and ion lines can be used for the qualitative identification of elements present in a sample and for the quantitative analysis of such elements from low ppb to higher ppb. AES is a multielement technique with the ability to determine metals, metalloids, and some non-metal elements simultaneously. The temperature of the excitation source of AES to achieve detection limits must exceed 6,000 K. AES is most useful for the easily excited alkali metals and alkaline earth metals. The process of AFS involves the emission of a photon from a gas-phase atom that has been excited by the absorption of a photon, as opposed to excitation by thermal or electrical means. AFS also involves the absorption of incident radiation and measures the radiation emitted from the excited state (Marshall 1997).

There are a lot of papers in the literature on major, minor, and trace element analyses in food by AS. In general, AAS analyzes a range of metals at various concentrations from high ppm (such as Calcium, Potassium, Magnesium, and Sodium) to low ppb (such as, Cadmium, Cobalt, Copper, Iron, Mercury, Manganese, Nickel, Lead, Selenium, and Zinc). The aim of metal content being analyzed in foods and beverages is the determination of nutrition content, food safety, and for the purpose of categorization and authentication (Intawongse and Dean 2006).

Fiorino et al. (1976) measured As, Se, Sb, and Te in food with AAS. The food or biological tissue sample (1–3 g, dry weight) is weighed into a 100-ml Kjeldahl flask, and 30 ml of a (4:1:1) mixture of concentrated, reagent grade HNO_3 , H_2SO_4 , and HC_1O_4 is added to the flask. At least one reagent blank consisting of 30 ml of the ternary acid mixture is prepared with each set of 10–12 samples. The sample oxidation is carried out and the sample–acid mixture is heated. After the sample foaming subsides, the temperature is raised to produce steady boiling. Tüzün (2003) determined the concentrations of heavy metals (Pb, Cd, Fe, Cu, Mn, and Zn) in fish samples using graphite furnace AAS after dry ashing and wet ashing methods. Ghaedi et al. (2008) reported the preconcentration of Cr^{3+} , Fe^{3+} , Cu^{2+} , Ni^{2+} , Co^{2+} , and Zn^{2+} in real samples such as cow meat and fish samples. Dolan and Capar (2002) analyzed aluminum, arsenic, boron, barium, calcium, cadmium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, molybdenum, sodium, nickel, phosphorus, lead, selenium, strontium, thallium, vanadium, and zinc with inductively coupled plasma–AES with ultrasonic nebulization.

Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) spectroscopy studies the structure of molecules. NMR instruments and experimental methods permit determination of the three-dimensional structure of proteins as large as 900,000 Da. NMR instruments coupled to liquid chromatographs and mass spectrometers for separation and characterization of unknowns are used commercially. NMR involves the absorption of radiowaves by the nuclei of some combined atoms in a molecule that is located in a magnetic field.

NMR can be considered a type of absorption spectroscopy, not unlike UV/VIS absorption spectroscopy. Radiowaves are low-energy electromagnetic radiation. NMR spectroscopy is the preferred method to determine the chemical structures of macromolecules and measures the absorption of electromagnetic radiation in the radiofrequency region of 4 to 900 MHz (Skoog et al. 2007). However, the weak interaction strength between sample spins and inductive detectors, and low-thermal polarization, restricts the sensitivity to large ensembles (Müller et al. 2014).

Ali et al. (2015) showed a reduction in myowater in chicken breast meat with NMR operating at a resonance frequency for protons of 22.6 MHz. One gram of sample ($1 \times 1 \times 1$ cm) was placed in an NMR tube. Lim et al. (2015) showed molecular mobility of high-amylose maize starch and high-amylose maize starch acylated with acetate, propionate, and butyrate. Measurement of the molecular mobility of the starch granules by NMR spectroscopy with Carr–Purcell–Meiboom–Gill experiments showed that T_2 long was reduced in acylated starches and that drying and storage of the starch granules further reduced T_2 long. Dugo et al. (2015) used the NMR technique for geographical characterization of extra-virgin olive oils in Italy.

X-Ray Spectroscopy

X-ray absorption, emission, and fluorescence spectra are performed in the qualitative and quantitative determination of elements in solid and liquid samples. X-ray spectrometry can analyze short-range order with elemental specificity for disordered heterogeneous materials (Török and Labar 1998). X-rays use electromagnetic radiation with a wavelength range from 0.005 to 10 nm (0.05–100 Å). X-rays have shorter wavelengths and higher energy than UV radiation. X-rays are generated when a high-speed electron is stopped by a solid object or by electronic transitions of inner core electrons. They are absorbed by samples and the intensity is attenuated. The degree of absorption depends on the wavelength of the X-rays, the thickness, density, atomic number, and the local structure of the absorber (Kawai 2004).

Perring and Blanc (2007) developed a rapid and efficient method for the determination of iron in commercial infant cereals using an energy-dispersive X-ray fluorescence (EDXRF) technique. The total time of analysis for iron is less than 20 min. Tolra et al. (2011) studied to understand localization of Al in tea leaf tissues using low-energy X-ray fluorescence spectro-microscopy (LEXRF) because the localization of Al in plant tissues can help in the understanding of the processes of Al uptake, transport, and tolerance in plants.

Chromatography

Chromatography is essentially a physical method of separation. The separated components are distributed between two phases: the stationary phase and mobile phase. Chromatographic separations have three features: they are physical

methods of separation; two distinct phases are involved; and separation occurs with differences in the distribution constants of the individual sample components between the two phases. What is important is that either the rate of migration or direction of migration of the two phases are different. A convenient classification of chromatographic techniques can be made in terms of the physical state of the phases employed for the separation. When the mobile phase is a gas and the stationary phase a solid or liquid, the separation techniques are known as gas-solid chromatography (GSC) or gas-liquid chromatography (GLC), respectively (Poole 2002).

The chromatographic process is described by very complex theoretical equations related to the performance of columns in terms of the height equivalent to a theoretical plate, H , and the linear mobile phase velocity, u . This could be calculated from column dimensions and volume flow rates, or more simply measured directly using the retention time of an unretained analyte, t_M , and the measured column length, L . Three constants are used to describe chromatographic column efficiency (Van Deemter Equation) (Robinson et al. 2005).

$$H = A + \frac{B}{u} + Cu \quad (3)$$

A applies to columns packed with support particles. It becomes zero for open tubular columns when the mobile-phase velocity is slow enough for the flow to be laminar. In a packed column, the paths of individual analyte molecules will differ as they take different routes through the spaces between the particles. B accounts for the spreading of molecules in both directions from the band center along the length of the column as a result of random-walk diffusion. The C term displayed is actually divided into contributions due to mass transfer in the mobile phase, C_m , and in the stationary phase, C_s , where $C = C_m + C_s$ (Robinson et al. 2005).

Various terms can be used in chromatography, one of which is retention time (t_R). t_R shows the time required for a molecule to pass through a chromatographic column. The retention time of unretained solute (t_M) is used to determine the retention time of solute which isn't retained by the stationary phase and proceeds constantly through the mobile phase of the chromatographic column (Hısil 2011). The adjusted retention time (t'_R) is a better measure of chromatographic retention than t_R . It is calculated as:

$$t'_R = t_M + t'_R \quad (4)$$

The ratio represents the amount of time spent by the solute in the stationary phase related to the time it spends in the mobile phase (Hısil 2011).

$$k = \frac{t'_R}{t_M} \quad (5)$$

The number of theoretical plates (N) refers to column efficiency. W_B is the width of the peak (Hısil 2011).

$$N = 16 \left(t_R / W_B \right)^2 \quad (6)$$

$$N = \left(t_R / \sigma \right)^2 \quad (7)$$

$W_{1/2}$ is the peak width at half height (Hisil 2011).

$$N_{eff} = 5.54 \left(t'_R / W_{1/2} \right)^2 \quad (8)$$

Height equivalent to a theoretical plate (HETP) shows column efficiency (Hisil 2011):

$$HETP = L / N \quad (9)$$

where L is the length of column and N is the theoretical plate numbers.

When the column length is divided by the effective plate number (N_{eff}), height equivalent to one effective plate (H_{eff}) can be found (Hisil 2011):

$$H_{eff} = L / N_{eff} \quad (10)$$

Gas Chromatography

In gas chromatography (GC), the components of a vaporized sample are separated as a consequence of being partitioned between a mobile gaseous phase and a liquid or a solid stationary phase held in a column. These types are named according to stationary phase as GLC and GSC, respectively. GLC finds widespread use in all fields of science; its name is usually shortened to GC (Skoog et al. 2007). In GC, the first step consists of isolation of analytes from the sample. These analytes are then partitioned between a gaseous mobile phase and a liquid phase and, finally, identification and quantification of analytes (Hajslova and Cajka 2008).

The sample preparation method plays a crucial role in obtaining the required parameters of a particular analytical method. Some types of prior sample preparation are isolation, concentration, grinding, homogenization, reducing the particle size of component, inactivation of enzyme systems, and storage under frozen conditions of sample. In sample preparation for GC analysis, different sample preparation methods are used: headspace analysis, distillation, preparative chromatography (e.g., solid-phase extraction, column chromatography on silica gel), solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE), single drop microextraction (SDME), hollow-fiber liquid-phase microextraction (HF-LPME), dispersive liquid-liquid microextraction (DLLME), and gas purge microsyringe extraction (GP-MSE), or some combination of these basic methods (Qian 2010).

The basic components of a typical instrument for performing GC consists of a supply of carrier gas, a sample injection system, the separation column, the detector, and a data recorder (Skoog et al. 2007).

The mobile-phase gas in GC is called the carrier gas. It must be chemically inert, pure, and without water. In addition, specialized traps can be purchased to reduce or remove hydrocarbons and oxygen in the carrier gas (Eiceman 2010). Helium, argon, nitrogen, and hydrogen are the types of mobile-phase gas used (Hajslova and Cajka 2008).

The injection port containing a soft septum provides a gas-tight seal. Different injection ports are used depending on the various sample and instrumental requirements: split, splitless, programmed temperature injection, and cold on-column (COC) injector (Grob 1987).

Most gas chromatographs are equipped with ovens to keep the column at temperatures from 40 °C to 350 °C. Temperature-programmed ovens are preferred to allow separations of chemicals spanning a range of vapor pressures in a single analysis (Eiceman 2010; Qian 2010).

The GC column may be classified as either packed or capillary. Packed columns are often stainless steel and filled with inert support: diatomaceous earth (Chromosorb[®]), graphitized carbon, Teflon[®], inorganic salts, and glass beads, etc. (Merritt and Settle 1981). Capillary columns are not filled with inert support. Instead, GC includes a stationary phase which is a high-viscosity, low-volatility liquid (Cordero et al. 2012) or a thin film of liquid-phase coats the inside wall (McNair and Miller 2009). Capillary columns consist of three columns: WCOT (wall-coated capillary column), SCOT (support-coated open tubular column), and PLOT (porous layer open tubular column).

Detectors are utilized to sense the effluents from the column and provide a record of the chromatography in the form of a chromatogram. The detectors used for the GC analysis are the flame ionization (FID), thermal conductivity (TCD), electron capture (ECD), flame photometric (FPD), pulsed flame photometric (PFPD), and photoionization (PID) detectors (Wong et al. 2013).

GC can successfully be used in food application fields such as flavor and aroma characterization; study of the composition and authentication of origin; qualitative determination of volatile compounds from vegetable matrices; essential oils and various extracts; fat analysis and characterization; and residue and contaminant determination (Cordero et al. 2012). Analysis of food constituents with GC are shown in Table 1.

Liquid Chromatography

Liquid chromatography (LC) is the most widely used of all of the analytical separation techniques and is classified by the separation mechanism or by the type of stationary phase: partition chromatography, adsorption or liquid–solid chromatography, ion exchange or ion chromatography, size-exclusion chromatography, affinity chromatography, and chiral chromatography (Skoog et al. 2007).

Table 1 Gas chromatography in the food industry

Analyte	Sample	Detection method	References
Esters, terpenoids, aldehydes, alcohols, ketones, furans, aromatic compounds, lactones, acid	Strawberry	TOF-MS	Samykanno et al. (2013)
Neutral sugar	Pitaya	FID	Montoya-Arroyo et al. (2014)
Pesticides	Food (vegetables)	TOF-MS	Dalluge et al. (2002)
Polycyclic aromatic hydrocarbons (PAH)	Crude oil	FID-MS	Zhao et al. (2014)

FID flame ionization detector, *MS* mass spectrometry, *TOF* time of flight

Size-exclusion chromatography is often used for solutes with molecular masses greater than 10,000. However, it has now become possible to use reversed-phase chromatography to analyze such compounds. For lower molecular mass ionic species, ion-exchange chromatography is widely preferred, while small polar but non-ionic species are best tested using reversed-phase methods. Once adsorption chromatography was used for separating non-polar species, structural isomers, and compound classes such as aliphatic hydrocarbons from aliphatic alcohols, adsorption chromatography with solid stationary phases was replaced by normal-phase chromatography due to problems with retention reproducibility and irreversible adsorption. Affinity chromatography is used for isolation and preparation of biomolecules, and chiral chromatography is employed for separating enantiomers (Hisil 2011; Yetim and Cam 2012).

Pumping pressures of several hundred atmospheres are required to achieve reasonable flow rates. Because of these high pressures, the equipment for high-performance liquid chromatography (HPLC) tends to be more elaborate and expensive than equipment for other types of chromatography. There are many advantages of HPLC over traditional low-pressure column LC: higher speed, wide variety of stationary phases, improved resolution, greater sensitivity, and easier sample recovery (Reuhs and Rounds 2010).

The main components of a basic HPLC system are a pump, injector, column, detector, and data system (Pomeranz and Meloan 1994). The pump is one of the major components as in order to obtain better separations, smaller particles must be packed into the column. High pressures are required for effective separation (Reuhs and Rounds 2010).

HPLC is a very powerful and versatile technique for analyzing and purifying food and beverages. Typical food analytes quantified by ion chromatography and CE include sugars, aspartame, phospholipids, vitamins, caffeine, organic acids, ionic species, peptides, and proteins. More sensitive fluorescence detectors for HPLC instruments enable lower limits of detection for vitamins, proteins, aspartame, and mycotoxins (McGorin 2000). Analysis of food constituents with LC are shown in Table 2.

Table 2 Liquid chromatography in the food industry

Analyte	Sample	Detection method	References
Biogenic amine	Different coffee	LC-UV	Restuccia et al. (2015)
Isoflavone	Legumes	UHPLC-MS/MS	Vila-Donat et al. (2015)
Polyphenols	Wine	HPLC	Arribas et al. (2012)
Sodium benzoate, potassium sorbate	Food and beverages	HPLC-UV; LC-MS/MS	Gören et al. (2015)
Terpenoid-based color substances	Food products	HPLC-DAD	Cserhádi and Forgács (2001)

DAD diode array detector, *LC* liquid chromatography, *MS/MS* tandem mass spectrometry, *UPLC* ultra high-performance liquid chromatography, *UV* ultraviolet

Supercritical Fluid Chromatography

Supercritical fluid chromatography (SFC) is a promising technique for the analysis of food components without previous sample extraction and derivatization. SFC can provide the molecular weight separating range of GC. SFC is characterized by a high resolution and flow rate because of the properties of supercritical fluids. Analysis of hydrophobic compounds can be performed using SFC. Supercritical fluid can be determined because as a liquid is heated, it starts to form a vapor phase. The vapors being formed become more dense, and with continued heating the liquid and vapor densities become closer to each other until the critical temperature point is reached. At this same point, the liquid line or phase boundary disappears. The small optimal column internal diameters combined with their low flow rates has impeded instrument development and the reliance on carbon dioxide as virtually the only convenient mobile phase for use with flame-based detectors has restricted the applications of SFC to compounds with adequate solubility in carbon dioxide, a relatively non-polar solvating medium (McNally 2006).

Lee et al. (2014) determined the profile of several regioisomeric triacylglycerols in palm and canola oils with SFC coupled with triple quadrupole mass spectrometry (MS). Qiao et al. (2014) used SFC to separate seven pairs of 25*R/S*-ergostanes from *Antrodia camphorata*. Taguchi et al. (2014) analyzed the application of SFC for fat-soluble and water-soluble vitamins.

Paper Chromatography

Paper chromatography is a liquid-liquid partitioning technique. The advantages of paper chromatography are that a small amount of sample is required, a high level of resolution, ease of detection, and simplicity. Paper consists of cellulose, while the stationary phase consists of water. The sample is spotted with a capillary glass tube

or micro-pipet on the paper. A diameter of 2 mm is preferable and achieves good separation. The mixture or sample is highly water-soluble or has the greatest hydrogen bonding capacity and moves slower. Separation takes place using ascending or descending mobile flow. While in ascending paper chromatography the bottom of the paper is placed into the mobile phase, in descending chromatography the solvent is placed in a trough at the top of the developing chamber. In this system, both capillary and gravity elution occurs on the bottom of the tank (Grillini 2006). When separation with paper chromatography is difficult, two-dimensional chromatography can be performed. It consists of developing a square sheet of paper in two directions (Kilz and Radke 2015).

Paper chromatography is chosen for analysis of highly polar compounds: sugars, amino acids, and natural pigments (Fuleki and Francis 1967). Ghosh et al. (2015) investigated the occurrence of starch hydrolytic products during haria preparation. The major endproducts were determined to be glucose, maltose, malto-triose, malto-tetraose, and malto-pentose. Liu et al. (2014b) combined paper chromatography with a paper-based CL analytical device for the determination of dichlorvos in vegetables. This study showed that chromatographic method was suitable for rapid and sensitive detection of trace levels of organophosphate pesticides in food samples (Bhatia et al. 2014).

Thin-Layer Chromatography

TLC is one of the most popular and widely used separation techniques because of its ease of use, cost effectiveness, high sensitivity, speed of separation, as well as its capacity to analyze multiple samples simultaneously for separation, isolation, identification, and quantification of components in a mixture. It is simple, fast, reproducible, and can achieve high resolution (Grillini 2006). The size of microscope slide plates is 20 × 20 cm and the mixture of sample is applied on this plate with a glass capillary or a micro-pipet. As in PC, the development solvent used depends on the materials to be separated; thus, the determination of the appropriate solvent or mixture of solvents is made by trial and error. The ascending technique, where the solvent migrates in a vertical fashion along the plate through capillary action, is the most common. The inner wall of the tank is often covered with chromatography paper in order to saturate the tank with solvent comprised of hexane, carbontetrachloride, benzene or toluene, chloroform, diethylether, and ethanol. The solvent front is marked and the plate is allowed to dry (Belanger, et al. 1997). Bakhtiyari et al. (2015) showed the chromatogram of the biogum produced from sucrose and molasses with TLC. Agatonovic-Kustrin et al. (2015) developed a simple method with a TLC method combined with high-resolution digital plate images to visually compare multiple wine samples simultaneously on a single chromatographic plate and to quantify levels of gallic acid, caffeic acid, resveratrol, and rutin.

Mass Spectrometry and Hyphenated Instruments

The combination of separation techniques has created powerful tools. MS hyphenates capillary GC and LC and the development of novel ionization techniques caused extensive spreading of MS to food analysis. Also, the relatively inexpensive quadrupole, ion trap mass analyzers, and the rediscovery of time-of-flight mass analyzers are used with this sophisticated instrumental technique in both research as well as routine applications (Cajka et al. 2009).

A mass spectrometer consists of three fundamental parts: an ion source, a mass analyzer, and a detector. First, the analyzed sample is introduced into the ion source of the instrument. These ions are then separated into the analyzer according to their ratio of m/z (mass-to-charge ratio). The separated ions are detected and the signal-containing information is stored as a mass spectrum (Hisil 2011).

The sample is introduced to the ion source after separation in GC or HPLC. There are several ionization methods: electron ionization and chemical ionization for GC-MS; electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI) for LC-MS. Some direct ionization techniques, such as matrix-assisted laser desorption ionization (MALDI), desorption electrospray ionization (DESI), desorption atmospheric pressure chemical ionization (DAPCI), and direct analysis in real time (DART), can be employed for sample characterization. Mass analyzers used now are quadrupole, quadrupole ion trap, linear quadrupole ion trap, magnetic sector, time-of-flight, Fourier transform ion cyclotron resonance (FT-ICR) analyzer, orbitrap. The detector monitors the ion current. Tandem mass spectrometry (MS/MS) has been presented as both a hyphenated technique and as a replacement for chromatography-MS (Yetim and Cam 2012).

GC-MS analysis has been used to identify the volatiles of apples, beer, carrots, cooked meats, etc. Páleníková et al. (2015) developed a method for determination of the pesticide level in soya-based nutraceutical products with GC-MS/MS. Lopez et al. (2015) applied a straightforward GC-MS method to determine the occurrence of 14 flavoring compounds (e.g., coumarin, estragole, methyl-eugenol, (R)-(+)-pulegone, thujone, alkenylbenzenes, *trans*-anethole, and myristicin, etc.) in foods such as liquids, semi-solids, dry solids, and fatty solids. Rodríguez-Gómez et al. (2014) developed and validated two accurate and sensitive methods with GC-MS/MS and ultra high-performance LC (UHPLC)-MS/MS for the determination of endocrine-disrupting chemicals such as bisphenol A and its main chlorinated derivatives, five benzophenone-UV filters, and four parabens in human breast milk. Zhuang et al. (2014) used a combined data matrix consisting of HPLC-diode array detector (HPLC-DAD) and inductively coupled plasma-MS (ICP-MS) measurements of samples from the plant roots of the *Cortex moutan*. Liu et al. (2015) developed an UHPLC-quadrupole time-of-flight (Q-TOF)-MS/MS method to identify peptides derived from the protein hydrolysate of *Maetra veneriformis*. Karioti et al. (2014) demonstrated the efficiency of one- and two-dimension

NMR spectroscopy along with HPLC-DAD-MS analyses in characterizing the content of a dietary supplement; two samples of olives; the leaves, flowers, and fruit of hawthorn; plants in flower of fumitory; Shepherd's purse, etc. Nebot et al. (2014) presented a rapid analytical method based on HPLC coupled to a tandem mass spectrometer (HPLC-MS/MS) for quantification of four tetracyclines (tetracycline, chlortetracycline, doxycycline, and oxytetracycline) in 31 prepared baby food samples containing vegetables and beef. Wu et al. (2014) used a novel sensitive and selective HPLC-MS/MS method for analysis of 11 bioactive components (six steroidal saponins and five flavonoids). Patras et al. (2014) identified several flavonoids from alcoholic extracts of raw fermented cocoa beans from Cameroon using a detailed HPLC-ESI-MS. Chen et al. (2014) used HPLC coupled with MS and Q-TOF MS for the rapid identification of lignans in flaxseed. Freitas et al. (2014) described sulfonamides, trimethoprim, tetracyclines, macrolides, quinolones, penicillins, and chloramphenicol in gilthead sea bream (*Sparus aurata*) using UHPLC-MS/MS.

Electrophoresis

Electrophoresis is based on the differential migration of charged components in an electric field. The basic requirements of electrophoresis are a semi-conducting medium and an electric field. The semi-conducting medium can be a paper soaked in an electrolyte or a gel placed in an electrolyte.

Conventional Electrophoresis

Conventional electrophoresis comprises vertical or horizontal and flat gel or disk gel electrophoresis. The horizontal system has more advantages than the vertical electrophoresis with respect to simpler handling, buffer strips instead of large buffer volumes, and efficient cooling. Flat gel electrophoresis also has an advantage over disk gel electrophoresis because a large number of samples can be analyzed at one time (Kane et al. 1999). In conventional electrophoresis, components are separated based on particular size, the small molecules migrating faster than the larger ones. Detection is a major problem because separated materials are trapped in a matrix and are not easily quantified. Gel electrophoresis is performed either by disk electrophoresis or by flat electrophoresis (Cancalon 2000).

Capillary Electrophoresis

In the CE methodology, the separation is performed in a capillary filled with a carrier electrolyte and loaded with analyte. Following application of electrical energy at both ends of the capillary, the analyte migrates and is sensed by the detector. CE comprises a group of electrophoretic techniques: capillary zone

electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), capillary isotachopheresis (CITP), and micellar electrokinetic capillary chromatography (MECC) (Oliveira et al. 2014). CZE separates analytes according to their charge-to-size ratio under the influence of an electric field in a buffer-filled capillary. Typical separations are performed in a 10–50 μm inner diameter, 10–50 cm long fused silica capillary at a potential of 10–30 kV. The basic apparatus for CGE is similar to that of CZE, the major difference between the two techniques being that while a sieving matrix is used in CGE, a background electrolyte solution is employed in CZE (Zhu et al. 2012). In the case of CIEF, the fused-silica capillary is coated with linear polyacrylamide to eliminate electroosmotic flow and reduce protein adsorption to the walls (Jensen et al. 2000) with a pI (isoelectric pH) difference as small as 0.005 pH units (Chen et al. 2003). A polyampholyte mixture in free solution is utilized by CIEF (Jensen et al. 2000). CITP is a preconcentration step to increase detection sensitivity prior to real separation. CITP uses the analytes' conductivity. The analyte zones are between a leading and a terminating electrolyte. Analytes go to discrete zones according to their mobilities (Suntornsuk 2010). MECC is a very suitable method for the determination of some components, because it allows rapid, selective, and accurate analysis. The fundamental principle of MECC is that an ionic surfactant used as a pseudostationary phase (PSP) provides separation of electrically neutral and charged solutes. The separation occurs primarily on partitioning of analytes with a hydrophobic micelle effects (Pucci and Raggi 2005). The advantages of CE in terms of high efficiency, good repeatability, fast analysis, and low consumption of electrolytes and samples make this technique a good alternative to other more established techniques for determination of components (González and González 2012).

Schocker and Becker (2001) used three different methods to separate low molecular mass proteins to identify food allergens and peptides of hazelnut: sodium dodecyl sulfate–polyacrylamid gel electrophoresis, immunoblotting, and protein staining. In this study of hazelnut protein, sodium dodecyl sulfate–polyacrylamid gel electrophoresis led to the clearest peptide separation pattern. Alenazi et al. (2015) demonstrated the ability to analyze milk samples after dilution with a background electrolyte. Bisphenol A was easily separated from all milk constituents on the basis of different electrophoretic mobility values. Vulcano et al. (2015) described secoiridoids in olive oil, oleocanthal, and oleacein with CE because it only needs minute volumes of sample and buffer. Both compounds were quantified by CE with ease and precision and their natural matrix was confirmed. An et al. (2015) studied the amount of putrescine, histamine, tyramine, phenylethylamine, and spermidine in oysters with CE coupled with electrochemiluminescence (CE-ECL) during storage at two different temperatures (0 °C and 4 °C). The results showed that spermidine and putrescine became dominant with an increase in the storage time. In contrast, the histamine, tyramine, and phenylethylamine levels increased slightly throughout the storage period in all of the experimental conditions. Liu et al. (2015) developed a new method for the determination of food colorants in beverage samples using diamino moiety functionalized silica nanoparticles (dASNPs) as both adsorbents in dispersive SPME (dSPME) and

PSPs in CE separation. Vergara-Barberán et al. (2014) developed a method to classify olive leaves and pulps according to their cultivar using protein profiles with CGE. Chmiel et al. (2014) determined the quantification of K^+ , Na^+ , Ca^{2+} , Mg^{2+} , and ammonium in berries of different blue honeysuckle cultivars with a capillary isotachopheretic (cITP) method. Wang et al. (2012) used micellar electrokinetic capillary chromatography to determine azoxystrobin, kresoxim-methyl, and pyraclostrobin in fruit and vegetables.

Conclusion and Future Directions

The range of instrumental methods is broad and they have applications in all areas of chemistry. Naturally, many different methods have been used and in some cases even developed by chemists. In this chapter, an overview is given in which the methods have been classified as spectroscopic, chromatographic, and electrophoretic. The instrumental methods have complementary roles in chemistry and include software packages that are available for experimental design, and are sometimes even included in laboratory instruments.

After several decades of instrumental analysis equipment being on the market, the technology and its applications have improved significantly. An end to the possible uses of this technology has not been reached, and there are always new challenges for further improvements in their performance and for the scope of the applications to be extended.

In the future, substantial progress and improvement in technologies employing the MS technique will be seen in food analysis. The range of analytical methods involving MS are reported to effectively detect, identify, quantify, and confirm various naturally occurring as well as xenobiotic substances in the food chain. Finally, hyphenated instruments are going to become the preferred method for routine usage in the future.

Cross-References

- ▶ [Classical Wet Chemistry Methods](#)
- ▶ [Sampling and Sample Preparation](#)

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Part III

Chemical Composition of Food and Food Commodities

Cristina M. Rosell and Raquel Garzon

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Abstract

Cereals have always occupied a preferential place in the food pyramid that gathers dietary guidelines. Despite society lifestyle changes, cereal-based goods are still the main players in the human diet, although their worldwide contribution to dietary patterns is different. Regarding cereals' role in the daily diet, three different categories could be considered worldwide: (i) cereals as staple/basis food, (ii) cereal-based goods as carriers of micronutrients or fortification, and (iii) cereal-based foods tailored for specific needs. Bread is the main bakery consumed product worldwide, although the concept of bread comprised thousands of different products regarding their processing, shapes, composition, and so on. Although with far less consumption, other bakery products include

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cookies, cakes and muffins, croissants, and pastries. An overview about the chemical composition of cereals and cereals products, as well as their role in human nutrition and health, is presented.

Introduction

Cereals constitute the raw commodities of the bakery products; because of that, a special consideration is given to the chemical composition of cereals and the impact of processing on that composition. Cereals are the edible seeds of the grass family, *Gramineae*. The global importance of cereal crops to the human diet has been in parallel to the written history of man and agriculture. Bakery products are mainly fermented cereal-based products subjected to a high-temperature process for increasing their organoleptic properties and shelf life. Cereals are the most important group of food crops produced in the world. In the last 50 years, cereal production has experienced a progressive linear increase, responding to the greater demand owing to population increase. Despite changes in lifestyles and cereal utilization, half of the cereal production is destined to human food (Fig. 1); only a slight modification has occurred in cereal utilization; in the 1960s, 51 % of the cereal production was utilized for human consumption, whereas in the twenty-first century, 52 % of the production is dedicated to food and food manufacture. Even better picture of the cereal contribution to the human diet could be obtained from the yearly consumption per capita that reached the maximum point, 150 kg/person year, in the 1990s, but it decreased to 147 kg/person year during the last two decades, owing to the shift to animal-based foods when income increases.

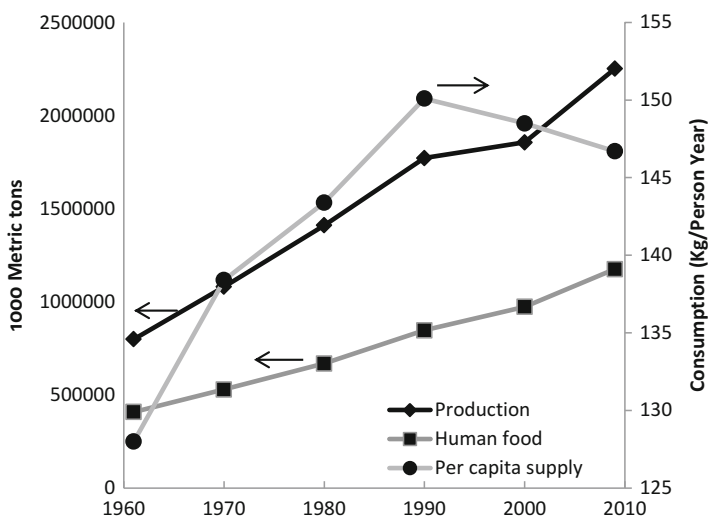


Fig. 1 Cereals as human food over the last 50 years

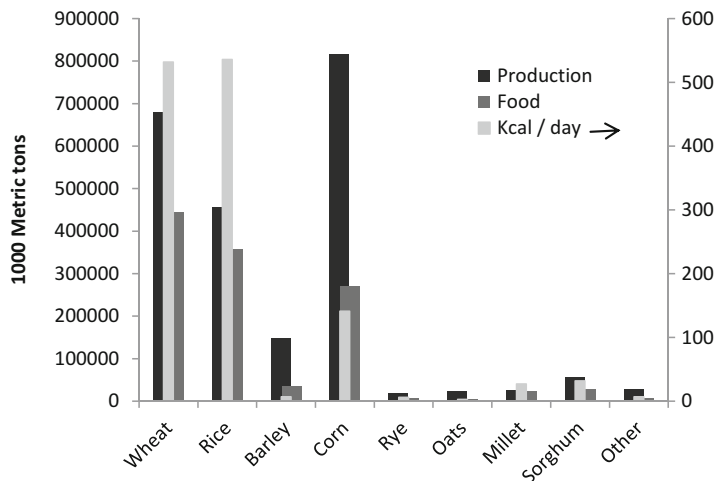


Fig. 2 Cereal production and contribution to the daily food intake in 2009

Cereal-based products are really ancient products that comprise a diversity of foods mainly obtained from wheat, rice, corn, millet, teff, and other minor cereals. Major cereal crops produced worldwide include wheat, rice, corn, and barley. Corn (or maize) represents the greatest (36 %) cereal crop for agriculture, but it has relatively less importance than wheat and rice because only one-third of its production is destined to human consumption, in comparison with the 66 % and 79 % of the wheat and rice used as human foods, respectively (Fig. 2). Some other minor cereals include sorghum, millet, oat, rye, and some pseudocereals as quinoa and amaranth.

Cereals still remain on the basis of the human nutrition because they lead to nutrient-dense baked goods. However, the weight of the cereals as an individual source of nutrition varies with region, depending upon the importance of a grain to the development of that culture. Cereal grains, such as wheat, rice, corn, barley, sorghum, millet, and oats, account for most of the food consumed by humans. Rice is more important to the development of Eastern cultures, where its cultivation is concentrated, and wheat is more significant to Western ones. Globally, wheat nourishes more people than any other grain, and it is a major part of the diet for one-third of all people. The majority of wheat is milled into flour and it is the basis of more foods than any other grain.

Cereal crops are energy dense, providing about 10–20 times more energy than most succulent fruits and vegetables. Nutritionally, they are important sources of dietary protein, carbohydrates, the B complex of vitamins, vitamin E, iron, trace minerals, and fiber. It has been estimated that global cereal consumption directly provides about 32 g of protein per person and day and 5.9 of fat per person and day, being the highest contribution in the developing countries (Table 1).

However, cereals are mainly consumed after milling or processing which significantly change their composition, due to removal of the outer parts, and consequently the composition of the derived baked products.

Table 1 Contribution of cereals to human nutrition in different world regions (Data source: FAOSTAT 2014)

	Food/capita/year (kg)	Kcal/capita/day	Proteins/capita/day (g)	Fat/capita/day (g)
World		1,292	32.0	5.9
Africa	151	1,283	34.0	8.4
Northern America	109	831	24.4	3.6
Central America	153	1,283	32.9	11.5
South America	118	972	23.3	3.1
Asia	155	1,421	33.5	5.9
Europe	131	1,002	29.9	4.0
Oceania	98	792	22.9	3.1

Apart from the nutritional contribution, cereals have important nutraceutical and health benefits that go beyond the provision of nutrients. The consumption of cereal based foods products produces feelings of satiety; and their regular consumption in the main meals appears to be key drivers of healthier dietary patterns (Aisbitt et al. 2008). Nevertheless, consumption of cereal-based foodstuff has been attached to several misconceptions: first the association of cereals, mainly wheat-based goods, with food intolerance or allergy and second the link between cereals and fattening effect (Aisbitt et al. 2008). Therefore, unless following the advice from a registered dietitian or other health professional, consumers must be skeptical to all those currents and do not eliminate unnecessarily this whole food group because they provide a range of macro- and micronutrients and fiber. Dietary fiber, beta-glucan, resistant starch, carotenoids, phenolics, tocotrienols, and tocopherols have been related to disease prevention like cardiovascular diseases and strokes, hypertension, metabolic syndrome, type 2 diabetes mellitus, obesity, as well as different forms of cancer (Yu et al. 2012).

Cereals have a variety of uses as food. Owing to their protein functionality, only two cereals, wheat and rye, are suited to the preparation of leavened bread. Because of that, this chapter will be mainly referred to baked goods derived from those crops, although mention will be made to corn due to its use for producing tortillas, which are extensively consumed in Mexico and their commercialization has been extended around the world.

Worldwide Consumption of Bakery Products

The almost ubiquitous consumption of cereals all over the world confers cereals a prominent position in international nutrition. Cereal-based products account great part of our daily diet through the consumption of bread, breakfast cereals, cookies, snacks, cereal bars, cakes, and so on. The total consumption of bread, viennoiserie, and patisserie was estimated as nearly 39 million tons in the 27 EU countries.

Worldwide bread consumption accounts for one of the largest consumed foodstuff, with an average consumption ranging from 41 to 303 kg/year per capita, which becomes an essential part of the human diet, enjoyed at various times of the day. Bread, through the different forms, is the most widely consumed food worldwide. It is handy and very convenient for the on-the-go consumers, available in any place and during all year around, affordable, and from nutritional point of view a source of energy in the form of starch, besides the supply of dietary fiber and a range of vitamins and minerals.

Bread has changed in many ways since our ancestors, going from a grainy flatbread to an aerated texture (Fig. 3). Bread is the product of fermenting and baking a mixture of whole wheat or refined flour, water, salt, and yeast or baking powder, as the basic ingredients. With the years, innovation has been focused on industrial processing (Rosell 2009), and, more recently, trends drive to obtain nutritious bread in response to the health concerns of the consumers.

A study for the European Commission in 2010 found that the European bread market was around 32 million tons in the 27 EU countries and the market share of the industrial bakers versus the craft bakers was approximately 50/50 although great differences were encountered among countries. Bread consumption patterns differ widely within the EU but most countries have an average consumption of 50 kg of bread per person per year.

Sliced pan bread is greatly extended in the USA, but Europe's consumers prefer crispy breads with crusty surface like French baguette. The opposite sensory characteristics could be found in the steamed bread that is consumed in Asian countries. In India, flatbread called chapatti is consumed in the main meals, whereas flatbread in Mexico is made of corn and it is named tortilla. In Finland and Germany, a very dark rye bread, made of 100 % rye flour, is the most common bread. In Venezuela, the arepas are considered a staple part of the diet, and they are eaten for breakfast, as a snack, or together with a meal. In Brazil, pão de queijo, small, round, cheese-stuffed bread balls, are traditionally served for breakfast. It seems that the term bread comprises thousands of different types of breads around the world and even there is a big diversity within each country.

Bread consumption patterns differ widely around the world. For instance, within the EU, the average consumption is 106 g of bread per person per day, in contrast with the 40 g per person per day of fine bakery wares and around 10 g per person per day of breakfast cereals (EFSA Comprehensive European Food Consumption Database, published in March 2011). Bread consumption in Western Europe is stable although it varies greatly between countries. Germans and Austrians show the highest consumption of bread at around 80 kg/year, although the greatest annual consumption registered in the Guinness World Records belongs to Turkey in 2000 with 199.6 kg per person followed by Serbia and Montenegro with 135 kg and Bulgaria with 133.1 kg. In opposition, the UK and Ireland have an annual consumption of less than 50 kg/year.

Bread is also a staple food in the diet of American consumers, principally for adult men and women 55 years and older, households with low annual incomes,

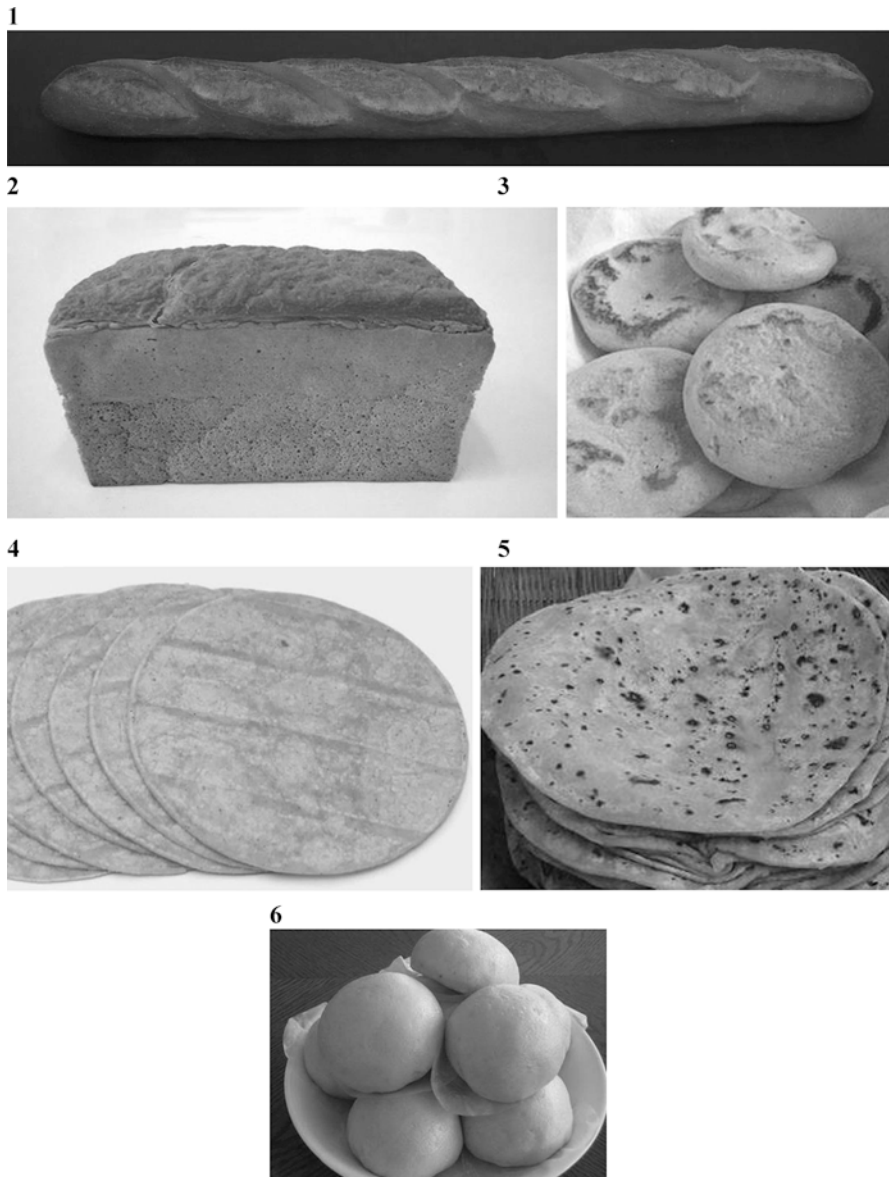


Fig. 3 Bread types worldwide. 1. White bread; 2. Pan bread; 3. Pita bread; 4. Tortilla; 5. Chapatti; 6. Chinese steamed bread

households led by a homemaker with some high school education, single and married active seniors, and Black/non-Hispanic ethnic groups.

Lately, the driving force for increasing bread consumption is the innovation in ethnic breads and the production of greater varieties of whole wheat breads with

oats, bran, seeds, etc. that are more nutritious products to attract health-conscious consumers (Lambert et al. 2009). There is also a growing trend for increased production of morning goods and specialty breads with lots of opportunities for innovation. Nevertheless, some popular diets, such as the Paleo and gluten-free diets, have limited the consumption of wheat breads. Changes in demand for bread have induced a growing demand for low-carbohydrate foods, whole grains, and gluten-free products. Companies have successfully attracted these health-conscious consumers by introducing products such as low-calorie “sandwich thins” and gluten-free products. This shift in consumer preferences has been attributed to more sophisticated consumer tastes and their desire for healthier and more nutritious food, in line with lowering sodium, besides the looking for more exotic or ethnic varieties and new formats, like thinner slices.

Chemical Composition of Cereals and Changes Associated to Milling and Proofing

The chemical composition of bakery products is mainly dependent on the commodity used for their production; because of that, the chemical composition of the different cereals that are used as raw materials in bakeries is considered crucial. All cereal grains have a fairly similar structure and nutritive value although the shape and size of the seed may be different (McKevith 2004). Cereal grains are formed of bran or the outer layers, endosperm and germ. Cereals are the major providers of food carbohydrates, representing about 50 % of all the carbohydrates consumed worldwide.

Cereals are quite similar in gross composition, being low in protein and high in carbohydrates. Regarding composition, cereals consist of 12–14 % water, 55–75 % carbohydrates, 1.5–6 % lipids, and 7–12 % protein; mineral composition varies from 1.4 % to 2.5 % and the fiber content ranges from 0.8 % to 4.1 %. Barley, sorghum, rye, and oat proteins have lower digestibility (77–88 %) than those of rice, corn, and wheat (95–100 %). The biological value and net protein utilization of cereal proteins are relatively low due to deficiencies in essential amino acids and low protein availability. Cereals provide B-group vitamins and minerals like phosphorous, potassium, magnesium, calcium, and traces of iron. Cereals are low in saturated fat but they are a source of polyunsaturated fats, including omega-3 linolenic acid, and also cholesterol-free.

Whole-grain intakes and specially cereal fiber have been repeatedly associated in the epidemiological literature with reduced mortality and risk of chronic disease including obesity, cardiovascular diseases, and type 2 diabetes (Smith and Tucker 2011; Borneo and León 2012).

Nevertheless, cereals are not consumed as grains. They undergo milling to obtain flour that has particular functionality for creating and developing an infinity of breads that differ in shapes, structure, and sensory characteristics. The chemical constituents of cereals are not evenly distributed in the grain. Bran that represents 7 % of the grain contains the majority of the grain fiber, essentially cellulose and pentosans, it is a source of B vitamins and phytochemicals, and 40–70 % of the

minerals are concentrated in this outer layer. The endosperm, the main part of the grain (80–85 % of the grain), is composed mainly of starch, it has lower protein and lipid content than the germ and the bran, and it is poor in vitamins and minerals. The germ, the small inner core, represents around 21 % of the grain, and it is rich in B-group vitamins, proteins, minerals like potassium and phosphorus, healthful unsaturated fats, antioxidants, and phytochemicals. Cereals are rich in glutamic acid, proline, leucine, and aspartic acid and are deficient in lysine. The amino acid content is mainly concentrated in the germ.

Milling provokes the reduction in nutrients and micronutrients that are mainly concentrated in the outer layers and germ. In the conversion of cereal grains to white flour, bran and germ are removed and with them the majority of the fibrous layers of the grain, B vitamins, phytochemicals, 50–80 % of the grain minerals, and lipids. Globally, twenty or more ingredients, such as thiamine, riboflavin, niacin, pyridoxine, folate, pantothenate, biotin, vitamin E, calcium, copper, iron, potassium, magnesium, manganese, phosphorus, zinc, chromium, fluorine, molybdenum, and selenium, are lost to the extent of 50–90 % during the milling process (Rosell 2008). Conversely, white flour contains mostly starch, protein, and low content of fat, vitamins, and minerals.

The consumption of processed or refined products may reduce the health benefits of the original food. In wheat-based processed foods, the removal of external layers and germ is parallel to the reduction in nonessential phytochemicals such as carotenoids, polyphenols, phytosterols/stanols, and dietary fibers. In consequence, those refined products have lost the potential ability to reduce the risk of major chronic diseases of humans, such as cancer, cardiovascular diseases, and Parkinson's disease.

Wheat is the most commonly used cereal for producing bakery goods. Wheat and wheat-based products have been the basis of the human diet for many centuries, so any process that leads to a decrease in the nutritional value of these products results in a reduction in the daily supply of fiber, vitamins, and minerals. The increasing awareness of the potential benefits of high-fiber diets has prompted a growing interest in the consumption of whole-grain breads or bran breads. The complex carbohydrates, mainly starch, are the major components of wheat (61–65 %), but it is also an excellent source of dietary fiber (9–12 %), composed mainly of cellulose, complex xylans, lignin, and β -glucans, which are located in the outer layers of the grain. The protein content of wheat is one of the highest in cereals; it ranges between 10 % and 15 %. The predominant amino acids are glutamic acid, proline, leucine, and aspartic acid and are mainly concentrated in the germ. The fat content is very low (1.7–2.0 %) and is mainly polyunsaturated with an absence of cholesterol. With respect to the micronutrient composition, wheat is also a source of B vitamins, namely, thiamine (B₁), riboflavin (B₂), and niacin (B₆), and minerals. Important amounts of calcium, phosphorus, iron, sodium, magnesium, and potassium are found in the aleurone layer of wheat.

Cereals and other plant foods may contain significant amounts of toxic or antinutritional substances. Most cereals contain appreciable amounts of phytates and enzyme inhibitors, and some cereals like sorghum and millet contain large amounts of

polyphenols and tannins. Some of these substances reduce the nutritional value of foods by interfering with the mineral bioavailability and digestibility of proteins and carbohydrates. Special consideration must be paid to phosphorus, since it is forming the complex phytic acid or myo-inositol hexaphosphate, which it is not readily available and is considered an antinutritional compound because of its adverse effects on the bioavailability of minerals. Other health-promoting compounds mainly concentrated in the germ and bran are the phytochemicals. These phytochemicals include lignans, a phytoestrogen that can lower the risk of coronary heart disease and may protect against hormonally linked diseases such as breast and prostate cancers.

Other phytochemicals include saponins, phytosterols, squalene, oryzanol, and tocotrienols, which lower blood cholesterol, and some phenolic compounds that have antioxidant effects.

The consumption of wheat is recommended due to its excellent nutritional profile, consisting of: (i) complex carbohydrates; (ii) dietary fiber; (iii) low fat content (without containing cholesterol); (iv) minerals, especially calcium, phosphorus, iron, and potassium; and (v) B vitamins.

Milling Impact on the Nutritional Composition of Cereals

Whole wheat flour contains all the constituents and nutrients from the grain; thus, no losses are produced when milling whole grains. Nevertheless, during the twentieth century, consumers move their preferences to white bakery products, which were a sign of high quality; in consequence, refining became a common practice for millers. Milling removes the external layers and germ of the grains and the extraction rate is indicative of the milling yield. Higher extraction rate means high recovery of flour from the initial grain; thus, more nutrients are recovered in the resulting flour. However, for obtaining white flour, extraction rates must be lowered to 65–70 %, increasing the starch concentration in the flour and with the subsequent losses of the rest of constituents. Wheat processing like milling can result in a significant change in the nutritional value of the ultimate wheat products. The nutrients concentrated in the bran layers and germ will be removed during the traditional or conventional milling. The most affected compound during conventional milling is the crude fiber followed by the ash (minerals) and vitamins, having a detrimental effect on the nutritional value. Without the bran and germ, about 25 % of the grain proteins are lost, along with 66 % of fiber, 92 % of selenium, 62 % of folate, and up to 99.8 % of phytochemicals (Rosell 2012). Some fibers, vitamins, and minerals may be added back into refined cereal products, which compensates for losses due to refining, but it is impossible to recover the phytochemicals lost in the processing. Other alternatives to improve the nutritive value of cereals include traditional genetic selection, genetic engineering, nutrient and micronutrient fortification, complementation with other proteins (particularly legumes), milling modifications, heating, and germination.

Agronomic fortification has been a very useful alternative for increasing the level of micronutrients in the edible parts of the plants, which is known as

biofortification. Biofortification focuses on the agricultural modifications as a public health intervention, which could have a direct impact on the diet of the low-income consumers, who are often at highest risk for micronutrient deficiencies. HarvestPlus is a Challenge Program of the Consultative Group on International Agricultural Research (CGIAR) in which the objective is to design, produce, test, and disseminate staple food crops that are biofortified with iron, zinc, and provitamin A. Under this program, a steady progress in breeding for biofortified crops has been achieved, with satisfactory results regarding levels of zinc and provitamin A carotenoids and iron in several staple food crops, including maize, rice, wheat, pearl millet, potatoes, and bananas or plantains.

Biofortification can be accomplished by either mineral fertilization or plant breeding. Biofortification by mineral fertilization is a common practice in some countries that applied selenium-containing fertilizers as a short-term solution for improving the selenium content of wheat. Selenium is an essential micronutrient for humans and may reduce the risk of degenerative diseases including cancer, but it is deficient in at least a billion people worldwide. Wheat is a major source of dietary selenium in humans. The intake of selenium through the baked goods obtained from agronomically biofortified wheat increases the plasma selenium concentration but without modifying substantially the selected biomarkers of degenerative disease risk and health status. Biofortification through plant breeding consists of the development of micronutrient-enhanced crop varieties through conventional breeding. Nevertheless, the implementation of this type of biofortification requires the identification of genetic resources, to determine the interaction of genotype and environment, to define the desired level of micronutrient increase, and finally to assess the cost-effectiveness. So far, it seems a sustainable and cost-effective approach for reaching biofortification in zinc and iron. Past efforts have focused on increasing crop yields, but today the enhancement of the nutritional quality of the crops has become an urgent task because about half of the world population suffers from malnutrition with respect to iron, zinc, and selenium.

Another agronomical approach for enhancing the nutritional value of wheat flour is the expression of a heat-stable phytase, which is more resistant to baking or processing temperatures and can degrade phytate more efficiently. Phytase is an esterase that catalyzes the stepwise hydrolysis of phytates to phosphate and inositol via penta- to monophosphates, thus progressively reducing the ability of phytates to complex with minerals (binding with minerals renders phytates nonabsorbable in the intestines). Animal feeding experiments confirmed a significant improvement in zinc bioavailability from transgenic wheat containing a heat-stable phytase.

Germination or sprouting has been traditionally applied at a household level to improve the nutritional, functional, and sensory properties of grains such as pulses and cereals. Pulses are a particularly rich source of vegetable proteins, dietary fibers, vitamins, and minerals, but their nutritional value is limited by the presence of antinutrients that reduce digestibility and micronutrient bioavailability, and by germination the level of antinutrients is substantially reduced. Only recently the benefits of germination have been recognized, and industrial processes have been developed to germinate grains and enable the change in their chemical composition.

Specifically, sprouting of grains for a limited period causes increased activities of hydrolytic enzymes, degradation of beta-glucans, improvement in the contents of certain essential amino acids and B-group vitamins, and a decrease in dry matter, starch, and antinutrients. Proteins and starch improve their digestibilities due to their partial hydrolysis during sprouting. Nevertheless, the conditions for sprouting must be carefully defined for each type of cereal. The flour from sprouted grains is nutritionally improved and it might be expected that resulting processed foods will have a substantial nutritional advantages.

An alternative to whole-grain flour is the use of different milling processes for obtaining nutritionally improved flours. Debranning or cereal-grain pearling is increasingly accepted by the milling and baking industry as a key stage in cereal processing. Pearling is the process prior to milling that removes effectively only the bran layers from the cereal grains with the application of abrasion and friction. This selective and limited removal allows nutritious parts, such as the aleurone layer to remain in the intact kernels. This pretreatment potentially could also improve milling yields of superior flour quality; besides, it lowers the capital investment costs. Nevertheless, the antioxidant capacity of pearled grains significantly decreases as the degree of pearling increases, which goes to the by-products that have higher antioxidant capacity compared to the pearled grains. The concentration of grain antioxidants is dramatically reduced during the refining process because phenolic compounds are concentrated in the outermost layers. Bran fractions, the by-product resulting from pearling, may be used as a natural source of antioxidants in the production of functional food ingredients or for the enrichment of certain products. Sequential pearling is applied to maximize the health benefits of the wheat flours, which could be used for partial replacement of the refined wheat flour (up to 10 %). The antioxidant and dietary fiber contents of the bread can be increased by enriching with pearled fractions, without affecting significantly the technological properties. Although in those strategies it must be considered the possible risk of micotoxins content when very external layers are not completely removed.

Nutritional Changes Occurring Along the Breadmaking Process

Different alternatives have been developed for adapting breadmaking to the consumer demands and for facilitating the baker's work (Rosell 2011). Breadmaking stages include mixing the ingredients, dough resting, dividing and shaping, proofing, and baking, with great variation in the intermediate stage depending on the type of bread. Breadmaking is a dynamic process with continuous physico-chemical, microbiological, and biochemical changes, motivated by the mechanical-thermal action and the activity of the yeast and lactic acid bacteria together with the activity of the endogenous enzymes. During mixing, fermenting, and baking, dough is subjected to physical changes till yielding bread, in which gluten proteins are mainly responsible for bread dough structure formation, whereas starch is mainly implicated in the final textural properties and stability. During mixing is formed a

continuous protein network where gluten, a non-pure protein system, has the main contribution.

Yeast and lactic acid bacteria participate along proofing and the initial stage of baking. Therefore, wheat flour, yeasts, and bacterial population of sourdoughs are the sources of different endogenous enzymes in breadmaking processes and exert an important effect on dough rheology and on the technological quality of bread (Rosell 2011). Different processing aids, namely, enzymes, are also used in breadmaking to improve the quality of the baked products by reinforcing the role of gluten, providing fermentable sugars, and/or contributing to stabilize the hydrophobic-hydrophilic interactions (Rosell and Collar 2008).

Fermented foods contribute to about one-third of the diet worldwide. Cereals are important substrates for fermented foods in all parts of the world and are staples in the Indian subcontinent, Asia, and Africa (Guyot 2012). Fermentation causes changes in food quality indices including texture, flavor, appearance, nutrition, and safety. Some benefits derived from fermentation include improvement in palatability and acceptability by developing improved flavors and textures; preservation through formation of acidulants, alcohol, and antibacterial compounds; enrichment of nutritive content by microbial synthesis of essential nutrients and improving the digestibility of protein and carbohydrates; removal of antinutrients, natural toxicants, and mycotoxins; and decreased cooking times.

The content and quality of cereal proteins may be improved by fermentation. Natural fermentation of cereals increases their relative nutritive value and available lysine. Bacterial fermentations involving proteolytic activity are expected to increase the biological availability of essential amino acids, whereas yeast fermentations mainly degrade carbohydrates. Starch and fiber tend to decrease during fermentation of cereals. During breadmaking, the total amino acid content (particularly for ornithine and threonine) increases by 64 % during mixing and undergoes a decrease of 55 % during baking, with glutamine, leucine, ornithine, arginine, lysine, and histidine being the most reactive amino acids. In general, wheat doughs started with lactic acid bacteria show a gradual increase of valine, leucine, and lysine along the fermentation, and also proline but only during the initial hours of proofing. Additionally, the action of proteinases and peptidases from lactic acid bacteria on soluble polypeptides and proteins results in the increase of short-chain peptides that contribute to plasticize the dough and give elasticity to gluten.

Fermentation also modifies the mineral content of the product, improving its bioavailability. Changes in the vitamin content of cereals with fermentation vary according to the fermentation process and the raw material used in the fermentation. The yeasted breadmaking process leads to a 48 % loss of thiamine and 47 % of pyridoxine in white bread, although higher levels of those vitamins could be obtained with longer fermentations (Batifoulier et al. 2005). Native or endogenous folates show good stability to the baking process, and even an increase in endogenous folate content in dough and bread compared with the bread flour was observed by Osseyi et al. (2001). Nevertheless, breadmaking process with yeast fermentation is beneficial for reducing the levels of phytate content with the subsequent increase in magnesium and phosphorus bioavailability (Rosell 2012).

Free amino acids in wheat flour and dough play an important role in the generation of bread flavor precursors, through the formation of Maillard compounds during baking. In fact, leucine, proline, isoleucine, and serine reacting with sugars form typical flavors and aromas described as toasty and bread-like, while excessive amounts of leucine in fermenting doughs lead to bread with unappetizing flavor. But it must be stressed that the biochemical changes that occur during breadmaking are highly dependent on the process, that is, time and temperatures for leavening, resting, and baking or cooking (Dewettinck et al. 2008). In fact, in the case of steamed bread, a decrease in the amount of amino acid takes place during production, especially alanine and tyrosine.

A special remark must be done regarding acrylamide (probable carcinogen) that has been found in starchy baked foods. No link between acrylamide levels in food and cancer risk has been established and based on the evidence to date, but some concern has risen with the first mentions. However, the Scientific Committee on Food of the European Union (EU) has endorsed recommendations made by the Food and Agriculture Organization/World Health Organization addressed to research the possibility of reducing levels of acrylamide in food by changes in formulation and processing.

Chemical Composition of Bread

Bread is the bakery specialty most frequently consumed, but different bread types result from diverse recipes, processing, shapes, and so on (Fig. 3). Analysis of the UK Government's National Diet and Nutrition Survey in 2012 suggests that bread still contributes more than 10 % of an adult's daily intake of protein, thiamine, niacin, folate, iron, zinc, copper, and magnesium; 20 % of our fiber and calcium intake; and more than 25 % of our manganese intake. Thus, eating bread can help consumers to meet their daily requirements for many nutrients, including micronutrients such as zinc and calcium.

A long-standing belief of the consumers is that bread fattens. This encourages many people to restrict, or even eliminate, bread from their diet. Nevertheless, eating patterns that include whole-grain bread could not be associated with overall obesity or excess abdominal adiposity and may be beneficial to ponderal status. Regarding dietary patterns that include refined bread, a possible relationship with excess abdominal fat has been encountered. Whole-grain cereals of various kinds are recommended due to their healthy and nutritious composition (Collar 2008).

Refined grains and cereals do not contain sufficient nutrient for maintaining the health of sedentary populations. Refined cereals, such as white flour, generally have higher glycemic index than its whole-grain counterpart; thus, consuming refined cereals causes a sharp rise in blood sugars, demanding a strong response from the pancreas. A diet full of high-glycemic-index foods has been linked to the development of diabetes. Generally, dietary pattern of people who eat large amounts of refined cereals is poor in more nutritious foods like fruits and vegetables, which increases the risk of certain diseases, such as some types of cancer. Sometimes,

fiber removed during milling is added back to refined products or they are enriched with vegetable fibers, but it still remains unknown whether those resulting products have similar beneficial properties and functionality to products from whole wheat cereals.

It is recommended that cereals should be consumed at least 4–5 serves daily and that at least half of these serves should be whole grain; even as little as one serving daily reduced health risks. It must remind that the protective components (such as fiber, antioxidants, and phytoestrogens) are found in the outer layers of grains (Collar 2008).

As has been mentioned before, the basic ingredients for making bread are flour, water, yeast, and salt. Nevertheless, changes in lifestyle have conducted to modify recipes for extending shelf life using preservatives but also for improving texture with conditioners, leavening agents, shortenings, and humectants (Table 2). Conditioners include chemical compounds like emulsifiers (sodium stearoyl lactylate and monoglycerides), enzymes (alpha-amylase, lipase, xylanase, and so on), ascorbic acid, azodicarbonamide (not allowed in some countries), and calcium peroxide. Although bread is fermented by yeast and also sourdough (provides lactic acid bacteria), it is common for some kind of bread to add leavening agents, also known as raising agents or baking powders, among those sodium bicarbonate, sodium aluminum sulfate, disodium diphosphate, sodium hydrogen carbonate, monocalcium phosphate, and/or sodium acid pyrophosphate, calcium sulfate, and calcium phosphate. Other chemicals frequently used are the preservatives for extending the shelf life, like calcium propionate, potassium sorbate, methyl parabens, and/or citric acid. The combination of those ingredients led to breads with diverse nutritional composition (Table 2). The energy contribution of bread could go from 147 to twice that value in some type of breads like packaged chapatti or tortillas. Steamed bread is the one with lower energy supply per serving, due to its high moisture content. Part of those calories, from 6 % to 22 %, comes from fat, but no *trans* fats are present in bread composition. It is also important the absence of cholesterol, which was expected since cereals do not contain it. Refined flours are mainly carbohydrate, and the amount of proteins is limited; in consequence, the same pattern is observed in bread products. Carbohydrates constitute around half of the composition of breads, with the exception of steamed bread that has 50 % of that, due again to its high moisture content. The amount of dietary fibers in breads obtained from refined flour is relatively low (1–4 %). Protein content in wheat bread ranges from 3 to 9 g/100 g. Regarding minerals, bread is a major contributor to sodium intake in many countries. The salt content in bread is not really high, but due to its daily frequent consumption, it provides around 22 % of the mean salt intake from foods. In fact, in the UK, bread and breakfast cereals provide over one-third of salt in children's diets. Excessive sodium intake has been strongly associated with high blood pressure and hypertension, which can increase the risk to suffer heart disease and stroke.

Because of that, different national campaigns have been promoted to reduce the content of salt in breads as a way to reduce overall sodium intake. For instance, the Irish Bread Bakers Association (IBBA) was involved in an ongoing voluntary salt

Table 2 Ingredients used in bread recipes and nutritional composition

Ingredients	White bread (1)	Pan bread (2)	Pita bread (3)	Tortilla (4)	Chapatti (5)	Chinese steamed bread (6)
Wheat flour	x		x		x	x
Enriched unbleached flour		x		x		
Vegetable shortening				x		x
Sugar		x	x			x
Yeast	x	x	x			x
Leavening				x	x	x
Salt	x		x	x	x	
Soybean oil		x				
Sunflower oil			x			
Rapeseed oil					x	
Preservatives		x	x	x		
Conditioners		x		x		
Enzymes			x			
Rice flour				x		
Fumaric acid			x			
Calcium sulfate		x				
Low-fat yogurt					x	
Amount per 100 g						
Calories	240	250	257	279	284	147
Calories from fat	14	18	29	57	52	32
Total fat (g)	1.60	18	3	7	5.8	3.59
Saturated fat (g)	0.4			3	0.5	0.707
Trans fat (g)						
Cholesterol (mg)						
Sodium (mg)	650	571	457	525	330	549
Total carbohydrate (g)	47	54	54	51	49.4	25.06
Dietary fiber (g)	4	4	3		2.1	0.9
Sugars (g)	4	11	3		1.6	0.09
Protein (g)	8	7	9	8	7.4	3.3

Numbers following the name of the bread are used to identify their picture in Fig. 3

reduction program in partnership with the Food Safety Authority of Ireland (FSAI), which resulted in a drop by a minimum of 10 % in the breads.

There is now considerable evidence for the beneficial role played by dietary fiber in health and disease (Smith and Tucker 2011). Dietary fiber absorbs water and increases bowel bulk, resulting in a softer and larger bulk and more frequent bowel action. This provides a good environment for beneficial bacteria while decreasing the levels of harmful bacteria and the buildup of carcinogenic compounds. Cereal fiber or whole grains offer protection against heart disease. Regularly eating cereals that are rich in soluble fiber has been found to significantly reduce the amount of cholesterol in the bloodstream and reduced the risk of heart disease by 25–28 %, stroke by 30–36 %, and type 2 diabetes by 21–30 %.

Conversely, high-insoluble-fiber diet has been associated with decreased risk of developing colon cancer and diverticular disease. There is scientific evidence that people who eat whole grains regularly have lower risk of obesity, as measured by their body mass index and waist-to-hip ratios, and lower risk of many chronic diseases.

Whole grains may be eaten whole, cracked, split, ground, or milled into flour. Those forms can be incorporated in bread dough recipes to increase nutritional composition. Whole wheat breads and cereals are recommended as the best source of energy and fiber (Harris and Kris-Etherton 2010). When a food label indicates that the package contains whole grain, the “whole grain” part of the food inside the package is required to have virtually the same proportions of bran, germ, and endosperm as the harvested kernel does before it is processed, although labeling recommendations are dependent on countries’ regulations. FDA defined in 1999 that “For purposes of bearing the prospective claim, the notification defined ‘whole grain foods’ as foods that contain 51 % or more whole grain ingredient(s) by weight” (extract). The Whole Grains Council (2006) allows certifications with the basic stamp of products that contain 8 g of whole grain per serving where 51 % of the grains are whole grain. The “100 % stamp” must only be tagged in products that contain 16 g of whole grain per serving, and all the grains in the product must be whole grain.

Incorporation of whole-grain flours increased free and bound phenolics and antioxidant capacity, apart from soluble, insoluble, and total dietary fiber fractions and total minerals. Despite the growing concern about healthy dietary guidelines, the impact of the whole-grain products in the human nutrition is still low (Siró et al. 2008). Whole grains make up about 10–15 % of grain products on supermarket shelves, within a huge presence of refined grain foods. Despite whole meal breads provide higher energy and fiber content, no great differences could be observed in the energy and fiber content due to the disparity of ingredients used for making bread (Table 3). Even fat content could be rather similar to that of refined breads. Protein content is higher than the one presented in refined breads because aleurone is also included in wholemeal wheat. Sometimes, cereal bran is added to refined wheat flour to increase the nutritional value of the flour, particularly the dietary fiber content, nonetheless bran stability somewhat limits its use in breadmaking. Different treatments like extrusion and steam cooking of bran have been suggested for improving its technological properties and also its stability during storage. Among stabilized cereal brans, stabilized rice bran is preferred due to its sweet and palatable flavor, which positively affects the sensory evaluation of the bran-containing breads. Wheat germ despite its nutritional properties is considered a by-product of the milling industry due to a high risk of rancidity. But again, certain thermal treatments such as extrusion can reduce that drawback and constitute a suitable treatment to stabilize wheat germ in bread dough or alternatively it can be used as defatted wheat germ. Extruded bran can be incorporated in wheat breads up to 10/100 g flour without significantly affecting the technological and sensorial quality of the breads.

Very often, the dietary fiber content of breads is increased by adding dietary fibers from different sources, like fruit extracts, resistant starch, beta-glucans, and

Table 3 Proximate composition of bread types containing different grains

Amount per 100 g	3 grains	Multigrain	White bread	Large enriched bread	Whole grain and flaxseed	Whole wheat
Calories (kcal)	316	256	289	250	263	279
Total fat (g)	12	3	4	3	4	3
Total carbohydrates (g)	42	47	53	53	45	49
Dietary fiber (g)	5	7		3	8	7
Proteins (g)	13	12	8	6	11	12
Sodium (mg)	395	442	632	563	421	488
% daily value						
Vitamin A						
Vitamin C						
Calcium	11	5	5	13	11	5
Thiamine	39		26	31	16	14
Riboflavin	53		16	19	5	9
Niacin	11		16	19	16	19
Folic acid	16		21	25	5	9

so on. Oat and oat or barley products containing beta-glucans are associated with many health claims including reducing blood cholesterol levels, although the physiological efficacy is dependent on the level and molecular weight of beta-glucans. Numerous commercial fibers are available in the market, which differed in solubility, particle size, hydration properties, and viscosity, among other characteristics.

Bread, like other cereal-based products, is rich in carbohydrates and produces high glycemic response. The replacement of part of wheat flour by fibers offers the chance to improve the nutritional balance of bread at the expense of readily digestible carbohydrates. In general, dietary fiber enrichment is responsible for deterioration in the expected and perceived liking of breads, although the effect might be alleviated using high-quality wheat flours.

Other trend goes to enrich bread by using protein from different sources. Legumes have been proposed as flour sources for complementing the essential amino acid balance of the cereal proteins. Legumes are rich in proteins, dietary fiber, complex carbohydrates, resistant starch, vitamins, and minerals. This practice improves the amino acid profile and increases the protein content of the bakery products made from blended flours. This approach has been selected in some countries with minor production of wheat for replacing it in bread production with the added nutritional benefit. Faba beans are among the legumes used for producing protein-rich flours. Levels of legumes used in breadmaking may reach up to 15 % wheat flour replacement without affecting dramatically the rheological properties of the dough and the sensory characteristics of the breads. That addition could lead up to 20 % increase in the protein content with minor effect on other constituents like fat.

Even with some types of breads like flatbreads, flours milled from green lentils, navy beans, and pinto beans can replace up to 25 % of the wheat flour. For instance, pita breads can be prepared with navy and pinto bean flours with coarse particle sizes with adequate color, texture, and sensory acceptance. This type of breads has been enriched with milk, eggs, other cereals, legumes, syrup, dried fruits, leafy vegetables, cassava, green banana, flaxseed flour, sesame, blackseeds, species, and dried or fresh herbs in order to increase the nutritional content in protein, vitamins and minerals, and fibers.

Other small seeds have been also added to nutritionally improve the quality of breads. For instance, fennel seeds are a source of many nutrients, like sugars, minerals, essential fatty acids, vitamins, protein, fiber, and many flavonoids. Bread with fennel seed content between 5.0 and 7.0 % shows good acceptability besides an increase in the antioxidant activity (i.e., total phenolic content, ferric reducing antioxidant power, and 2,2-diphenyl-1-picrylhydrazyl radical scavenging). Full-fat or partially defatted flaxseed meals have been added in the recipe of bread and flatbread for increasing the insoluble and soluble dietary fiber contents and amino acid (like isoleucine) and fatty acid composition (palmitic acid and stearic acid). However, when adding flaxseed, it is necessary to select the adequate processing conditions to keep the desired health attributes (Mercier et al. 2014). In fact, the benefits of flaxseed enrichment depend on the mechanical and physical properties of cereal products, enrichment level, and processing history. Flaxseed lipids are really stable under most processing and storage conditions, owing to the significant antioxidant properties of lignans; nevertheless, there is still scarce information about the impact of home handling on lipid oxidation. Concerning product shelf life, it has been also reported that cereal products enriched with flaxseed show similar or improved shelf life compared to their counterparts with no flaxseed enrichment; presumably, flaxseed may hinder starch retrogradation, contribute to moisture content retention, and delay microbial growth. Nevertheless, most cereal products containing flaxseed show lower organoleptic properties, and recommendations suggested that enrichment up to 15 % can be carried out with less impact on the sensory pattern.

Lately, consumers' interest in the role of nutrition for health and well-being seems a priority. Therefore, today, the main concern of the industry is to innovate, meet, and satisfy consumer requirements. In the baking industry, that trend has prompted the development of baked goods, keeping in mind the healthy concept. Some very novel and innovative improvements developed for enhancing the healthy benefits of breads are the processing of probiotic breads, inclusion of microencapsulated oils containing omega-3 fatty acids, and so on. In recent decades, studies on human nutrition have emphasized the importance of omega-3 and omega-6 intakes. With that purpose, different sources of those fatty acids have been investigated to be used in breadmaking. Flaxseed is a good source for such fatty acids and it has been successfully applied to obtain pan breads and Brazilian cheese roll enriched with flaxseed flour with good acceptance. Therefore, the cheese roll enriched with flaxseed presents a good alternative for aggregating nutritional and functional benefits to the conventional product. Alternatively,

flaxseed oil can be used with the purpose of enriching with omega-3 in bread formulations, with the subsequent improvement of the nutritional value of wheat flaxseed-enriched breads. Also the substitution of shortenings with microencapsulated n-3 polyunsaturated fatty acids reflects an alternative for improving healthy aspects of breads.

There is a growing market for foods that contain probiotic bacteria, and a wide variety of probiotic strains are being added to an array of foods. The ability of probiotic strains to survive the conditions of the manufacturing processes (e.g., temperature, pH, oxygen, etc.) is the main impediment food manufacturers must overcome. The development of functional breads containing viable microorganisms has been a challenge due to the high temperature reached during baking, which results in significant losses in probiotic viability. Successful probiotic breads have been obtained using thermostable *Lactobacillus* and also incorporating *Lactobacillus acidophilus* in microcapsules applied to bread surface through edible coatings; specifically, those have been sprayed on partially baked breads to minimize the impact of the baking temperature on the microorganism viability. The heat stability of probiotic *Bacillus subtilis* R0179 has permitted its addition in bread and cookie formulations with adequate survival. Another probiotic that has been recommended for probiotic breadmaking is *Lactobacillus rhamnosus* R0011, which, despite its heat sensitivity, when sprayed on baked bread shows good stability over the shelf life of the product. In the USA, *B. subtilis* and *L. rhamnosus* are entitled for structure/function claims, which can be used in baked products.

Probiotic bread was launched in the USA by a baking company claiming that a health and wellness bread contains probiotics, which contributes to the health and balance of the digestive system; besides, it was made with whole wheat flour, flaxseeds, sunflower seeds, chia seeds, and millet for added nutritional value.

Composition of Other Bakery Products

Apart from bread, other common bakery products include muffins, cookies, crackers, pastries, and croissants. Converse to the basic bread composition, sweet bakery products are obtained from very rich recipes, with numerous ingredients. Those products are in general hypercaloric due to their high content of sugar and fatty ingredients. Some sweet products (cookies, croissants, pastries, and muffins/cakes) have been selected for reviewing ingredients used in their recipe and also for showing their nutritional composition (Tables 4, 5, and 6).

Cookies, also referred as biscuits in some parts of the world, have a great diversity of composition. A variety of ingredients is used, which determine their sensory perception but mainly texture during fracture. Table 4 shows different kinds of biscuits; they mainly differ in fat content and therefore in their texture when cutting. Wheat flour is usually the main ingredient of this kind of products, followed by fat (from animal or vegetable sources) and sugar. Sugar could be added as sucrose, glucose, or fructose syrup. This type of products is leavened with baking powders,




generally ammonium bicarbonate and sodium bicarbonate. Minor ingredients comprise salt, dairy ingredients, and flavors like vanilla. Cookies have very low moisture content (4–8 %) and water activity, which inhibits microbial growth during their storage. Regarding their nutritional composition, as can be expected from the fat-rich recipe, they are highly caloric (from 400 to 500 kcal/100 g) (Table 4). No *trans* fats are necessary for processing cookies. Cholesterol is present whenever fats from animal sources are used in the recipes. Cookies have higher content of carbohydrates than bread due to the high sugar content, and fiber could greatly vary depending on whether fibers were incorporated in the recipe. Protein content is usually derived from the cereal flour and some traces of dairy ingredients. Legume flours are also added for cookie making, with significant improvement of their nutritional quality.

Other caloric products are croissants and pastries, which require a high amount of fat for reaching a layered structure. Examples of composition are given in Table 5. Wheat flour is still the main ingredient closely followed by fat (non-hydrogenated or partially hydrogenated vegetable margarine). In addition, croissants contain sugar, mono- and diglycerides, lecithin, sugar, yeast, salt, and preservatives, whereas pastries could contain mono- and diglycerides, lecithin, and syrup. Energy provided by 100 g of croissants or pastries is around 400 kcal, with a great contribution of fats to that caloric value. High cholesterol content could be present when animal fats are used. The amount of carbohydrates is low compared with the rest of bakery products and proteins generally from the flour content.

Muffins and cakes admit thousands of variations in their recipes, either due to raw materials or fillings, toppings, and so on. In this type of products, egg proteins and sugar play a really important role for determining their internal structure and, in consequence, their mouthfeel and taste. Some examples of composition can be displayed in Table 6. In muffins and cakes, the main ingredient is wheat flour closely followed by oil and eggs. Since they are rich in fat and oils, emulsifiers are usually present in their composition to improve texture. Lecithin, mono- and diglycerides, and sodium stearoyl lactylate are used as emulsifiers. Preservatives and also gums like xanthan gum, guar gum, or modified celluloses are commonly present for extending the shelf life and the freshness perception. Consumption of these products supply a high amount of calories mainly from fat and sugars, and the cholesterol content is dependent on the type of fat used in their processing. Usually, they supply high amounts of sodium and very low levels of fibers.

Non-bread bakery products are usually rich in fats and sugars; thus, they are conceived for consumer's pleasure and healthiness is somewhat secondary. Nevertheless, even in this type of products, consciousness about improving their nutritional profile is being sited. Tendencies are mainly focused on improving the fats composition and reducing the caloric intake from sugars. Usually, sweet bakery products contain a high amount of saturated fats with *trans*-fatty acids, which are needed for the handling and texture of laminated doughs. However, in the last decade, there is sufficient scientific information supporting the prejudicial effect of the *trans*-fatty acids on the blood lipid metabolism and the development of arteriosclerosis and cardiovascular diseases. In consequence, great efforts have been concentrated in looking for functional replacements of those fatty

Table 4 Ingredients used in cookie recipes and their nutritional composition

	Basic cookies 	Butter cookies 	Cookies – digestive 
Ingredients			
Wheat flour	x	x	
Whole wheat flour			x
Vegetable fat	x		x
Butter		x	
Sugar	x	x	x
Glucose syrup	x		x
Fructose syrup	x		x
Milk powder	x		
Baking soda	x	x	x
Salt	x	x	x
Aroma	x		
Sodium bisulfite	x		
BHA (butylhydroxyanisole)	x		
Vanilla		x	
Eggs		x	
Soy lecithin		x	

(continued)

Table 4 (continued)





	Basic cookies	Butter cookies	Cookies – digestive
Ingredients			
Amount per 100 g			
Calories (kcal)	433	519	483
Calories from fat	159	222	189
Total fat (g)	18	26	21
Saturated fat (g)	5	15	10
<i>Trans</i> fat (g)			
Cholesterol (mg)		56	<5
Sodium (mg)		370	0.65
Total carbohydrate (g)	72	67	66
Dietary fiber (g)	7		3
Sugars (g)	19	22	18
Protein (g)	7	4	6

Table 5 Ingredients used in croissant and pastry recipes and their nutritional composition



	Croissants 	Puff pastry sheets 
Ingredients		
Enriched bleached wheat flour	x	
Unbleached enriched wheat flour		x
Vegetable margarine	x	
Vegetable shortening		x
Water	x	x
Sugar	x	
Monoglycerides	x	x
Diglycerides	x	x
Soy lecithin	x	x
Citric acid	x	
Potassium sorbate	x	
Flavor	x	
Vitamin A palmitate	x	
Vitamin D	x	
Beta-carotene color	x	x
Soybean oil	x	
Yeast	x	
Salt	x	x
Sodium propionate	x	
Fructose corn syrup		
Wheat gluten		x
Amount per 100 g		
Calories (kcal)	438	415
Calories from fat	168	244
Total fat (g)	21	27
Saturated fat (g)	12	15
<i>Trans</i> fat (g)		
Cholesterol (mg)	67	
Sodium (mg)	467	341
Total carbohydrate (g)	46	34
Dietary fiber (g)	3	<2
Sugars (g)	11	2
Protein (g)	8	7

Table 6 Ingredients used in croissant and pastry recipes and their nutritional composition

	Muffin	Cake
		
Ingredients		
Wheat flour	x	x
Sugar	x	x
Soybean oil	x	x
Canola oil	x	
Eggs	x	x
Water	x	x
Cultured buttermilk	x	
Margarine	x	
Baking powder	x	x
Dextrose	x	
Whey milk	x	
Salt	x	x
Monoglycerides	x	x
Diglycerides	x	x
Sodium stearoyl lactylate	x	x
Xanthan gum	x	
Sodium bicarbonate	x	
Natural flavor	x	x
Artificial flavor	x	x
Citric acid	x	
Soy lecithin	x	x
Food starch modified		x
Partially hydrogenated soybean oil		x
Cottonseed oil		x
Palm oil		x
Nonfat milk		x
Wheat gluten		x
Dextrose		x
Cornstarch		x
Soy flour		x
Sodium propionate		x
Calcium sulfate		x

(continued)

Table 6 (continued)

	Muffin	Cake
		
Ingredients		
Cellulose gum		x
Buttermilk powder		x
Beta-carotene color		x
Potassium sorbate		x
Coconut oil		x
Amount per 100 g		
Calories	364	400
Calories from fat	162	188
Total fat (g)	17	21
Saturated fat (g)	3	4
<i>Trans</i> fat (g)	0	0
Cholesterol (mg)	56	65
Sodium (mg)	343	365
Total carbohydrate (g)	48	51
Dietary fiber (g)	1	<1
Sugars (g)	31	28
Protein (g)	5	5

acids. The usual fat replacers can be classified into three categories: carbohydrate based, protein based, and fat based. Inulin, polydextrose, and oligofructose have been proposed as fat mimetics in cookie making or inulin in muffin making. Another alternative is the use of edible emulsions of water in fat in the presence of a humectant for obtaining low-fat products.

In addition, fat- and sugar-reduced cookies have been also developed. With that purpose, fat mimetics have been used for partial fat replacement and polyols, like lactitol, sorbitol, and maltitol, for sugar replacement. With this strategy, up to a 50 % fat replacement could be obtained, but those replacements usually result in hard and brittle cookies which do not have a proper expansion after baking. In doing those fat replacements, it must be taken into account that before new ingredients can be used in food, they must either be self-affirmed to be generally recognized as safe (GRAS) or approved for such use by the Food and Drug Administration (FDA) under a food additive petition. A GRAS substance is defined as one generally recognized by scientific experts to be safe for specific uses on the basis of an

extensive history of use or on the basis of published scientific evidence. These procedures seek to ensure the safety of foods for consumers of all ages. The majority of fat reduction ingredients currently used are considered to be GRAS.

Among other alternatives for improving the nutritional profile of those products is the use of wheat germ up to 20 % for cake making. Wheat germ addition increases the ash, protein, fat, and mineral contents (Ca, Cu, Fe, Mg, Mn, P, K, and Zn) of the cakes, although the addition of emulsifiers is advisable for keeping physical and sensory quality.

Bakery Products in Fortification Programs

Bakery foodstuffs constitute staple food in many countries, and as such, they have been selected as the best carriers of micronutrients in fortification programs when some special requirements are detected in the population (Baurenfiend and DeRitter 1991). Properly used fortification can be a strategy to control nutrient deficiencies (Allen et al. 2006). Fortification may be the easiest, cheapest, and best way to reduce a deficiency problem. Nevertheless, some concerns related to proliferation and excessive promotion of the fortified foods have risen, because that could lead to a simultaneous replacement of the non-fortified foods in the diet and to avoid situations of excessive intake of certain micronutrients (Rosell 2008). The pros and cons of fortification need to be balanced in each circumstance (Rosell 2004).

Fortification can be defined as the addition of one or more vitamins and/or minerals to a food, regardless of its usual content in the food, in order to prevent or correct a demonstrated deficiency of one or more vitamins and/or minerals in the population or specific population groups or to improve the nutritional status of the population and dietary intakes of vitamins or minerals due to changes in dietary habits, and that addition must be based on generally accepted scientific knowledge of the role of vitamins and minerals in health (Rosell 2004, 2008). Additions are carried out based on generally accepted scientific knowledge of the role of vitamins and minerals in attaining good health. Foods are fortified for the following reasons:

- To prevent or correct a demonstrated deficiency of one or more vitamins and/or minerals in the population or specific population groups
- To improve the nutritional status of the population and dietary intakes of vitamins or minerals due to changes in dietary habits

Enrichment is a term usually interchanged with fortification that should be equivalent to restoration of the vitamin and mineral levels lost during manufacturing, storage, and handling.

Cereal fortification methods have been developed to restore the nutrients that have been removed during milling and to improve the nutrient intake level of a

specific population (Poletti et al. 2004). In addition, some efforts have focused on enhancing the nutritional quality of the crops through biofortification strategies, because half of the world population suffers from malnutrition with respect to iron, zinc, and selenium. For instance, selenium is an essential micronutrient for humans, and it is deficient in at least one billion people worldwide (Lim et al. 2013). Plants and plant-derived products transfer the soil-uptaken selenium to humans; thus, the cultivation of plants enriched in selenium can be an effective way to improve the selenium status on humankind (Pérez-Massot et al. 2013). Therefore, controlled agronomic biofortification of wheat crops for flour and bread production could provide an appropriate strategy to increase the intake of some minerals.

Fortification of flour is usually carried out in mills, where the nutrient mixture is blended with the flour. Milling produced a uniform distribution of fortificants with no significant separation during packaging or transportation. In doing so, it is critical the definition of the nutrients and the levels to be added must be based on the nutritional needs and deficiencies of the population, the common consumption of the fortifiable flour, the sensory and physical aspects, and economic cost. The addition of B vitamins, iron, and calcium is a common practice in some developed countries (Rosell 2008, 2014). However, the nutrient added must have good stability during storage, and in defining the levels to be added must be considered the extraction rate during milling.

Flour fortification has become mandatory in numerous countries, where specific legislation has been set up to define the list of foods acting as carriers, the minerals and vitamins to be added back, the levels, and also the chemical compounds allowed to supply those minerals and vitamins (Rosell 2008, 2012, 2014). In fact, enriched flours might contain, apart from the wheat flour, niacin, reduced iron, thiamine mononitrate, riboflavin, and folic acid. The success of fortification programs always depends on good control, so they should be set up, regulated, and enforced by the national governments.

There are some criteria or principles for fortification that should be met for those when planning to fortify one or more foods to improve nutritional status. They apply mainly to fortification as a strategy to tackle micronutrient deficiencies. Those criteria are (i) known nutrient deficiency in the population, (ii) wide consumption of the food to be fortified among the at-risk population, (iii) suitability of the food and nutrient together, (iv) technical feasibility, (v) limited number of food manufacturers, (vi) no substantial increase in the price of the food, and (vii) legislation. When a fortification program is established, continuous monitoring and control of the fortification, in order to determine the success of the strategy, are necessary.

Food fortification offers an important strategy to help control, in particular, the three main micronutrient deficiencies, namely, deficiencies of iodine, vitamin A, and iron (Ranum et al. 2001). In developing countries, the greatest priority should be given to fortification with these nutrients. With iodine, fortification alone, in the form of salt iodization, is often the only strategy used. With vitamin A and iron, fortification should be used in combination with, not to the exclusion of, other interventions (Hurrell et al. 2010). It must be stressed that also compounds

interactions might enhance or shield the effectiveness of the fortification. For instance, inhibitors of iron absorption include phytate, polyphenols, soy protein, and calcium, and enhancers include animal tissue and ascorbic acid. Organic acids, such as citric acid, malic acid, and tartaric acid, are promoters of Fe bioavailability. Sensory analysis must be always considered because differences in the overall quality of breads prepared with fortified flour with the addition of chemical sources of minerals might affect sensory characteristics.

Fortification has been also very effective to reduce the deficiency prevalence of the following micronutrients: niacin, thiamine, riboflavin, folate, vitamin C, zinc and calcium. Only phytate and soy protein inhibit zinc absorption, although also the consumption of black tea simultaneously with fortified bread significantly reduces the zinc absorption. The supplementation of B vitamins to flour is faced with the problem of low stability of these compounds during storage. Hence, higher effectiveness is obtained when B vitamins are added at the bakery rather than at the mill.

B vitamins (thiamine, B₁; riboflavin, B₂; and niacin, B₃) are supplemented to cereal flours in developed countries to ensure the adequate intake of those nutrients through, for instance, the consumption of fortified bread. Pyridoxine or vitamin B₆ is also supplemented to wheat for obtaining fortified bread, although bioavailability decreases by 5–10 % when it is used in whole wheat bread (Leklem et al. 1980). Vitamin B₁₂-fortified bread is a good source of this vitamin (Selhub and Paul 2011); with its consumption even the elderly can ensure the efficient absorption of vitamin B₁₂ (Rusell et al. 2001). Other less exploited but convenient and efficient strategy of increasing the levels of vitamins is the use of selected natural riboflavin-overproducing strains. For instance, riboflavin-overproducing strains of *Lactobacillus plantarum* can be used in breadmaking (by means of sourdough fermentation) to enhance bread vitamin B₂ content.

Bread has also been fortified with vitamin D using cholecalciferol, showing good dispersion in the breads and stability. This fortified bread increases serum 25-hydroxyvitamin D concentration as effectively as the cholecalciferol supplement, without affecting serum intact parathyroid hormone concentration or urinary calcium excretion (Natri et al. 2006). Therefore, fortified bread is a safe and feasible way to improve vitamin D nutrition.

Folic acid is another B vitamin added to cereals due to its great contribution to the population health. In 1996, the American Food and Drug Administration concluded that 1,000 µg folate/day is the safe upper limit of folate intake for the general population. The fortification level was established at 140 µg folic acid/100 g for enriched cereal-grain products; that fortification provides 10 % of the daily value per bread serving. This level of fortification was chosen to assist women of reproductive age in increasing their folic acid intake by a daily average of 140 µg folic acid. Currently, folic acid mandatory fortification is already used in more than 50 countries, including the USA and Canada, where research suggests it reduces the rate of neural tube defects by 25–50 %. It seems that this fortification will be imminently mandatory in the UK.

In addition, it has been described that the consumption of bread fortified with 2.5 % elemental calcium concentration is an effective way for ameliorating the

hyperphosphatemia without inducing hypercalcemia (Babarykin et al. 2004). Therefore, calcium-fortified bread can be consumed as a treatment of uremic hyperphosphatemia. Calcium-fortified pita bread was similar to its regular counterpart (Ziadeh et al. 2005).

Special Bakery Products Developed for Targeted Groups

There is some population with food ailments associated to cereals, mainly gluten. This type of products requires special attention because their composition is completely different than the ones obtained directly from cereals. This section will describe the chemical composition of gluten-free breads and other types of breads that have been developed to meet the requirements of targeted groups.

To be in a healthy state, the maintenance of energy balance is necessary. Positive energy balance in favor of the energy ingested leads to obesity, regardless of the composition of excess energy. The frequency of obesity has increased dramatically in many developed and developing countries. There is great concern among health authorities because of the relationship between obesity and some other diseases like diabetes, cardiovascular disease, and other chronic diseases of lifestyle. A tactic to fight that trend is to increase carbohydrate consumption (in replacement of fat) because carbohydrate provides only 4 kcal per gram compared with 9 kcal per gram for fat. High-fiber foods and whole-grain breads and cereals can be an effective part of any weight loss program (Collar 2008) since they have slower digestion and induce a feeling of satiety.

When considering cereals and health, it is important to introduce that a percentage of population shows intolerance to gluten intake like celiac disease. Peptides released during gluten digestion are responsible for the intolerance in genetically predisposed individuals. Up to date, the unique effective treatment is dietary therapy, avoiding the intake of gluten-containing foods (García-Manzanares and Lucendo 2011). A general consensus has been reached that the cereals considered gluten-free safe are rice and corn (Rosell and Marco 2008). Wheat, spelt, kamut, rye, triticale, barley, and probably oats and hybrids of these grains must be eliminated from the diet. Gluten-sensitive individuals can tolerate buckwheat, millet, amaranth, quinoa, teff, and/or sorghum. To mimic the viscoelastic properties of gluten, a large number of flours and starches as well as many ingredients such as gums, enzymes, and proteins from different sources have been used for making bread resembling the structure, mouthfeel, acceptability, and shelf life of gluten breads (Rosell 2007, 2013).

The absence of gluten in natural and processed foods, despite being the cornerstone treatment for celiac disease, may lead to nutritional consequences, such as deficits and imbalances. Nutrition counseling for celiac disease has focused on the foods to avoid when sticking to gluten-free diet, but the nutritional quality of gluten-free products must enter into the picture. There are growing concerns over the nutritional adequacy of the gluten-free dietary pattern because it is often characterized by an excessive consumption of proteins and fats and a reduced

intake of complex carbohydrates, dietary fiber, vitamins, and minerals (Penagini et al. 2013). As a consequence, the long-life adherence to gluten-free products has been associated to undernourished and also mineral deficiencies that could lead to anemia, osteopenia, or osteoporosis. In recent years, attention has focused on the nutritional quality of gluten-free products available in the market, which are of lower quality and poorer nutritional value than their gluten-containing counterparts. Gluten-free breads are mainly starch based and contain low amounts of vitamins, minerals, and in particular dietary fiber, but with great variation among marketed products (Rosell 2014). Gluten-free breads have very low contribution to the recommended daily protein intake, with a high contribution to the carbohydrate dietary reference intake. In general, gluten-free breads show great variation in the nutrient composition, being starch-based foods low in proteins and high in fat content, with high glycemic index. Besides that, gluten intolerance, like celiac disease, induces an intestinal lesion that leads to various deficiencies of nutrients, vitamins, and dietary minerals, being ferropenia, vitamin B₁₂, folic acid, and fat-soluble vitamin deficiencies especially frequent. This proximal location in the small intestine often results in malabsorption of calcium, iron, folic acid, and fat-soluble vitamins. Patients newly diagnosed or inadequately treated often have low bone mineral density, which appears to be directly related to the intestinal malabsorption. Osteomalacia or osteopenia is secondary to the reduced calcium absorption, caused by atrophy of the intestinal villi, and/or to a vitamin D deficiency, leading to secondary hyperparathyroidism; because of that, osteoporosis is a frequent complication accompanying celiac disease. Those patients are particularly prone to have low bone density and high risk of fractures. Therefore, a redesign of the gluten-free bakery goods is needed for obtaining gluten-free baked products with similar nutritional composition to that of their gluten counterparts. Those products would allow celiac patients and/or population with other allergic reactions and intolerances caused by proteins or another component of cereals to meet dietary guidelines without changing their dietary pattern.

In the last decade, new recipes and ingredient composition are proposed for obtaining nutritional balances or enriched gluten-free breads. Additionally, the supplementation of gluten-free bread with proteins has been a technological strategy for improving the protein network and for mimicking the viscoelastic properties of gluten proteins in wheat-containing breads. Protein enrichment can be obtained using legume flours, egg proteins, or dairy proteins at different percentages (0 %, 10 %, 20 %). Eggs are frequently avoided because gluten-intolerant patients are very often intolerant to albumin. Dairy powders can be added up to 12 % because higher levels provoke great detrimental effect on the technological properties. Legume flours are useful for protein and fiber enrichment of bakery foodstuff, like gluten-free cakes. Nevertheless, their effect on the technological and sensorial quality is dependent on the legume origin. Legumes significantly enhance the protein content and its availability, and the total dietary fiber content, besides its incorporation, reduces the glycemic index of the resulting product. Moreover, lentil-containing cakes led to low-density batters and high specific volume.

Gluten-free bakery products have not been immune to the fiber enrichment trend. With that aim, different fibers have been added to this type of products. Usually, whole wheat grains are not used for making gluten-free products due to technological limitations for obtaining low dense and porous structures; because of that, fibers from different sources are being preferred for enriching these products. Fibers from cereal bran, legumes' outer layer, and processing by-products of apple and potato industry have been used for enriching wheat cakes and muffins. Rice bran up to 10 % or therein fractions have been proposed for increasing the protein, fat, and dietary fiber contents; moreover, they supply insoluble and soluble dietary fiber. Soluble dietary fibers also contribute to the technological quality of gluten-free breads, improving color, specific volume, softness of crumb, and crumb porosity. Insoluble and soluble fibers or their blends are recommended for making gluten-free layer cakes enriched in soluble and insoluble fibers of acceptable quality, without compromising excessively the specific volume and the crumb texture. Due to health benefits derived from the intake of fiber-containing foods and the recommendation to still balance the consumption of soluble and insoluble fibers, a blend of insoluble fiber with soluble fibers like inulin or guar gum is advisable for replacing up to 20 g/100 g of gluten-free flour when making enriched gluten-free layer cakes.

Gluten-free flours have not been subjected to regulations regarding fortification programs; because of that, numerous gluten-free products are mineral and vitamin poorer than their wheat counterparts. The fortification of gluten-free bread with different sources of minerals like calcium has been a topic of research, owing to the impact of calcium on the health of gluten-intolerant patients. Calcium lactate, calcium citrate, calcium chloride, and calcium carbonate are among the proposed sources of elementary calcium for gluten-free breads. All those sources led to fortified breads, calcium carbonate followed by calcium citrate is the most recommended salt for obtaining calcium fortification of gluten-free breads based on the sensory acceptance of breads. The same approach has been carried out with different iron compounds, although elemental iron seems to be more stable and with less impact on sensory and technological characteristics of the gluten-free breads.

Other allergies are related to the intake of dairy, peanuts, tree nuts, egg, soy, fish, or shellfish – the allergens that with wheat/gluten account for 90 % of all food allergic reactions in the USA. To give response to those consumers with some type of allergies, there are products in the market free from lactose and dairy compounds, eggs, and so on. The presence of those products is still very limited and this market is not a strong driven force as occurs in the gluten-free products.

Conclusion and Future Directions

The market of baked goods is always increasing and thousands of new products are launched yearly. Cereal baked goods are still an important source of nutrients worldwide, owing to their presence in almost all daily meals. Those products are the

main sources of complex carbohydrates and proteins, besides B vitamins and minerals like calcium, iron, potassium, magnesium, manganese, phosphorus, and zinc. Nevertheless, a great part of those is lost during milling; because of that, whole wheat products are recommended. When considering baked products, a distinction must be made between bread and the rest of baked products, since their chemical composition is considerably different, moreover, on the fat content.

Despite the availability of the increasing number of bakery products, recipes are changing and the nutritional profile of the baked products has been changing over the last decades. This fact together with the changes underwent in the consumers' lifestyle and also the increasing detection of diverse ailments obey to reconsider carefully the recipes or formulations used for making baked products. Lately, enrichment and fortification are gaining popularity for providing more nutritious baked products. With this purpose, additional raw materials like pseudocereals, legumes, and other protein sources are being included in the recipes. In addition, having nutrition awareness in mind, recipes have also been modified for healthier ingredients or additives, which is really important in baked goods rich in fat constituents. Simultaneously, the special requirements needed by consumers when subjected to dietetic therapies make necessary the design and development of tailored baked goods that meet sensory desires and nutritional requests. It is envisaged that during the next decade, nutrition is still going to govern the driven forces of the food market and nutrition claims will try to attract consumers.

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Cross-References

- ▶ [Chemical Composition of Cereals and Their Products](#)
- ▶ [Chemical Composition of Eggs and Egg Products](#)
- ▶ [Chemical Composition of Fat and Oil Products](#)
- ▶ [Chemical Composition of Sugar and Confectionery Products](#)

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Abstract

Chemical constituents are summarized for a selection of important beverages: fruit juices, carbonated nonalcoholic drinks (including mineral/springwater, soda water, soda pop, cola drinks, root beers, and tonic water), functional beverages (sports, health, energy, and relaxation drinks), and alcoholic beverages (wine, cider, beer, rice wine, spirits, and their flavorings). Major chemical

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constituents reviewed include pigments, colorants, carbohydrates, sweeteners, acids, volatile compounds, phenolic compounds, terpenoids and steroids, nitrogen compounds (especially amines, amino acids, and proteins), minerals, vitamins, ethanol (for alcoholic beverages), carbon dioxide (for carbonated drinks), and preservatives. General relationships between chemical content and methods of processing are emphasized for several key beverages.

Introduction

Knowledge of the chemical composition of a beverage is important for both understanding the organoleptic character of the beverage and for maximizing its commercial value. While it is impossible in a work of this kind to be totally comprehensive, this chapter summarizes the main chemical constituents of common drinks in two parts: fruit juices, nonalcoholic carbonated drinks, and nonalcoholic functional beverages in the first part and alcoholic beverages in the second.

Fruit juices and alcoholic beverages (many of which are derived from fruit juices) are discussed in terms of the common categories of natural chemical constituents that they contain. These include pigments, carbohydrates, acids, volatile compounds, phenolic compounds, amino acids, peptides, proteins, and other organic nitrogen compounds, minerals, vitamins, and bitter/astringent compounds. Additionally, ethanol and carbon dioxide contents are discussed for relevant beverages. Also considered are legal additives, such as colorants, flavorants, sweeteners, and preservatives, as well as potentially toxic substances that are formed naturally during processing (e.g., ethyl carbamate in wines and distilled spirits). The major processing methods for several kinds of beverages are described, tabulated, or illustrated in outline, as these are a major influence on the chemical composition of the final products and hence on their organoleptic character.

Nonalcoholic carbonated beverages and functional drinks (apart from unflavored mineral or springwater) are treated from the point of view of purified water to which has been added a selection of chemical ingredients. These include carbon dioxide, salts, sugars and/or low-calorie sweeteners, colorants, flavorants, vitamins, amino acids, and special active ingredients, such as central nervous system (CNS) or smooth muscle stimulants (e.g., caffeine, creatine) or relaxants (e.g., melatonin, L-theanine), either as pure substances or as part of plant extracts (e.g., guarana, ginseng, green tea). In contrast to other sections, most of the data in the Nonalcoholic Functional Drinks section comes from product labels or company Web sites and hence are anonymous, in order to avoid advertising.

In some cases, component concentration data may be only approximate values, being results, for example, of semiquantitative chromatographic (e.g., GC/MS) determinations that use just one internal standard. Nevertheless, such data can still give a broad understanding of chemical composition.

Agricultural, industrial, and microbiological contaminants originating from raw materials are not generally included, except in a few important cases.

Chemical Composition of Nonalcoholic Beverages and Functional Drinks

Fruit Juices

Several methods are available for the commercial production of fruit juices. The simplest of these is pressing, which gives a cloudy product with a shelf life of only a few days, even at chill temperature storage. The shelf life can be extended somewhat by the use of a light pasteurization step. Freshly pressed juice (such as apple, lime, or orange) is considered by many to have the best flavor. However, most commercial fruit juices are the results of more elaborate processes that involve filtration and/or concentration steps (evaporation, usually at reduced pressure), followed by restoration (i.e., adding back flavor compounds, pulp) or reconstitution (i.e., diluting with water) toward the end of the process. Fruit juices that have been processed without evaporation and reconstitution steps are called NFC (not from concentrate) juices. Restoration or reconstitution is performed in such a way as to give a product that is the same as the original pressed juice so that the essential composition and quality factors of the juice are maintained (Heredia et al. 2013 and references therein). These juices are generally pasteurized and aseptically packaged, giving them much longer shelf lives at ambient storage (Ashurst 2012 and references therein). Apart from the above, fruit juices, usually in the form of concentrates or sometimes in decolorized or deionized forms, are important ingredients of soft drinks, some carbonated varieties of which are discussed in Nonalcoholic Carbonated Beverages. Additionally, many fruit juices are obtained expressly for winemaking. Some of their chemical components survive into the wine, but many are lost or changed.

This section deals solely with the chemical composition of pure fruit juices, obtained by any of the above processes and without the inclusion of additives, such as sugar or preservatives, which is forbidden in many countries. The focus is on more common juices made from fruit grown in temperate climates, but depending on available data, certain aspects of the chemical composition of some less common juices are included, particularly those that are frequently converted to wine or are ingredients of soft drinks.

Pigments

The major pigments of red-purple fruit juices, such as those of black currant, blueberry, red/black cherry, black grape, blood orange, or strawberry, are anthocyanins, mostly anthocyanidin 3-*O*-glycosides, but there are many variations. Figure 1 displays the six fundamental anthocyanidins (the aglycone part of anthocyanins), along with examples of glycosides and acylated glycosides that constitute anthocyanin fruit juice pigments. Table 1 displays the major anthocyanins found in selected juices, along with total anthocyanin content (TAC), either as a typical value or as a range. TAC is often quoted as mg cyanidin or cyanidin 3-*O*-glucoside equivalents per L or per 100 g fresh weight.

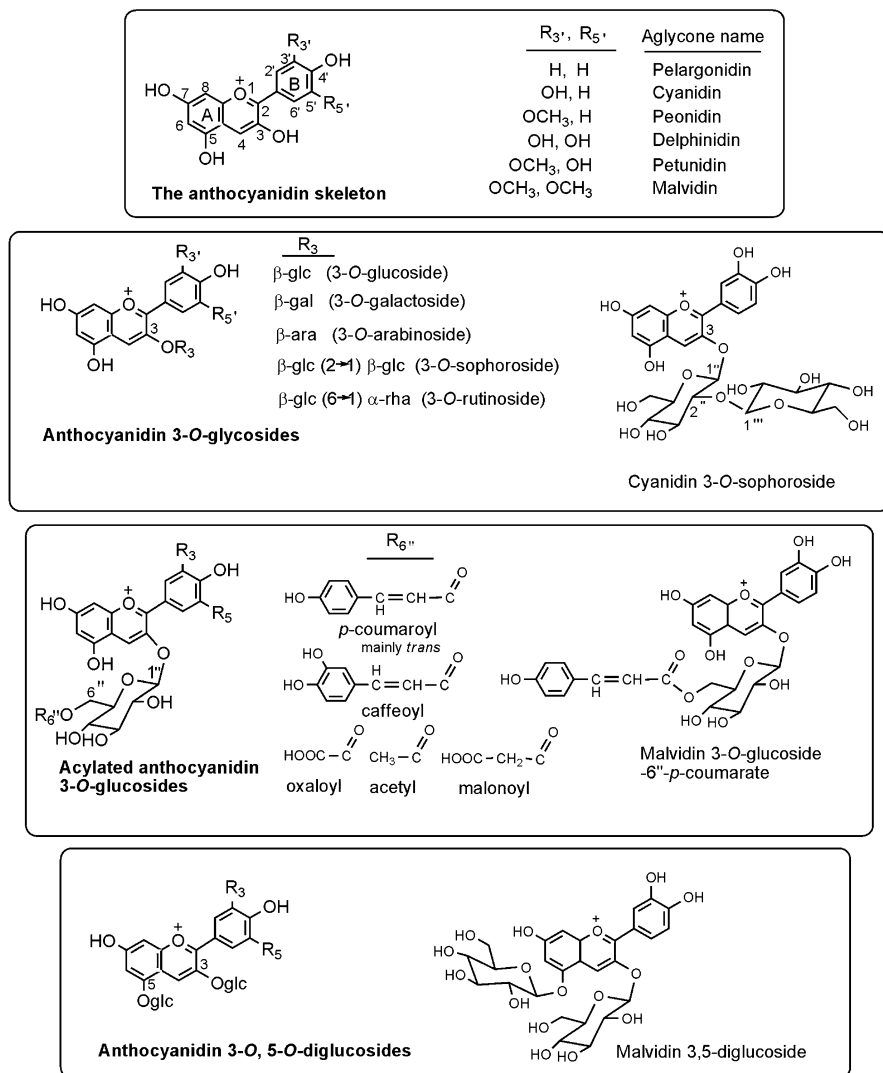


Fig. 1 Structures of some fruit juice anthocyanins

The most common anthocyanins are cyanidin glycosides, except for black grape juice where the bulk of the anthocyanins (50–90 %) are malvidin derivatives. Strength of color and TAC depend on several factors, including the variety and condition of the fruit and the juicing process, especially with regard to skin contact and heat, enzyme, or other treatments.

Additional to monomeric anthocyanins, lower concentrations of anthocyanin oligomers may be present in grape juice, depending on the extent of extraction from

Table 1 Major anthocyanin pigments in red-purple fruit juices^a

Juice	Major anthocyanins	Typical total anthocyanin content ^b
Bilberry/blueberry <i>V. myrtillus</i> / <i>Vaccinium corymbosum</i>	Malvidin 3- <i>O</i> -galactoside, delphinidin 3- <i>O</i> -glucoside, cyanidin 3- <i>O</i> -glucoside, delphinidin 3- <i>O</i> -arabinoside, petunidin 3- <i>O</i> -glucoside, malvidin 3- <i>O</i> -glucoside	3,800
Blackberry <i>Rubus</i> spp.	Cyanidin 3- <i>O</i> -arabinoside, cyanidin 3- <i>O</i> -dioxaloylglucoside, cyanidin 3- <i>O</i> -malonylglucoside, peonidin 3- <i>O</i> -glucoside	1,256–1,978
Black currant <i>Ribes nigrum</i> L.	Cyanidin 3- <i>O</i> -rutinoside, delphinidin 3- <i>O</i> -rutinoside, delphinidin 3- <i>O</i> -glucoside, Cyanidin 3- <i>O</i> -glucoside, petunidin 3- <i>O</i> -glucoside, peonidin 3- <i>O</i> -rutinoside	2,620
Blood orange <i>Citrus sinensis</i> (L.) Osbeck	Cyanidin 3- <i>O</i> -glucoside, cyanidin 3- <i>O</i> -6''-malonylglucoside, delphinidin 3- <i>O</i> -glucoside	43–291
Cherry (red/black) <i>Prunus cerasus</i> L. (sour) <i>Prunus avium</i> L. (sweet)	Cyanidin 3- <i>O</i> -glucosylrutinoside ^c cyanidin 3- <i>O</i> -rutinoside, cyanidin 3- <i>O</i> -sophoroside ^d , pelargonidin 3- <i>O</i> -rutinoside, cyanidin 3- <i>O</i> -glucoside, peonidin 3- <i>O</i> -rutinoside	350–634
Grape (black) <i>Vitis Vinifera</i> , <i>V. labrusca</i> , <i>V. riparia</i> , etc.	Malvidin 3- <i>O</i> -glucoside, malvidin 3- <i>O</i> -galactoside-6''-acetate, malvidin 3- <i>O</i> -, 5- <i>O</i> -diglucoside ^e , delphinidin 3- <i>O</i> -glucoside, malvidin 3- <i>O</i> -galactoside-6''- <i>p</i> -coumarate, petunidin 3- <i>O</i> -glucoside, peonidin 3- <i>O</i> -glucoside	800–1,600
Raspberry (red) <i>Rubus idaeus</i> L.	Cyanidin 3- <i>O</i> -sophoroside, cyanidin 3- <i>O</i> -glucoside, pelargonidin 3- <i>O</i> -glucoside	351–491
Strawberry (red) <i>Fragaria x ananassa</i> Duch	Pelargonidin 3- <i>O</i> -glucoside, cyanidin 3- <i>O</i> -glucoside pelargonidin 3- <i>O</i> -rutinoside	39.4–136.1 442

^aData from McKay et al. (2011, pp. 419–435) and references therein, unless specified otherwise

^bTAC (mg cyanidin 3-*O*-glucoside equivalents/kg fruit mass or mg/L juice)

^{c,d}Data from Damar and Ekşi (2012); ^emain anthocyanin in sour cherry juice (140–321 mg/L);

^d(2.6–21.5 mg/L) in sour cherry juice

^eFound at high levels in the juice of *V. riparia* and *V. rupestris* grapes and hybrids of these with *V. vinifera*. It is absent or very minor in juice from *V. vinifera* varieties

the skins during the juicing process. These are flavan–flavylum dimers and trimers with C(4)–C(8) links (type A) or C(2)–O–C(7) and C(4)–C(8) links (type B) (Buglass and Caven-Quantrill 2013 and references therein). Also, low levels of vitisins (pyranoanthocyanins formed by reaction of anthocyanins with carbonyl compounds, such as acetaldehyde and pyruvic acid) have been found in blood orange juice (Hillebrand et al. 2004). Vitisins are generally much more evident in young red wines (see Fig. 10).

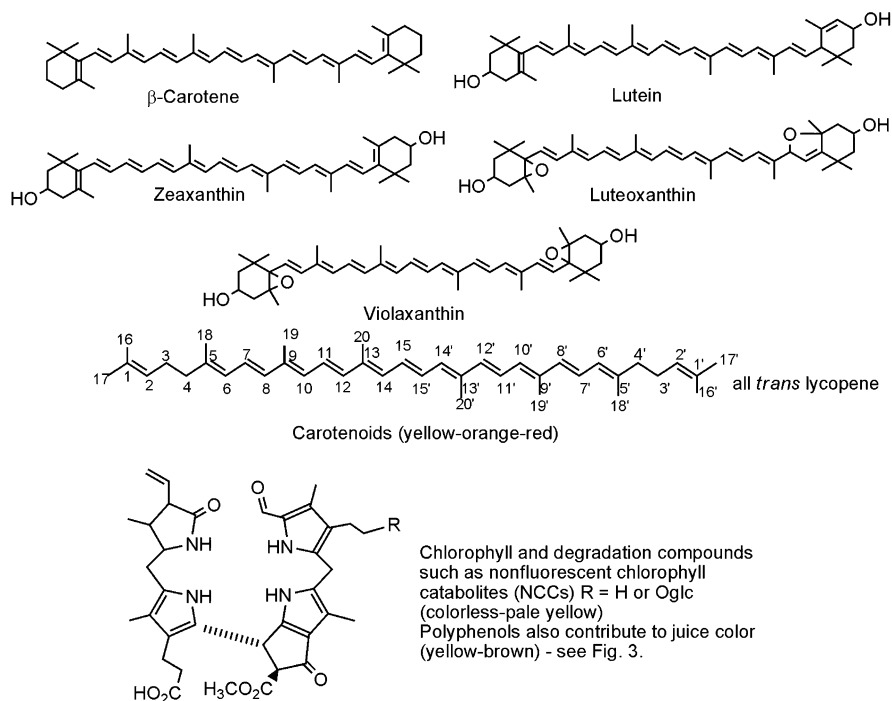


Fig. 2 Structures of some non-purple fruit juice pigments

Polyphenols (flavan-3-ols and their condensation oligomers, flavanones, flavones, and flavonols) (see Fig. 2 and Table 2 for examples), carotenoids (Fig. 2), and possibly chlorophyll and chlorophyll degradation products, are the major pigments of pale fruit juices. The latter have been found in apple, pear, and white grape juice, whereas certain carotenoids are also evident in red grape juice and wine, as minor pigments (McKay et al. 2011, pp. 419–435 and references therein).

Flavanone 7-*O*-glycosides are the most abundant flavonoids in all citrus juices (Tripoli et al. 2007 and references therein). Neohesperidoside flavanones (naringin, neohesperidin, and neoeriocitrin) are mainly present in bergamot, grapefruit, and bitter orange juices, whereas flavanone rutinosides (hesperidin, narirutin, and didymin) are present in bergamot, orange, mandarin, and lemon juices. See “Phenolic Compounds” for further discussion.

The major pigment of tomato juice and red grapefruit juice is the carotenoid all-*trans*-lycopene (Fig. 2): it comprises 80–90 % of the carotenoid content of freshly pressed tomato juice, but its concentration gradually diminishes with storage time, via conversion to a number of geometric isomers. Heat treatment during tomato juice processing leads to lesser formation of lycopene geometric isomers (5-*cis*-, 9-*cis*-, and 15-*cis*-lycopene) over a 56-day storage time (Vallverdú-Queralt et al. 2013). The main pigments of cherry tomatoes are all-*trans*-lycopene (80–90 %), β -carotene (4.3–12.2 %), and α -carotene, phytoene, and phytofluene (together, ~6.8 %) (Lenucci et al. 2006).

Table 2 Major pigments of non-purple fruit juices^a

Category	Examples	Comments
Carotenoids	α -Carotene, β -carotene (all- <i>trans</i>), (13- <i>cis</i>)-carotene	Carotenoids are present in many fruit juices, such as those of white grapes, apricot/peach, and citrus fruits, especially oranges. Some are very minor pigments of black grape juice. Lycopenes are prominent in tomato juice. Isomerizations can occur on heat treatment processes
	Oxygenated carotenoids: 2', 3'-anhydrolutein, α -cryptoxanthin, β -cryptoxanthin, lutein, (13- <i>cis</i>)-lutein, (13'- <i>cis</i>)-lutein, zeaxanthin	
	Epoxy and furanoyl carotenes: antheraxanthins, auroxanthins, luteoxanthins, mutatoxanthins, and violaxanthins	
	Lycopenes (mainly all- <i>trans</i> lycopene)	
Phenolic compounds ^b :		In many juices, including apple and pear juice
Chalcones	Butein, chalconaringenin	Apple and pear juice
Dihydrochalcones	Phloretin, phloridzin	In many fruit juices
Flavan-3-ols	(+)-Catechin, (–)-epicatechin	Especially citrus fruit juices
Flavones (and isoflavones) and flavonols	Apigenin, tangeretin (usually as glycosides)	In many fruit juices
	Kaempferol, quercetin (and glycosides)	
Flavanones and flavanols	Hesperitin, naringenin (usually as glycosides), Dihydroquercetin	Especially citrus fruit juices
Flavan-3,4-diols	Cyanidol	Especially pear juice
Procyanidin oligomers	Dimers, trimers, and upwards, of (+)-catechin, (–)-epicatechin	Especially in cider apple and perry pear juice
Chlorophyll and degradation products	Nonfluorescent chlorophyll catabolites (NCCs) and other chlorophyll breakdown products	In many pale juices, including apple, white grape, and pear juice

^aSome of these (e.g., some carotenoids and flavonoid phenols) are also present in purple juices (e.g., black grape juice), where provision of color is dominated by anthocyanins

^bSimple phenols and phenolic compounds of the hydroxybenzoic acid and hydroxycinnamic acid type (including quinic acid derivatives) contribute more to taste but may provide color indirectly via nonenzymic browning reactions

Carotenoids are also major pigments in orange juice, 5–23 mg/L being typical total carotenoid concentrations (Meléndez-Martínez et al. 2009). The main carotenoids of orange juice appear to be (9-*cis*)-violaxanthin, (all-*trans*)-violaxanthin (+*cis*-isomers), (9-*cis*)- or (9-*trans*)-antheraxanthin, zeaxanthin, mutatoxanthin epimers, and β -cryptoxanthin (others being <1 mg/L concentration) (Meléndez-Martínez et al. 2009).

Volatile Components

Hundreds of volatile compounds have been detected in fruit juices. Many of these (e.g., alcohols, carbonyl compounds, carboxylic acids, lactones, norisoprenoids) are secondary metabolites of biochemical pathways involving amino acids,

carotenoids, or fatty acids. Others (e.g., esters, ethers, and terpenoids) occur during ripening of fruit (Heredia et al. 2013). Some abundant or important volatile compounds (key odorants) of selected juices are given in Table 3. The odor threshold value (OTV) (or perception threshold) of a volatile is defined as the minimum concentration (often in water) at which the volatile can be detected organoleptically. OTVs of some key volatiles are given in Table 3. Odor activity value (OAV) (=concentration/OTV) was designed to indicate the extent of contribution of a compound to the global aroma: OAV > 1 has been suggested as a necessary condition for probable significant contribution.

Usually only a fraction of the many volatile compounds in a particular juice are odor active (i.e., with OAV > 1) at the concentration levels found in that juice. Aroma reconstitution methods can suggest the number and levels of odor-active compounds needed to reproduce the juice aroma. However, these results may not be universally accepted, for a number of reasons, including aroma perception subjectivity and omission of key (usually minor) compounds. Nonvolatile components (e.g., polyphenols), as well as scarce volatiles, can influence the perception of more abundant volatile compounds. Also, biological factors (e.g., genetics, agricultural practices, climate) and technological factors (e.g., method of juice preparation, storage) can influence global juice aroma.

In some cases, one or two volatile compounds can be highly suggestive of the whole juice aroma. Examples include 4-hydroxy-3,5-dimethyl-3(2*H*)-furanone (strawberry), *p*-1-menthene-8-thiol (grapefruit), methyl anthranilate (grape, *Vitis labrusca*, etc.), aromatic alcohols and carbonyls (juice of stone fruit, such as apricot, cherry, and peach), 4-mercapto-4-methyl-2-pentanone (grape, *V. vinifera* var. Sauvignon blanc), 1-(*p*-hydroxyphenyl)-3-butanone (raspberry), allyl carboxylates (pineapple), and 4-methoxy-2-methyl-2-butanone (black currant).

In general, however, many other compounds (often at low levels), as well as key odorants, contribute to global juice aroma/ flavor and should be included, as far as possible, in aroma reconstitution experiments.

Many fruit juice volatile compounds (especially alcohols and including terpenols) exist partially or entirely as glycosides, which are odorless until they are hydrolyzed to release the odorant aglycone. This can occur during fermentative conversion of juice to wine or by the addition of β -glucosidase or pectinase enzymes during juice preparation (see Fig. 13) (Buglass and Caven-Quantrill 2013).

Phenolic Compounds

This subsection focuses on non-anthocyanin, nonvolatile phenolic compounds, many of which contribute to color and/or are important contributors of bitter and/or astringent sensations in the taste and mouthfeel of fruit juices. Important classes of phenolic compounds, with examples, can be found in Table 4, while selected structures are displayed in Fig. 3. In general, juices from fruit that are specifically grown to produce cider, perry (pear cider), or wine have the highest total phenolic content (TPC), which is measured in gallic acid equivalents (GAE) and is usually quoted in mg GAE/L or mg GAE/100 g FW (fresh weight). See Table 5.

Table 3 Principal aroma/flavor compounds of fruit juices^a

Fruit juice	Characteristic aroma compounds
Apple (<i>Malus domestica</i> Bartch. or <i>M. sylvestris</i>)	Alcohols: 1-butanol, 1-hexanol, 2-methyl-1-butanol; carbonyls: <i>cis</i> -2-hexenal; esters: butyl hexanoate, ethyl butanoate, ethyl 2-methylbutanoate, hexyl acetate, hexyl butanoate, hexyl hexanoate, 2-methylbutyl acetate; norisoprenoids: β -damascenone
Apricot (<i>Prunus armeniaca</i> L.)	Alcohols: 2-phenylethanol; carbonyls: benzaldehyde; terpenoids: linalool, α -terpineol, 4-terpineol
Blackcurrant (<i>Ribes nigrum</i> L.)	Carbonyls: 1-octen-3-one; esters: ethyl butanoate, ethyl hexanoate, methyl butanoate; norisoprenoids: α -damascenone; pyrazines: 2-methoxy-3-isopropylpyrazine; terpenoids: cineole, linalool, 4-terpineol; sulfur compounds: 4-methoxy-2-methyl-2-butanethiol
Citrus juices:	Carbonyls: 4,5-epoxy- <i>trans</i> -2-decenal, 1-hepten-3-one, <i>cis</i> -3-hexenal; esters and lactones: ethyl butanoate, wine lactone; sulfur compounds: <i>p</i> -1-menthene-8-thiol, 4-mercapto-4-methyl-2-one
Grapefruit (<i>Citrus x paradisa</i> Macfad.), lemon (<i>Citrus limon</i> (L.) Burm. f.), Orange (<i>Citrus sinensis</i> (L.) Osbeck)	Alcohols: ethanol, methanol; carbonyls: 2-methyl-3-buten-2-ol; 4-methyl-2-pentanone, perillaldehyde; esters: ethyl acetate terpenoids: carvone, geranial, limonene, linalool, neral, γ -terpinene, α -terpineol, terpinen-4-ol
Grape (<i>Vitis vinifera</i> L., <i>V. labrusca</i> , <i>V. riparia</i> , <i>V. rupestris</i> , etc.)	Alcohols: hexan-1-ol, 2-phenylethanol; carbonyls: 2,3-butanedione, decanal, 2- <i>cis</i> -6- <i>trans</i> -nonadiol; esters: ethyl butanoate, ethyl 2-methylbutanoate, methyl anthranilate ^b ; furans: 4-hydroxy-2,5-dimethyl-3(2H)-furanone; pyrazines: 3-isobutyl-2-methoxypyrazine, 3-isopropyl-2-methoxypyrazine; terpenoids and norisoprenoids: β -damascenone, geraniol ^c , linalool ^c sulfur compounds: 3-(methylsulfanyl)propanal
Peach (<i>Prunus persica</i> (L.) Bartch var. <i>persica</i>)	Carbonyls: benzaldehyde; esters and lactones: γ -decalactone, ethyl acetate, hexalactone, methyl acetate; norisoprenoids: β -damascenone
Pear (<i>Pyrus communis</i> L.)	Esters: ethyl 2,4-decadienoate; terpenoids: <i>trans</i> , <i>trans</i> - α -farnesene
Pineapple (<i>Ananas comosus</i> (L.) Merr.)	Esters and lactones: allyl hexanoate, ethyl and methyl 2-hydroxybutanoate, ethyl and methyl 2-hydroxyhexanoate, ethyl and methyl 2-methylbutanoate, methyl 2-methylpropanoate, γ -nonalactone, δ -octalactone, γ -octalactone; furans: 4-hydroxy-2,5-dimethyl-3(2H)-furanone; terpenoids and isoprenoids: <i>trans</i> - β -damascenone
Strawberry (<i>Fragaria x ananassa</i> Duch.)	Carbonyls: 2,3-butanedione, <i>cis</i> -3-hexenal, esters: ethyl and methyl butanoate, methyl 2-methylpropanoate; furans: 4-hydroxy-2,5-dimethyl-3(2H)-furanone
Tomato (<i>Lycopersicon esculentum</i> Mill.)	Alcohols: <i>cis</i> -3-hexenol 3-methylbutanol; carbonyls: hexanal, <i>cis</i> -3-hexenal, <i>trans</i> -2-hexenal, 6-methyl-5-hepten-2-one; norisoprenoids: α -ionone; thiazoles: 2-isobutylthiazole

^aData from McKay et al. (2011), Heredia et al. (2013) and references therein

^bImportant in *V. labrusca*, *riperia* and other American species (and some hybrids)

^cPrevalent in muscat and/or traminer varieties of *V. vinifera*

Table 4 Important non-anthocyanin phenolic compounds in fruit juices

Class	Examples	Comments
Hydroxybenzoic acid type	<i>p</i> -Hydroxybenzoic acid, gallic acid, gentisic acid	Usually present as glycosides in apple, grape and pear juice
Hydroxycinnamic acid derivatives	Caffeic acid, <i>p</i> -coumaric acid, ferulic acid, sinapic acid	In many fruit juices, including black and white grape juice Caffeic and <i>p</i> -coumaric acids are often present as quinic acid derivatives (e.g., chlorogenic acid \equiv caffeoylquinic acid) – these are constituents of apple and pear juice Glucosides and tartrate esters (e.g., caftaric acid) are found in grape juice
Chalcones; dihydrochalcones	Butein, chalconaringenin, licochalcone, okanin; phloretin	Phloridzin (phloretin 2'-glucoside) is a component of apple and pear juices
Flavan-3-ols	(+)-Catechin and (–)-epicatechin	Present as aglycones in many fruit juices, such as apple, citrus, grape, and pear juices
Flavones	Apigenin, diosmetin, luteolin, nobiletin, sinensetin, tangeretin	Both types are mostly present as glycosides. Flavones are common in citrus fruit juices.
Flavonols	Isorhamnetin, kaempferol, myricetin, quercetin, rhamnetin	Also polymethoxy derivatives are found in orange juice. Flavonol glycosides (e.g., quercetin-3- <i>O</i> -rutinoside or rutin) are components of most fruit juices
Flavanones; Flavanonols	Didymin, heridictyol, hesperitin, isosakuranetin, narigenin; Dihydroquercetin	Flavanones are important components of citrus fruit juices, usually as neohesperidosides (7- <i>O</i> - β -glc-(2-1)- α -rha) or rutinosides (7- <i>O</i> - β -glc-(6-1)- α -rha)
Flavan-3,4-diols	Cyanidinol	Especially prevalent in perry pear juice
Condensed tannins: procyanidins and condensed proanthocyanidins (>10 flavan monomer units)	Dimers, trimers, and upwards of (+)-catechin and (–)-epicatechin, with 4–8 (sometimes 4–6) links or with a 4–8 and a 2–7 ether link	In apple, pear, and grape juices in particular, especially in black grape juice and cider apple and perry pear juices after some skin contact
Stilbenes	Piceatannol, resveratrol	In juice of blueberry and related fruits (e.g., huckleberry). Also in black grape juice
Hydrolyzable tannins (glucosidic esters of gallic acid, ellagic acid, and other phenolic acids)	Castalin, castalagin, ellagitannin, vescalin, vescalagin	Ellagitannin is found at low concentrations in some fruit juices (especially blackberry, raspberry, and strawberry); higher levels if seeds are ruptured during juicing

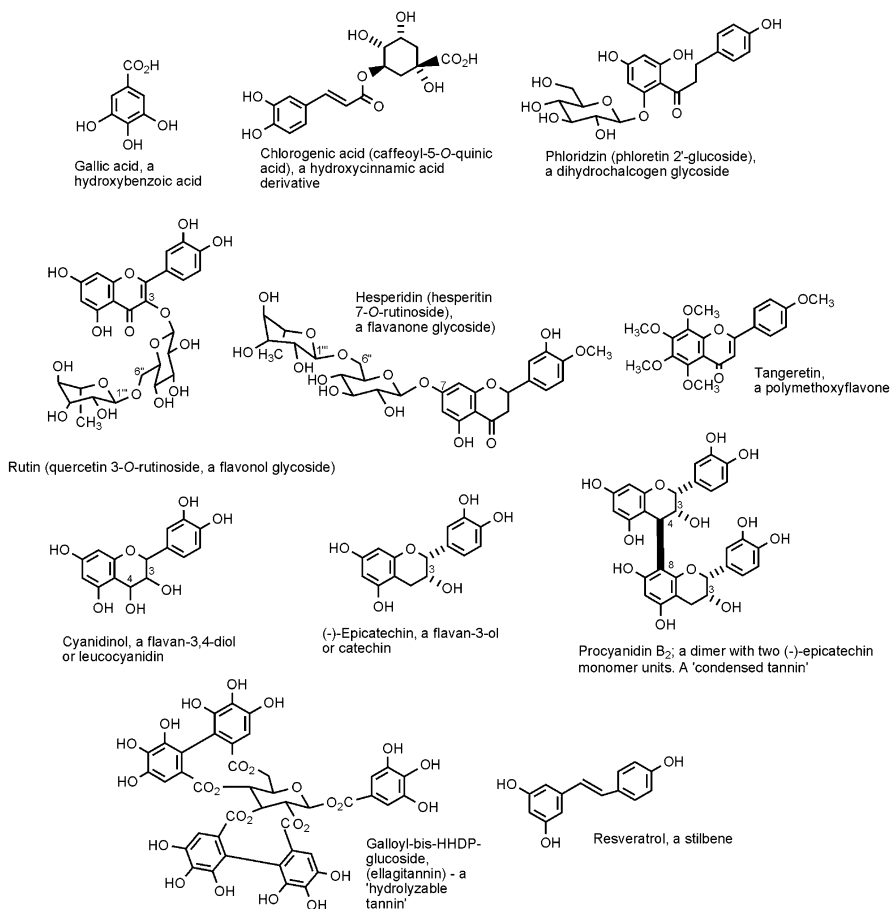


Fig. 3 Structures of some fruit juice phenolic compounds

The simplest phenols, hydroxybenzoic acids, are usually present as glycosides (mostly glucosides) in fruit juices: free forms are more prevalent in wine, especially red wine. Hydroxycinnamic acids are present as glucosides and also as esters of tartaric acid (e.g., *p*-coumaryl tartaric acid or coumaric acid and caffeoyl tartaric acid or caftaric acid) or quinic acid (e.g., coumaroylquinic acid; Fig. 3). Many hydroxycinnamic acids, in their various forms, are significant components of apple, grape, pear, and other fruit juices, where some are involved in “browning” (see Fig. 11). Also, caffeic and *p*-coumaric acids can combine with anthocyanidin 3-*O*-glucosides to form small quantities of acylated anthocyanins in black grape and other purple fruit juices (Fig. 1).

Chalcones and dihydrochalcones are comparatively rare, but phloretin and phloridzin in particular are important phenolic constituents of apple and pear juices. Monomeric flavonoid phenols are generally widespread throughout fruit juices, the

Table 5 Total phenolic content (TPC) and phenolic composition of selected fruit juices

Juice	TPC (mg GAE/L)	Major phenolic components (mg/L)
Apple ^a (from specific eating apples)	154.4–178.0	Hydroxycinnamic acids (56.8–67.7), dihydrochalcones (9.8–35.2), flavan-3-ols (7.6–54.6), procyanidins (32.3–46.8), flavonols (1.6–3.6)
Apple ^b (from German cider apples)	261.2–970.0	Hydroxycinnamic acids (138.5–592.6), dihydrochalcones (33.5–171.0), flavan-3-ols (32.8–249.1), procyanidins (32.0–143.4), flavonols (4.9–26.7)
Apple ^c (commercial juices)	109.9–495.0	Hydroxycinnamic acids (68.6–259.0), dihydrochalcones (9.4–75.8), flavan-3-ols (14.0–102.1), procyanidins (tr.–60.9), flavonols (tr.–13.5)
Cherry (sweet) ^{d,e}	44.3–87.9	Chlorogenic acid (0.60–2.61), <i>p</i> -coumaric acid (0.77–7.20), epicatechin (0.43–3.70), neochlorogenic acid (4.74–11.9), rutin (2.06–5.78)
Grape ^f	~300 (white)	Hydroxybenzoic and hydroxycinnamic acids as glycosides (100–200 in black grape juice) (10–20 in white grape juice), flavonols as glycosides (~100 in black grape juice) (1–3 in white grape juice), flavan-3-ols (~1,000 in black grape juice) (100–200 in white grape juice), procyanidins (~1,000 in black grape juice) (100–300 in white grape juice), flavanonols as glycosides (2.3–116), flavan-3,4-diols (2.3–116 in white grape juice), flavones (3–93 in white grape juice only)
	~2,000 (black)	
Grapefruit ^g	–	Flavanones: narirutin 4'-glucoside (9–15), naringin 4'-glucoside (16–21), rhoifolin 4'-glucoside (0–16), neoeriocitrin (0–4), narirutin (136–163), naringin (420–480), neohesperidin (6), rhoifolin (0–23), naringin-6'-malonate (24–33), poncerin (8–10)
Grapefruit ^h	441.09–725.71	Hydroxybenzoic acids: gallic acid (3.18–4.53), <i>p</i> -hydroxybenzoic acid (0.87–2.45), protocatechuic acid (1.87–3.70), vanillic acid (0.58–5.30)
		Hydroxycinnamic acids: caffeic acid (4.15–6.90), chlorogenic acid (3.12–5.17), <i>p</i> -coumaric acid (13.70–16.30), ferulic acid (14.09–26.46), sinapic acid (9.21–13.44)
		Flavanones: didymin (4.30–12.48), hesperidin (8.47–10.25), naringin (270.21–464.13), narirutin (63.80–120.06), neohesperidin (14.72–24.24), poncirin (16.98–26.02)
Orange ⁱ		Flavanones: naringenin 7-rutinoside-4'-glucoside (3.1), hesperetin 7-rutinoside-3'-glucoside (5.1), naringenin 4'-methyl-7-rutinoside (9.2), naringenin 7-rutinoside (33.2), hesperetin 7-rutinoside (86.3)

(continued)

Table 5 (continued)

Juice	TPC (mg GAE/L)	Major phenolic components (mg/L)
Tomato ^j	92.8–128.9	Hydroxycinnamic acids: caffeic acid (0.15–0.47), caffeic acid glycoside (0.22–0.68), chlorogenic acid (0.84–1.56), cryptochlorogenic acid (0.47–0.95), dicaffeoylquinic acid (0.15–0.27), ferulic acid glycoside (1.92–4.52) Flavanones: naringenin (3.60–7.04), naringenin 7- <i>O</i> -glucoside (0.13–0.58) Flavonols: kaempferol 3- <i>O</i> -glucoside (0.57–1.17), kaempferol 3- <i>O</i> -rutinoside (1.59–3.57), Quercetin (0.13–0.55), rutin (5.03–8.91)

^aKahle et al. (2005); *Malus domestica*: Fuji, Golden Delicious, Granny Smith, Red Delicious

^bKahle et al. (2005); *Malus sylvestris*: Bittenfelder, Bohnapfel, Boskoop, Brettacher, Kaiser Alexander, Kaiser Wilhelm, Winterrambur

^cKahle et al. (2005); probably mostly *M. domestica* blends

^dUsenik et al. (2008); *Prunus avium* L.

^eTPC in mg GAE/100 g FW; composition in mg/100 g FW

^fRibereau-Gayon et al. (2000, pp. 129–186); Amerine and Ough (1980, pp. 175–199); *V. vinifera*

^gHsu et al. (1998); Actually juice of two common *Citrus grandis* Osbeck x *Citrus x paradisi* Macfad. crosses: *Melogold* and *Oroblanco*

^hKelebek (2010); (*Citrus x paradisi* Macfad.) *Handerson*, *Rio red*, *Ruby red*, *Star ruby*

ⁱTomás-Navarro et al. (2014); actually juice of Citrus hybrid between mandarin and sweet orange (*Citrus sinensis* L. var. “Ortanique”)

^jVallverdú-Queralt et al. (2013)

major flavonoid classes being flavones, isoflavones, flavonols, flavanones, flavanonols, flavan-3-ols (catechins), and flavan-3,4-diols (leucoanthocyanins or anthocyanogens). Of these, flavan-3-ols and flavonols are almost ubiquitous in fruit juices, probably partly because they are located in both the pulp (or pericarp) and skins of fruit, whereas others, such as flavones, are concentrated in skins or peel. Flavanones are usually the most abundant flavonoids of citrus juices, while flavones, located mostly in the peel, are usually present only at low or trace levels (or are undetectable), although polymethoxylated flavones exist in tangerine and some other citrus juices. Flavanonols (e.g., dihydroquercetin or taxifolin) are found in grape juice, and flavan-3,4-diols are significant constituents of pear juice. The majority of flavonoid phenols exist mainly as *O*-glycosides in fruit juices: exceptions include flavan-3-ols, which are always present as aglycones, and a few flavones in citrus peel that exist as *C*-glycosides.

Oligomeric and polymeric flavonoid phenols (condensed tannins) are also important in many juices, including apple, bilberry/blueberry, cherry, grape, and pear juices. They are generally known as procyanidins and mostly consist of (+)-catechin and/or (–)-epicatechin monomer units. Type B (the most common) has C (4)-C(8) or C(4)-C(6) interflavan bonds, whereas type A has an addition ether bond between C(5) or C(7) on the lower flavonoid unit and C(2) on the upper unit. Procyanidins exist largely as aglycones, with dimers and trimers being most common, but polymers of up to 17 units exist in cider apple juice.

Flavan-3-ols, procyanidins, and other polyphenols are also capable of forming oligomers with anthocyanins or their derivatives, but these are more important in wine (Fig. 10). Hydrolyzable tannins such as ellagitannins (Fig. 3) are rare in juices (e.g., cherry, raspberry, and strawberry juices) but are more common in oak-aged wine.

Carbohydrates

Free sugars are crucial to the palatability of fruit juices, but carbohydrates in general, including their derivatives (such as sugar acids and reduced monosaccharides or polyols), are present in juices in many different guises, as outlined in Table 6. Total free sugars are often estimated from measurement of total soluble solids, using either a hydrometer or refractometer.

Table 7 lists typical values for common fruit juices, using the °Brix (Balling or Plato) scale, which is probably the most widely used scale for this purpose. It is an approximate measure of mass of sugar (in g) per 100 g juice – % sugar (w:w).

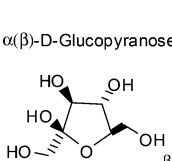
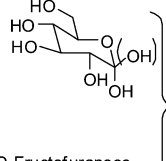
It can be seen from Table 8 that the most abundant fruit juice free sugars are the disaccharide sucrose (saccharose), along with the monosaccharides D-fructose and D-glucose, the first two being especially prevalent. Other free sugars exist in fruit juices but usually at low to very low levels: these include the monosaccharides L-arabinose, D-galactose, L-rhamnose, D-ribose, and D-xylose; the disaccharides lactose, maltose, melibiose, and trehalose; and the trisaccharide raffinose. Juice of *V. vinifera* grapes contain only minute amounts of sucrose and no trehalose, whereas most other fruit juices (including those of other *Vitis* species, such as *V. labrusca*) contain considerable, even dominant, amounts of sucrose.

With respect to relative sweetness, if sucrose has a rating of 1, fructose rates 1.73, glucose rates 0.74, and the pentoses (L-arabinose, etc.) rate about 0.4 so that, if the total sugar content of two juices is the same, the juice with the higher fructose–sucrose–glucose ratio will be sweeter.

Depending on the processing methods, varying amounts of pectic substances, oligomers and polymers of β-D-glucose, and oligomers and polymers of L-arabinose and D-xylose are present (Table 6). All of these are “fibrous” and are “soluble” or “insoluble” according to the extent of polymerization and branching, the insoluble materials forming part of the cloud. They arise from the breakdown of pectic and cellulosic structural materials of the fruit. If β-glucanase and xylanase enzymes (for cellulose/arabinoxylan degradation) and pectinase (pectolase) enzymes (for pectin degradation) have been used in the juicing process and if the juice has been filtered, then it will contain only relatively small amounts of soluble oligomers and monomers (including low-trace levels of rare sugars and derivatives – see Table 6). Freshly pressed juice, however, will contain a significant amount of fibrous material (dietary fiber) – see Table 10.

Polyols (sugar alcohols), such as sorbitol, are present in apple, cherry, and pear juices in particular but not in grape juice that has been prepared from healthy fruit. However, grapes that have been infected by the fungus *Botrytis cinerea* will give juice that contains low levels of glycerol, mesoinositol, and sorbitol. Under the right conditions, this is favorable for the production of certain types of wine, but table grapes grown to produce juice for drinking should always be healthy.

Table 6 Carbohydrates in fruit juices

Carbohydrate context	Comments
<p>Free sugars</p> <div style="display: flex; align-items: center;"> <div style="margin-right: 20px;"> <p>$\alpha(\beta)$-D-Glucopyranose</p>  </div> <div style="margin-right: 20px;"> <p>β-D-Fructofuranose</p>  </div> <div style="font-size: 3em; margin-left: 10px;">}</div> </div> <p>Sucrose: α-D-glc-(1-2)-β-D-fruf</p> <p>Gluconic acid, <i>meso</i>-inositol, mannitol and sorbitol (sugar alcohols)</p>	<p>These are the major free sugars of fruit juices. Fructose is probably the most important overall. Glucose and fructose are by far the main free sugars of grape juice, but sucrose is abundant in many fruit juices, such as apple, citrus, peach, pear and strawberry juices. β-D-galactose is found at low levels in some juices (e.g. grape juice, ~ 0.1 mg/L). Other disaccharides; lactose, maltose, melibiose, and raffinose are present at very low levels in many juices.</p> <p>Gluconic acid is the major sugar acid of fruit juices. Apple and pear juices are relatively rich in sorbitol. <i>meso</i>-Inositol is found in grape juice.</p>
<p>Glycosidic units</p> <p>β-L-Arabinose, β-D-glucopyranose, β-D-galactose α-L-rhamnose, β-D-xylose (monosaccharides)</p> <p>Rutinose (β-D-glc-(6-1)-α-L-rha), neohesperidose, (β-D-glc-(1-1)-α-L-rha), sophorose, (β-D-glc-(2-1)-β-D-glc), sambubiose (β-D-glc-(2-1)-β-D-xy) (disaccharides)</p>	<p>These are the main monosaccharide sugars of the wide range of glycosides of odorous alcohols (including terpenols) and phenols (including simple phenols and flavonoid phenols) found in all fruit juices. The sugar units are usually linked to the aglycone by ether (C-O-C) bonds, but sometimes by C-C bonds.</p>
<p>Sugar components of pectic substances</p> <p>α-D-Galacturonic acid and methyl galacturonate</p> <p>β-L-Rhamnose, α-L-arabinose</p> <p>β-D-Apiose, α-L-fucose, 2-O-methyl-α-L-fucose, β-D-glucuronic acid, β-deoxy-D-lyxo-heptulosaric acid, 2-keto-3-deoxy-D-manno-2-oxctulosic acid, β-3-C-carboxy-5-deoxy-L-xylose and 2-O-methyl-α-D-xylose</p>	<p>These units form the backbone of fruit pectic substances.</p> <p>L-rhamnose forms part of the rhamnoglacturonan regions of pectic substances; L-arabinose occurs in side chains and in regions of the main chain.</p> <p>These rare sugars are found in rhamnoglacturonan II regions of pectic substances. These regions are resistant to pectinase action and may be the only pectic substances found in fruit juices made using total liquefaction by pectinase enzymes.</p>
<p>Sugar components of cell wall polysaccharides</p> <p>β-D-glucopyranose, β-D-xylose, α-L-arabinose</p>	<p>These are the monomers of cellulose, which together with pectic substances and lignins forms the cell walls of fruit. Glucose polymers are called β-glucans and xylose polymers are known as β-xylans. Polymers of β-D-xylose and α-L-arabinose are sometimes known as pentosans.</p>

Uronic acids (sugar acids), principally galacturonic acid, are found in many fruit juices, including grape juice, where total uronic acid concentration is generally 100–300 mg/L.

Acids

Like sugars, acids are also crucial to the palatability of fruit juices – without them they would taste flat and sugary, many would have different colors, and they would all be highly prone to microbiological spoilage. Table 7 displays the total acidity of

Table 7 Total soluble solids and total acid content of selected fruit juices^a

Fruit	Apple ^b	Apricot	Bilberry	Blueberry	Blackberry	Black currant
Total soluble solids (°Brix) ^c	11–17	13–14	8–12	11–13	8	10
Total acidity (g/L)	2–10	6–15	8	3	12	30
Fruit	Cherry	Grape (table)	Grape (wine)	Grapefruit	Guava	Mango
Total soluble solids (°Brix)	13–18	14–20	18–32	8–11	8–10	11–15
Total acidity (g/L)	10–26	3–6	6–9	12–14	5	11–14
Fruit	Orange	Peach	Pear ^b	Pineapple	Strawberry	Tomato
Total soluble solids (°Brix)	11–13	11–15	13–17	12–18	8	5–7
Total acidity (g/L)	10–13	4–9	3–10	9–12	12	3–5

^aTypical values or ranges. Data from Amerine and Ough (1980, pp. 45–73), Chinnici et al. (2005), Shamsudin et al. (2005), Cheng et al. (2007), McKay et al. (2011, pp. 419–435), Li et al. (2012)

^bLower °Brix values and lower total acidities tend to be associated with cider apples and perry pears

^cTotal soluble solids (an approximate measure of sugar content) ~g sugar/100 g juice

selected juices, where it can be seen that there is a wide acidity range, from mildly acidic apple or grape juices to highly acidic black currant juice.

Table 8 shows that the most common and most abundant fruit juice acids are citric and malic acids, but in general juice organic acids are mostly low-molecular-weight mono-, di-, and tricarboxylic acids with pK_a values in the range 3.01–5.74. They all exist largely as free (molecular or undissociated) acids, but those with lowest $pK_a(1)$ values, especially L-(+)-tartaric acid (3.01), citric acid (3.09), and L-(–)-malic acid (3.46), will be present in partial salt form (see “Salts” subsection). To these we can add the inorganic acids phosphoric and sulfuric acids, which will be present mainly as dissociated salts, ascorbic acid (see “Vitamins” subsection), the sugar acids, and the very weakly acidic phenolic acids mentioned in previous subsections.

Other carboxylic acids found in juices include tartaric acid (especially important in grape and pomegranate juice), acetic, isocitric, pyruvic, quinic, succinic, and some other acids of the citric acid cycle/glycolysis pathway.

Amino Acids, Peptides, and Proteins

Of the total nitrogen content of ripe fruit juices, as determined by the Kjeldahl or Dumas methods, normally less than 10 % is present as inorganic nitrogen (mainly NH_4^+); amino acids and peptides make up about 30–40 % each and the rest is made up of mainly proteins, along with small amounts of amines, amino sugars, *N*-heterocycles, and *N*-containing vitamins.

Amino acids include the “magic twenty” protein components, plus citrulline, hydroxyproline, norvaline, and ornithine (all L- α -amino acids), as well as β -alanine and γ -aminobutanoic acid (GABA). Table 9 displays the total amino acid content

Table 8 Major acids and free sugars of selected fruit juices

Juice	Apple	Apricot	Grape	Grapefruit	Mandarin	Mango
Acids (g/L)	M (1.4–5.6) ^a	M (2.3–5.1) ^a	T (4.0–8.0) ^b	C (19–24) ^c	C (12–17) ^d	M (6.9–10) ^e
	C (tr.–1.1)	C (2.1–3.2)	M (2.0–5.0)	M (1.8–3.0)	M (3.8–8.0)	C (1.6–2.6)
	S (0.2–0.8)	S (0.1–0.8)	S (0.5–1.0)	S (0.2–0.6)	S (0.8–3.7)	S (0.9–1.6)
		Q (tr.–0.4)	U (0.1–0.3)			T (0.1–0.9) A (0.16)
Sugars (g/L)	Fr (60) ^g	Su (35–69) ^a	Fr (80–130) ^b	Su (29–35) ^c	Su (28–68) ^d	Fr (50–53) ^e
	Gl (17)	Gl (45–63)	Gl (70–120)	Fr (22–27)	Fr (9.2–22)	Su (33–80)
	Su (25)	Fr (33–44)		Gl (22–25)	Gl (6.9–20)	Gl (20–28)
	So (5)					
Juice	Peach	Pear	Pomegranate	Strawberry		
Acids (g/L)	M (1.5–5.6) ^a	C (1.5–2.0) ^a	C(2.0–10.0) ^f	C (0.5–1.0) ^g		
	C (1.0–1.9)	M (0.5–1.2)	M (3.0–3.5)	M (0.1–0.2)		
	S (0.1–1.5)	S (0.4–1.0)	T (0.2–0.6)	Q (t-0.2)		
		Q (t-0.07)		S (0.02)		
Sugars (g/L)	Fr (22–75) ^a	Fr (48–68) ^a	Fr (40–50) ^f	Gl (2.9–6.4) ^g		
	Su (20–77)	So (15–28)	Gl (40–50)	Fr (1.8–3.5)		
	Gl (37–43)	Gl (17–20)		Su (0.9)		
		Su (1.7–15)				

Key: A Acetic, C Citric, Q Quinic, S Succinic, T Tartaric, U Uronic (sugar acids). Sugars: Fr Fructose, Gl Glucose, Su Sucrose, So Sorbitol, tr. trace

^aChinicci et al. (2005)

^bRibéreau-Gayon et al. (2000, pp. 3–40; 55–80)

^cKelebek (2010)

^dSdiri et al. (2012)

^eLi et al. (2012)

^fMena et al. (2012)

^gGündüz and Özdemir (2014)

and the most abundant amino acids in selected fruit juices. They are predominantly L-isomers, but it is evident that the levels of individual amino acids in juice depend on processing methods. Generally lower levels have been found in pomegranate, strawberry, and tomato juices prepared from concentrates or by using some kind of heat treatment than in freshly squeezed juices (Vallverdú-Queralt et al. 2013). Likewise, concentrations of D-isomers (e.g., D-PRO) are higher in heat-processed juices (Tezcan et al. 2013).

Oligopeptides (up to tetrapeptides), such as glutathione, and polypeptides (<10,000 Da molecular weight) constitute a considerable part (~30–40 %) of the organic nitrogen content of juices but have not been extensively studied. Proteins are present in juices in concentration up to ~300 mg/L. Grape juice has several of molecular weight 10,000–100,000 Da but with the majority being in the 20,000–50,000 Da range. Many of the proteins are typical of fruit proteins and include chitinases, lipid transfer proteins, pathogen-related proteins, ripening-

Table 9 Total amino acid content and major α -amino acids of selected fruit juices

Juice	Total amino acid content (mg/L)	Major amino acids (most abundant on left). All L isomers, except where specified otherwise
Apple ^a	~800	ASN, ASP, NVAL, SER, GLU, ALA, PRO, ILEU, THR ~ VAL, LEU ~ PHE
Grape ^b	1,500–4,000	PRO, ARG, GLU, SER, ALA, α -ABA, THR, ASP
Pear ^c	~2,700	ASP, GLU, LEU, LYS ~ SER ~ VAL, ALA, GLY ~ ILEU ~ PRO, PHE, ARG, CYS ~ HIS, MET, TYR
Pomegranate ^d	733–3,374	SER, L-PRO, ALA, D-PRO, GLU, TRP, ARG, ASP, ASN, LEU
Strawberry ^e	418.7–464.5	ALA, SER, ASP, GLU, THR, VAL, GLY ~ ILEU, TYR, LUE ~ PHE, LYS
Tomato ^e	445.0–768.8	GLU, PHE, SER, HIS, ASP, LYS, ILEU, ARG, VAL, ALA, MET, GLY

^aPhenomenex Inc. (2014) Application data (NVAL Norvaline)

^bBuglass and Caven-Quantrill (2013) and references therein; α -ABA, α -aminobutanoic acid

^cUSDA National Nutrient Database for Standard Reference Release

^dTezcan et al. (2013); one juice also contained D-LEU

^eVallverdú-Queralt et al. (2013)

related proteins, thaumatin-like proteins, and vacuolar invertase 1, G1N1. Similar proteins are found in wine (see Table 13; Wigand et al. 2009).

Inorganic Cations and Anions (Electrolytes)

Fruit juices can be significant sources of mineral nutrients, which are present as salts of various inorganic and organic acids (Table 10). The macronutrients are Ca, K, Mg, N, Na, and P, the metals existing as their cations, N as NH_4^+ (ammonium), and P as phosphate anions. Micronutrients, often called trace elements, include B, Cu, Fe, Mn, Se, Si, and Zn.

If juice is evaporated to dryness, the dry extract (usually ~15–30 g/L juice) contains all the nonvolatile organic matter, plus inorganic compounds. When the dry extract is combusted at 525 °C, in a stream of air, all organic salts (acetates, tartrates, etc.), except ammonium salts, are converted to inorganic salts, mostly carbonates, the resulting ash usually being ~1.5–3.0 g/L juice. The total inorganic salt content can be estimated by titration.

Potassium is the most abundant ion, followed by calcium and phosphate, then N (as NH_4^+), and magnesium. Sodium is normally found at lower levels in juice (1–4 mg/100 g juice), an exception being pomegranate juice, which has ~9 mg/100 g juice (Table 10). The most abundant inorganic anions (after phosphates) are chloride and sulfate. Of the micronutrients, Fe is usually found at higher levels than Zn, although some juices, such as pomegranate juice, are good sources of dietary B and Mn.

Many minerals may persist into alcoholic beverages during fermentation and other processes, although some, like Ca and P, may be partially removed by precipitation, while others, such as Ca, NH_4^+ , SO_4^{2-} , Na, Cu, Fe, and others, may accumulate (deliberately or by accident) at certain stages of the process.

Table 10 Total mineral, vitamin, and dietary fiber content of selected fruit juices^a

Juice	Mineral content (mg/100 g juice)	Vitamin content (/100 g juice)	Total dietary fiber (g/100 g juice) ^b
Apple	K = 101, Ca = 8, P = 7, Mg = 5, Na = 4; Fe = 0.12, Zn = 0.02	Vit. C = 0.9 mg, niacin = 0.073 mg, vit. B ₆ = 0.018 mg, riboflavin = 0.017 mg, vit. E = 0.01 mg	0.5
Blood ^c orange	K = 171.2, P = 8.8, Mg = 6.7, Ca = 5.9, Na = 1.3; Fe = 0.25, Zn = 0.12, Cu = 0.113, Mn = 0.076, Se = 0.001	–	–
Clementine	K = 177, Ca = 30, P = 21, Mg = 10, Na = 1; Fe = 0.14, Zn = 0.06	Vit. C = 48.8 mg, niacin = 0.636 mg, thiamin = 0.086 mg, vit. B ₆ = 0.075 mg, riboflavin = 0.030 mg, vit. E = 0.20 mg, folate = 0.024 mg,	0.3
Grape	K = 104, P = 14, Ca = 11, Mg = 10, Na = 5; Fe = 0.23, Zn = 0.08	Vit. C = 0.12 mg, niacin = 0.12 mg, vit. B ₆ = 0.04 mg, pantothenic acid = 0.04 mg, Vit. A = 0.4 µg (8 I.U.), vit. K = 0.4 µg	–
Grapefruit	K = 143, Ca = 12, P = 12, Mg = 9; Fe = 0.08, Zn = 0.07	Vit. C = 33 mg, niacin = 0.269 mg, vit. B ₆ = 0.043 mg, thiamin = 0.037 mg, riboflavin = 0.020 mg, folate = 0.012 mg, vit. A = 0.005 mg (101 I.U.)	–
Lemon	K = 103, P = 8, Ca = 6, Mg = 6, Na = 1; Fe = 0.08, Zn = 0.05	Vit. C = 38.7 mg, niacin = 0.091 mg, vit. B ₆ = 0.046 mg, thiamin = 0.037 mg, folate = 0.020 mg, riboflavin = 0.015 g, vit. E = 0.15 mg, vit. A = 0.005 mg (101 I.U.)	0.3
Orange	K = 200, P = 17, Ca = 11, Mg = 11, Na = 1; Fe = 0.20, Zn = 0.05	Vit. C = 50.0 mg, niacin = 0.400 mg, thiamin = 0.090 mg, vit. B ₆ = 0.040 mg, riboflavin = 0.030 mg, folate = 0.030 mg, vit. A = 0.010 mg (200 I. U.), vit. E = 0.04 mg, vit. K = 0.1 µg	0.2
Pear	K = 130, Ca = 12, P = 12, Mg = 8, Na = 8, Zn = 0.08	Vit. C = 34 mg, niacin = 0.300 mg, riboflavin = 0.030 mg, thiamin = 0.010, vit. B ₆ = 0.010 mg, folate = 0.004 mg, vit. E = 0.09 mg, vit. K = 0.003 mg	0.4

(continued)

Table 10 (continued)

Juice	Mineral content (mg/100 g juice)	Vitamin content (/100 g juice)	Total dietary fiber (g/100 g juice) ^b
Pineapple	K = 130, Ca = 13, Mg = 12, P = 8, Na = 2; Fe = 0.31, Zn = 0.11	Vit. C = 43.8 mg, Niacin = 0.636 mg, vit. B ₆ = 0.100 mg, thiamin = 0.058 mg, riboflavin = 0.021 mg, vit. E = 0.02 mg, folate = 0.018 mg, vit. A = 2.5 µg (51 I.U.)	0.2
Pomegranate	K = 214 Ca = 11.0, P = 11.0, Na = 9.0; Mg = 7.0, Fe = 0.080, Zn = 0.080, Mn = 0.080	Vit. E = 0.36 mg, pantothenic acid = 0.28 mg, niacin = 0.24 mg, vit. C = 0.080 mg, vit. B ₆ = 0.040 mg, folate = 0.024 mg, vit. K = 0.010 mg	–

^aData from USDA (2014) National Nutrient Database for Standard Reference Release, unless stated otherwise. Metals as cations, P as phosphate

^bFresh whole fruit generally possess higher levels of fiber, followed by freshly pressed juice (e.g., 1.5–2.4 g/L for citrus fruits)

^cCautela et al. (2009)

Vitamins

Like the parent fruits, juices are good sources of a wide range of vitamins. Table 10 displays the main vitamin content of selected juices, where it can be seen that vitamin C (ascorbic acid) is the most abundant and most widespread vitamin, having high levels in citrus juices (typically ~50 mg/100 g juice). High levels are found in black currant and guava juices too (181 and 110 mg/100 g juice, respectively). Niacin is usually the next most abundant vitamin (~0.2–0.4 mg/100 g juice), followed by vitamin B₆ (pyridoxine), thiamine (B₁), riboflavin (B₂), vitamin E, pantothenic acid, folate, and vitamin K. An exception is pomegranate juice, which has pantothenic acid as the main vitamin, and it has relatively high levels of folate and (particularly) vitamin K (e.g., ~0.010 mg/100 g juice – about 25 times the concentration in grape juice).

Vitamin levels are generally higher in freshly pressed juices – processing steps, such as pasteurization and evaporation, are known to deplete the juice vitamin content somewhat. Likewise, vitamin levels tend to be lower in alcoholic beverages made from fruit juices, although some, especially B vitamins and vitamin C, can be added legally at certain production stages.

Other Components

Nonvolatile triterpenoids, such as limonin, nomilin, nomilinic acid, obacunone glucosides, and aglycones, are found in citrus juices. During fruit maturation, total glucoside concentration rises, while total aglycone level drops, often to below taste perception levels (~6 mg/L juice): glucosides are tasteless, but

aglycones are bitter and can be a cause of consumer rejection. Total limonoid glucoside levels vary between about 60 mg/L for some grapefruit hybrid juices, through ~190 mg/L for grapefruit juices, and ~320 mg/L juice for some sweet orange juices (Hsu et al. 1998). Some grapefruit cultivar and hybrid citrus juices have total aglycone levels above the taste threshold.

Pentacyclic triterpenoids (again nonvolatile), such as maslinic acid, oleanolic acid, ursolic acid, and derivatives, are found in the waxy skins of many fruits, including apples and pears. See Fig. 25. They are potent *in vitro* anticancer agents. Ursolic acid is present at levels up to 1.6 g/kg fruit in apples, and cloudy juices contain low levels of these compounds, depending on the processing (Fuller et al. 2011, pp. 1093–1110 and references therein).

Nonalcoholic Carbonated Beverages

Carbonated drinks form an important part of the soft drinks industry. They can be divided into the following major categories:

- Mineral water and soda water
- Fruit drinks
- Cola- and root beer-type drinks
- Tonic water

Apart from (unflavored) mineral water and most soda water, all categories have added compounded flavor mixtures, sugar, or low-calorie sweeteners, and some have added colorants. The carbonation level of most drinks is around 3 vols CO₂ per vol liquid, which gives internal pressures of ~2 atm at 4 °C and ~2.5–3 atm at 21 °C.

Carbonated Mineral Water and Soda Water

Carbonated mineral water is sourced from natural springs or from deep subterranean aquifers, where it is carbonated by natural CO₂ at above atmospheric pressure. Nowadays, the CO₂ is usually removed at source and then added back when the water is bottled, typically at 4 °C and under CO₂ pressure of 1.2 atm. During its long contact with rock, the water acquires many mineral ions, its mineral profile depending largely on local geology (Table 11). Bottled mineral water must conform to national standards of purity and must be consistent with regard to pH and mineral content. It is permissible to remove undesirable constituents, such as volatile sulfur compounds (e.g., in hot volcanic springwater), Fe²⁺/Fe³⁺, or Mn³⁺, prior to treatment with ozone and/or UV light (to kill microorganisms) before bottling.

Water that flows through limestone or chalk tends to be alkaline, with high HCO₃⁻, Ca²⁺, and Mg²⁺ content – and sometimes with high sulfate and Na⁺ content too – whereas water flowing through basalt or other volcanic rock tends on the acidic side, with lower total dissolved solids (TDS) (Table 11).

Table 11 Major mineral content (mg/L) of some bottled carbonated mineral waters^a

Dolomite (Italy) (limestone)	Vergèz Languedoc (France) (limestone)	Eiffel (Germany) (dolomite limestone)	Fiji (volcanic)	Cachet Spring (French Alps) (granite)
pH = 7.8	pH = 5.46	pH = 6.0	pH = 7.7	pH = 7.2
Ca ²⁺ = 180	Ca ²⁺ = 155	Ca ²⁺ = 348	Ca ²⁺ = 17	Ca ²⁺ = 80
Cl ⁻ = 180	Cl ⁻ = 25	Cl ⁻ = 40	Cl ⁻ = 5	Cl ⁻ = 6
HCO ₃ ⁻ = 238	HCO ₃ ⁻ = 445	HCO ₃ ⁻ = 1,816	HCO ₃ ⁻ = 140	HCO ₃ ⁻ = 360
Mg ²⁺ = 52.3	Mg ²⁺ = 6	K ⁺ = 11	K ⁺ = 0	K ⁺ = 1
Na ⁺ = 57	Na ⁺ = 11	Mg ²⁺ = 108	Mg ²⁺ = 13	Mg ²⁺ = 26
Sulfates = 459	Sulfates = 38	Na ⁺ = 118	Na ⁺ = 18	Na ⁺ = 6
NO ₃ ⁻ = 2.2	NO ₃ ⁻ = 18	NO ₃ ⁻ = 18	Sulfates = 0	Sulfates = 12
Si (silicate) = 7.5		Sulfates = 38	Si (silicate) = 94	
TDS ^b = 960	TDS = 688	TDS = 2,479	TDS = 220	TDS = 330
Tÿ Nant (Wales) (granite, sedimentary)	Clairvic spring, (Auvergne, France) (volcanic)	Napa Valley (US) (hot spring)	Vichy (Allier, France) (volcanic)	Catalàn (Girona) (Spain) (volcanic)
pH = 6.8	pH = 7.0			pH = 6.82
Ca ²⁺ = 22.0	Ca ²⁺ = 11.5	Ca ²⁺ = 2	Ca ²⁺ = 103	Ca ²⁺ = 54.1
Cl ⁻ = 14.0	Cl ⁻ = 13.5	Cl ⁻ = 200	Cl ⁻ = 235	Cl ⁻ = 601.5
HCO ₃ ⁻ = ?	HCO ₃ ⁻ = 71	HCO ₃ ⁻ = 25	HCO ₃ ⁻ = 2,989	HCO ₃ ⁻ = 2,135
Mg ²⁺ = 11.5	K ⁺ = 6.2	K ⁺ = 14	K ⁺ = 66	K ⁺ = 48
Na ⁺ = 22.0	Mg ²⁺ = 8	Mg ²⁺ = 0	Mg ²⁺ = 10	Mg ²⁺ = 9.2
Sulfates = 4.0	Na ⁺ = 11.6	Na ⁺ = 170	Na ⁺ = 1,172	Na ⁺ = 1,110
NO ₃ ⁻ < 0.1	Sulfates = 8	Sulfates = 110	Sulfates = 138	Sulfates = 138
Dry residue = 165 (at 180 °C)	Si (silicate) = 31.7			(Si) silicate = 76.8
	TDS = 131	TDS = 521	TDS = 4,713	TDS = 3,052

Sulfates = SO₄²⁻ and HSO₄⁻

^aThe identity of each mineral water is given by the location of the source, not by brand name

^bTotal dissolved solids

Flavored carbonated mineral water has enough added fruit essences (e.g., lemon, lime, or apple) to give just a light flavor, which means that the flavor and bittering component content will be low.

Nonalcoholic “spritzers” are more substantially flavored sparkling water. They lie between flavored mineral water and fruit drinks, having typical carbohydrate content, protein content, and fiber content of 60–70 g/L, ~1 g/L, and ~1 g/L, respectively.

Soda water (sparkling water or seltzer water) refers to any water (even domestic tap water) that is artificially carbonated, often at CO₂ pressures as high

as ~8 atm, at 8 °C. Commercial examples are usually filtered or purified water (which must conform to national purity standards) that contains low levels of added salts (e.g., sodium or potassium bicarbonate or citrate, potassium sulfate, or disodium phosphate). This is the kind of soda water that is used in “mixers,” say with whisky. Additionally, some manufacturers add sucrose syrup and vanilla essence and/or other extract (such as quillaja) to make a sparkling drink sometimes known as “cream soda”.

Fruit Drinks

Carbonated fruit drinks (“pop,” “lemonade,” “soda pop”) constitute a major part of the soft drinks industry. They are made with purified water (or even springwater or natural mineral water in some cases), fruit juice (typically 2–10 % v:v), possibly compounded flavors containing fruit extracts or essential oils, sugar syrup and/or low-calorie sweetener, and possibly coloring. The fruit juice is used in concentrated form (~6× original strength), obtained by heat evaporation, membrane concentration, or cryoconcentration (Ashurst 2012 and references therein). Essences, essential oils, and extracts are used as ingredients in compounded flavor formulations and, as such, are individually likely to constitute no more than ~0.1 % (w:v) of the fruit drink. Compounded flavor mixes are especially important in the manufacture of cola- and root beer-type drinks. The range of volatile flavor compounds and bitter compounds for a particular fruit drink is similar to that in the pure fruit juice or alcoholic beverage containing that fruit (Table 3 and Fig. 25), but citrus drinks have lower levels of terpene hydrocarbons, as most of these are removed from the essential oil before compounding (Ashurst 2012).

Nowadays, high-fructose corn syrup and sucrose syrup are the main sugar source, sometimes in combination with a very small quantity of low-calorie sweetener or intense sweetener. In diet (low-calorie) versions of soft drinks, a combination of low-calorie sweeteners (e.g., saccharin and aspartame), always at very low concentration, is often used. See Fig. 4 for examples of low-calorie sweeteners and intense sweeteners.

Beverage colorants, deemed “natural” in most countries, are extracts or concentrates of fruit or vegetable and include annatto, beet juice, grape skin extract, β-carotenes, paprika, and saffron. Also included is cochineal, of animal origin. Synthetic colorants include Brilliant Blue (FD&C 1, E135), Indigotine (FD&C 2, E132), Erythrosine (FD&C 3, E127), Allura Red (FD&C 40, E129), Tartrazine (FD&C 5, E102), and Sunset Yellow (FD&C 6, E110). Caramel, in its various forms (E150a-d) is the most widely used beverage colorant (Buglass and Caven-Quantrill 2012), especially regarding cola and similar drinks. Plain caramel (E150a), made from sugar and acid (or alkali or salts) (but with no ammonia or sulfite), will supply low levels of monomeric and polymeric *O*-heterocycles (furans and pyrans), whereas ammonia caramels (E150c, d) supply additional low levels of monomeric and polymeric *N*-heterocycles (imidazoles and pyrazines).

Preservatives used in soft drinks include benzoates, dimethyl dicarbonate (DMDC), sorbates, and sulfites, depending on national regulations, and with maximum levels generally around 200 mg/L.

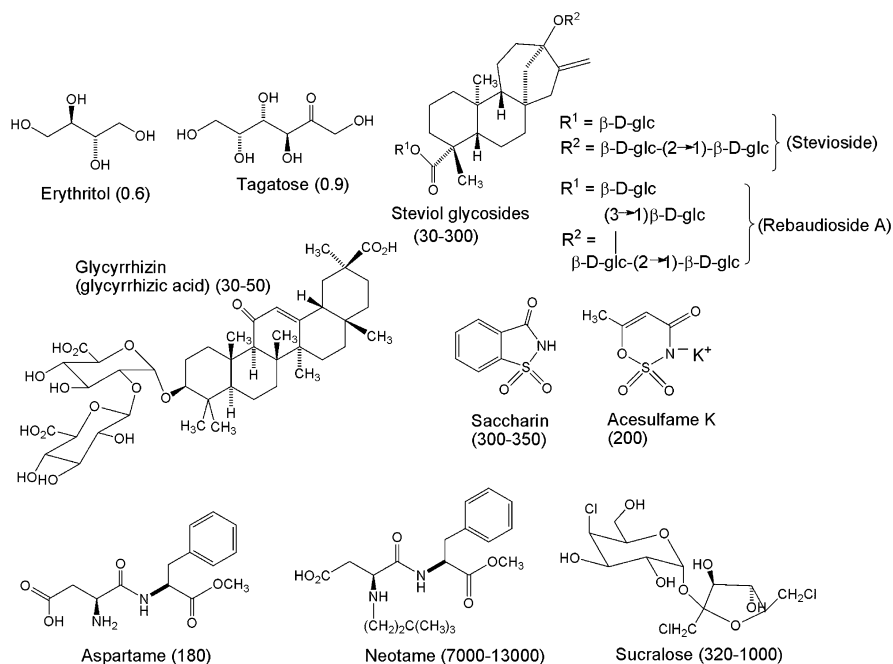


Fig. 4 Natural and synthetic low-calorie sweeteners used in soft drinks (approximate sweetness relative to sucrose = 1)

Cola- and Root Beer-Type Drinks

Modern cola drinks are made of sweetened, acidified, colored carbonated purified water, with a compounded flavor mix consisting of a source of caffeine, essential oils of citrus, cinnamon, and vanilla, along with gum arabic as an emulsion stabilizer. Sometimes other essential oils, such as nutmeg, are included. Caffeine content is usually 100–200 mg/L. The acidulant is phosphoric acid and/or citric acid, the sweetener is either sucrose syrup or high-fructose corn syrup (HFCS), and the colorant is caramel. Diet versions use low-calorie sweeteners (Fig. 4), and clear cola drinks (without caramel) are also available.

Cocaine, one of the original bitter agents, has not been used in cola drinks for many decades, because of its addictive nature. Some cola manufacturers use catuaba bark (*Trichilia catigua*) extract, which gives bitter but nonaddictive tropane alkaloids, catuabines A–D.

The compounded flavor mix for root beers is more complex than that for cola drinks (Table 12), but like cola, the combination of essential oils, extracts, and other ingredients differs between manufacturers and are trade secrets. Root beers usually possess a more spicy and bitter character than cola drinks.

Historically, the major flavor component was extract of *Sassafras albidum* root, but safrole, this oil's major component, has been shown to be carcinogenic

Table 12 Ingredients and selected organoleptic components of root beers^a

Ingredient (part of plant used)	Botanical name	Major organoleptic components
Sassafras (root)	<i>Sassafras albidum</i>	Safrole, terpenoids, saponins
Sarsaparilla (root)	<i>Smilax regelii</i> , <i>S. glycyiphylla</i>	Terpenoids, polyphenols, saponins
Wintergreen (leaf, berry)	<i>Gaultheria procumbens</i>	Methyl salicylate
Licorice (root)	<i>Glycyrrhiza glabra</i>	Anethole, glycyrrhizin
Birch sap	<i>Betula lenta</i> , <i>B. nigra</i>	Sugars
Black cherry (bark)	<i>Prunus serotina</i>	Lignins, saponins
Spruce (sap, needle)	<i>Picea rubens</i> , <i>P. mariana</i> , <i>P. sitchensis</i>	Bornyl acetate, camphene, δ -3-carene
Burdock (root)	<i>Arctium lappa</i>	Acids, polyphenols, polyacetylenes, inulin and other fibrous carbohydrates, arctigenin, arctiin
Dandelion (root)	<i>Taraxacum officinale</i>	<i>o</i> - and <i>m</i> -xylene, 2-ethyl-1-methylbenzene, heneicosane, tricosane, taraxacin
Root beer plant (root)	<i>Piper auritum</i>	Safrole, terpenoids, polyphenols
Cinnamon (bark)	<i>Cinnamomum verum</i>	Estragole, ethyl cinnamate
Nutmeg (fruit)	<i>Myristica fragrans</i>	Eugenol, elemicin, myristicin
Aniseed (fruit)	<i>Pimpinella anisum</i>	Anethole, ethyl cinnamate
Star anise (fruit)	<i>Illicium verum</i>	Anethole
Ginger (root)	<i>Zingiber officinale</i>	<i>ar</i> -Curcumene, zingiberene, zingiberol, gingerol
Clove (fruit)	<i>Syzygium aromaticum</i>	Acetyleneugenol, eugenol, eugenin
Mint (leaf)	<i>Mentha</i> spp.	(-)-carvone, menthol, menthone
Fennel (seed)	<i>Foeniculum vulgare</i>	Anethole, anisaldehyde, apiole, dillapiole, limonene, (+)-carvone
Fenugreek	<i>Trigonella foenum-graecum</i>	Saponins, fibrous carbohydrates
Cassia (bark)	<i>Cinnamomum aromaticum</i>	Benzaldehyde, chavicol, cinnamaldehyde
Soapbark	<i>Quillaja saponaria</i>	Polyphenols, saponins
Allspice (corn or seed)	<i>Pimenta dioica</i>	Eugenol, caryophyllene
Balsam	<i>Abies balsamea</i>	Benzyl benzoate, benzyl cinnamate, cinnamic acid
Hop (flower)	<i>Myroxylon balsamum</i>	Humulene, myrcene
	<i>Humulus lupulus</i>	

^aOnly a certain combination of these will be used by a particular manufacturer, usually as extracts or essential oils. This is not an exhaustive list. See also Table 19

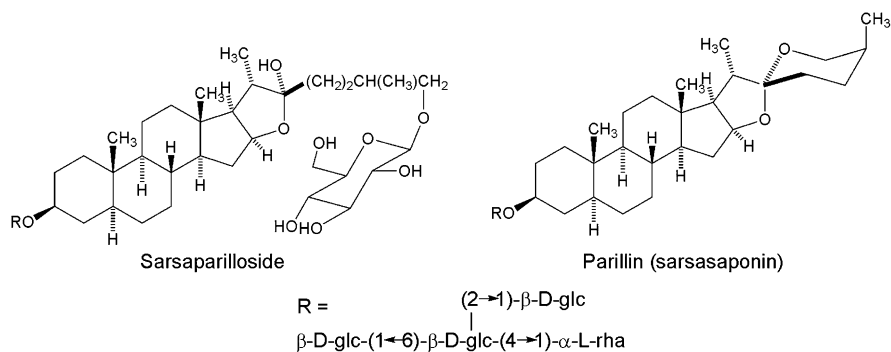


Fig. 5 Bitter saponins (triterpenoids) in sarsaparilla (*Smilax regelii*), a flavoring ingredient of root beers. These and other saponins aid foaming

in mice (there is no evidence for carcinogenicity in man). Nowadays, either “safrole-free” sassafras root extract or some substitute is used in commercial examples. Sweeteners are as for other soft drinks, but molasses or honey is sometimes used. “Sarsaparilla” and “dandelion and burdock” are carbonated soft drinks, like root beers, but are made with much more limited flavor mixes, as implied by their names. Table 12 lists a few compounds of organoleptic importance in the essential oils or extracts used in these two drinks, and Fig. 5 gives the structures of two of many bitter saponins (triterpenoid or steroidal glycosides) found in sarsaparilla root extract; these compounds also aid the foaming properties of such drinks. Root beers and related drinks are usually colored with caramel. Ginger beer (or ale) is a carbonated drink flavored with ginger essence or powder (and sometimes other essences), sucrose or HFCS, or low-calorie sweeteners; they are not usually colored. See Table 12 for major chemical components of ginger.

Note that root beers and ginger beer can be made as alcoholic versions (the originals were probably alcoholic), using a yeast culture and added citric acid for balance. Filtration after mixing thoroughly with yeast, fermenting for a short time at $\sim 20^\circ\text{C}$, then cooling to $\sim 4^\circ\text{C}$, and stoppering give an effervescent, mildly alcoholic drink (0.5–2 % ABV, depending on fermentation time). Whether these drinks are classified as soft or alcoholic depends on national regulations.

Tonic Water

Tonic water or Indian tonic water is a sweetened carbonated drink flavored with cinchona bark extract or quinine. Nowadays, the quinine content is low (the United States Food and Drug Administration – USFDA – specifies a maximum level of 83 mg/L), so the drink is ineffective as a prophylactic against malaria (requiring $\sim 2,000$ mg per day for adults), which was its original purpose. It is widely used in cocktails or mixers (e.g., with citrus juices or gin).

Table 13 Carbohydrate, mineral, and protein/amino acid content of sports drinks^a

Component	Concentration range (mg/L)	Usual level or range (mg/L) in drinks that contain them
Sugars	0–76 (g/L)	~60 (g/L)
Na ⁺	170–3,750	420–845
K ⁺	0–450	84–250
Mg ²⁺	0–420	–
Ca ²⁺	0–340	25–125
Cl ⁻	0–1,270	340–380
Protein	0–85	~33
Amino acids	0–15 (g/L)	–

^aData from labels of selected drinks

Functional Nonalcoholic Beverages

Functional drinks are those that claim to provide some sort of specific physiological function, along with health benefits. Only ready-to-drink products (a large and growing market) are discussed in detail here. They can be subdivided into the following general categories (with a certain amount of overlap):

- Sports, performance, and recovery drinks
- Health beverages
- Energy and rejuvenation beverages
- Relaxation drinks

Fermented functional drinks, such as kefir, soy beverages, fermented whey, kombucha (fermented tea), and fruit vinegars (made from fruit wines), although some of the most nutritious of such beverages, are not dealt with here.

Sports, Performance, and Recovery Drinks

These generally still (non-carbonated) drinks are designed to promote rehydration and to be a source of fuel for rapid metabolic conversions that are required during vigorous exercise. They claim to prevent dehydration and to increase athletic performance. The first generation of such drinks were purified water that contained electrolytes and/or carbohydrates and sometimes fruit flavorants and colorants, but modern versions can contain, besides these, a combination of amino acids, proteins, vitamins, lecithin, L-carnitine, chromium picolinate (a regulator of carbohydrate metabolism), and herb extracts. Sports drinks can be classified as hypotonic, isotonic, or hypertonic, depending on concentrations of minerals (lower, similar, and higher levels, respectively, of minerals in comparison with the human body). Caffeine is not a component of sports drinks but is a major component in most energy beverages. Table 13 summarizes the sugar, mineral (electrolyte), and protein content of sports drinks, where it can be seen that Na⁺ is the major electrolyte (~460–930 mg/L), being needed to replenish the sodium lost in sweat.

Electrolyte-only sports drinks are sometimes called rehydration drinks – their function is to replenish lost moisture and minerals. Most sports drinks contain sugars, often a mixture of fructose, glucose, and sucrose, for optimum metabolism during strenuous exercise (Reents 2007). Many modern sports drinks contain amino acids or protein (Table 13), in response to observations that their presence aids athletic performance. Also present in some sports drinks are additional ingredients listed above – it is here that there is some overlap with health beverages.

Health Beverages

Many drinks are called “healthy,” but this subsection considers only ready-to-drink products that are marketed specifically as “health drinks” for normal individuals and are not necessarily associated with sporting activities. Some companies provide concentrates for mixing with water to make a “health drink.” Some of these can be highly complex mixtures of powders of seaweed, cereals, fruit, vegetables, herbs, or mushrooms. These will supply a wide range of phytochemicals (carbohydrates, fats, amino acids, proteins (including enzymes), terpenoids, phenolic compounds, minerals, vitamins, and others) associated with particular components, along with dietary fiber.

Typical ready-to-drink health beverages consist of purified water, with added minerals (but not Na^+) and vitamins: they are generally devoid of carbohydrates, fats, amino acids, and proteins. They contain flavorings (e.g., natural flavorings or those from fruit/vegetable concentrates), citric acid (acidulant), calcium lactate (electrolyte), possibly caramel or other colorant, and low-calorie sweeteners, such as a sucralose/acesulfame-K combination (Fig. 4). The vitamin content (typically A, B complex, C, D, and E) per bottle (usually 237 mL) often supplies ~15–70 % of the daily requirement.

Some health drinks (“calorie-burning drinks”) focus on people who seek to lose weight. These drinks frequently contain relatively high levels of the polyphenol epigallocatechin gallate (EGCG) or tea extract (EGCG is a component of tea) or chlorogenic acid (a coffee component). The polyphenols reduce the effectiveness of carbohydrates and other sources of calorific nutrition, and additionally, they are themselves effective antioxidants and may enhance beneficial gut flora activity.

Energy/Rejuvenation Beverages

This category of functional (often carbonated) drinks is quite separate from sports drinks. Energy drinks provide a wide range of central nervous system (CNS) and/or muscle stimulants, the most common of which is caffeine (a methylxanthine), often (at least in part) derived from natural extracts, such as guarana or yerba mate extract. These extracts are a source of many other phytochemicals, including other methylxanthines that can have quite different physiological functions to caffeine (Fig. 6).

The caffeine content of energy drinks ranges from about 150 mg/L to over 3,500 mg/L, with typical values of 200–400 mg/L, whereas cola and related drinks typically have 100–150 mg/L caffeine content. Although the caffeine and other non-calorific stimulants in energy drinks create a feeling of alertness and confidence, these substances are not a source of metabolic energy like carbohydrates, for example.

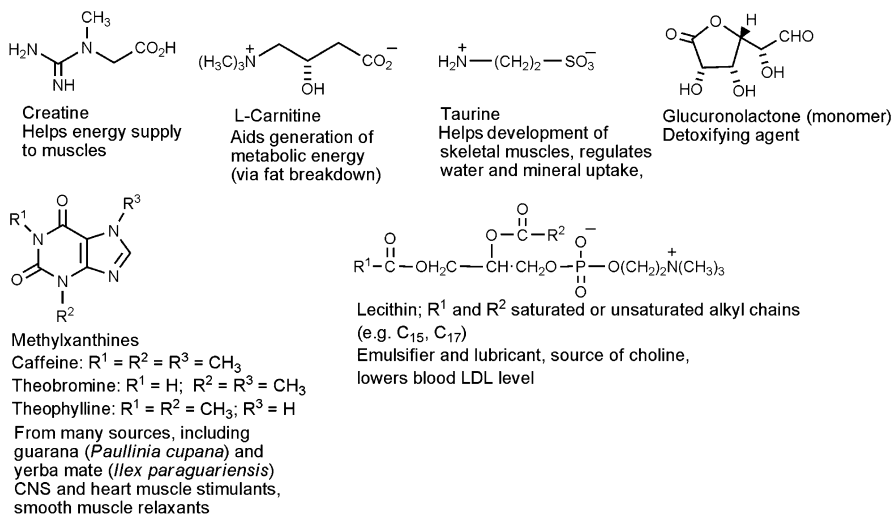


Fig. 6 Active chemical constituents frequently used in energy beverages

Apart from caffeine, common ingredients of energy drinks include high-fructose corn syrup or sucrose syrup, carnitine, creatine, glucuronolactone, inositol, maltodextrose, and/or taurine, as well as extracts of açai berry (*Euterpe oleracea*), catuaba bark (*Trichilia catigua*), *Epimedium* spp., ginkgo (*Ginkgo biloba*), ginseng (*Panax* spp. mainly), guarana (*Paullinia cupana*), *Gymnema sylvestre*, *Polygonum cuspidatum*, white thistle (*Silybum marianum*), and/or yerba mate (*Ilex paraguariensis*). The extracts can supply a wide range of natural chemicals, including alkaloids, amino acids, carbohydrates (including dietary fiber) terpenoids, steroids, flavonoid and other polyphenolic compounds, and vitamins, many of which are antioxidants; selected examples can be found in Fig. 7, where it can be seen that terpenoids, steroids, and polyphenolic compounds are usually found as glycosides.

These extracts, or in some cases individual components, have been found to have physiological activity; for example, ginkgolides aid mental concentration, ginsenosides can be either CNS stimulants or relaxants (Qi et al. 2011), catuabines (from *Trichilia catigua*) are CNS stimulants, and icariin (from *Epimedium* spp.) is a smooth muscle relaxant. The concentrations of individual plant extract-derived physiologically active components in functional beverages will be in general very low, so with normal usage, an “overdose” situation will not occur. Nevertheless, energy drink labels carry warnings of the type: “Consumption of more than two cans in a day may be harmful to your health. Not to be used for pregnant women, breast feeders, children under the age of 16, people with heart disease, high blood pressure, diabetes, allergy to caffeine, and athletes during exercise.”

Also, drinkers of functional beverages should assess the likelihood of antagonism between beverage active ingredients and current medication or other aspects of diet, such as ethanol intake. “Diet” versions contain low-calorie sweeteners, such as sucralose and/or acesulfame-K.

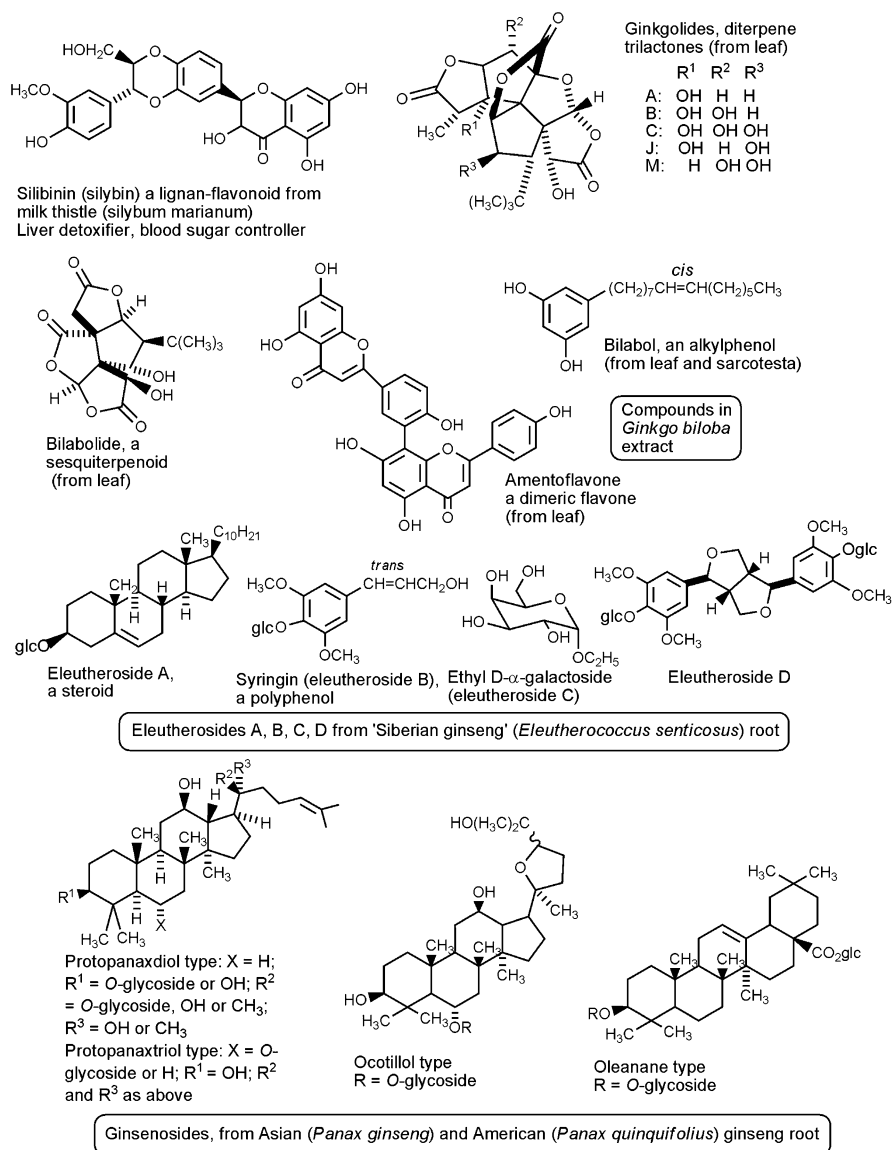


Fig. 7 Characteristic chemical components of some plant extracts used in energy beverages

Relaxation Drinks

Currently (2014) having a relatively modest share of the functional beverage industry, relaxation drinks are designed to provide the consumer with a feeling of relaxation that accompanies loss of anxiety. This is achieved mostly by including in the drink components that interact with inhibitory neurotransmitter receptors, such

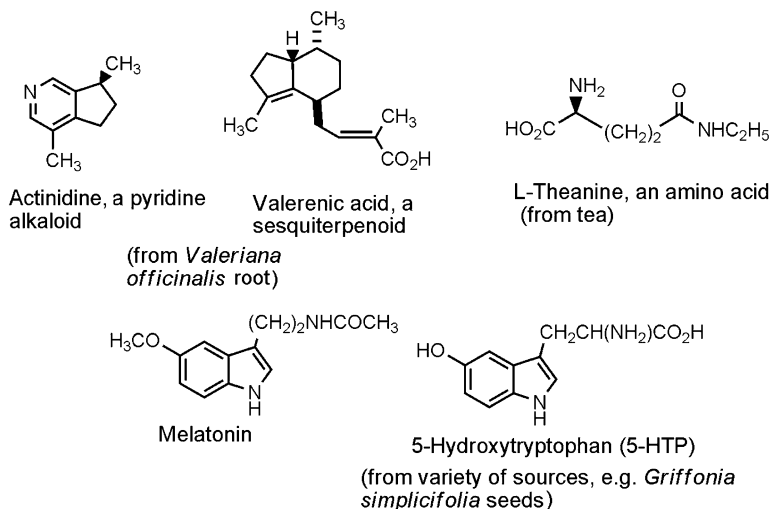


Fig. 8 Structures of some active components of relaxation drinks

as GABA and serotonin receptors, or those that block excitatory neurotransmitter receptors, especially glutamate receptors.

Typical relaxation drinks consist of a purified water base with flavorant, sweetener, and possibly colorant, along with one or more active ingredients, which include L-theanine (from tea), valerian (*Valeriana officinalis*) root extract (which contains alkaloids like actinidine, sesquiterpenoids like valerenic acid, γ -aminobutyric acid (GABA) and many other compounds), 5-hydroxytryptophan (5-HTP) (precursor of serotonin), and melatonin (Fig. 8).

Chemical Composition of Alcoholic Beverages

This section deals with compositions of major fermented alcoholic drinks – wine, cider, beer, and related cereal-based beverages – as well as distilled products: brandy, fruit spirits, whisk(e)y and related spirits, flavored spirits (such as gin and arak), and liqueurs.

Many changes of composition occur when drinks such as wine, cider, beer, and rice wine are produced from their respective raw materials or precursors – grape or fruit juice, apple juice, hopped or flavored wort, or rice porridge. Some of the original components disappear completely, but some survive through to the final product. Totally new components appear as a result of alcoholic, malolactic, and other fermentations and also because of various other production practices. The most notable new component is, of course, ethanol, followed in many beverages by glycerol. Typical ethanol concentrations of major alcoholic beverages are shown in Table 14, and these are further discussed in the relevant subsections. When fermented drinks are distilled to produce spirits, most nonvolatile components are

Table 14 Ethanol, water, and nonvolatile component composition of alcoholic beverages^a

Component	Beverage				
	Wine	Beer	Cider	Rice wine	Spirit
Water (g/L)	810–910 ^c	920–950	900–960	950–960	340–680
Ethanol (g/L) ^b	80–110 ^c	30–50	20–75	50–144	280–560 ^f
Carbohydrate (total) (g/L)	1.0–100	15–37 ^d	0.14–25	–	Trace ^g
Free sugars (g/L)	1.0- > 100	Trace-30	Trace-20	17.92–27.48 ^e	Trace ^g
Fiber (g/L)	Trace	Trace-10	Trace-3.0	–	Trace
Amino acids, peptides, and proteins (g/L)	0.2–3.0	2.0–6.0	0.2–0.5	0.60–1.05 ^e 76.33–102.93 ^h	Trace–3.0
Caloric value (Cal/L)	640–1,000	280–450	300–400	–	2,240

^aTypical values or ranges. Data from Fuller et al. (2011, pp. 961–992) and references therein, unless specified otherwise

^bThese figures multiplied by 0.125 will give the % alcohol by volume (%ABV)

^cNot including fortified wines and vins de liqueur: these would have ~128–176 g/L ethanol

^dIncludes α -glucans (dextrins), many of which are non-fermentable

^eData from Shen et al. (2012) and references therein, for clear rice (“yellow”) wine

^fThe most common ethanol content is 320 g/L (=40 % ABV). Some liqueurs and other spirit-based drinks have 128–240 g/L

^gCask-aged spirits may contain wood-derived carbohydrates

^hData in g/kg from Kang et al. (2014) for cloudy makkoli

lost (Table 14) and many aroma compounds become more concentrated in the highly ethanolic product. The organoleptic impact can be enhanced or altered by various production processes, such as cask aging and inclusion of flavorings.

Wine

Wine is strictly the fermented juice of freshly crushed and pressed grapes: it is this beverage that is the main topic of this subsection. However, wine made from other fruits (“fruit wine” or “country wine,” often via rather different processes) is important in some areas and hence is given some consideration later. A summary of the basic (grape) winemaking processes is given in Fig. 9. For a particular wine, its chemical composition is derived from the exact details of its processing, as well as from the genetic identity of its grapes and the agricultural and climatological background to the their cultivation. Likewise, deviations from the basic schemes of Fig. 9 (such as fortification with spirit, drying of grapes, biological aging, oxidative aging, and heat treatments) can make significant differences to the composition. The following paragraphs include outlines of how these factors can influence wine composition.

Wine Color

The anthocyanin and related pigments of black grape must (Fig. 1, Table 1) are also present at high levels in new red wine (total anthocyanin content: 100–1,500 mg/L,

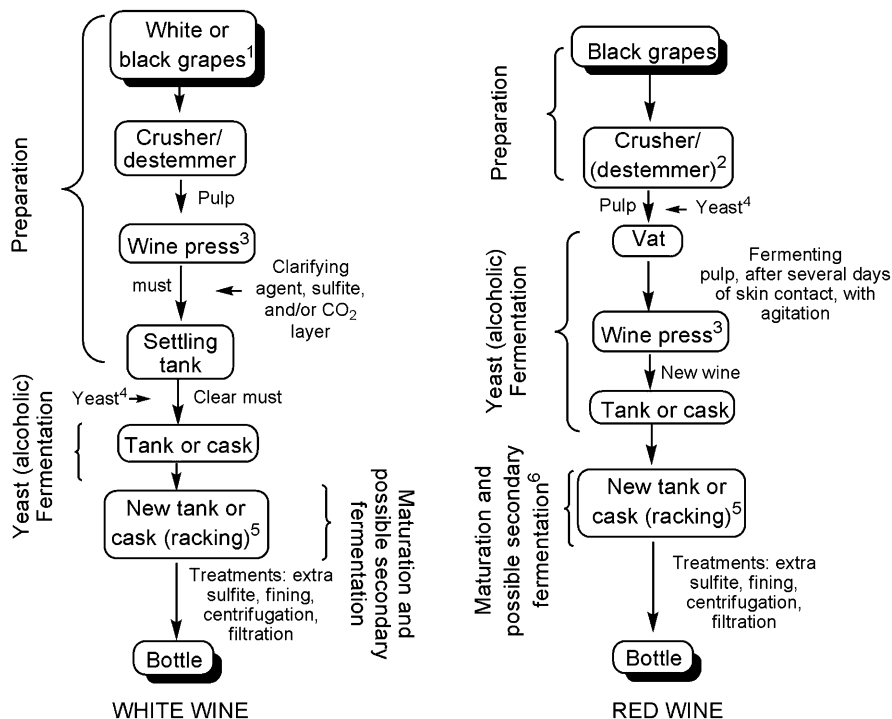
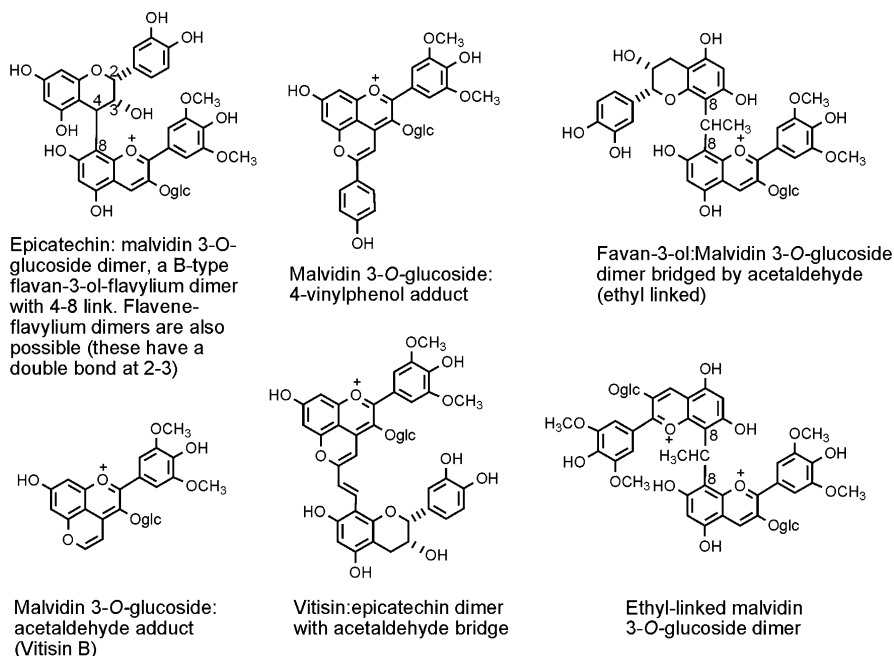


Fig. 9 The basic production processes for white and red wine. 1 If black grapes used, pressing the crushed grapes in immediate. 2 Some red wines are made without removing the stems. 3 Free run juice/wine is sometimes fermented separately from press juice/wine. 4 Pure yeast cultures tend to be added to white grape must made in cool climates, otherwise the natural yeasts from the skins are often allowed to ferment the must, especially that from warmer climate black grapes. 5 The number of rackings are according to tradition or the will of the winemaker, as is the duration of maturation. Sulfite levels are checked at each racking. 6 Nearly all red wines and some white wines undergo secondary (malolactic) fermentation

total phenol content: 0.5–4 g/L). However, during maturation these rather reactive pigments are progressively converted to a large number of more stable pigments, through a variety of reactions that can involve small molecules like acetaldehyde, as well as other monomeric or oligomeric polyphenols. These reactions include the formation of:

- Castavinols via the addition of 1,2-diketones (like diacetyl) across the 2 and 4 pyrylium ring positions
- Pyranoanthocyanins (vitisins) by addition of carbonyl compounds such acetaldehyde and pyruvic acid across the 5-OH position of ring A and the pyrylium ring 4 position
- Adducts with 4-vinylphenol, again involving the 5-OH position of ring A and the pyrylium ring 4 position



See Figs. 1 and 3 for examples of other pigment and nonpigment polyphenols in fruit juice. Some of these, such as anthocyanin glycosides, procyanidins, other oligomers/polymers, and hydrolyzable tannins are also present in red wine

Fig. 10 Some pigment polyphenols in red wine (Buglass and Caven-Quantrill 2013 and references therein)

- Dimers with an ethyl link (acetaldehyde bridge) by reaction between two anthocyanin monomers (at ring A 8 position) and acetaldehyde
- Oligomers by reaction between an anthocyanin (or vitisin) and a flavan-3-ol or procyanidin oligomer, with or without ethyl or acetaldehyde bridges

Some of these products are illustrated in Fig. 10. Many of these new pigments absorb light of ca. 520 nm and hence are purple-red, explaining why red wines remain red after 2 years maturation in bulk before bottling, despite almost complete disappearance of the original anthocyanins during this time. However, due to the larger oligomers and the procyanidins formed concurrently by condensation of flavan-3-ols (Figs. 1 and 11), they tend to be orange-yellow, and so red wines gradually turn through brick red to brown as they age further. Additionally, the bigger polymers precipitate (possibly as polyphenol-protein complexes) after long maturation times, eventually leaving a phenol-depleted, paler, browner wine.

Like white grape juice, the main contributors to white wine color appear to be certain polyphenols (up to 300 mg/L), carotenoids (up to 750 $\mu\text{g/L}$), and possibly chlorophyll and degradation products (a few ng/L). White wines, like many pale juices, can undergo enzymic or nonenzymic browning, especially at low sulfite

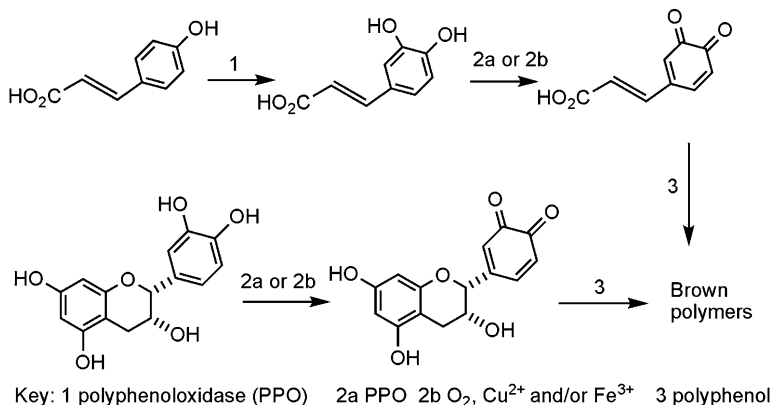


Fig. 11 White wine enzymic and nonenzymic browning mechanism outline (illustrated for (–)-epicatechin and *p*-coumaric acid)

levels. This is caused by the oxidation of catechol or galloyl units of polyphenols to *o*-quinones, which then form brown polymers by reacting with other polyphenols (Fig. 11).

Ethanol, Carbohydrate, and Carbohydrate Derivative Content

Summaries of ethanol and carbohydrate content of wines are given in Table 14. Wine ethanol content varies from around 6 % ABV (6 % v:v) for some semi-sparkling sweet wines (e.g., Lambrusco and Moscato), through 8–12 % for wines from cooler climates, to 13–16 % for those of warmer climates and special wines made from dried grapes (e.g., Amarone della Valpolicella and Vin Santo), finally up to ca. 22 % for some fortified wines. For dry or semidry wines (<10 g/L residual sugars), the %ABV is directly related to ripeness (sugar content) of the grapes, but sweet wines (30– > 100 g/L residual sugars) can have low (~8 % for Trockenbeerenauslesen or ~2 % for Tokaji Aszú Essencia) or high (13.5–15.5 % for Sauternes) ethanol levels.

Like grapes, the most abundant residual wine sugars are fructose and glucose, and since glucose is more easily fermented by yeast, fructose is the dominant sugar of sweet wines. Sucrose is present only at trace levels, if at all. Law generally forbids the addition of raw sugar (e.g., sucrose) to wine, but concentrated sterile grape juice (süssreserve) can be used as a sweetener of certain wine categories (e.g., Qualitätswein bestimmte Anbaugebiete – QbA wines of Germany).

Small quantities (0– ~130 mg/L) of other monosaccharides, notably arabinose, galactose, rhamnose, ribose, and xylose are present in wine: some of these are the result of the degradation of grape pectic substances during fermentation, especially if exogenous pectinases have been used. Trehalose is the dominant disaccharide of wine (trace levels to ~600 mg/L), particularly in wines made from grapes infected by *Botrytis cinerea* (“botrytised” or “nobly rotted” grapes), such as Sauternes and Quarts de Chaume. Other disaccharides are normally found at lower than the 5 mg/L level.

Wine polysaccharides (up to ~750 mg/L) exist as neutral pectic substances, yeast mannoproteins, and protein conjugates, the most abundant being arabinogalactan proteins (~300 mg/L). Other pectic substances of grape juice are hydrolyzed during fermentation to monosaccharides and galacturonic and glucuronic acids, at levels of 200–1,400 mg/L, although concentrations up to 7,500 mg/L have been recorded for botrytised wines (Amerine and Ough 1980, pp. 45–73 and references therein). Gluconic acid is usually found in wine at concentrations less than 500 mg/L but up to ~6,000 mg/L in wine from botrytised grapes.

Chemically, polyhydric alcohols, glycerol, butanediols, mannitol, mesoinositol, and sorbitol are reduced sugars: the first named is normally the most abundant organic compound (1–20 g/L) in wine, after ethanol. Its presence improves the appearance and mouthfeel of wine due to increased viscosity. It is produced mainly by yeast metabolic processes, but again it is more abundant in botrytised wine, whose grape juice may already have up to 5 g/L before fermentation. Average levels of mannitol and sorbitol in wine are ~100 mg/L and ~300 mg/L respectively, but once again these are higher in wine from botrytised grapes.

Volatile Compounds

Wine odor and flavor are of utmost importance. Volatile compounds that give rise to wine aroma and bouquet, together with various tastants (acids, sugars, salts, phenolic compounds, and others) contribute much to flavor, often simply described as “taste.” Human perception of odor is complex and is not considered here, so odor descriptors of volatile wine substances (Table 15) refer to basic perception at levels found in wine. This is because descriptors vary enormously with concentration and also according to the presence or absence of co-components, both volatile and nonvolatile.

Wine volatiles have basically three origins, as outlined in Fig. 12: grape must (“varietal aroma”), fermentation (yeast and possibly bacterial metabolic products) and chemical reactions, and wood contact (mainly oak) during maturation (“oak aroma”). Particular volatile components can have more than one origin – for example, certain volatile phenols like *p*-ethylphenol and *p*-vinylphenol can come from MLF (via the shikimate pathway) or from contact with toasted oak. Note that many wines have no oak contact – they are fermented and matured in stainless steel or other non-wooden vessels.

During fermentation of grape must, the levels of many volatiles, like certain alcohols, carbonyl compounds, and terpenoids, decrease and are augmented with or replaced by high levels of acetate and ethyl carboxylate esters (especially C₆, C₈, C₁₀), some terpenoids, and certain norisoprenoids (derived from juice carotenes).

Many volatiles exist in grape juice as odorless glycosides or L-cysteine conjugates (Fig. 13). Alcoholic fermentation may release some odorous aglycones due to the action of yeast *O*-glycoside hydrolases, but this can be enhanced legally by the addition of pectolytic enzymes during fermentation. Additionally, odorous sulfur compounds (Fig. 13) are released from their cysteine conjugates seconds after swallowing due to the action of saliva lyases.

Although a single volatile compound may influence aromas of particular wines (see Table 15 for many examples) to a recognizable degree, in reality, this

Table 15 Summary of volatile components of wine and examples of some key odorants^a

Chemical class	Examples with typical concentrations or ranges ($\mu\text{g/L}$, unless otherwise stated)	Examples of key odorant (OTV in ng/L) [aroma descriptors]	Typical example of wine source
Acetals	Acetal (1,1-diethoxymethane) ($4.5\text{--}6.4 \times 10^4$)	Acetal (4,000–42,000) [fruity]	Sherry, vin jaune
Alcohols	Ethanol (6–16 % ABV), 2-methyl-1-propanol (10^5), 3-methyl-1-butanol (isoamyl alcohol) (2×10^5), 2-phenylethanol (5×10^4)	2-Phenylethanol (2.0×10^6) [Rose]	Muscat
Carbonyls	Acetaldehyde ^b (up to 2.8×10^5), diacetyl (20–5,400)	Diacetyl (6,500–15,000)	Chardonnay
		Phenylacetaldehyde [floral, honey]	Pedro Ximénez, Sauternes
Carboxylic acids	Acetic acid (5×10^5), formic acid (5×10^4)		
Carboxylate esters	Ethyl acetate (up to 5×10^5), ethyl hexanoate (600–1,800), ethyl octanoate (1,100–1,700), diethyl succinate (100–1,400), isoamyl acetate (40–6,100), 2-phenylethyl acetate (200–5,100)	Isoamyl acetate ($2.5 \times 10^5\text{--}4.1 \times 10^6$) [banana, fruity]	Tempranillo
Lactones	γ -Butyrolactone (10^3), dodeceno- γ -lactone (140–270), <i>cis</i> , <i>trans</i> -oak (whisky) lactones, wine lactone (100)	<i>trans</i> -Oak lactone (490,000) [coconut, toasted oak]	Oak-aged wines
Norisoprenoids (C_{13})	<i>trans</i> - β -Damascenone (0.005–6.5), β -ionone (up to 2.5)	<i>trans</i> - β -Damascenone (40–60) [sweet, floral]	Pedro Ximénez, Muscat
Organosulfur compounds ^c	3-Mercaptohexanol (0.15–3.5), 3-mercaptohexyl acetate (up to 0.5), 3-mercapto-3-methylbutanol (0.02–0.15), 4-mercapto-4-methyl-2-pentanol (0.015–0.15), 4-mercapto-4-methyl-2-pentanone (up to 0.15), phenylmethanethiol (0.005–0.02)	3-Mercaptohexanol (60) [fruity, sulfury]	Sauvignon Blanc, Cabernet Sauvignon
		3-Mercaptohexyl acetate (4) [tropical fruit]	Verdejo
		4-Mercapto-4-methyl-2-pentanone (0.6) [sulfury, black currant]	Gewürztraminer, Sauvignon blanc, Scheurebe
Phenols	2-Methoxyphenol (3,600), 2-methoxy-4-vinylphenol ($4,500\text{--}2.5 \times 10^4$), vanillin (4.5×10^4)	Vanillin (2×10^5) [vanilla]	Oak-aged wines

(continued)

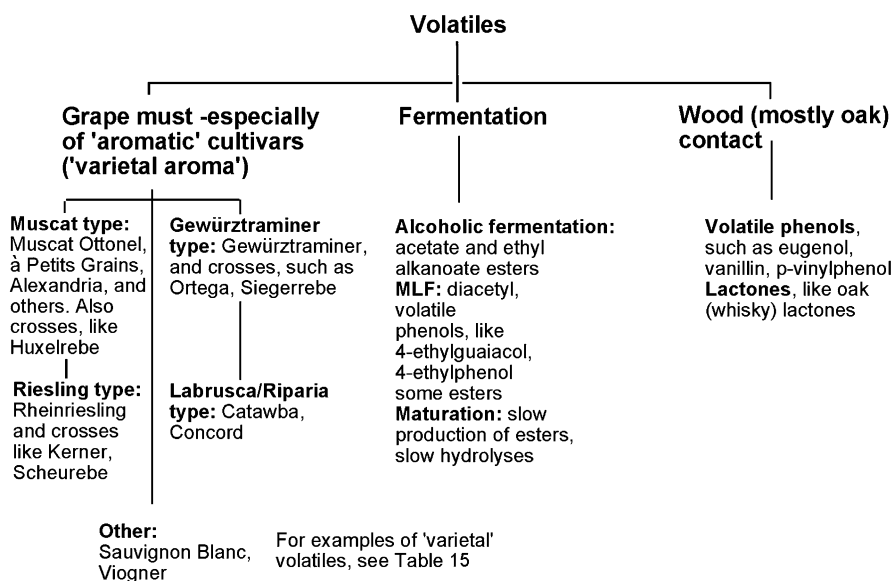
Table 15 (continued)

Chemical class	Examples with typical concentrations or ranges ($\mu\text{g/L}$, unless otherwise stated)	Examples of key odorant (OTV in ng/L) [aroma descriptors]	Typical example of wine source
Pyrazines	2-Methoxy-3-ethylpyrazine, 2-methoxy-3-isobutylpyrazine (up to 0.05), 2-methoxy-3- <i>sec</i> -butylpyrazine, 2-methoxy-3-isopropylpyrazine	2-Methoxy-3-isobutylpyrazine (15) [herbaceous, green pepper]	Cabernet Sauvignon
Terpenoids	Citronellol (2–12), geraniol (5–506), hotrienol (25–127), linalool (6–473), rotundone, α -terpineol (3–87)	<i>cis</i> -Rose oxide (200) [rose, spicy]	Gewürztraminer
		Rotundone (6) [green pepper]	Shiraz (Syrah)

^aData from Buglass and Caven-Quantrill (2013), Ribéreau-Gayon et al. (2000, pp. 129–186) and references therein

^bIn most wines, much of the acetaldehyde is bound to SO_2 , as the bisulfite addition compound

^cPresent in grape must and wine mainly as L-cysteine conjugates

**Fig. 12** Origins of wine volatile compounds

compound usually exists in many wines, so that it is the overall volatile component profile that ultimately defines the full aroma of each wine.

Acids

Acids are essential to wine quality: they provide the wine with a sourness that balances fruity, sweet, salty, and bitter/astringent organoleptic sensations.

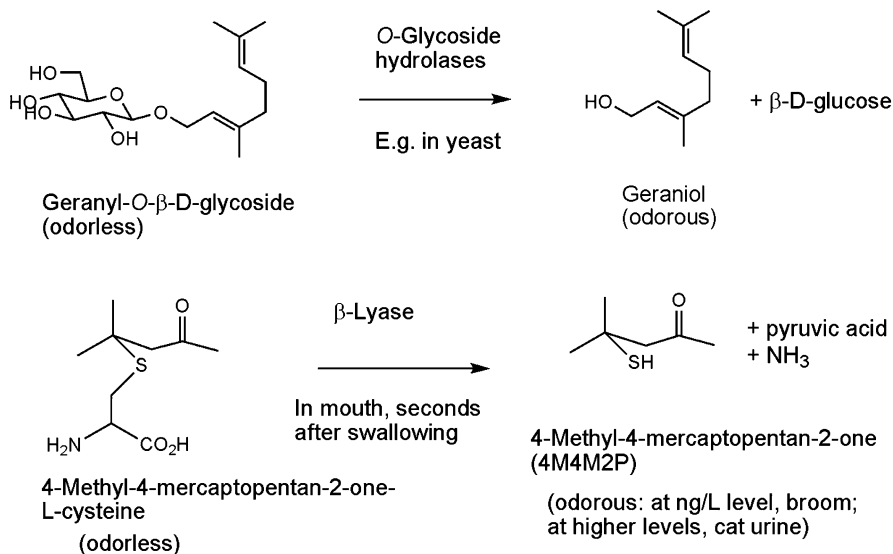


Fig. 13 Release of odorous compounds by enzyme action

Moreover, they endow the wine with longevity and contribute to wine color and, in time, to wine flavor. Of course, too much or too little acidity can be unpleasant and will give a wine with unbalanced “taste.” Wine acids are derived from grape juice and/or yeast metabolic pathways that operate during fermentation, although low levels of phenolic acids are wholly or partially derived from oak contact. The levels of the major original grape acids are generally lowered somewhat during alcoholic fermentation, either as a result of yeast activity (L-malic acid, ~5–20 %) or because of precipitation of salts (L-tartaric acid, ~10 %).

The major wine acid is L-(+)-tartaric acid, usually found in concentrations of 2–4 g/L. Wine that has not undergone malolactic fermentation (MLF) – most white wine and some red wine – also has significant levels of L-(–)-malic acid (2–4 g/L). Succinic acid is also a significant wine acid (~1 g/L), as is D-lactic acid, at lower levels (usually < 500 mg/L) and, also at low levels, acids from various yeast metabolic pathways (e.g., citric, fumaric, pyruvic, and shikimic acids). L-Lactic acid is abundant (1–4 g/L) only in wines that have experienced MLF, in which case the malic acid levels of such wines will be very low (often a few hundred mg/L or less). Sugar acids are discussed in the previous paragraphs.

Acetic acid is the main volatile acid of wine: low levels (well below 1 g/L) are beneficial to wine flavor, but higher levels are suggestive of spoilage, mostly by *Acetobacter* microorganisms.

Amino Acids, Peptides, and Proteins

The total amino acid and ammonium (NH_4^+) content of wine (< 1.5 g/L up to 50 mg/L, respectively) is generally lower than that of the original grape juice (1.5–4 g/L up to

Table 16 Summary of identified proteins in Portugieser red wine (From Wigand et al. 2009)^a

Band (experimental molecular weight/kDa)	Theoretical molecular weight/kDa	Identification	Origin
1 (9)	11.7, 11.8	Lipid transfer proteins (LTPs) ^b	Grape
2 (12)	11.7, 11.8	LTP isoform 4 + LTP	Grape
3 (25)	24.0–27.2	Three thaumatin-like proteins (TLPs) ^c + endochitinase ^d class IV	Grape
4 (37)	71.5	Hydrolyzed vacuolar invertase 1 GIN 1 ^e	Grape
	48.0	Protein TOS1 precursor	Yeast
5 (47)	71.5	Hydrolyzed vacuolar invertase 1 GIN 1	Grape
	23.2–48	Three cell wall and matrix precursor proteins	Yeast
6 (61)	23.2–53.0	Four fungal protein precursors	Yeast
	71.5	Hydrolyzed vacuolar invertase 1 GIN 1	Grape
7 (77)	71.5	Vacuolar invertase 1 GIN 1	Grape
	23.2	Cell wall protein precursor	Yeast ^f
8 (150)	53.0	Glycosidase precursor	Yeast ^f
	53.0	Endochitinase precursor	Yeast ^f

^aDetermined using SDS-PAGE electrophoresis and LC-MS

^bLTPs are defense proteins located in grape skins and hence will be absent or at lower levels in rose or white wines. They are possible allergens

^cTLPs are implicated in haze formation; they are possible allergens

^dEndochitinases are grape antifungal defense proteins and possible allergens

^eVacuolar invertases are found in grape pulp and are responsible for hexose accumulation during ripening

^fAll the higher-molecular-weight yeast proteins are likely to be glycoproteins

300 mg/L, respectively), because of net utilization of these substances by yeast during alcoholic fermentation and (where appropriate) by bacteria during malolactic fermentation. MLF is known to deplete the wine of arginine, glutamic acid, histidine, and tyrosine; moreover if *Pediococcus cerevisiae* is the MLF agent, histidine is decarboxylated to the biogenic amine histamine (see “[Other Components](#)”).

Protein content of wine varies from a few mg/L to over 400 mg/L. Most proteins are in the molecular weight range 12–65 kDa and are mostly grape-derived, possible allergenic, defense proteins. Higher-molecular-weight proteins, probably extensively glycosylated (e.g., mannoproteins), are generally of yeast origin, and sometimes fining proteins (e.g., egg albumen) can be found in wine. Heating (see “[Fortified Wines](#)”) and fining with bentonite reduces protein content. Table 16 lists the proteins found in a red wine, along with brief descriptions of their origins and characteristics.

Minerals and Vitamins

Cations and anions in wine help to maintain its acid–base buffer capacity and some, like K⁺, Mg²⁺, Ca²⁺, and Na⁺, endow a certain saltiness of taste, which is more

Table 17 Mineral and vitamin content of alcoholic beverages^a

Beverage	Wine (mean value)	Beer	Cider	Rice wine ^b
Minerals^c				
K (mg)	90–1,840 (880)	300–500	727	549–713
P (mg)	10–820 (291)	110–200	80	–
Ca (mg)	6–208 (88.9)	4–140	70	108–166
Mg (mg)	21–173 (96.9)	50–100	50	183–224
Na (mg)	6–309 (37.5)	50–100	50	28–61
Cl (mg)	5–596 (72.2)	120–350	–	–
S (mg)	70–4,390 (823)	107–400	–	–
B (mg)	2–112 (20)	–	–	–
Fe (mg)	Trace–90 (7.9)	Trace–0.6	2.1	0.18–2.2
Zn (mg)	Trace–8.8 (1.6)	Trace–3.0	0.5	3.5–5.1
Mn (mg)	0.14–17.4 (2.0)	Trace–0.3	2.5	5.2–6.4
Cu (mg)	Trace–3.7 (0.52)	Trace–0.7	–	0.59–0.70
Se (µg)	Trace–3.0	Trace–3.4	0.8	–
Vitamins				
B ₁ (thiamine) (mg)	2–58 (8.7)	0.03–0.09	–	–
B ₂ (riboflavin) (mg)	0.08–0.25 (0.16)	Trace–0.4	0.03–0.5 ^d	–
B ₃ (niacin) (mg)	0.8–2.1	2.0–8.0	0.1–1.0 ^d	–
B ₅ (pantothenate) (mg)	0.47–1.87 (0.89)	0.3–1.0	–	–
B ₆ (pyridoxine) (mg)	0.22–0.82 (0.46)	0.1–0.9	–	–
B ₁₂ (cyanocobalamin) (µg)	Trace–1.6 × 10 ⁻⁴	Trace–0.8	–	–
Vitamin C	Trace	Trace	–	–
Folate (µg)	Trace–12	6.2–68.2 ^c	–	–
Biotin (µg)	Trace–4.6 (2.1)	Trace–10	–	–
Choline (mg)	48–64	77–123	–	–
Betaine (mg)	2.4–3.2	60–88	0.3–1.1	–
Nicotinic acid (mg)	0.99–2.19 (1.73)	–	–	–

^aTypical values or ranges for non-distilled drinks in mg/L or µg/L. Data from Fuller et al. (2011, pp. 961–992) or Amerine and Ough (1980, pp. 153–174) or Hough et al. (1982, pp. 776–838) and references therein, unless specified otherwise. Minerals and vitamins are effectively lost on distillation, so spirits are not included in the table. Spirit-based drinks, such as some alcopops, cocktails, and fruit liqueurs, may have some minerals and vitamins returned

^bData from Shen et al. (2012): Chinese “yellow wine,” values rounded down to 2 or 3 significant figures

^cMetals as cations, P as phosphates, S as sulfates, B as borates

^dData from Goverd and Carr (1974)

^eData from Owens et al. (2007)

obvious in dry white wines. Typical mineral content of wines are displayed in Table 17. Certain grape juice electrolytes (e.g., calcium, sulfate, and phosphates) are depleted during processing by precipitation, but at the same time, levels of others may increase: Na⁺ from sodium metabisulfite (preservative), ion exchangers, and concrete vats; calcium sulfate from use of gypsum in sherry making; and diammonium phosphate from added yeast nutrient. Also, grapes treated with Cu,

Mn, and Zn fungicides (Bordeaux mixture, mancozeb, and zineb, respectively) may give wine with higher concentrations of these ions. Additionally, sodium chloride levels may be higher in wines originating from coastal vineyards, and iron and copper ions can be picked up by contact with these metals during processing, but this is uncommon these days. Very low levels of contaminant toxic metal cations (e.g., Cd, Cr, Hg, Ni, Pb, Sn) might be present, but concentrations of all minerals will be well below their toxicity levels, as required by law.

Wine is not well endowed with vitamin content, although vitamin B₁ is present at higher levels than grape juice, due to yeast autolysis or possibly because it has been added (along with diammonium phosphate) as a yeast nutrient before fermentation. Table 17 summarizes the vitamin content of wine.

Other Components

Biogenic amines, principally histamine and tyramine, are formed in wine as a result of decarboxylation of α -amino acids during alcoholic and malolactic fermentation. Figure 14 gives an outline of this, with examples and other information. These amines are of interest because of their potentially allergenic character toward some predisposed individuals (Önal 2007).

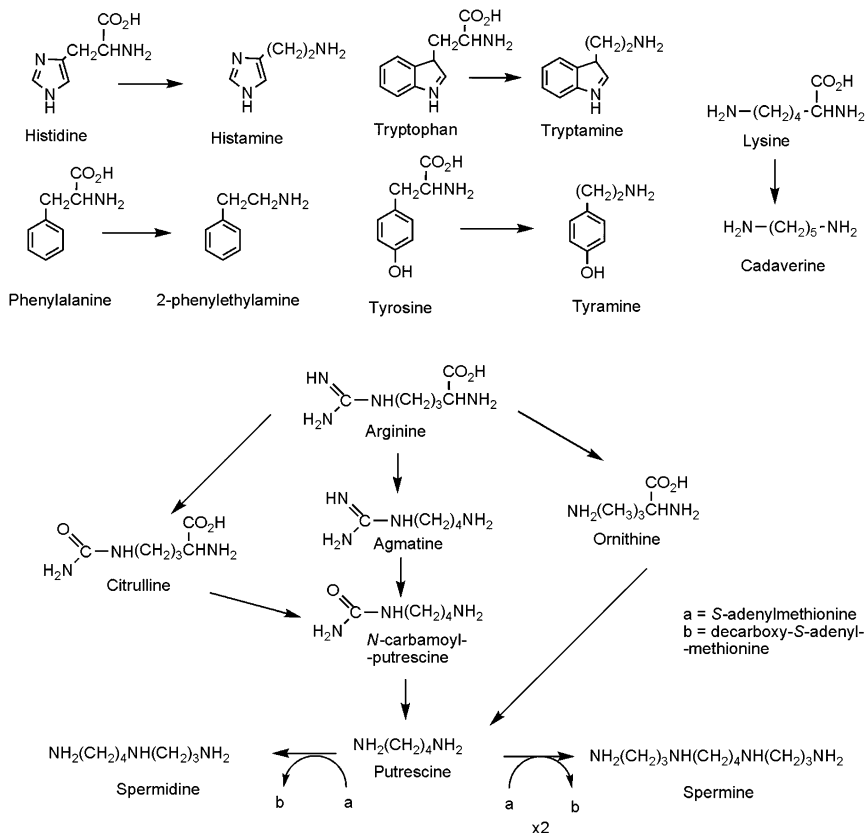
Ethyl carbamate (urethane), a mild carcinogen, is found in wine at levels 0–24 $\mu\text{g/L}$, although higher concentrations are found in fortified, heat-treated wines, Asian rice wines, and especially spirits. It is formed by reaction of urea (a fermentation byproduct) with ethanol (Fuller et al. 2011, pp. 1093–1110).

Sulfite exists in wine naturally, but because it is very widely used as a preservative in the wine industry, it is generally present in wine at concentrations of ~100–~400 mg/L . The most active form is molecular SO_2 , but much of this is bound to carbonyl compounds and phenols in wine. Like amines, sulfite is potentially allergenic. Legal total sulfite levels depend on national regulations but generally should not exceed ~260 mg/L for dry wines and ~400 mg/L for sweet wines.

Carbon dioxide, a major fermentation product, is essential in the production of sparkling and semi-sparkling wines – in lesser amounts it can add an attractive mouthfeel to young wines (“pétillant” or “spritzig” wines). Full sparkling wines (vins mousseux) such as cava and champagne contain 11–12 $\text{g CO}_2/\text{L}$ wine or ~6 $\text{L CO}_2/\text{L}$ wine, which is equivalent to a 750 mL bottle internal pressure of ~6 atm at ~10 °C. Semi-sparkling wines (crémant, perlé, spumante) have bottle CO_2 pressures of 2 atm or less, while for pétillant or spritzig wines, this will be just over 1 atm.

Fortified Wines

These are wines with added grape spirit. They range from fino and manzanilla sherry; sercial and verdelho madeira, where small quantities of spirit are added late in the process, through port; bual and malmsey madeira; vins doux naturels (VDN); Australian liqueur muscats, where larger amounts of spirit are added early to stop fermentation, to vins de liqueur, where spirit is added to the grape must (no fermentation). Ethanol content (%ABV) of fortified wines is from ~16



Occurrence in red wines (mg/L)
 Histamine (0~8.2), 2-phenylethylamine (0~0.37), tryptamine (0~2.0), tyramine (0~7.9)
 Lower levels in MLF wines when conducted by *Oenococcus oeni*; higher levels if *Lactobacillus* or *Pediococcus* spp. perform MLF

Fig. 14 Formation and distribution of biogenic amines in wine (Önal 2007 and references therein)

(fino and manzanilla sherry) – through ~17.5 (vins de liqueur) and 18–20 (most port, oloroso sherry, madeira, and VDN) – to 22+ (aged tawny port).

Several specific features essential in the manufacture of the best-known fortified wines have direct impact on the chemical composition of such wines, as summarized in Table 18.

Aromatized Wines

Aromatized wines are those flavored by the inclusion of aromatic substances of plant origin. They are also generally fortified with grape spirit or mistelle (a mixture of grape must and grape spirit). The best known is vermouth (16–18 % ABV), originating in northern Italy but now made in many countries. The flavorings, added

Table 18 Summary of influence on composition of special features in the production of some fortified wines^a

Fortified wine type	Special features of production	Influence on wine composition (typical concentrations in mg/L)
Fino and manzanilla sherry	1. Grapes dusted with gypsum (“yeso”) to aid formation of flor	1. Increased acidity
	2. “Biological aging” in part-filled cask: flor velum forms on surface, depletes O ₂ , prevents some oxidations, and utilizes amino acids	2. Pale color and higher levels of acetaldehyde, other carbonyls, acetal, 2,3-butanedione (acetoin), lower alcohols (e.g., isoamyl alcohol), lower fatty acids, and some lactones
Oloroso sherry	1. As above	1. As above
	2. Oxidative aging for long periods in cask: wines undergo enzymic and nonenzymic browning	2. Deeper color due to more flavonoid polymers. Low levels of acetaldehyde and higher levels of acetate and ethyl alkanoate esters, as well as some furans, methional, and sotolon ^b
Madeira	Estufagem: wines undergo heat treatment. Best wines kept in casks in south facing loft for 2 years or more. Undergo Maillard and related reactions, like Strecker degradation	Deeper color due to more flavonoid polymers. Some Maillard and Strecker products at high levels – HMF ^c (~361), methional, phenylacetaldehyde, γ -(methylthio)butanoic acid (~5.7), 2-phenylpyruvic acid (~9.6). Also 1,3-dioxolanes formed from acetaldehyde and glycerol
Ruby port	1. Short fermentation on pulp (with continual agitation), stopped by mixing with grape spirit; one with high aldehyde content preferred	1. Persistent deep red color due to formation of more stable vitisins, aldehyde-bridged oligomers, and others (Fig. 10). Survival of β -carotenoids, sulfur compounds, and other fermentation-sensitive compounds in young wine
	2. Standard (oxidative) cask aging for ~2 years	2. Slow development in bottle (e.g., slow conversion of β -carotenoids to odorous norisoprenoids)
Tawny port	1. As above	1. As above
	2. Much longer highly oxidative cask aging, with frequent forced racking (high exposure to O ₂)	2. Brown coloration and faster conversion of β -carotenoids to odorous norisoprenoids, faster depletion of sulfur compounds, formation of 5-HMF ^c and sotolon ^b (~1)

^aFrom McKay et al. (2011, pp. 383–418) and references therein

^bSotolon = 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone

^c5-HMF = 5-hydroxymethyl-2-furaldehyde

to a white wine base, are extracts or essential oils of herbs, bitter roots, and bark (collectively known as “botanicals”). Red vermouth contains caramel, and mistelle is used to sweeten and fortify sweet vermouth. The chemical composition of vermouth is similar to the white wine base but with a large number of extra

components (mostly at the $\mu\text{g/L}$ or ng/L levels) derived from the botanicals and (for red vermouth) caramel. Examples of notable botanical components and their plant origins are given Table 19, but note that a particular compound may be present in several botanicals and may even be present in the base wine.

Resinated wines are white (and occasionally rosé) wines flavored with pine resin: *retsina* (of European Union (EU) designated origin Greece and Cyprus; 12.0–12.5 % ABV), fermented with pieces of Aleppo pine (*Pinus halepensis* Miller) resin, is the most famous example. Typically, *retsina* has significantly higher levels of terpenoids (pinenes, caryophyllene, cembrene, limonene, myrcene, terpinolene, and many others), as well as polyphenols (flavan-3-ols, *p*-coumaric acid, gallic acid, tyrosol, and others) that are mainly derived from the resin.

Fruit Wines

In a few cases, fruit wines can be made like grape wines: by crushing and pressing the fruit (with or without fermentation on the pulp), after minor adjustments of acidity and sweetness. However, in most cases, the fruit pulp is too low in sugar and too high in acid (see Table 7) and hence has to be considerably diluted with water and then brought up to balance with added sugar, acids, and (most likely) grape tannins, prior to fermentation. Concentrated grape juice might also be used to make these wines, and therefore much of their chemical composition originates from adjuncts (e.g., malvidin pigments from black grape juice, grape tannins, and citric acid and/or tartaric acid). Pectinase enzymes are very likely to have been used in the processing, thus releasing some less common sugars, uronic acids, and a little methanol. Table 20 lists some phenolic compounds and volatile compounds of selected fruit wines.

Cider

“Cider” is defined here as fermented apple juice (USA: hard (apple) cider), a drink that is popular in many European countries and is becoming well known worldwide. It can be produced from cider apples (*Malus sylvestris*) or dessert/culinary apples (*Malus domestica*), in which case the more strongly alcoholic versions are sometimes called apple wine. Cider from cider apples is usually notably higher in polyphenolic content, giving it more body (tannic taste and mouthfeel) than other cider.

Different methods of production have a direct bearing on the chemical content of cider, and so will be outlined here. The two extreme production methods are the traditional and factory or industrial processes (Fig. 15), although in practice, many small producers may use some intermediate process. Traditional cider is made from the juice of freshly crushed and pressed apples, often using “spontaneous” fermentation, a minimum of added sulfite, often involving malolactic fermentation (MLF), and generally without filtration or pasteurization but often including some kind of

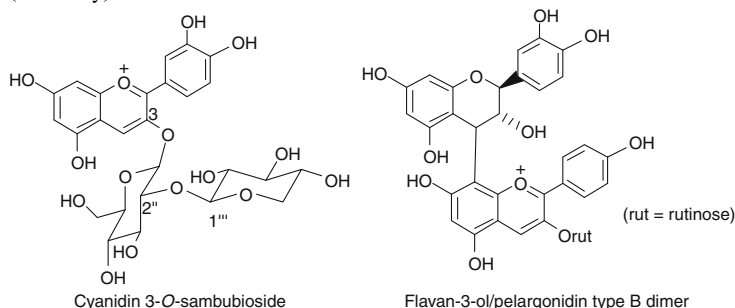
Table 19 Some flavor and bitter components of vermouthe

Flavor components: monoterpene hydrocarbons							
Camphene (coriander, juniper)	<i>p</i> -Cymene (oregano)	Limonene (citrus)	Myrcene (hop)	α - β -Pinene (citrus, juniper)	Sabinene (coriander, juniper)	α -Terpinene (hyssop, juniper)	γ -Terpinene (oregano)
Oxygenated monoterpenoids							
Camphor	1,8-Cineol	Citronellol	Linolool	Pinocamphone	α -Terpineol	4-Terpineol	α -, β -Thujone
(coriander sage)	(hyssop, mugwort)	(citrus, rose)	(coriander, hop, hyssop)	(hyssop)	(hyssop, juniper)	(juniper)	(mugwort, wormwood)
Aromatic monoterpenoids							
Anethole	Estragole	Eugenol	<i>p</i> -Thymol	α -Bisabolol	β -Caryophyllene	Chamazulen	
(aniseed, star anise)	(basil, cinnamon)	(basil, cloves, cinnamon)	(oregano, thyme)	(chamomile)	(chamomile)	(chamomile)	
Bitter components							
Cratogenic acid (cloves), oleonic acid (cloves), ursolic acid (basil, oregano, thyme) – all pentacyclic triterpenoids							
Amarogentin (gentian), eugenetin, eugenin (cloves), gentiopicroside (gentian), deoxypodophyllotoxin (juniper), quinine (<i>Cinchona</i> spp.), santonin (wormwood), sweroside (gentian)							

Botanical names: aniseed (*Pimpinella anisum*), basil (*Ocimum basilicum*), chamomile (*Chamomilla recutita*), cinnamon (*Cinnamomum verum*), clove (*Syzygium aromaticum*), coriander (*Coriandrum sativum*), gentian (*Gentiana* spp.), hop (*Humulus lupulus*), juniper (*Juniperus communis*), hyssop (*Hyssopus officinalis*), mugwort (*Artemisia vulgaris*), oregano (*Origanum vulgare*), rose (*Rosa* spp.), sage (*Salvia officinalis*), star anise (*Illicium verum*), thyme (*Thymus* spp.), wormwood (*Artemisia pontica*)

Table 20 Some polyphenols and volatile compounds of selected fruit wines^a**Polyphenols (wine where found)**

Cyanidin 3-*O*-sophoroside (Fig. 1) (raspberry), cyanidin 3-*O*-sambubioside (elderberry), 5-carboxypyranocyanidin (strawberry), 5-methylpyranocyanidin (black currant), flavan-3-ol/pelargonidin type B dimer (strawberry), ellagitannins (strawberry), piceatannol (a stilbene) (blueberry)

**Volatile compounds****Orange wine^b**

Ethyl butanoate (307), 3-methylpentanol (166), linalool (1,640), *g*-butyrolactone (491), 3-(methylthio)-propanol (166), geraniol (22.5), 2-phenylethanol (27,261)

Clementine/mandarin (*Citrus reticulata* Blanco) wine

Ethyl octanoate, ethyl decanoate, ethyl hexanoate, isoamyl acetate, 2-phenylethanol, ethyl acetate, limonene, ethyl succinate, ethyl dodecanoate, 5-hydroxymethylfurfural, furfural, 5-methylfurfural, 2-methyl-1-propanol, 4,5-dimethylfurfural, furfuryl alcohol

Strawberry wine^c

Ethyl octanoate, ethyl decanoate, ethyl hexanoate, amyl acetate, isoamyl alcohol, ethyl dodecanoate, ethyl 9-decenoate, *trans*-nerolidol, ethyl cinnamate, octyl acetate, methyl decanoate, decanoic acid, octanoic acid

Black raspberry wine^d

Isoamyl alcohol (657–1,158), isobutanol (270–464), methanol (266–302), ethyl acetate (87.1–278), 1-propanol (75.2–145), acetaldehyde (24.8–43.2), isoamyl acetate (9.64–46.24), methyl acetate (9.11–15.8), 1-butanol (0–3.61), benzaldehyde (0–3.47), furfural (1.34–2.21), ethyl hexanoate (1.32–1.84), *trans*- β -damascenone (1.25–1.47), 2-phenylethyl acetate (0.89–1.24), acetone (0–1.21), ethyl furoate (0.01–0.23)

^aFrom McKay et al. (2011, pp. 419–435) and references therein, unless specified otherwise

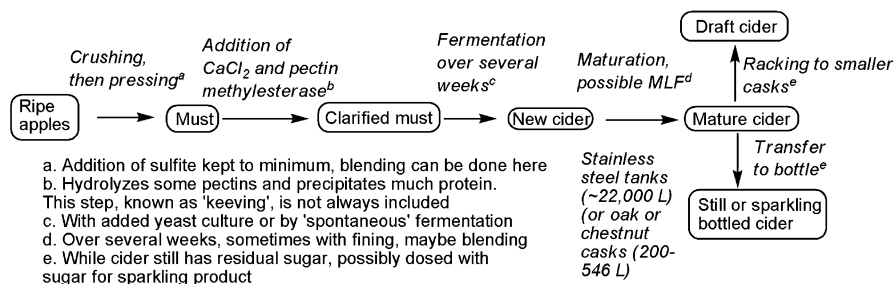
^bFrom Selli et al. (2008). Most potent odorants out of 35 listed in mg/L

^cFrom Kafkas et al. (2006)

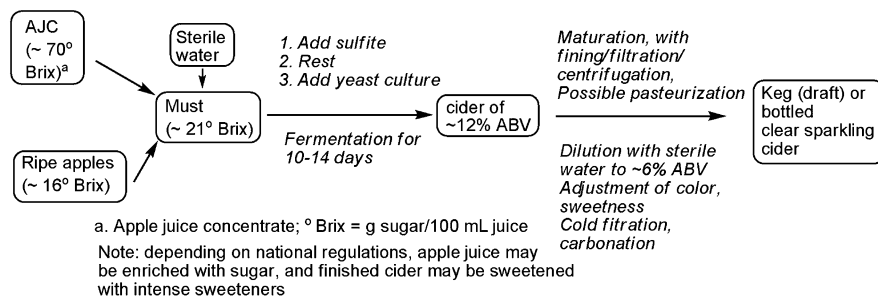
^dFrom Lim et al. (2012). In mg/L rounded to 3 or 4 significant figures. Range for wines made from juice or juice-pulp or juice-pulp seeds

fining. Hence the product can be hazy (like west of England “scrumpy” and French “cidre fermier”) or near bright to bright (like some English cask cider, French “cidre bouché,” and cider from northern Spain).

Factory cider is generally made with much greater control and refinements, and in many cases, diluted apple juice concentrate (AJC), obtained locally or bought on the world market, forms part of the must. MLF is often actively discouraged. The product is usually bright, with less pronounced aromas and flavors.



Outline of traditional cidermaking



Outline of factory cidermaking

Fig. 15 Outline schemes for traditional and factory cider production (Based on McKay et al. 2011, pp. 231–265)

Ethanol Content

The usual range of ethanol content of cider (Table 17) is from ~2–2.5 % ABV (for sweet cider of Brittany and Normandy) to 5–9 % for most dry European cider. Added sugar (according to national regulations) can raise the % ABV to 12 or more (e.g., for apple wine).

Polyphenol and Acid Content

An outline polyphenol content of English cider is given in Table 21, where probably the lower ends of the ranges correspond to cider produced from non-cider apples, which usually have much lower phenolic content.

The most notable polyphenols are caffeoylquinic acid and *p*-coumaroylquinic acid (hydroxycinnamic acids), phloridzin (phloretin 3-*O*-glucoside) and phloretin 2'-*O*-(2''-xylosylglucoside) (dihydrochalcones), flavan-3-ols, procyanidins (from dimers up to ~60-mers), and flavonols (Fig. 16). Anthocyanins present in the skins of some cider apples are usually present only at very low levels.

The major acids of cider produced without MLF are L-malic, citric and succinic acids, whereas in MLF cider, malic acid is almost depleted and high levels (up to 5 g/L) of L-lactic acid may be observed. Other acids include D-lactic acid, sugar acids, fumaric acid, shikimic acid, and volatile acids (see later).

Table 21 Phenolic components of English cider^a

Total hydroxycinnamates	Total dihydrochalcones	Total flavonols	Total flavan-3-ols	Total procyanidins	Total phenolic content
10–584 ^b	4–93 ^c	2–26 ^d	7–224 ^e	8–722 ^f	44–1,559

^aData (for 23 ciders of wide range of styles), in mg/L, from Marks et al. (2007). Lower values are associated mainly with ciders produced from either apple juice concentrate or dessert apples

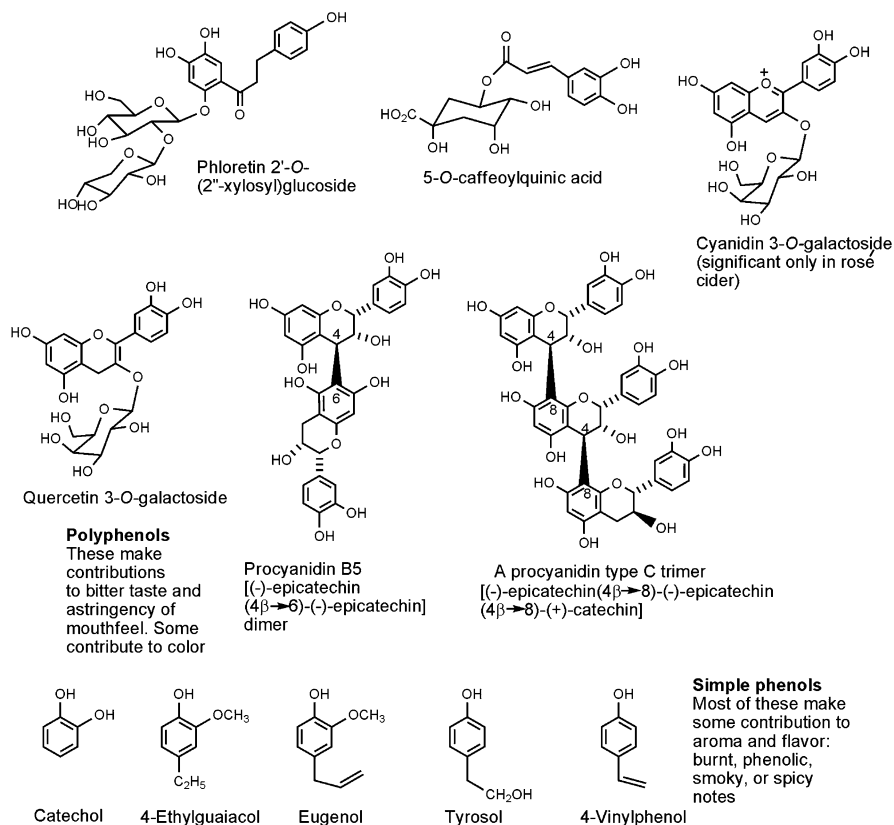
^bMost abundant hydroxycinnamate: 5-*O*-caffeoylquinic acid (12–437 mg/L)

^cMost abundant dihydrochalcone: phloridzin (2–71 mg/L)

^dMost abundant flavonol: quercetin 3-*O*-galactoside (1.1–12.5 mg/L)

^eIncluding procyanidin B₂. Most abundant flavan-3-ol: (–)-epicatechin (1–106 mg/L)

^fMostly dimers and trimers

**Fig. 16** Some notable phenolic substances in cider

Carbohydrates

Much cider is dry, so residual sugar levels are often low, composed mainly of fructose, sorbitol, and sugars derived from pectin degradation. Acid pectic substances, in particular, are usually highly degraded, especially if keiving has been

Table 22 Volatile compounds of Fuji apple wine^a

Compound	Concentration (mg/L)	Flavor descriptor	Compound	Concentration (mg/L)	Flavor descriptor
Alcohols (not including ethanol)			Esters		
2-Butanol	184.85	Fusel oil	Ethyl acetate	54.8	Fruity
Isoamyl alcohol	232.00	Fusel oil	Ethyl butanoate	2.19	Pineapple
1-Hexanol	2.18	Grassy	Ethyl decanoate	1.50	Fruity
2-Phenylethanol	43.50	Rose	Ethyl hexanoate	0.72	Floral
Acids			Ethyl lactate	4.63	Winey
Acetic	282.93	Vinegar	Ethyl octanoate	1.09	Fruity
Hexanoic	4.75	Sweaty	Diethyl succinate	0.24	Winey
Octanoic	6.11	Sweaty	Isoamyl acetate	16.66	Fruity

^aData from Wang et al. (2004). Apple wine (12.0 % ABV) was prepared from Fuji dessert apples, with added sugar, cultured yeast, and pectin methylesterase. Note that no other lower carboxylic acids were found, and phenols were present only at trace levels

used in the manufacturing process (Fig. 15). Typically, they are present up to ~44 mg/L, while neutral pectins and polysaccharides are usually at higher levels (up to ~244 mg/L) (Mangas et al. 1999).

Volatile Compounds

As with wine, many volatile compounds of cider are derived from alcoholic (and possibly malolactic) fermentation, and others originate from the fruit. The most flavor active compounds in cider are given in Tables 22 and 23, the former relating to apple wine type (~12 % ABV) from a dessert apple variety, the latter to traditional cider produced from French cider apples.

It can be seen that the traditional cider is richer in volatile phenols (phenolic, spicy notes), probably derived from MLF, and lower carboxylic acids (cheese notes). Additional to the compounds listed in Tables 22 and 23 is an acetal (green cider notes) formed by reaction between octane-1,3-diol (released from its glucosides during fermentation) and acetaldehyde, a fermentation byproduct (McKay et al. 2011, pp. 231–265 and references therein; Fig. 17).

Organic Nitrogenous Compounds, Minerals, and Vitamins

Thanks to the cidermaking process (especially keeving and fermentation), cider is low in amino acids, peptides, and proteins (Table 17). Proteins, possibly mostly

Table 23 Volatile compounds in French cider (cidre), with aroma descriptors^a

Alcohols	Carboxylic acids	Esters		Miscellaneous
		Acetates	Ethyl Alkanoates	
3-Methyl-1-butanol (fusel oil)	Butanoic acid (rancid)	Butyl (pineapple)	Butanoate (pineapple)	γ -Butyrolactone (butter)
2-Phenylethanol (rose)	2-Methylbutanoic acid (blue cheese)	Heptyl	Dodecanoate (floral)	α -Farnesene (citrus, herb)
		Hexyl (apple)	Heptanoate (fruit, wine)	2,6-Dimethoxyphenol (medicinal)
		2-Methylpropyl (green apple)	Hexanoate (banana)	2-Methylnaphthalene (camphor)
		3-Methylpropyl (apple)	2-Methylbutanoate (green apple)	Oct-1-en-3-one (mushroom)
		2-Phenylethyl (rose, honey)	3-Methylbutanoate (green apple)	Phenol (phenolic)
			2-Methylpropanoate (fruit)	2-Phenylacetaldehyde (pungent, floral)
		Pentanoate (apple),	1,2,4,5-Tetramethylbenzene (rancid)	
		Propanoate (pineapple)	4-Vinyl-2-methoxyphenol (spicy, phenolic)	
		Tetradecanoate (sweet, wax)		
		Other alkanoates:		
		3-Methylbutyl octanoate		
		2-Methylbutyl 2-methylbutanoate (berry fruit)		

^aOther than ethanol. Data from Villière et al. (2012). Most of these compounds have been detected in cider from many other countries, made from many apple cultivars

Table 24 Important stages in the brewing of beer^a

Stage	Purpose	Comments
1 (a) Malting	(a) To stimulate production of hydrolytic, redox, and acid regulatory enzymes and to promote a limited degradation of cereal starch, protein, and cellulose. Known as modification	(a) Warm, moist conditions cause germination of the barley grains, which is then stopped by drying
(b) Drying	(b) To stop germination/modification	(b) With hot air. Gives pale malts with malty, biscuity flavors. High degree of enzyme activity
(c) Kilning (roasting)	(c) To produce colored malts, depending on temperature, duration of heating, and initial moisture content of malt	(c) In slotted, rotating drums. Special flavors (e.g., chocolate, coffee) result from caramelization and Maillard reactions. Low or zero enzyme activity
2. Mashing of crushed malted cereal grains	To convert starch to soluble sugars, proteins to smaller peptides and amino acids, and cellulosic polymers to smaller polymers and oligomers: β -glucans and arabinoxylans (pentosans)	Temperature of $\sim 65^\circ\text{C}$ gives $\sim 80\%$ fermentable sugars. The partially or non-fermentable sugars are called α -glucans or dextrins. When the cereal/water porridge is filtered, it is called wort
3. Boiling the wort with hops or hop products	To give biochemical and microbiological stability, haze stabilization (by precipitating protein–polyphenol complexes), and to change the flavor profile (by inclusion of hops, promoting thermal reactions, and loss of unwanted compounds, like DMS ^b)	Hops are the traditional flavoring. Boiling is needed to convert α -acids to bitter <i>iso</i> acids. Many other volatile and nonvolatile compounds are extracted from hops
4. Cooling the hopped wort	To precipitate and remove insoluble material and to prepare for fermentation	Insoluble material is called trub. It is mainly proteins, lipids, and polyphenols
5. Fermentation of the hopped wort	To cause biochemical changes that produce beer	Most beers are fermented with added yeasts (<i>Saccharomyces cerevisiae</i> for top fermentation at $\sim 17^\circ\text{C}$ or <i>S. uvarum</i> at $\sim 9^\circ\text{C}$ or lower for bottom fermentation) ^c
5. Conditioning the new beer	To mature the beer to give a better-flavored product (including removal of unwanted compounds, such as diacetyl) ^d and to maximize solubilization of CO_2	Traditional lager is matured at $\sim 5^\circ\text{C}$ for many weeks. Ale can be matured at this temperature, but in cask, usually at $\sim 15^\circ\text{C}$ for 2–3 weeks. Many modern lagers are conditioned at $\sim -2^\circ\text{C}$ for a few days. For most beers, fining, filtration, pasteurization, artificial carbonation or priming, and other steps are included before release for sale

^aAssuming basic cereal origin is barley, as for most beers

^bDimethyl sulfide

^cMany modern lagers are fermented close to ale temperatures for a few days and then matured at very low temperatures, to speed up turnover

^dDiacetyl is a strongly odorous fermentation byproduct – it is reabsorbed by yeast and reduced to a flavorless product

Ethanol, Water, and Carbohydrates

Typical ethanol, water, and carbohydrate contents of beer are given in Table 17. Ethanol content of most beers is in the range 3.5–6.5 % ABV, but some strong ales and lagers have up to 12 % ABV: higher values than this indicate that the beer has been freeze-distilled. Much water (“liquor”) is used for brewing: it must conform to national purity and safety standards (low heavy metal, nitrate, organic compound content, high clarity, acceptable pH, no pathogenic bacteria, etc. – see Fuller et al. 2011, pp. 1076–1092 and references therein), whether it comes from a private well or some public source. Depending on the desired style of beer, the liquor is often adjusted for acidity and mineral content prior to mashing. For example, permanently hard water (with high CaSO_4 and MgSO_4 content) is needed for pale ale, very soft water is best for Pilsner, and temporarily hard water (rich in $\text{Ca}(\text{HCO}_3)_2$ and $\text{Mg}(\text{HCO}_3)_2$) suits dark beers, like Münchener, porter, and stout. The mineral contents of these beers differ accordingly.

During mashing at $\sim 65^\circ\text{C}$, linear amylose starch and branched amylopectin starch are hydrolyzed by α - and β -amylases and limit dextrinase mainly to oligomers (α -glucans or dextrans $\sim 20\%$), maltose, and glucose (Fig. 18; Table 25). The latter two are fully fermentable by brewer’s yeast, but many oligomers are either only slowly fermentable or (those with α -glc(1–6) α -glc links) non-fermentable and so remain in the finished beer, giving it “body” and a little sweetness. Lower mash temperatures produce more complete hydrolysis, which ultimately results in a strong, drier, “low-carb” or “diet” beer. Higher mash temperatures give less degradation, which produces a weak, sweet “high-carb” beer, like Malzbier, if partially fermented at a very low temperature.

Also during mashing, cereal cell walls are enzymatically degraded to polymeric (insoluble) and, especially, oligomeric (soluble) β -glucans and arabinoxylans (pentosans) (Fig. 18), many of which are linked to polyphenols like ferulic acid. High levels of these compounds in unfinished beer can lead to filtration problems prior to packaging, so many brewers add exogenous pullulanase or pectolase enzymes to effect maximum degradation. However, these soluble fibrous compounds have health benefits (McKay et al. 2011, pp. 132–210 and references therein) – see Table 28 for fibrous carbohydrate contents of beers.

Carbon Dioxide

Carbon dioxide is a major fermentation byproduct and as such is used to provide the “sparkle” and foam (“head”) of many beers, such as bottle- and cask-conditioned beers (mostly ales). However, most beers have at least some exogenous CO_2 added up to the time of packaging, and it is these beers – brewery conditioned canister (keg), bottled, and canned beers – that have the highest CO_2 content. Cask-conditioned beers contain <1.0 – 1.5 L CO_2 /L beer; draft keg beers have 2.2 – 2.8 L CO_2 /L beer, and bottled or canned beers contain 2.4 – 3.0 L CO_2 /L beer (McKay et al. 2011, pp. 132–210), corresponding to internal pressures of ~ 1 atm to ~ 3 atm at $\sim 10^\circ\text{C}$.

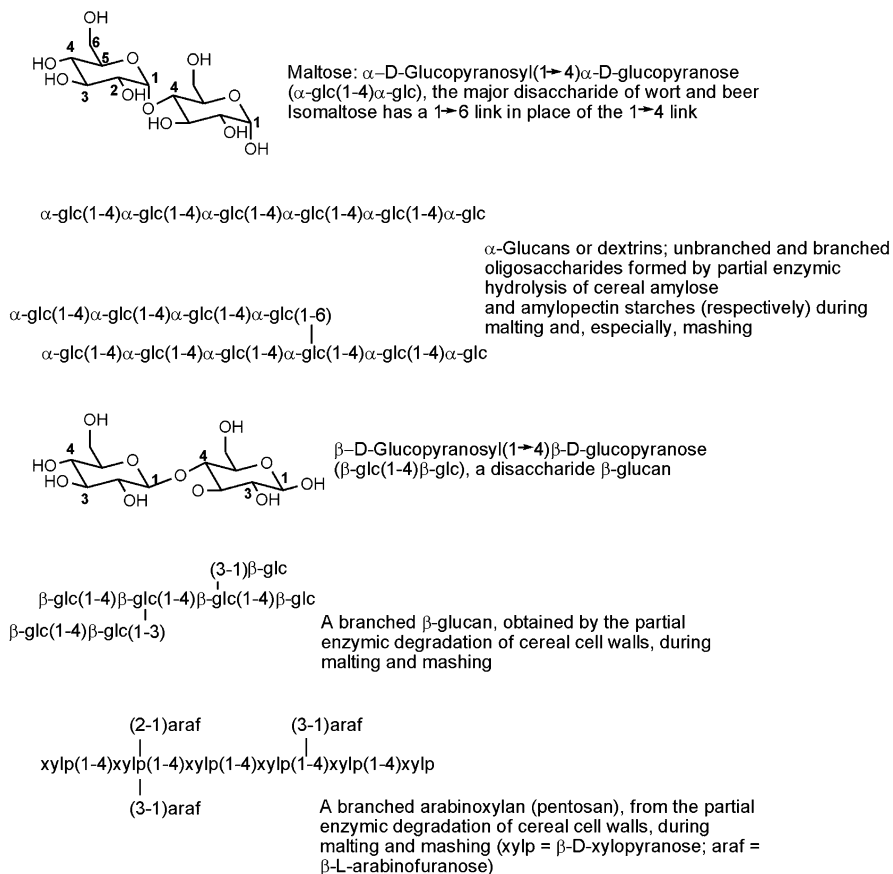


Fig. 18 Carbohydrates of wort and beer

Bitter Compounds

The flower (cone) of the female hop plant (*Humulus lupulus*) is easily the most widely used beer flavorant/preservative, being first documented in brewing in the eighth century AD. Hop cones when boiled with wort release phenolic norisoprenoid α - and β -acids (along with many other substances, including the prenylated dihydrochalcone xanthohumol) into the wort, where isomerization of α -acids to iso- α -acids, oxidation of β -acids, and many other reactions occur (Fig. 19), although isomerization of α -acids is only about 30 % complete in 1 h.

Many of these compounds contribute to the familiar bitter taste of beer, but it is the iso- α -acid stereoisomers that are considered to be the most important. Total iso- α -acid content of beer normally ranges between ~15 mg/L and ~60 mg/L and of particular styles as follows: brown ale (15–30 mg/L), pale ale (20–55 mg/L), Pilsner (20–40 mg/L), stout (~30 mg/L), extra stout, and strong beers (up to 60 mg/L). Generally the most abundant iso- α -acids are the *cis*-isomers,

Table 25 Carbohydrates in beer^a

Monosaccharides, disaccharides, and α-glucans (dextrans)										
Carbohydrate	Glucose ^b	Fructose	Maltose ^c	Isomaltose	Trehalose	Maltotriose	Maltotetraose	Oligomers		
Degree of polymerization	1	1	2	2	2	3	4	5–9		
Molecular weight (D)	180.2	180.2	342.3	342.3	342.3	504.5	666.6	828.8–1477.4		
Mean no. branches/molecule	–	–	–	–	–	–	0	1–2		
β-Glucan and arabinoxylan content^d										
Beer type	Standard filtered beer									
Content (g/L)	0.5–3.0									
	Bottle- or cask-conditioned beer									
	Up to 10.0									
	Strong ale or wheat beer									
	Up to 6.0									

^aDerived from cereal. Data from Duarte et al. (2003) or McKay et al. (2011, pp. 132–210) and references therein^bThe major monosaccharide^cThe major disaccharide^dDietary fiber

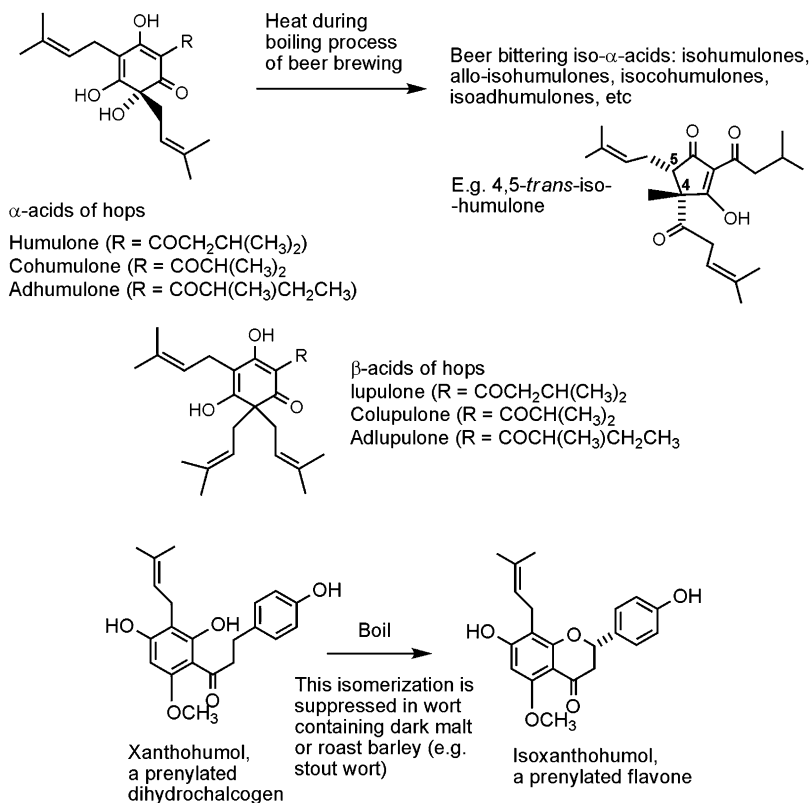


Fig. 19 Major bittering substances in beer and their origins

Table 26 Iso- α -acid content of Pilsner beers^a

Iso- α -acid	<i>c</i> -ich	<i>t</i> -ich	<i>c</i> -ih	<i>t</i> -ih	<i>c</i> -iah	<i>t</i> -iah
Content (mg/L)	9.7	2.7	7.9	2.2	2.5	0.7

c *cis*, *t* *trans*, *ich* isocohumulone, *ih* isohumulone, *iah* isoadhumulone

^aData from Jaskula et al. (2007): liquid–liquid extraction of isoacids from beer, followed by quantitation by HPLC. Mean of replicate values for three beers

with *cis*-isocohumulone and *cis*-isohumulone being the most abundant of all (Table 26), and the cohumulones are the most intensely bitter. Because of high variations in α -acid and volatile terpenoid content of hop varieties, hops are divided into three categories: aroma or noble (low α -acid; ~5 % w:w), intermediate or dual purpose, and bittering (high α -acid; 8–15 % w:w).

Volatile Compounds

Also released into the wort from hops during the boil are numerous volatile compounds, many of which are important contributors to beer hop aroma. These

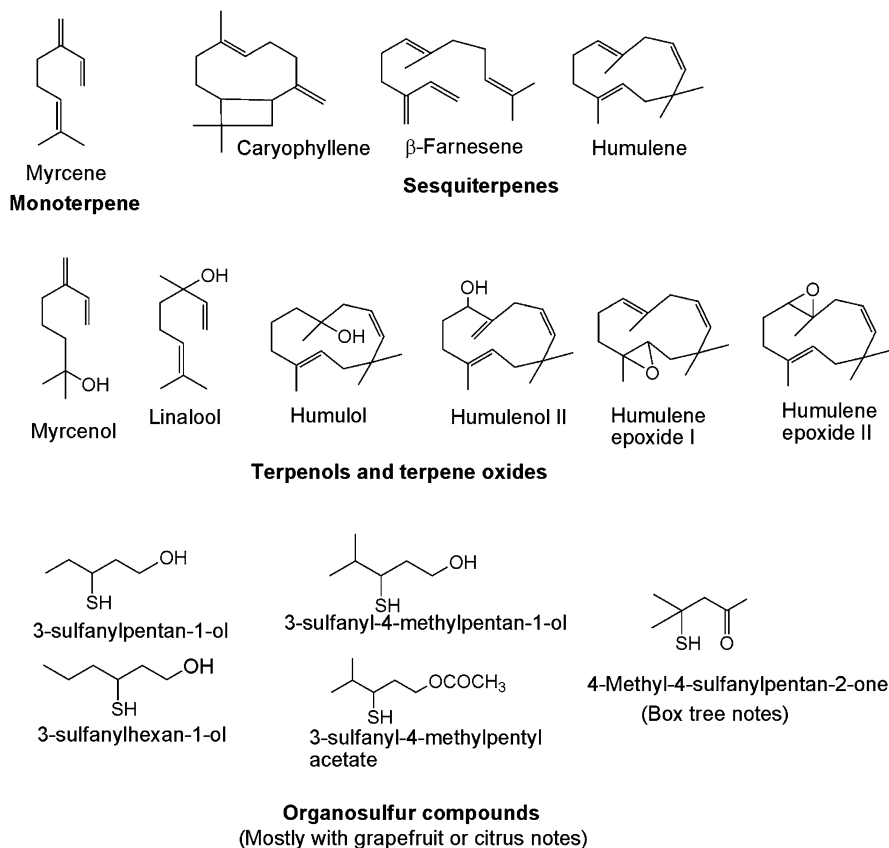


Fig. 20 Some volatile compounds of hops

include large numbers of fatty acids, carbonyl compounds, esters, and furans, but of special interest are the terpenoids (particularly oxygenated sesquiterpenoids) and sulfur compounds (Fig. 20), since these are thought to be the biggest contributors.

During the boil, some of these terpenoids undergo a variety of transformations and some will be lost via evaporation, so to compensate for this, some brewers add a portion of hops late in the boil or hop oil at later stages of the process. The most abundant terpenoids in Bavarian beer (in $\mu\text{g/L}$) were humulenol I (1150), linalool (450), humulol (220), and humulene epoxide I (125), followed by Karahana ether (60), 7,7-dimethyl-6,8-dioxabicyclo[3,2,1]octane (50), T-cadinol (45), humulene epoxide II (40), β -fenchyl alcohol (40), and α -terpineol (40) (Hough et al. 1982, pp. 422–453 and references therein).

Although much of the primary beer aroma is derived from hops, some volatile compounds originating from wort or produced by yeast alcoholic fermentation also make important contributions. These include ethanol, CO_2 , some lower alcohols, some esters, lower carboxylic acids, and caramelization/Maillard and related

products (various *O*- and *N*-heterocycles). The latter have special importance if caramalts, crystal malts, roast malts, or roast barley are used in the brew, but they are also present generally, as a result of the boiling stage. A summary of typical beer volatile compounds is given in Table 27.

Amino Acids, Peptides, Proteins, and Other Nitrogen Compounds

Beer generally contains a rather greater quantity and wider range of these compounds than cider or wine, even though much is lost during boiling (as “trub”) and fermentation. The total nitrogen (*N*) content of beer is in the range 300–4,000 mg/L (mostly up to ca. 2,000 mg/L), with α -amino *N* content making up about 6–15 % of this, the remainder being ammonium, peptides, polypeptides, proteins, nucleic acid *N* components, biogenic amines, and *N*-heterocycles (see “Volatile Components”). They originate mostly from wort (cereal) and yeast. Table 28 displays the range and mean values of amino acids in 35 beers (Kabelová et al. 2008), where it can be seen that L-proline is easily the most abundant, because of its resistance to metabolism during fermentation.

Around 30 proteins or polypeptides have been found in beer, mostly glycopeptides or glycoproteins in the molecular weight range 5,000–12,000 Da, but also some of ~40,000 Da, one of which is related to “barley protein Z” (which binds β -amylase), and a few in the range 100,000–150,000 Da. The latter are thought to have a positive influence on foam quality, whereas some of the smaller glycoproteins are believed to have a negative influence on beer clarity. The small quantities of hordeins (the prolamins of barley gluten, with molecular weights of 30,000–50,000 Da) that are also present (especially in less refined beers) are of interest because of their potential effect on gluten-sensitive individuals. Biogenic amines, especially histamine and tyramine, in beer (3.02–3.23 mg/L and 3.61–7.4 mg/L, respectively – similar levels to red wine) are likewise of interest, because of their potentially allergenic nature (Fuller et al. 2011, pp. 1093–1110).

Individual nucleotides, nucleosides, and their nitrogenous bases (pyrimidines and purines) are present in the ranges (mg/L) 1.1–73.3, 7.0–139.0, and 0.2–41.0, respectively, the total content of these species being much higher than in cider or wine.

Phenolic Compounds

According to the predominant brewing methods, phenolic compound levels in beer are low, even though malt and hops are rich in polyphenols and certain volatile phenols are formed during fermentation. This is largely because too high a phenolic content in the finished beer can lead to hazes (polyphenol–protein colloids) and premature staling, which produces unacceptable aroma and flavor (Table 29).

Much of the malt polyphenols exist as cross-links between the polymeric carbohydrate chains that form the grain cell walls. Some of these, and much of the free polyphenols (some from hops), are lost in the boiling step as “trub,” along with proteinaceous material and lipids. One important hop-derived prenylated polyphenol, xanthohumol, partially survives the brewing processes, some of it being isomerized to isoxanthohumol during boiling (Fig. 13). Xanthohumol levels in beer are in the range (0.126–0.200 mg/L), and both compounds have been shown

Table 27 Typical volatile compounds in beer^a

Compound	Typical or range of concentrations (µg/L)	Odor threshold value (OTV) ^b (µg/L)	Odor activity value (=concn/OAV) ^c	Comments
Ethanol	$3.5\text{--}6.5 \times 10^7$	1.0×10^5	350–650	Highest levels in ales
CO ₂	$\text{Tr.}\text{--}37.2 \times 10^3$	150	0.72	
Acetaldehyde	20–580	0.5	0.40–2.41	
Diacetyl	0.36	7.0×10^4	0.29–0.85	
Hex-2-enal	$0.5\text{--}6.0 \times 10^4$	6.5×10^4	0.24–2.1	
1-Propanol	$0.6\text{--}9.8 \times 10^4$	33	0.25–3.1	
2-Methylpropanol	$0.8\text{--}4.1 \times 10^4$	3.3×10^4	0.05–0.8	
2-Methylbutanol	$0.28\text{--}1.69 \times 10^5$	1.6×10^3	0.33–0.88	
3-Methylbutanol (isoamyl alcohol)	$1.9\text{--}5.5 \times 10^4$	2.0×10^4	0.87	
2-Phenylethanol	0.1–0.4	1.75×10^5		
Dimethyl sulfide	345–3,175	1.5×10^3		
Methionol	$0.8\text{--}6.9 \times 10^4$			
Ethyl acetate	$0.4\text{--}4.9 \times 10^3$			
Isoamyl acetate	$0.1\text{--}1.6 \times 10^4$			
SO ₂	$0.57\text{--}1.45 \times 10^5$			Lambic and Gueuze beers have higher levels
Acetic acid	1.3×10^3			
3-Methylbutanoic acid	$0.41\text{--}9.63 \times 10^2$			Higher values for blond ales and wheat beers
4-Vinylphenol ^d 4-Vinylguaiacol ^d	$0.053\text{--}3.76 \times 10^3$			
Humulene I	1,150			
Linalool	450			
Humulol	220			
Humulene epoxide I	125			
2-Acetylfuran	$0.4\text{--}9.7 \times 10^4$	8.0×10^4	0.05–1.2	Higher levels in dark beers
5-Hydroxymethylfurfural	$0.5\text{--}7.8 \times 10^3$	1.0×10^6		None have OAV > 1, but collectively they give burnt/coffee aroma
2-Methylpyrrole	1.8×10^4	20–10 ³		
Pyrazines ^e	$\text{Tr.}\text{--}4.19 \times 10^2$			

^aData from Hough et al. (1982, pp. 776–838), unless stated otherwise

^bIn water or beer: these values can vary enormously according to experimental design and hence should be used as a guide to odor potency

^cOAV > 1 indicates individual contribution to aroma, but values lower than 1 for family groups of compounds (e.g., pyrazines) can be important

^dData from Vanbeneden et al. (2008)

^eMethylpyrazine, tetramethylpyrazine, pyrazine, and 2-ethyl-3,5-dimethylpyrazine, in particular

Table 28 L-Amino acid content of beers^{a†}

L-Amino acid	ASP	ARG	ALA	GLU	GLY	HIS	ILEU	LEU	LYS
Range (mg/L)	1.5–26.4	3.8–92.2	11.1–63.6	0.3–32.9	6.2–130	2.2–33.9	1.5–21.3	2.5–41.9	1.8–36.2
Mean (mg/L)	9.2	33.5	40.2	10.9	22.3	14.9	8.9	15.4	12.2
L-Amino acid	MET	PHE	PRO	SER	THR	TYR	VAL		
Range (mg/L)	1.1–10.4	2.9–46.4	31.8–250.4	0.6–25.7	1.5–59.2	2.9–50.6	2.7–39.9		
Mean (mg/L)	4.1	19.3	146.3	9.4	10.1	22.0	21.6		

^{a†}Data from Kabelová et al. (2008) from analysis of 35 European beers

Table 29 Compound giving rise to aged aromas and other organoleptic perceptions of stale beer^a

Compound	Aroma (other)	Major origin and comments
<i>trans</i> -2-Nonenal	Cardboard (astringent mouthfeel)	From oxidation of linoleic acid and aldol condensation of lower aldehydes (from oxidation of lower alcohols). Detected at low levels because of low odor threshold value (OTV) (0.1 mg/L)
Oxidized flavonoid phenols (<i>o</i> -quinones)	Grassy, metallic	Flavonoids may be oxidized by oxidized melanoidins (Maillard reaction products)
3-Methylpropanal (isovaleraldehyde)	Grassy	The <i>o</i> -quinone groups can cause hazes via polyphenol–protein colloids
Hexanal	Cheesy, goaty	General oxidation of 3-methylpropanol (a fusel alcohol)
3-Methylpropanoic acid	Grassy, currant	Oxidation of unsaturated long-chain fatty acids
Strecker aldehydes (e.g., phenylacetaldehyde)		Oxidation of isohumulone (from hops) Reaction between amino acids and 4,5-epoxy-2-alkenols (breakdown products of oxidized unsaturated long-chain fatty acids)

^aInformation from McKay et al. (2011, pp. 132–210), and references therein

to have highly estrogenic and anti-inflammatory properties. Also of health benefit are the small quantities of tumor-inhibiting isoflavones (biochanin A, daidzein, formononetin, and genistein) found in beer.

Volatile phenols, such as 4-vinylphenol (smoky, phenolic notes) (Table 27), can be found at beneficially low levels in ales (especially wheat beers) fermented at relatively high temperatures and in beers produced from smoked malt (Rauchbier and some porter beers).

Lipids

Beer contains only trace amounts of lipids (glyceride esters, fatty acids up to C₁₈, and steroids), since much of the original lipid content of malt is removed in the trub at the boiling stage. High lipid content reduces foam quality and oxidation of unsaturated long-chain fatty acids, like linoleic acid, can ultimately give *trans*-2-nonenal, a major “staling” compound of beer (cardboard notes) (Table 29).

Minerals and Vitamins

The mineral content of beer is very variable, since in practice many different brewing liquors are used, and there are many water treatments available to transform the liquor into one that is more suitable for brewing a particular beer style, including boiling, ion exchange, and reverse membrane osmosis (all performed prior to mashing). Nevertheless, beer is a rich source of minerals and some typical mineral contents are shown in Table 17.

Beer is generally rather richer in vitamins than cider or wine (Table 14). In particular, it is a rich source of folate, containing up to 68.2 µg/L (as 5-MTHF) (Owens et al. 2007).

Some Other Components

Malt and hops contain a wide range of both volatile and nonvolatile weak carboxylic and phenolic acids, while others are formed during fermentation, but usually at lower levels than those in cider or wine so that the total acidity of beer is normally not more than 1–2 g/L (as sulfuric acid), corresponding to pH 3.9–4.6. High concentrations of D- and/or L-lactic acid, along with a higher than normal level of acetic acid (and ethyl acetate), are indicative of the involvement of lactic acid bacteria and yeasts such as *Hansenula* and *Pichia* spp. during fermentation. Lambic and Gueuze beers, produced by spontaneous fermentation by such organisms, contain up to 3,500 mg/L lactic acid and 1,200 mg/L acetic acid. If *Brettanomyces* spp. are involved in fermentation (as in some Belgian ales, like Lambic beers), then short-chain fatty acids are produced in significant concentrations, giving pleasant cheesy notes.

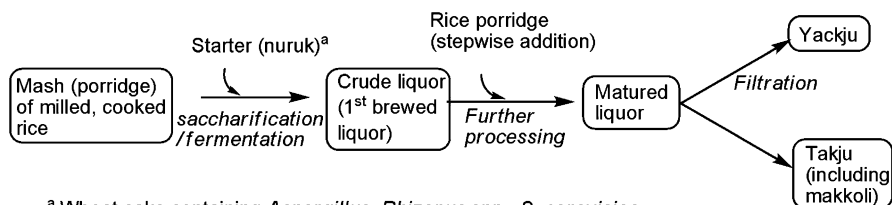
A number of malodorous products are produced during the “staling” of beer, toward the end of its shelf life. This can happen prematurely if the levels of O₂, polyphenols, and/or lipids are too high in the new beer, so modern brewing processes strive to keep the levels of these substances low, especially in the later production stages. Oxidation is the basic cause of staling and occurs by a variety of routes, many being catalyzed by Cu²⁺/Cu⁺, Fe³⁺/Fe²⁺, and Mn³⁺/Mn²⁺ redox systems. The major compounds associated with stale beer are shown in Table 29.

Many beers do not have added sulfite (preservative), but those that do are subject to national regulations regarding maximum total allowed sulfite levels (e.g., 200 mg/L in the EU and 80 mg/L in the USA).

Rice Wine

Rice wine, in its many variations, is enjoyed all over eastern Asia, and some forms of it (like Japanese sake) have some popularity in the USA and Europe. Rice wine production is more like that of beer than wine, but hops are not used in rice wine manufacture and the saccharification of starch and fermentation steps occur in the same vessel (occurring either simultaneously or sequentially) (McKay et al. 2011, pp. 211–230 and references therein). As a typical example, Fig. 21 shows the outline of the traditional production process for makkoli (makgeolli), a popular Korean cloudy rice wine. In all cases, a mash of cooked milled rice and water (porridge) is treated with certain amylolytic and fermentative fungi and lactic acid bacteria (LAB) growing on a suitably nutritious medium (the “starter”). Details of the starter are given in Fig. 21.

Rice wine can be cloudy or clear, usually unflavored but sometimes flavored with fruit juice or syrup, flowers, herbs, or mushrooms. The cloudy drinks, in particular, are a rich source of carbohydrate (including dextrans and fibrous β-glucans), amino acids, proteins, and minerals (Tables 14 and 17). The ethanol content of rice wine (Table 14) varies from 6 % to 7 % ABV for cloudy versions, like makkoli, to 16–20 % ABV for most clarified types, including Chinese “yellow wine” and sake. Ethanol (brewer’s alcohol) may be legally added to some kinds of



^a Wheat cake containing *Aspergillus*, *Rhizopus* spp., *S. cerevisiae* and lactic acid bacteria (LAB). Sometimes *S. cerevisiae* added separately
 nuruk:rice porridge ~ 1.5:8.5

Note different types of starter for Asian rice wines:

Japan	China	Vietnam
Koji is steamed rice containing <i>Aspergillus oryzae</i> ; <i>S. cerevisiae</i> added separately	Qu is wheat or red rice cake containing <i>Aspergillus</i> , <i>Rhizopus</i> spp., and <i>Monascus purpureus</i> ; <i>S. cerevisiae</i> added separately	Men is rice and cassava cake containing <i>Amylomyces rouxii</i> , <i>R. oryzae</i> , <i>S. cerevisiae</i> , and others

Fig. 21 Outline scheme for the manufacture of traditional Korean rice wine (Based on McKay et al. 2011, pp. 211–230)

sake. Also, because of the activities of LAB, rice wine generally possesses high levels of lactic acid (D- and/or L-isomers) – indeed in many modern styles of sake, lactic acid may be added with the starter.

The spectrum of microorganisms in the starter influences the flavor of rice wine (superimposed on a cereal-like alcoholic taste) via its volatile compound composition, as does the degree of polishing of the rice: highly polished rice gives a fruity product (e.g., much sake), whereas unpolished rice yields a product with earthy, grainy notes as well as fruity notes (e.g., some Chinese rice wine). The volatile compounds of rice wine are typified by two examples. Table 30 demonstrates the fruity-wine character of makkoli made from polished rice, while Table 31 shows the more aromatic characters of some Chinese rice wines, where benzenoid compounds, furans, *N*-heterocycles, phenols, and sulfur compounds are in evidence, as well as acids, alcohols, carbonyl compounds, and esters.

Spirits

If any alcoholic beverage (5–12 % ABV) is boiled, and if its vapor is condensed, then the result will be a crude distilled spirit containing ~22–30 % ABV. Most of the nonvolatile components in the wine (pigments, polyphenols, carbohydrates, acids, amino acids, peptides, proteins, and minerals) are left behind in the still, whereas the distillate is composed of ethanol, water, and many volatile flavorants (“congeners”; Fig. 22). The volatiles of most spirits (irrespective of identity) generally fall into the categories: esters, aromatic compounds, terpenoids, alcohols, acetals, aldehydes, phenols, ketones, furans, carboxylic acids, other heterocyclic

Table 30 Volatile compounds in makgeolli (makkoli), a Korean rice wine^a

Alcohols	Acetate esters	Ethyl alkanoate esters
Isobutanol (24.2–43.4)	Isobutyl (0.2–0.6)	Hexanoate (0.63–1.08)
Isoamyl alcohol (196.2–365.5)	Isoamyl (10.3–17.2)	Octanoate (1.07–2.17)
2-Methylbutanol (66.4–123.1)	2-Methylbutyl (0.9–1.8)	Decanoate (1.21–2.98)
2-Phenylethanol (134.6–156.3)	2-Phenylethyl (2.4–4.1)	

^aData from Kang et al. (2014), in mg/L

Table 31 Volatile compounds of Chinese rice wines^a

Compound	Concentration (µg/L)	Compound	Concentration (µg/L)
<i>Alcohols</i>		<i>Esters, continued</i>	
2-Methylpropanol	n.d.–945	Ethyl 2-phenylacetate	24.7–630
2-Methylbutanol	n.d.–2,158	Ethyl 3-pyridine carboxylate	n.d.–477
3-Methylbutanol	n.d.–2,295	Isoamyl acetate	
1-Hexanol	n.d.–102	2-Phenylethyl acetate	n.d.–222
3-Ethoxypropanol	n.d.–173	<i>Carbonyl compounds</i>	
2,3-Butanediol	n.d.–297	3-Hydroxy-2-butanone	n.d.–64.4
1-Octanol	n.d.–91.6	Nonanal	n.d.–59.5
<i>Acids</i>		<i>Aromatics (not esters)</i>	
Acetic	42.9–503	Benzaldehyde	139–3,540
3-Methylbutanoic	n.d.–47.8	Acetophenone	n.d.–137
Hexanoic	n.d.–173	Benzyl alcohol	0.48–130
Octanoic	7.89–174	2-Phenylethanol	1,333–16,711
Decanoic	n.d.–18.9	<i>cis</i> -2-Phenyl-2-butenal	n.d.–968
<i>Esters</i>		<i>Lactones</i>	
Ethyl acetate	303–3,352	γ-Butyrolactone	n.d.–67.5
Ethyl benzoate	13.5–432	γ-Nonalactone	n.d.–220
Ethyl 3-methylbutanoate	n.d.–9,827	<i>Phenols</i>	
Ethyl hexanoate	n.d.–299	Phenol	n.d.–53.0
Ethyl lactate	77.0–4,560	4-Ethyphenol	n.d.–48.1
Ethyl octanoate	n.d.–115	<i>Sulfur compounds</i>	
		Dimethyl trisulfide	n.d.–70.2
		<i>Furans</i>	
		Furfural	117–2,831
		<i>N-Heterocycles</i>	
		Tetramethylpyrazine	n.d.–73.0

^aData from Luo et al. (2008): 10 different rice wine types. Values have been rounded off to 3–5 significant figures (n.d. not determined); only compounds that are present in a majority of samples are included

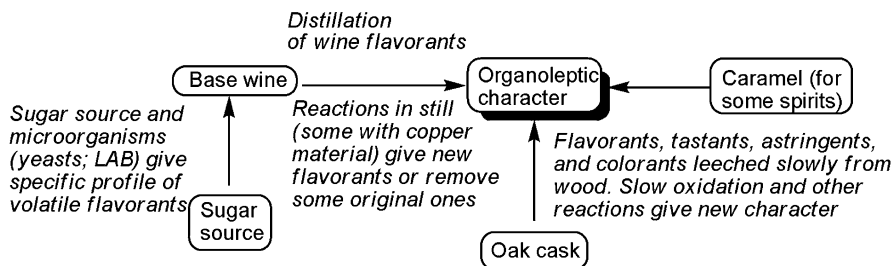


Fig. 22 Some origins of spirit organoleptic character (not including “flavored spirits” (like gin) or liqueurs)

compounds, and organosulfur compounds. Table 32 outlines the basic production methods for some important distilled spirits.

Ethanol and Water Content in Relation to Distillation Method

Distillation is performed in stills (Fig. 23), the simplest being pot stills (alembics or alambics), where at least two distillations (in batches) are needed to achieve a 65–75 % ABV distillate (with “heads” and “tails” management). Most pot still spirits are double or triple distilled. The same results can be reached using a columnar still (fitted with a fractionator or rectifier) in a single batch distillation. A continuous still (e.g., a Coffey still) can give a highly rectified spirit (~95 % ABV) in a continuous single distillation: a continuous flow of wine gives a continuous flow of spirit. However, the purer the spirit, the lower the concentrations of its volatile flavorants and the weaker the flavor. For this reason, the European Union specifies that rectified spirit should be less than 86 % ABV, but in practice most “new make” spirits are collected at much lower ethanolic strengths (e.g., ~68 % ABV for Scotch whisky; ~57 % ABV for Bourbon).

The alcoholic strengths of spirits are shown in Table 14: these figures refer to finished spirits, as purchased. Most spirits have ethanol contents in the range 37.5–46 % ABV; they are diluted with pure water to these values before bottling. In cask or maturation vessel, their ethanol contents would be ~60 % ABV or higher: Scotch malt whiskies bottled without dilution are called “cask strength” (57–63 % ABV). Liqueurs (USA: cordials) are usually of 16–40 % ABV, and eastern Asian spirits are normally of 20–40 % ABV, whereas highly rectified vodka or similar can be of 86 % + ABV.

Spirit Production Process and Chemical Composition of Spirit

The three aspects of production that have the greatest influence on the spirit composition (and hence organoleptic character) are summarized in Table 33. They are:

- Nature of raw materials and nature of fermentation (e.g., active microorganisms)
- Distillation (equipment, method, and reactions)
- Cask aging (extraction of compounds from wood, slow reactions, adsorption of some compounds on wood)

Table 32 Fermented beverage origin, type of still, distillation method, and maturation method for selected spirits

Spirit	Fermented beverage origin	Still type ^b	Distillation method	Maturation (min. aging in cask in years)
Armagnac	White wine	Continuous	Continuous	Oak cask (2)
Calvados ^a	Cider	Alambic charentais	Double	Oak cask (3)
Cognac	White wine	Alambic charentais	Double	Oak cask (2.5)
Fruit	Fruit wine	Pot or column	Double or single	Glass or porcelain mostly, but also oak or ash cask
Gin	Cereal wine (called “wash”)	Pot or column	Double or single	None usually; sometimes glass or cask
Pomace ^c	Grape or fruit pomace	Pot or column	Double or single	Oak cask (2)
Rum/ cachaça	Cane sugar wine or similar (called “beer”)	Pot or column or continuous	Double or single or continuous	Oak cask (1) or none
Soju/ shochu/ Chinese liquor	Rice wine (trad.)/wine from various sugar/starch sources	Mostly continuous, pot (trad.)	Continuous or double	Earthenware or none
Tequila/ mezcal	Maguey (<i>Agave</i>) pine wine	Pot	Double	Oak cask (2)
Vodka	Cereal wash/potato wine	Continuous	Continuous	None usually
Whisk(e)y	Cereal wash ^d	Pot, column, or continuous	Double/triple, single, or continuous	Oak cask ^e (3 for Scotch, 2 for Bourbon)

^aA kind of pot still. Other apple spirits are made using column or continuous stills

^bPot and column stills are batch stills, the latter having some kind of fractionator in the still head, giving a more refined spirit. Continuous (e.g., Coffey stills) give a continuous flow of usually highly refined spirit: Armagnac is an exception

^cPomace is the residual fruit pulp left behind when the fermenting wine is pressed from it

^dBourbon wort (51–75 % maize, remainder barley, rye) is fermented with crushed grains and distilled off the porridge wine. Scotch whisky wort (all barley for malt whisky, barley and wheat or maize for grain whisky) is filtered off the grains before fermentation and distillation

^eCharred oak casks for Bourbon, used Bourbon casks, ex-sherry casks, and others for Scotch whisky

Table 34 (Scotch whisky and Bourbon whiskey) shows how volatile composition depends on type of cereal, fermentation method, and distillation method, whereas Table 35 (*Agave* spirits) demonstrates how different species of the raw material can influence volatile composition, given similar distillation and other processing techniques. Concentrations of selected congeners in a particular spirit type (cider spirit) are given in Table 36. Table 37 (pear spirit) shows how the presence of wine lees (yeast and other deposits) can influence the distillate volatile composition.

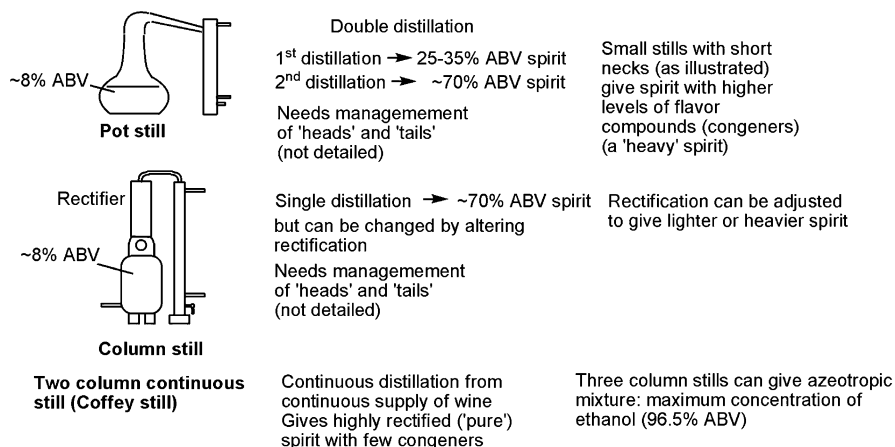


Fig. 23 Influence of still type and distillation method on chemical composition (and hence organoleptic character) of spirit

Generally, the greater the number of evaporation/condensation cycles during distillation, the lower is the congener level of the spirit (a “light” or “pure” spirit), hence, in general, continuous stills give the “lightest” spirits, while pot stills produce the “heaviest.” See Table 32 for examples of kinds of stills and distillation modes used to produce various well-known spirits.

For very good practical, as well as historical reasons, pot and columnar stills are usually constructed of copper (or at least have copper inner surfaces), in which case high (malodorous) levels of organosulfur compounds in the wine are much depleted to positive odor levels in the spirit, through reaction with the copper surface, giving nonvolatile copper sulfide. However, copper also catalyzes reaction between hydrogen cyanide (from urea in the wine) and ethanol to give ethyl carbamate, although its production can be limited by using high reflux rates and lower distillation temperatures.

Flavored Spirits

These are usually diluted highly rectified spirit or grain neutral spirit (GNS) either redistilled with flavoring agents (raw materials, essential oils, essences, etc.) or simply infused with flavoring agents (Buglass and Caven-Quantrill 2012 and references therein). The following are the best-known types (flavorings or “botanicals” in parentheses):

Gin (juniper berry, coriander, citrus peel, orris root, and others)

Vodka (fruit, such as cranberry, or herbs)

Akvavit (caraway, dill, and others)

Arak, tsipouro, ouzo, pastis, and others (aniseed and star anise mainly)

Rum, cachaca (fruit or spices)

Soju (herbs, flowers, roots, bark)

Table 33 Influence of steps in production process on chemical composition and organoleptic character of spirits^a

Raw materials and fermentation step	Distillation step	Cask aging
<p>Raw materials (e.g., cereals, fruit, vegetables sugar cane juice, etc.), along with fermentative microorganisms and fermentation conditions define wine and hence spirit flavor profile:</p> <ul style="list-style-type: none"> – Acidic wine tends to give better brandy (higher levels of esters) (fruity, sweet) – Peat-smoked malted barley gives malt whisky with higher levels of volatile phenols (smoky, spicy, medicinal) – Slow “spontaneous” fermentations tend to give spirit with higher level of flavor compounds – Brewer’s yeast gives more fruity esters, furanones (sweet, caramel), and methional (cooked potato) in spirit than distiller’s yeast – Activities of LAB give higher levels of ethyl lactate, volatile phenols and γ-lactones (from γ-hydroxy acids) 	<p>Type of still and method of distillation – see Fig. 18.</p> <p>A lower number of evaporation/condensation cycles tends to produce spirits with higher levels of acetaldehyde, acetals, ethyl acetate, and long-chain fatty acid ethyl esters, whereas a higher number of evaporation/condensation cycles tends to give a spirit richer in fusel alcohols, isoamyl acetate, 2-phenylethanol, and 2-phenylethyl acetate</p> <p>Reactions:</p> <ul style="list-style-type: none"> – Acetaldehyde and acetal formation – Isomerization of terpenoids – Formation of norisoprenoids from carotenes – Maillard reactions (to give furans and <i>N</i>-heterocycles). <p>Lower distillation temperature gives fewer furans (less caramel character)</p> <ul style="list-style-type: none"> – Depletion of some sulfur compounds in copper still 	<p>Extraction of compounds from wood: lactones^b (coconut, sweet), furans (toasty, nutty), <i>trans</i>-2-nonenal (woody), phenols^c (smoky, spicy), maltol and cyclotene^d (sweet, caramel), terpenoids (floral, herbaceous), pyrazines (coffee, chocolate, burnt)</p> <p>Also hydrolyzable tannins^e (bitter, astringent) and coumarins (bitter)</p> <p>Slow reactions: esterifications, hydrolyses, oxidations (e.g., aldehydes to acids)</p> <p>Adsorption on to wood: Cu²⁺ (from copper still) and some sulfur compounds</p> <p>Slow overall loss of ethanol or water, depending on original strength of spirit in cask</p> <p>For Scotch malt whisky: flavor compounds from the cask’s previous occupant (e.g., oloroso sherry, port, Sauternes)</p>

^aFrom Buglass et al. (2011, pp. 469–594) and references therein. For further information on distillation method, see Fig. 23

^bFor example, *cis*- and *trans*-whisky lactone

^cFor example, cresols, eugenol, guaiacol, phenol, vanillin, and others (lignin hydrolysis products and charring pyrolysis products)

^dWood toasting products

^eEllagitannins and gallic acid tannins mainly

Many of these flavored spirits have added sucrose syrup before bottling, giving about 20–50 g sucrose/L spirit, about 10 % of the sugar content of liqueurs (next subsection). Low-calorie sweeteners, like steviol derivatives, or occasionally maple syrup can be used in place of sucrose.

In these spirits, the flavorant, tastant, and bitter/astringent agents tend to dominate the organoleptic character of the drink; the volatile component profile reflects

Table 34 Volatile compounds of aged Scotch whisky and Bourbon whiskey

Scotch whisky ^a (aroma descriptor)	Bourbon whisky ^b (aroma descriptor)
Key odorants^c	
Ethyl esters: cyclohexanoate, decanoate, dodecanoate, hexanoate, 2-methylpentanoate, 3-methylpentanoate, octanoate (all fruity or waxy)	Norisoprenoid: <i>trans</i> - β -damascenone (fruit)
Acetate ester: isoamyl acetate (fruity)	Lactones: γ -nonalactone (peach), γ -decalactone (coconut), <i>cis</i> -whisky lactone (coconut)
Alcohols: 3-methylbutan-1-ol (malt), 2-methylpropan-1-ol (winey)	Phenols: eugenol (cloves), vanillin (vanilla)
Acids: decanoic (fat), hexanoic (cheese), octanoic (waxy)	
Other important odorants	
Phenols: <i>o</i> - <i>p</i> -cresols (phenolic), 4-ethylphenol (smokey), phenol (phenolic), guaiacol (coffee), vanillin (vanilla)	Alcohols: 3-methylbutan-1-ol (malt), 2-phenylethanol (rose),
Aldehydes: <i>trans</i> , <i>trans</i> -2,4-nonadienal, <i>trans</i> -2-nonenal, hexanal (all green)	Aldehydes: <i>trans</i> -2-decenal, <i>trans</i> -2-heptenal, <i>trans</i> , <i>trans</i> -2,4-nonadienal (all green/fatty)
Alcohols: 4-hepten-1-ol, 1-octen-3-ol, 2-nonenol (all mushroom, earthy)	Ester: ethyl 2-methylbutanoate (fruit)
Sulfur compounds: dimethyl trisulfide (onion), methyl (2-methyl 3-furyl) disulfide (meaty)	Lactone: <i>trans</i> -whisky lactone (coconut)
	Norisoprenoid: β -ionone (violet)
	<i>Plus ethanol and other alkanolate esters</i>

^aBuglass et al. (2011, pp. 469–514) and references therein

^bPoisson and Schieberle (2008)

^cKey odorants allocated on basis of odor activity values and/or by reconstitution/omission studies

the composition of the flavoring mixture. Thus gin made with a high proportion of juniper berries has a high α -terpineol, oxygenated sesquiterpenoid, and oxygenated diterpenoid (Fig. 24) content, whereas that made with a greater quantity of coriander has a higher level of linalool (Tables 19 and 38). Anethole is the dominant volatile of aniseed flavored spirits, having a mean concentration of ~ 2.5 g/L (Buglass et al. 2011, pp. 554–573 and references therein).

Liqueurs

Liqueurs (USA: cordials) are flavored and sweetened spirits of ethanol content 15–40 + % (v:v) and sugar content of at least 10 % (w:v). GNS is often the spirit base, but others, such as brandy, fruit spirit, or gin, are used in some liqueurs (e.g., cherry spirit in cherry liqueurs, gin in sloe gin). Sugar is added mostly as sucrose syrup (usually 200–300 g/L liqueur) but honey is also used for some liqueurs. Like flavored spirits, the combination of botanicals competes well with the ethanol and sugar for organoleptic attention and tends to dominate the taste of most liqueurs. The following are the major categories of liqueurs:

Table 35 Selected volatile compounds in *Agave* (maguey) spirits – tequila, mezcal, sotol, and bacanora^a

Compound conc. (mg/L)	Acetaldehyde	1-Butanol	2-Butanol	2/3-Methyl-1-butanol
Tequila (mixed)	80	10	600	1,350
Tequila (100 %)	80	5.5	695	2,450
Mezcal	90	9.5	630	1,800
Sotol	150	23	295	200
Bacanora	470	17	490	1,700
Compound conc. (mg/L)	2-Phenylethanol	Ethyl lactate	Methanol	
Tequila (mixed)	18	235	1,450	
Tequila (100 %)	63	170	2,150	
Mezcal	56	180	1,500	
Sotol	18	40	1,300	
Bacanora	47	35	2,700	

^aData from Lachenmeier et al. (2006). Mean values. Tequila made from *Agave tequilana* Weber var. *azul*; mezcal made from *A. angustifolia* Haw., *A. potatorum* Zucc., *A. salmiana* Otto, and others. Sotol made from *Dasyliirion* spp.; bacanora made from *A. angustifolia*. “Mixed” tequila is 51 % agave spirit. These were all produced using pot stills, but the fermentative microorganism profile probably differed throughout the samples

Table 36 Selected volatiles in cider spirit from Asturias (N. Spain)^a

Compound	Acetaldehyde	Acetal	Ethyl acetate	Methanol	2-Butanol	1-Propanol
Conc. range (mg/L)	231–339	72–140	513–893	764–1,430	24–124	415–550
Compound	2-Methyl-1-propanol	2-Propen-1-ol	1-Butanol	2-Methyl-1-butanol	3-Methyl-1-butanol	Furfural
Conc. range (mg/L)	253–354	21–493	80–147	433–523	2,190–2,573	12–23

^aData from Madrera et al. (2006), p. 5 commercial samples distilled in “alquitara,” traditional pot stills, usually used for grape marc spirits. Similar levels of these compounds have been found in other cider spirits, such as calvados

Fruit liqueurs (e.g., “cherry brandy,” sloe gin, curaçao)

Herb liqueurs (Kümmel, “monastery liqueurs,” ginseng, flower petal liqueurs)

Cocoa, coffee, and tea liqueurs

Nut liqueurs

Cream liqueurs

As well as flavorant herbs and spices, specific bittering agents, such as citrus peel, elecampane, gentian roots, cascarilla, cinchona, quillaja bark, and others, play an important role in the organoleptic impact of liqueurs, and some, like saffron, are added for color. Examples of flavor and bitter chemical components of herbs,

Table 38 Concentrations of selected volatile components of commercial gin^a

Compound	Concentration (mg/L) [mean]		
	London dry	Plymouth	Mahon
Monoterpenoids:			
α -Pinene	1.95–3.60 [2.56]	6.12	5.65
Sabinene	0.66–1.02 [0.94]	0.09	2.53
β -Myrcene	2.38–5.01 [4.00]	6.17	11.09
β -Phellandrene	0.20–0.54 [0.31]	0.46	0.64
γ -Terpinene	1.16–1.37 [1.26]	2.87	1.51
Citronellol	0.05–0.22 [0.13]	0.10	1.97
Myrtenol	0.02–0.04 [0.03]	0.02	0.37
Citronellal	0.08–0.17 [0.12]	0.06	0.41
Camphor	0.83–1.54 [1.17]	1.19	0.85
Linalool	10.96–36.99 [22.37]	16.83	1.93
α -Terpineol	1.13–1.89 [1.51]	3.80	9.03
Geranyl acetate	0.70–2.09 [1.38]	1.53	0.25
γ -Elemene	0.22–0.42 [0.35]	0.65	0.92
Sesquiterpenoids:			
Caryophyllene oxide	0.15–0.39 [0.23]	0.09	5.13
Torreyol	0.08–0.19 [0.13]	0.14	1.52
Spathulenol	0.05–0.08 [0.06]	0.04	1.29
Elemol	0.01–0.02	0.01	0.32
<i>trans</i> -Cadinol	0.03–0.07 [0.05]	0.08	0.97
<i>trans</i> -Muurool	0.03–0.08 [0.05]	0.12	1.27
Eudesmol	0.01–0.02	0.01	0.18
α -Cadinol	0.06–0.12 [0.09]	0.18	1.45
Total monoterpenoids	45.84	62.24	71.34
Total sesquiterpenoids	4.18	7.01	19.76

^aData from Vichi et al. (2005)

allowing greater opportunities for manufacturers to more effectively monitor their production processes at all stages and to improve their products. Over the past two or three decades, consumers and governments alike have become increasingly interested in health issues relating to beverages. Labeling legislation with regard to content and health warnings have been in force throughout the food industry for many years, and although there is considerable variation between countries and between food types, it is in general likely that legislation will be tightened over the next decades. At the same time it is likely that the trend toward the greater use of natural ingredients (which contain unidentified compounds or some of unknown or uncertain physiological activities) will continue. Also, data on physiological activity or toxicity of chemical components of beverages will become more complete and more reliable. Hence knowledge of chemical composition of beverages will maintain its importance.

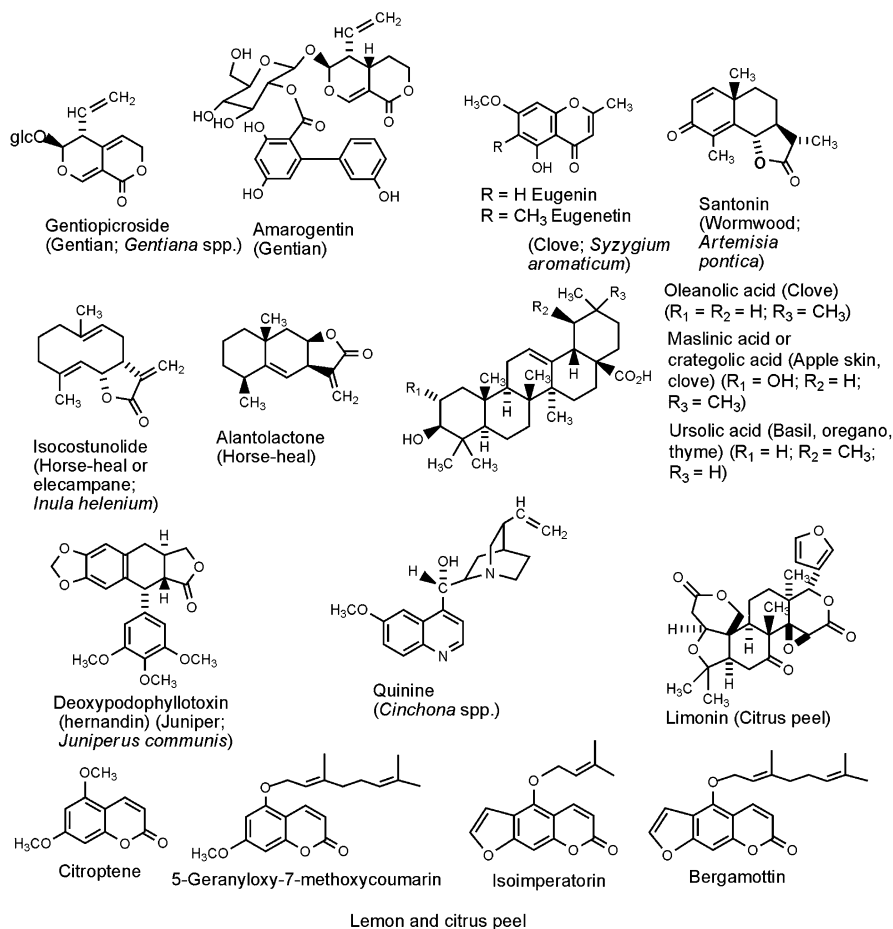


Fig. 25 Some bitter or astringent compounds of fruit and herb liqueur flavorings

Cross-References

- ▶ [Classical Wet Chemistry Methods](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Drying](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Freezing](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Heating and Cooking](#)

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Abstract

Humans have a history of cultivating cereal crops and utilizing their grains to prepare various types of food for thousands of years. The most popular cereal products available in the market include bread, cookies/biscuits, cakes, pasta, noodles, and extruded snacks and breakfast cereals. They are an important part of our daily diets and provide energy and essential nutrients for human health. Cereal grains contain starch and protein as the major components and lipid, non-starch carbohydrates, phytic acid, vitamins, and minerals as the minor components. Physical interactions and chemical reactions occur between these constituents during the processing and storage of cereal products, which determine their quality, storage stability, and nutritional value. With an increasing population of people suffering from celiac disease, diabetes, obesity, and other metabolic syndrome, there are opportunities and challenges for the food industry to develop healthier cereal products through utilizing novel ingredients and improving processing technologies. This book chapter offers a good review of chemical compositions of different cereal grains, processing technologies applied to produce various cereal foods, and future trends of research and product development in this area.

Introduction

Cereals are plants belonging to the Gramineae family, and they have been cultivated by humans for thousands of years for the edible components of their grains. Major cereal crops grown in the world include wheat [mainly common wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.)], maize (*Zea mays* L.), rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), sorghum [*Sorghum bicolor* (L.) Moench], oats (*Avena sativa* L.), millet [mainly pearl millet (*Pennisetum glaucum* (L.) R. Br.)], rye (*Secale cereale* L.), and triticale (*Triticale hexaploide* Lart.). Cereal grains and the food products derived from them are an important part of human diets, providing energy and nutrients for the growth and health of humans. Grains from different cereal crops vary in the shape, size, structure, and chemical composition. Important components and representative energy values of selected cereal grains are shown in Table 1. Large contents of

Table 1 Chemical compositions and energy values of selected cereal grains (USDA-ARS 2014)

Grain	Carbohydrate (%)	Protein (%)	Lipid (%)	Total dietary fiber (%)	Sugar (%)	Energy (kcal/100 g)
Wheat, hard white	75.90	11.31	1.71	12.2	0.41	342
Wheat, soft white	75.36	10.69	1.99	12.7	0.41	340
Durum wheat	71.13	13.68	2.47	— ^a	—	339
Maize, yellow	74.26	9.42	4.74	7.3	0.64	365
Rice, brown	76.17	7.50	2.68	3.4	—	362
Barley	77.72	9.91	1.16	15.6	0.80	352
Sorghum	72.09	10.62	3.46	6.7	2.53	329
Oats	66.27	16.89	6.90	10.6	—	389
Millet	72.85	11.02	4.22	8.5	—	378
Rye	75.86	10.34	1.63	15.1	0.98	338
Triticale	72.13	13.05	2.09	—	—	336

^aNo information is available from the database

available carbohydrate (primarily starch) and protein contribute to the relatively high calorie densities of cereal grains (329–389 kcal/100 g). This was one main reason that cereal grains were originally harvested, cooked, and consumed as staple foods by humans for energy supply. With the development of food processing technology, an increasing proportion of cereal grains has been used as raw materials to produce a wide variety of food products to enrich our diets. Grains of wheat, maize, and rice are most widely used by the food industry due to their diverse functional properties and steady supply.

Carbohydrate is the predominant constituent of cereal grains, the content of which ranges from 66.27 % to 77.72 % (Table 1). Starch is the major form of carbohydrate reserve in cereal grains, and it is found as insoluble granules. Starch is composed of two types of polysaccharide: amylose and amylopectin. Amylose is an essentially linear polysaccharide connected by α -1,4 glycosidic bonds, whereas amylopectin is a highly branched polysaccharide consisting of about 5 % α -1,6 branch linkages (Hizukuri 1986). Amylose contents of starches from different cereal varieties are different: waxy, normal, and high-amylose starch contains 0–8 %, 20–30 %, and more than 40 % amylose, respectively (Jane 2009). Amylose content and structures of starch molecules remarkably affect the physical properties and digestion rate of starch, which can have significant impacts on the texture, storage stability, and nutritional value of cereal products.

Protein is the second largest component of cereal grains (7.50–16.89 %) (Table 1). Protein is a vital constituent with respect to the functional property and nutritional value of cereal grains. Proteins present in cereal grains vary in the amino acid composition and molecular weight. Traditionally, proteins can be classified

into four types based on the solubility in different solvents (Delcour and Hoseney 2010; Osborne 1924): (1) Albumins are soluble in water, and the solubility is not influenced by a low salt concentration; (2) globulins are insoluble in water but soluble in 0.5–1.0 M salt solution; (3) prolamins are soluble in 60–70 % ethanol aqueous solution, and the prolamins of wheat, maize, barley, sorghum, and oats are called gliadin, zein, hordein, kafirin, and avenin, respectively; and (4) glutelins are soluble in dilute acid or alkaline solution, and the glutelins of wheat, rice, and barley are called glutenin, oryzenin, and hordenin, respectively. The gliadins and glutenins in wheat flour, after being mixed with a proper amount of water, interact with one another to develop gluten networks and contribute to the formation of a viscoelastic dough. This special property of wheat flour makes it suitable for the production of various bakery products. Ingestion of wheat gluten and related proteins from barley and rye, however, has been identified to cause celiac disease in humans, an autoimmune disorder characterized by inflammation, villous atrophy, and crypt hyperplasia in the small intestine (Alaedini and Green 2005). Therefore, in the past decades much research attention has been paid to the development of high-quality gluten-free cereal products by replacing wheat, barley, and rye flour with other cereal flours (e.g., rice) and/or adding functional food ingredients (e.g., food gum and modified starch) (Lamacchia et al. 2014).

Lipid is a minor component of cereal grains, the content of which ranges from 1.16 % to 6.90 % (Table 1). Lipids present in cereal grains include a diverse group of compounds that are soluble in organic solvents. The major constituents are triglycerides, phospholipids, and glycolipids. Lipids in flour, particularly whole-grain flour, are prone to hydrolytic rancidity (mainly by the action of lipase) and oxidative rancidity (mainly by autoxidation and the action of lipoxxygenase) (Doblado-Maldonado et al. 2012). Despite being a minor constituent, lipids play a critical role in the organoleptic quality and storage stability of cereal flours and derived products. Other minor components present in cereal grains include non-starch carbohydrates (e.g., cellulose, arabinoxylan, β -glucan, and arabinogalactan), phytic acid, vitamins, and minerals. Some minor components have significant impacts on the functionality of cereal flours. For example, arabinoxylan in wheat flour can affect the water absorption, hydration rate, and dough development (Courtin and Delcour 2002).

The concept of “dietary fiber” is introduced from a nutrition point of view. According to the definition given by the American Association of Cereal Chemists (AACC), dietary fiber refers to the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the small intestine with complete or partial fermentation in the large intestine (AACC 2001). Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. The total dietary fiber contents of cereal grains vary between 3.4 % and 15.6 % (Table 1), and they can be classified into insoluble (e.g., cellulose) and soluble dietary fibers (e.g., water-extractable arabinoxylan and β -glucan). Because dietary fibers can provide health benefits to consumers, such as laxation, blood cholesterol attenuation, and/or blood glucose attenuation (AACC 2001), increasing dietary fiber contents of cereal foods has been a hot research topic for decades.

Among all the food products derived from cereal grains, bread, cookies/biscuits, cakes, pasta, noodles, and extruded snacks and breakfast cereals are the most popular with consumers globally. For the manufacture of these products, cereal grains are usually milled to flour first and then processed with the addition of other ingredients. This book chapter aims to provide a comprehensive overview of the formulas and systems used to convert raw cereal-based materials into various food products and to discuss how their quality, sensory attributes, and storage stability are affected by the interactions between different constituents.

Bread

Bread is a popular staple food commonly made from wheat. Consumers in different regions have their own preferences for “good-quality” bread. For example, baguette in France is distinguishable by the long shape as well as the hard and crispy crust, whereas such crust is unacceptable for pan bread in the USA. The fine cell structure of sandwich bread in the UK cannot be found in flatbread in the Middle East (Cauvain 2007).

Bread Formulas

Ingredients used to make straight-dough bread following AACC Method 10-10B (AACC 2000) and different specialty breads (Heenan et al. 2008) are listed in Table 2. Flour, water, yeast, sugar, and salt are the essential ingredients for breadmaking. The other ingredients are added to improve the quality, sensory attributes, and storage stability.

Breadmaking Process

Different breadmaking systems, including straight-dough, sponge-and-dough, liquid-sponge, and short-time breadmaking, have been summarized and compared by Delcour and Hoseney (2010). Different methods produce breads with different textures and flavors. Essentially, the basic operations of breadmaking include dough formation, fermentation, and baking as shown in Fig. 1.

Dough Formation: Breadmaking starts with weighing the ingredients (Table 2) and mixing them to form a smooth and homogeneous dough. The amount of water needed is determined by the water absorption characteristic of the flour, which can be measured using a farinograph or mixograph (Ram et al. 2005). Water absorption determines the mobility of different constituents in the dough and affects the interactions between them in subsequent processing. Mixing homogeneously hydrates the flour and provides the mechanical force required for the dough development.

Fermentation: Fermentation is a process that yeast utilizes available sugars in the dough to generate CO₂ and ethanol. The addition of malt (containing α -amylase

Table 2 Ingredients needed for the preparation of different types of bread

Bread	Ingredient
<i>Straight-dough bread</i> (AACC 10-10B)^a	Flour (14 % moisture basis, 100 %)
	Water (variable, optimum)
	Yeast (5.3 %) ^b
	Sucrose (6.0 %)
	Salt (1.5 %)
	Shortening (3.0 %)
	Malt flour (optional, 0.2 %)
	Ascorbic acid (optional, 40–50 ppm)
	Potassium bromate (optional, 10–20 ppm)
	Soy flour and/or nonfat dry milk and/or whey solids (optional), shortening-sparing compounds (optional)
Maturing agents (optional)	
<i>Specialty breads^c</i>	
Croissants	Wheat flour, yeast, milk, water, sugar, salt, butter, egg
Bagel	Wheat flour, yeast, milk, water, sugar, salt, butter
Focaccia	Wheat flour, yeast, milk, water, sugar, salt, olive oil, egg
White loaf	Wheat flour, yeast, milk, water, sugar, salt, butter, egg
Sourdough loaf	Wheat flour, whole meal flour, yeast, wild yeast, milk, water, sugar, salt, butter, egg
Brioche	Wheat flour, yeast, milk, salt, sugar, butter, egg
Pugliese	Wheat flour, drum flour, yeast, milk, water, sugar, salt, butter
Ciabatta	Wheat flour, yeast, milk, water, sugar, salt, butter
Rye	Wheat flour, whole meal flour, coarse rye flour, yeast, milk, water, sugar, salt, butter, molasses

^aThe information is from AACC Method 10-10B (AACC 2000)

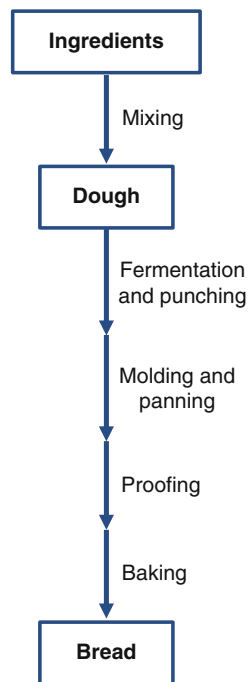
^bThe weights of other ingredients are on flour weight base

^cThe information is from Heenan et al. (2008)

and β -amylase) or α -amylase can release more sugars to accelerate the fermentation process. As the dough expands, the gluten molecules uncoil and expose more intermolecular hydrogen bonding opportunities, which enhances the strength of the dough. The fermented dough is usually punched to remove most of the CO₂, molded, portioned, and proofed before baking (Fig. 1). The punching step creates more but smaller gas cells via subdivision and redistributes the ingredients in the dough (Delcour and Hosenev 2010). The fermentation process affects the rheology of the dough and contributes to the crumb cell structure and flavor of the baked bread (Cho and Peterson 2010).

Baking: Baking refers to a process that the fermented dough is heated at temperatures between 200 °C and 250 °C for 20–45 min to obtain bread. In the early stage of baking, the activity of yeast drastically increases to generate more CO₂ until the yeast is killed at about 55 °C. Meanwhile, more CO₂ diffuses from the aqueous phase to the gas cells, and the gas expands as the temperature of the dough rises. These factors lead to a rapid increase in the dough volume in the early stage of baking, which is known as “ovenspring.” As heat is transferred from the external to the internal part of the dough, the temperature in the dough center rises, which

Fig. 1 A basic process for the making of bread



eventually gelatinizes starch and denatures protein. The gelatinized starch and denatured protein lose moisture and set the structure of the bread in the late stage of baking. Other important reactions taking place in the baking step include the Maillard reaction (between reducing sugars and amino acids, proteins, and/or other nitrogen-containing compounds) and caramelization of carbohydrates, which are responsible for the browning of the crust and the development of aroma (Cho and Peterson 2010; Purlis 2010).

Functions of Different Components in Breadmaking

Flour from common wheat is normally used for breadmaking, although flours from other crops, such as durum wheat and rye (Table 2), are also used in some regions. In North America, wheat is classified as soft and hard wheat according to the resistance of the kernels to crushing. Hard wheat varieties, the kernels of which are more difficult to crush, are used for breadmaking because of the higher protein content and stronger dough formation than soft wheat varieties.

After being mixed with an appropriate amount of water, wheat flour forms a cohesive and viscoelastic dough, which can retain the gas produced from yeast fermentation or chemical leavening and thus gives a light and aerated product after baking. The dough development is a result of the interactions between two major components of wheat gluten: gliadins (prolamin protein) and glutenins (glutelin protein). Gliadins and

glutenins account for 40–50 % and 30–40 % of the total protein in wheat flour, respectively (Shewry et al. 2009). Some critical structural features of gliadins and glutenins include the following: (1) Glutenins consist of high-molecular-weight and low-molecular-weight subunits, which can be linked via intermolecular disulfide bonds to form protein polymers with molecular weights of several million daltons (D'Ovidio and Masci 2004); (2) about 35 % of the total amino acids of the gluten proteins have hydrophobic side chains, which promotes hydrophobic interactions between protein molecules; (3) the predominant amino acid of gluten proteins is glutamine, which favors the hydrogen bonding between protein molecules; and (4) the gluten proteins are devoid of substantial charges, which suggests little charge repulsion between protein molecules. All these factors are accountable for the formation of a cohesive and viscoelastic wheat gluten structure (Delcour and Hosney 2010). Because of the large molecular weights, glutenin polymers form continuous networks that provide elasticity to the dough (Belton 1999). Oxidizing agents, such as ascorbic acid, potassium bromate (Table 2), potassium iodate, and azodicarbonamide, promote intermolecular disulfide bonding, and therefore, they can be used to enhance the dough strength for breadmaking. Reducing agents, such as cysteine and sodium metabisulfite, however, weaken the dough structure. The monomeric gliadins, on the other hand, are responsible for the viscous property of the dough. The gluten networks determine the crumb cell structure and affect the texture of bread after baking.

Despite being the predominant component of wheat flour (about 65–70 %), starch mainly acts as a filler in the dough formation. During baking, starch is gelatinized and becomes an important factor influencing the texture of bread. In general, it is believed that amylose gelation contributes to the initial firmness of bread after baking (Morita et al. 2002), whereas amylopectin retrogradation is partially responsible for the increase of crumb hardness during the storage of bread (Gray and BeMiller 2003; Zobel 1988).

In addition to hydrating different components in the wheat flour, water is a dispersing agent and medium for chemical reactions to occur. Yeast has leavening ability, producing CO₂ and ethanol from fermentable sugars. Sugars are the energy source for yeast, and they also impart sweetness and affect the crust color. In addition to functioning as a flavor enhancer, salt controls the yeast growth and thus the fermentation rate. Shortening has a lubricating function, which facilitates the expansion of gas cells in the dough for a larger loaf volume. Functions of different lipids in breadmaking have recently been reviewed by Pareyt et al. (2011).

Quality and Storage

Freshly baked bread can be recognized by its crispy crust, moist and soft crumb, and appealing aroma. During storage, the quality of bread deteriorates and becomes less palatable, which is known as staling. Major changes that occur in the staling process include a softer crust, a firmer and less elastic crumb, and loss of fresh flavor. Additionally, the bread is prone to mold growth if it is stored in a high-moisture environment.

Bread staling is a complex phenomenon because different changes occur to various components. Amylopectin retrogradation is generally accepted to be an important factor accountable for the increase of the crumb firmness during staling (Gray and BeMiller 2003; Zobel 1988). Another factor responsible for crust softening and crumb hardening is the migration of moisture from the interior of bread to the surface (Baik and Chinachoti 2000). The interactions between protein matrices and gelatinized starch have also been proposed to play a role in the staling of bread (Martin et al. 1991). The loss of fresh flavor in staled bread is attributed to the evaporation of desirable aroma compounds (e.g., 2-acetyl-1-pyrroline) and the retention of undesirable compounds [e.g., (*E*)-non-2-enal from lipid peroxidation] (Schieberle and Grosch 1992).

Because the staling of bread shortens the shelf life and causes substantial economic loss, retarding this process has attracted much research interest. Several approaches have been adopted to effectively control bread staling (Gray and BeMiller 2003): (1) adding α -amylases to the formula and the mechanisms for the use of α -amylases have been discussed in a recent review article (Goesaert et al. 2009), (2) adding emulsifiers (e.g., lecithins, monoglycerides, and sodium stearoyl lactylate) to delay starch retrogradation by forming single-helical complexes with starch molecules, and (3) storing bread at proper temperatures to minimize starch retrogradation, such as below $-20\text{ }^{\circ}\text{C}$ (below the glass transition temperature of starch) or above $30\text{ }^{\circ}\text{C}$. The use of suitable packaging materials can also retard bread staling as they slow down the loss of moisture and aroma compounds to the air.

Cookies/Biscuits

Cookies or biscuits are a group of small baked products made from wheat flour together with fat, sugar, water, and other ingredients. Different from bread that is usually consumed as a staple food, cookies/biscuits represent the largest category of baked snack products.

Formulas for Cookies/Biscuits

Formulas for the making of some representative cookies/biscuits have been summarized by Pareyt and Delcour (2008) as shown in Table 3. Flour, sugar, fat, and water are the essential ingredients for producing cookies/biscuits. By varying the proportions of different ingredients, cookies/biscuits with a variety of textures and shapes can be obtained. Contrary to breadmaking, soft wheat flour is commonly used for making cookies/biscuits. Sodium bicarbonate and ammonium bicarbonate are leavening agents, and sodium metabisulfite is added as a reducing agent. Other ingredients are incorporated to enhance the quality and sensory attributes of cookies/biscuits.

Table 3 Formulas for the making of selected cookies/biscuits (Pareyt and Delcour 2008)

Ingredient	Marie (type 1, %)	Marie (type 2, %)	Rich tea (%)	Cabin (%)	Gem (%)	Semisweet biscuits (sheet and cut, %)
Flour (9 % protein)	100	100	100	100	100	100
Sugar	19 ^a	20.8	25	10	17	19–25
Fat	13	16.1	20	5	12	13–20
Water	24	17.9	19	20	19	19–24
Syrup and/or malt extract	2	–	4	2	4	2–4
Nonfat milk powder	1.7	2.5	1.4	–	–	1.4–1.7
Salt	1	0.36	1	0.8	0.8	1
Acidulant	–	0.36	–	–	–	–
Sodium bicarbonate	0.4	0.54	0.6	0.8	0.8	0.4–0.6
Ammonium bicarbonate	1.5	0.54	0.4	0.8	0.4	0.4–1.5
Lecithin	0.26	–	0.4	0.1	0.24	0.26–0.4
Sodium metabisulfite	0.03	0.022	0.035	0.03	0.03	0.03–0.035

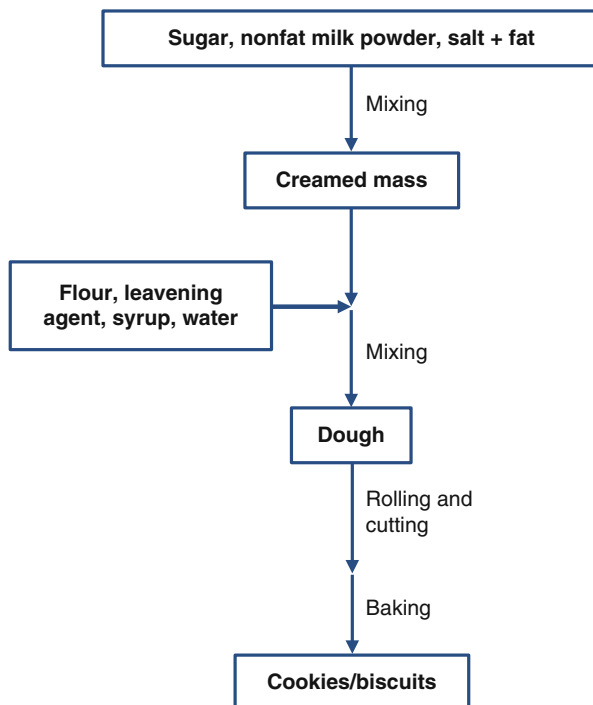
^aThe weights of other ingredients are on flour weight base

Preparation of Cookies/Biscuits

A typical process used to produce cookies/biscuits is shown in Fig. 2. The major operations include preparation of creamed mass, dough formation, and baking. The preparation of creamed mass begins with weighing and mixing dry ingredients, such as sugar, nonfat milk powder, and salt. The mixture is further blended with fat (e.g., shortening) to form creamed mass. Flour and other ingredients are weighed and mixed with a predetermined amount of creamed mass to form a dough. After rolling and cutting, the dough with a certain width and height is baked at temperatures between 180 °C and 220 °C for 8–15 min to obtain cookies/biscuits. In the early stage of baking, the dough spreads as a result of gravitational flow and leavening in the oven. At a certain time point, the spreading of the dough stops and the shape is fixed (Miller et al. 1997). In the final stage of baking, the Maillard reaction and caramelization occur, contributing to the browning color and flavor of the end product as discussed before.

Major differences between the making of cookies/biscuits and bread lie in the following: (1) Hard wheat flour that can form a strong viscoelastic dough is chosen for bread, whereas soft wheat flour that tends to form a weak dough is preferred for cookies/biscuits; (2) more sugar and fat but less water is used in the formula for cookies/biscuits (Tables 2 and 3); and (3) there are no fermentation and proofing processes involved in the making of cookies/biscuits. Consequently, cookies/biscuits and bread have very different textural characteristics, shapes, and flavors.

Fig. 2 A typical process for the making of cookies/biscuits



Functions of Different Components in Making Cookies/Biscuits

Soft wheat flour provides a matrix to embed other ingredients for the dough formation. Because of a relatively low protein content and weak strength, the dough from soft wheat flour has desirable extensibility for making cookies/biscuits. Reducing agents (e.g., cysteine and sodium metabisulfite, Table 3) can be added to break disulfide bonds between protein molecules to improve the extensibility. Starch, the predominant constituent of wheat flour, affects the dough rheology and the texture of baked cookies/biscuits. Wheat flour with a higher level of damaged or gelatinized starch has been reported to produce cookies with a smaller diameter (Donelson and Gaines 1998), which can be attributed to that the damaged or gelatinized starch absorbs more water and results in a higher viscosity of the dough. Therefore, wheat flour with a low content of damaged starch is preferred for making cookies/biscuits. Due to the high sugar level and low water content in cookie/biscuit dough, starch is not or only partially gelatinized during baking (Varrianomarston et al. 1980). The ungelatinized starch granules play a role of “filler” in the matrix of cookies/biscuits. Because starch is not fully gelatinized, starch retrogradation is not a major problem for the storage stability of cookies/biscuits.

Sugar not only contributes to the sweetness but also affects the structure and texture of cookies/biscuits. Sucrose with a smaller average particle size leads to

better spreading of the final product (Kissell et al. 1973). When sucrose content is high, cookies/biscuits with a hard texture are obtained because sucrose turns to a glassy structure upon cooling. Glucose syrup can be used to partially replace sucrose to prepare a softer product. Maltodextrins can be used to lower the sweetness and impart a more crispy structure (Finley et al. 1992). During baking, sugar competes with starch for water and thus has an influence on the degree of starch gelatinization. Sugar also contributes to the color and flavor as a result of the Maillard reaction and caramelization.

Fat dilutes the gluten networks and acts as a tenderizer to promote the extensibility of the dough. When fat is present at a high level, very little water is needed to obtain consistent softness. Fat also contributes to the sensory quality of cookies/biscuits. Endogenous lipids, despite being a minor component of wheat flour (~2.0 %, Table 1), have been shown to play a significant role in the dough rheology and the quality of cookies/biscuits (Papantoniou et al. 2003).

Water hydrates protein and starch and solubilizes the other ingredients in the formula for the dough development. The amount of water affects the rheology and spreading of the dough. It has been demonstrated that more water in the dough increases the spreading rate but shortens the time needed for setting the structure during baking. Therefore, final cookie diameter is not substantially affected (Miller et al. 1997).

Quality and Storage

Attractive quality characteristics that consumers look for in cookies/biscuits include good integrity, a tender texture, and appealing flavor. Because of the low moisture content (1–5 %), cookies/biscuits have a relatively long shelf life. During shipping and storage, cookies/biscuits should be protected from the absorption of moisture from the air and physical damage. The structure of cookies/biscuits makes them susceptible to physical damage. Consequently, cookies/biscuits are often packed in a primary container, such as a formed tray, and have flow wrap as the secondary packaging to avoid breakage and crumbling during shipping. As the product contains a high level of fat, rancidity from lipid oxidation is a major concern regarding the shelf life. The use of fat with good oxidative stability, addition of antioxidants [e.g., butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), α -tocopherol, and ascorbic acid], and proper packaging can effectively retard the reaction (Smith et al. 2004).

Cakes

Cake is another category of bakery goods enjoyed by consumers all over the world. Compared with bread and cookies/biscuits, the crumb of cake is characterized by its aerated structure and high moisture content. Generally, cakes can be classified into

Table 4 Formulas of foam-type and shortening-based cakes

Ingredient	Foam-type (AACC Method 10-15) ^a	Shortening-based (AACC Method 10-90) ^b
Flour	100 %	100 % (14 % moisture basis)
Sugar	285 % ^c	140 %
Fat	–	50 % (shortening)
Dried egg albumen	36.4 %	–
Dried egg whites	–	9 %
Nonfat dry milk	–	12 %
Leavening agent	1.4 % (monocalcium phosphate, monohydrate)	Variable, depending on the type
Salt	2.7 %	3 %
Water	268 % (added on 14 % moisture basis of flour)	Variable, optimum

^aThe information is from AACC Method 10-15 (AACC 2000)

^bThe information is from AACC Method 10-90 (AACC 2000)

^cThe weights of other ingredients are on flour weight base

two types according to the preparation method (Conforti 2006): (1) foam-type cakes that primarily depend on the foaming and aerating properties of egg for the crumb structure and volume, such as angel food cake, and (2) shortening-based cakes whose crumb structure is derived from the fat-liquid emulsion created during batter preparation, such as pound cake.

Cake Formulas

Formulas of foam-type and shortening-based cakes are shown in Table 4. The formulas of cakes contain larger proportions of sugar and water compared to those of bread (Table 2) and cookies/biscuits (Table 3). The flour used for cake making is commonly soft wheat flour that is chlorinated to pH 4.5–5.2 (Thomasson et al. 1995). High levels of egg and milk rich in proteins are incorporated into the formulas for both types of cake, and the proteins are vital for the buildup of the structure.

Preparation of Cakes

The processes for making foam-type and shortening-based cakes are depicted in Fig. 3. For foam-type cakes, the structure is built upon the foaming and aerating properties of egg proteins. Therefore, egg albumen is dissolved in water and then properly whipped to incorporate a desirable amount of air into the foam before mixing with other ingredients to obtain the batter. In contrast, for the shortening-based cakes, the ingredients are mixed in 2–3 subsequent mixing steps to form a fat-liquid emulsion in the batter (AACC Method 10–90, AACC 2000). Between

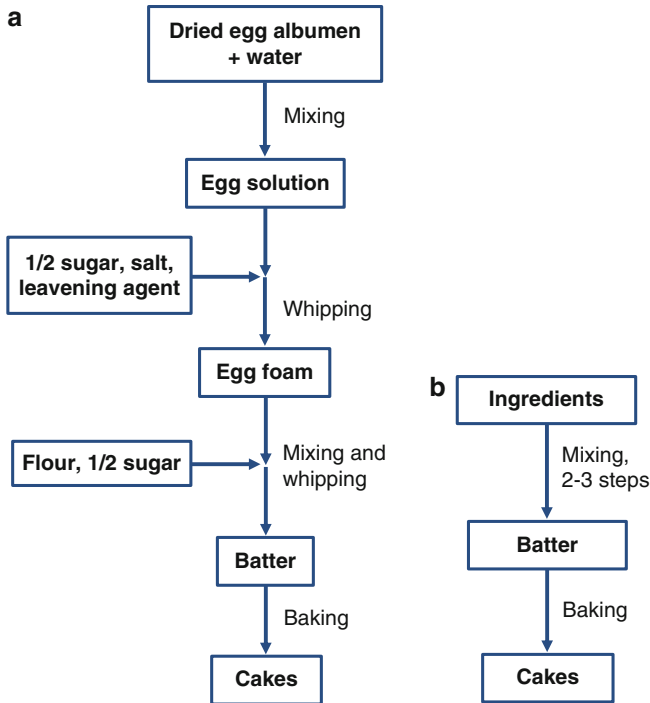


Fig. 3 Typical processes for the making of foam-type (a) and shortening-based (b) cakes. The making of foam-type and shortening-based cakes is based on AACC Methods 10–15 and 10–90 (AACC 2000), respectively

these two methods, foam-type batter allows the use of higher levels of sugar and water (Table 4). The batter is baked at temperatures between 175 °C and 200 °C for 30–45 min to obtain the cake.

Functions of Different Components in Making Cakes

In the breadmaking process, a large number of gas cells can be introduced to the dough through fermentation, punching, and proofing before baking (section “[Breadmaking Process](#),” Fig. 1). For the making of cakes, however, the gas cells are mainly created in the preparation of batter, and some cells are inevitably lost during mixing and baking. Thus, a batter with a suitable viscosity to maintain the gas cells is critical for an aerated and light final product. The batter viscosity is also important to prevent the separation of the dispersed phase that contains starch granules, fat, and air in order to obtain a cake with a uniform structure.

During baking, the major changes taking place include starch gelatinization as well as denaturation and coagulation of proteins, which lead to a significant increase in the viscosity of the batter and partially contribute to the setting of cake. One

important reason for the use of chlorinated soft wheat flour in cakes is that the chlorination treatment oxidizes starch and decreases its gelatinization temperature. Therefore, the starch can be gelatinized and swell at a lower temperature to increase the batter viscosity and set the structure at an earlier stage (Delcour and Hoseney 2010). This is quite important considering that the gelatinization temperature of starch is elevated by the high sugar content. The use of unchlorinated flour causes the cake to collapse in the oven because the starch is not properly gelatinized to support the structure. Chlorination results in additional changes in wheat flour, which are also important factors contributing to the cake structure (Sinha et al. 1997). An alternative method to improve the functional properties of wheat flour for cake baking is heat treatment (Thomasson et al. 1995). The so-called “heat-oxidized” cake flour is available in the market, and it meets the requirements for “clean label.” Also, flour with a lower protein content and a smaller particle-size distribution is generally preferred for the preparation of cake with a larger volume (Gaines 1985; Yamamoto et al. 1996). In addition to the components of flour, the denatured and coagulated egg proteins also help to build the structure.

Sugar contributes to the sweetness of cakes. Because sugar increases the gelatinization temperature of starch, the level of sugar has an impact on the texture of cakes. When sucrose is the only sugar used in the formula, the color of the cake tends to be pale because sucrose, a nonreducing sugar, cannot react with amino acids, proteins, and/or other nitrogen-containing compounds to impart browning color. A small amount of reducing sugar (e.g., glucose or fructose) or dry milk can be added to solve this problem.

Fat (usually shortening) is important for the formation of fat-liquid emulsion in the batter of shortening-based cakes, and it contributes to a tender structure, a moist eating quality, and a desirable flavor. When a high level of shortening is used, it results in a high viscosity of the batter to stabilize the dispersed phase. But if the level of shortening is too high, the plastic shortening melts as the temperature of the batter increases during baking, which causes a drastic decrease in the batter viscosity. This is detrimental to the cake quality. Therefore, an appropriate amount of shortening should be used for the optimum quality of the end product.

The addition of leavening agents (e.g., monocalcium phosphate, sodium bicarbonate, and sodium acid pyrophosphate) introduces more gas cells to the batter during whipping and early stage of baking, which contributes to a more aerated and light structure of the cake.

Quality and Storage

Aside from a high volume, other desirable characteristics of cakes include a tender texture, a moist mouthfeel, and a richness of flavor. Major changes that can adversely affect the quality of cake during storage include dry-out, starch retrogradation, and mold growth as a result of high moisture content. Other deteriorations, such as lipid oxidation and flavor loss, are also observed. Because of the fragile structure, physical damage is a major concern during the shipping of cakes.

The use of proper packaging is an effective method to prevent the dry-out, lipid oxidation, flavor loss, and physical damage of cakes. Addition of emulsifiers can be used to retard starch retrogradation (Gray and BeMiller 2003). The growth of molds can be controlled by lowering the water activity and/or adding certain preservatives (Smith et al. 2004). Sorbic acid, propionic acid, and/or their salts are added to the cake batter before baking for this purpose (Bennion et al. 1997; Smith et al. 2004).

Pasta

Pasta, originating from Italy, is a popular staple food mainly for its taste, convenience, and high nutritional value. Traditionally, pasta is made from semolina, coarsely milled flour (average particle size around 250 μm) from durum wheat (*Triticum durum* Desf.). Durum wheat is a separate species of wheat different from common wheat (*Triticum aestivum* L.). Kernels of durum wheat are characterized by the higher protein content (Table 1) and greater hardness than those of common wheat. Although flours from other botanical origins (e.g., rice and buckwheat) are also used for pasta production, our discussion in the chapter only focuses on the products made from durum wheat.

Pasta Formulas

Essential ingredients needed for the production of pasta include semolina and water. Important characteristics of semolina for high-quality pasta include high protein content and gluten quality, uniform particle size, and suitable contents of ash and pigments (mainly carotenoids and anthocyanins). Nonessential ingredients, such as egg, vegetables, and cheese, are also added to improve the texture, color, flavor, and nutritional value of pasta.

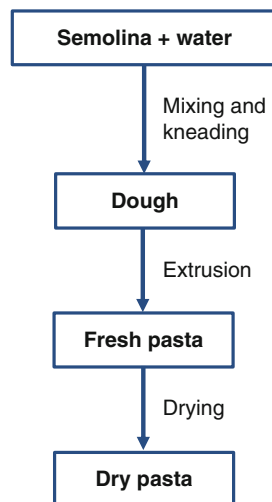
Preparation of Pasta

A common process used to produce pasta is shown in Fig. 4. The major operations are dough formation, extrusion, and drying.

Dough Formation: Semolina is mixed with water to reach a moisture content of 30–35 % and then kneaded to form a stiff and smooth dough. Different from the bakery products discussed above, the pasta dough is not subjected to fermentation or leavening treatment. In fact, the presence of air cells in the dough should be avoided because they reduce the brightness, translucence, and mechanical strength of the final product (Antognelli 1980). It is a common practice to knead the dough under vacuum condition to minimize air cell formation. The vacuum also prevents the oxidation of pigment compounds by lipoxygenase (Dexter 2004).

Extrusion: The formed dough is extruded to pass through a die to obtain fresh pasta with a certain shape. Single-screw extruder is usually used for the operation.

Fig. 4 A typical process for the making of pasta



Similar to other applications of extrusion technology, pasta is produced by conveying the raw semolina dough through the barrel under shear stress. The extrusion process applied to produce pasta is “cold extrusion,” in which the temperature is maintained below 50 °C to minimize starch gelatinization, protein denaturation, and heat damage to pigment compounds. The extruder barrel is commonly jacketed and cooled with water to keep the temperature low. Die and cutter are installed at the end of the extruder to obtain products with targeted shapes and sizes. The most popular products include long pasta, such as spaghetti and vermicelli, and short pasta, such as macaroni, farfalle, and ruote. Fresh pasta is sometimes sold in the market. But it is more common to dry the pasta products before distributing them to the market.

Drying: The fresh pasta is dried under controlled temperature and relative humidity to reduce the moisture content from approximately 30–35 % to 12–14 %. The drying step is critical to obtain pasta with uniform structure and color. Drying is a rate-determining step in the production of pasta. Traditionally, pasta is dried at 40–50 °C for 15–30 h to reach the targeted moisture content. High-temperature drying (60–100 °C) has become a more popular method in the industry due to improved productivity. Research has demonstrated that high-temperature drying leads to partial denaturation of wheat gluten and reduces starch granule swelling and amylose solubilization. Consequently, high-temperature drying improves cooking properties of pasta, such as longer al dente cooking time, increased firmness, lower water uptake, and decreased surface stickiness (Zweifel et al. 2003).

Functions of Different Components in Making Pasta

Semolina and water are the essential ingredients for the making of pasta. Semolina not only determines the structure of pasta but also imparts the color. After

hydration, mixing, and kneading, gluten proteins form networks surrounding starch granules. The networks are crucial for the structural integrity and firm texture of pasta. In general, a high ratio of glutenins to gliadins is preferred for a superior quality of pasta because of the greater gluten strength contributed by glutenins as discussed in the section “[Functions of Different Components in Breadmaking](#)” (Dexter and Matsuo 1980; Wasik and Bushuk 1975). The gluten protein networks become denser and more compact after extrusion and drying, which delays the penetration of water into the interior of pasta during cooking. Therefore, pasta has a longer cooking time than other similar products, such as noodles. Additionally, the protein networks hinder the enzymatic hydrolysis of starch in pasta, making it a low-glycemic staple food (Granfeldt and Bjorck 1991).

Hydration is one critical step for pasta making. The required amount of water is determined by the particle-size distribution of semolina. Because smaller particles absorb more water than the larger counterparts, a nonuniform particle-size distribution may lead to improper hydration of larger particles. This can cause white blotches in the final product (Manthey and Twombly 2005). Because the barrel temperature of the extruder is controlled below 50 °C, no starch gelatinization or protein denaturation occurs.

Quality and Storage

Pasta needs to be cooked before consumption. Water absorption, cooking loss, and texture are the basic criteria for judging the quality of cooked pasta. The cooked pasta is supposed to possess a firm “al dente” bite and a nonsticky surface. Dry pasta is commonly packaged using plastic bags or paperboard boxes and can have a shelf life up to 2 years under normal storage conditions. Major deteriorations in pasta quality during storage include changes in color and cooking properties, such as browning of pigments, easy cracking, and increased cooking loss. Due to its high moisture content, fresh pasta has a much shorter shelf life (1–2 days) than dry product. Suitable packaging (such as modified atmosphere packaging) and proper storage temperatures can be used to extend the shelf life of fresh pasta.

Noodles

Noodles, generally accepted to originate from China, are a major form of wheat consumption in East Asia (Fu 2008). Like pasta, noodles are another type of wheat-based product made from unleavened dough. The shape of noodles looks similar to that of some pasta products (such as spaghetti), but the raw materials and technologies used to produce them are quite different. A broad definition of noodle also includes those products made from rice flour or starch. Differences in the formulation and processing of wheat- and rice-/starch-based noodles are discussed in this section.

Table 5 Basic formulas of three types of wheat-based noodles (Adapted from Hou et al. 2010. Copyright © (2010), Wiley)

Ingredient	Chinese raw noodles	Japanese white salted noodles	Yellow alkaline noodles
Wheat flour (%)	100	100	100
Water (%)	28–32 ^a	32–40	28–34
NaCl (%)	1–2	2–5	1–1.5
K ₂ CO ₃ (%)	–	–	0.5
Na ₂ CO ₃ (%)	–	–	0.5

^aThe weights of other ingredients are on flour weight base

Formulas of Wheat-Based and Rice-/Starch-Based Noodles

Basic formulas of three types of wheat-based noodles are given in Table 5 (Hou et al. 2010). Wheat-based noodles are made from common wheat. Both hard and soft wheats are used for noodles, depending on the product type. NaCl (1–5 %, on flour weight base) is solubilized in water, and the resultant solution is mixed with wheat flour to form a dough. For the preparation of yellow alkaline noodles, *kansui* containing a low concentration of NaCl and alkaline salts (e.g., K₂CO₃ and Na₂CO₃) is used. Nonessential ingredients, such as egg, stabilizers (e.g., guar gum and locust bean gum), modified starches, and emulsifiers (e.g., lecithins and monoglycerides), are also added to the formulas to improve the appearance, texture, and flavor of the finished product. For the preparation of rice-/starch-based noodles, rice flour/starch and water are the essential ingredients. It is uncommon to include salt and other ingredients in the formulas of this kind of product.

Preparation of Noodles

Wheat-Based Noodles

The processing of different types of wheat-based noodles has been summarized by Hou (2001) (Fig. 5). The production starts with mixing wheat flour with salt solution or *kansui* to form a dough. Other ingredients, such as food gums, modified starches, and emulsifiers, can be added to improve the properties of the dough. The temperature of the added solution and the moisture content of the dough (about 28–35 %) are carefully controlled for easier handling of the dough. After mixing, the formed dough is rested for around 10–30 min for homogeneous hydration of dough particles and relaxation of gluten structure. The rested dough is then divided into two portions, and each piece is passed through a pair of sheeting rolls to form a noodle dough sheet. The two sheets are combined and passed through the sheeting rolls again to develop a smoother and stronger sheet. After resting the compounded dough sheet, it is subjected to further dough sheeting through a series of rolls (typically 4–6) with decreased roll gaps. It is crucial to sheet the dough in the same direction to achieve better alignment of gluten proteins for greater strength

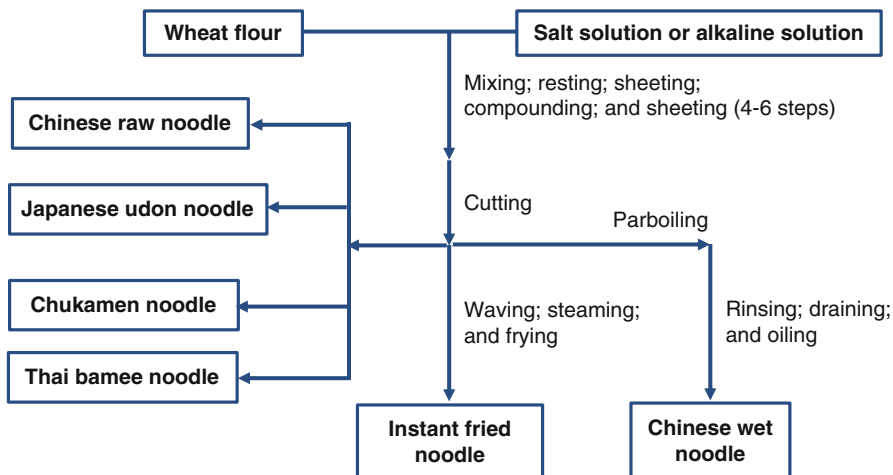


Fig. 5 Processing of different types of wheat-based noodles (Adapted from Hou 2001. Copyright © (2001), Wiley)

and improved physical integrity. The dough sheet is passed through the calibration rolls to reduce to a consistent thickness and then cut into noodle strands using a cutting machine. After dusting the noodle strands with fine flour or starch, the raw noodles can be sold as fresh. Examples of such products include Chinese raw noodles, Japanese udon noodles, chukamen noodles, and Thai bamee noodles. Similar to fresh pasta products, fresh noodles only have a shelf life of several days.

The fresh noodle strands are often further processed (secondary processing) to prepare products with a prolonged shelf life and unique organoleptic quality (Fig. 5): (1) instant noodles by waving, steaming, and subsequent deep-frying or air-drying to fix the structure and reduce the moisture content to 5–8 %; (2) Chinese wet noodles by parboiling followed by rinsing, draining, and oiling; (3) dry noodles by drying under controlled conditions; and (4) frozen noodles by freezing at a fast rate to reach 15–20 °C below 0 °C.

Rice-/Starch-Based Noodles

The processing of rice-/starch-based noodles (Fig. 6) is different from that of wheat-based noodles (Fig. 5). Because of the absence of gluten to form a cohesive and viscoelastic dough, the rice flour or starch needs to undergo thermal treatments to gelatinize the starch, which can then act as a binder to hold the shape of the noodles (Lu and Collado 2010). Popular rice-/starch-based products include “cut noodles” and “extruded noodles” (Fig. 6) (Lu and Collado 2010). The processing of “cut noodles” starts with the preparation of a rice/starch slurry, followed by steaming a thin layer of the slurry on a tray or a sheet. The gelatinized sheet is then sliced into strips to achieve the final product. “Extruded noodles” differ from “cut noodles” in that extrusion cooking is used to partially gelatinize the starch and shape the dough into threads or strings (Fig. 6). Like wheat-based noodles, fresh rice-/starch-based

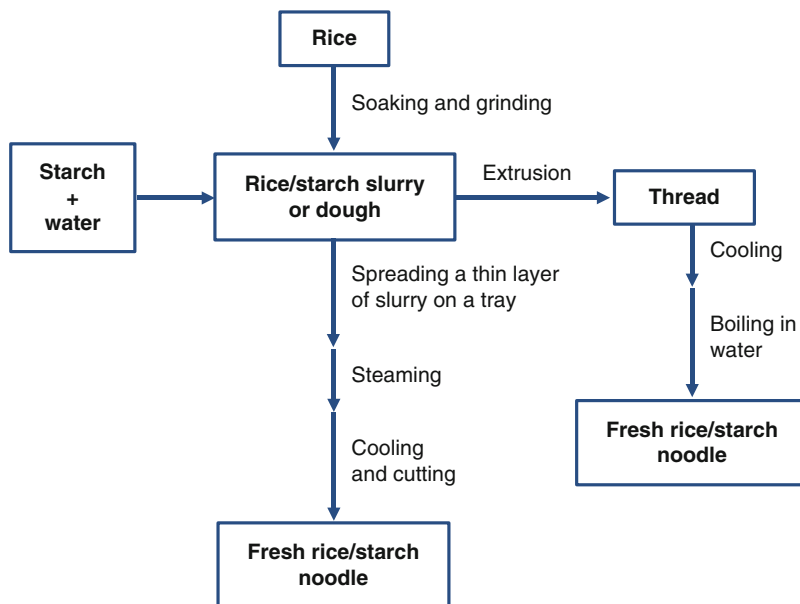


Fig. 6 Processing of fresh rice-/starch-based noodles

noodles are subjected to secondary processing, such as frying, drying, or fast-freezing, to manufacture a wider variety of products with a longer shelf life (Lu and Collado 2010). Rice-/starch-based noodles offer an alternative option of noodle products for celiac patients. Furthermore, rice-/starch-based noodles, particularly with a high degree of starch retrogradation, have a slow starch digestion rate and can provide potential health benefits to consumers (Panlasigui et al. 1992; Ranawana et al. 2009).

Functions of Different Components in Making Noodles

Wheat-Based Noodles

For the preparation of wheat-based noodles, wheat flour is the component for the buildup of the structure. The protein content and gluten quality of wheat flour determine the cohesiveness and elasticity of the formed dough, which eventually affects the texture of the noodles. Flour having a higher protein content and a larger proportion of glutenins generally produces noodles with a more chewy and elastic texture and greater hardness (Hou et al. 2013; Zhang et al. 2011). After cooking, starch in noodles is gelatinized and becomes an important factor influencing the texture of cooked noodles. Wheat flour with an increased amylose content in general results in increased hardness and cohesiveness of the cooked noodles (Zhang et al. 2011). The effects of damaged-starch content on the textural properties of noodles are not conclusive, depending on the level of starch damage and

noodle type (Oh et al. 1985; Zhang et al. 2011). Ash content of flour has a significant influence on the color of noodles. Most flours used for noodles require an ash content below 0.5 %.

The use of NaCl solution at a concentration of 1–5 % not only imparts salty taste but also strengthens and tightens the gluten structure to improve the texture of noodles (Hou 2001). The addition of *kansui* containing alkaline salts gives the noodles light yellow color and special flavor as well as toughens the dough to produce a firmer and more elastic final product (Hou 2001).

Rice-/Starch-Based Noodles

For the production of rice-/starch-based noodles, starch is the most important factor determining the texture. The gelatinized starch acts as an effective binder to hold the matrices together for the shape and texture of the noodles. To produce rice-/starch-based noodles, rice flour or starch with a relatively high amylose content (e.g., *indica* rice or mung bean starch) is preferred (Kasemsuwan et al. 1998; Lu and Collado 2010). Amylose, an essentially linear starch molecule, has a strong gelling ability and is responsible for the formation of strong starch networks in the noodles (Kasemsuwan et al. 1998). The gelatinized starch can be subjected to retrogradation for further reassociation between starch molecules, which enhances the network formation for a firmer product (Satmalee and Charoenrein 2009). The thermal treatment (steaming or extrusion cooking, Fig. 6) applied to gelatinize starch also causes protein denaturation. But the impacts of protein on the quality of rice-/starch-based noodles are minimal because of the low content (Table 1) and poor functionality of the protein.

Quality and Storage

Because of the diversity of noodle products, consumers in different regions have different preferences for the eating quality of noodles. For example, consumers in China in general prefer noodles with a chewy texture, whereas fresh noodles with a soft texture (such as udon) are more popular in Japan (Delcour and Hosney 2010). Fresh noodles, either wheat or rice/starch based, have a rather short shelf life (1–2 days). They are usually sold in local food markets or to the food-service industry. It is important to maintain the dough structure and to prevent the dry-out of the fresh noodles so that they can retain the good texture and fresh quality after cooking. One effective method to prolong the shelf life of fresh noodles without changing their eating quality is to freeze them to prepare frozen noodles. The fresh noodles are typically packaged first before being quickly frozen. It is critical to reduce the temperature from 0 °C to –5 °C at a fast rate to minimize the sizes of ice crystals (Hou et al. 2010). The temperature of the noodles is further brought to 15–20 °C below 0 °C. If the frozen noodles are stored properly, they can have a shelf life up to 1 year.

For instant and dry noodles, keeping the moisture content low is essential for maintaining a long shelf life. To prevent the absorption of moisture from the

environment, the noodles, together with a small bag of moisture absorbent, are packaged using materials with low moisture permeability (e.g., polyethylene, polyvinylidene chloride, and laminated plastic film) (Smith et al. 2004). Because of the high lipid content (about 15–25 %), major deterioration observed in instant fried noodles is the rancidity from lipid oxidation. Selection of frying oil with good oxidative stability, addition of antioxidants, and use of modified atmosphere packaging are effective methods to control the reaction. Instant noodles are commonly packaged with a separate seasoning bag, which can be added to the noodles before consumption and thus provides convenience for consumers.

Extruded Cereal Products

Extrusion is a continuous process in which a semiliquid material is cooked by a combination of heating, shearing, and pressure and then passed through a die at a predetermined flow rate to shape the product. The extrusion cooking discussed in this section is different from the cold extrusion applied to produce pasta (section “Preparation of Pasta”, barrel temperature < 50 °C) because the barrel temperature in extrusion cooking can reach 90–140 °C. The use of extrusion to produce snacks can date back to the late 1940s, and it is still a popular processing technology in the food industry due to its versatility, high productivity, and high energy efficiency. Extrusion technology is applied to produce snacks, breakfast cereals, bread products, meat analogue, and pet foods from various cereal grains.

Ingredients Used for Extruded Products

In extrusion processing, flour and water are the essential ingredients. Depending on the product, flours from various plant sources with different particle-size distributions are used. A combination of different flours or a blend of flour with starch and/or protein powder is also used in order to achieve the end product with a desirable expansion rate, good texture, and high nutritional value. In addition to hydrating the flour for the dough formation, water plays roles of plasticizer, heat conductor, and lubricant during extrusion. Nonessential ingredients, such as sugars, lipids, emulsifiers, and flavoring and coloring compounds, are also added to improve the quality of the product.

Extrusion Process

A typical extrusion process used to produce different foods is depicted in Fig. 7. After blending the dry raw material with water, the mixture is stored in the preconditioner for a period of time (from several minutes to hours) for homogeneous hydration. Preconditioning, however, is not required for all extrusion processing. The hydrated material is then transferred to the feeder and fed to the

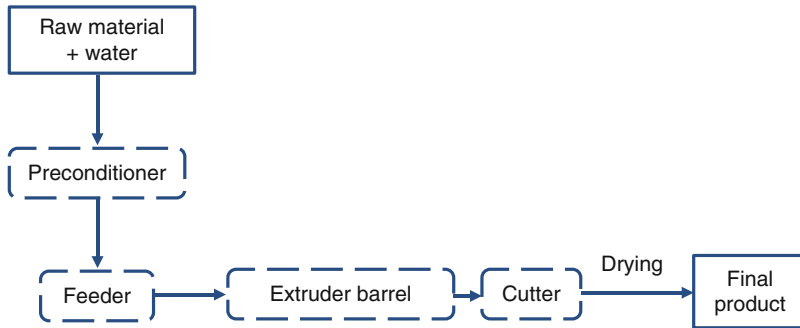


Fig. 7 Extrusion process applied to manufacture food products

extruder at a controlled rate. If necessary, more moisture can be added to the material in the extruder barrel.

In the extruder barrel, the hydrated raw material is further mixed and heated to form a dough, sheared, and conveyed to the end of the barrel. A high pressure is also built up inside the barrel. The input thermal (heating) and mechanical energy (shearing and pressure) causes physical and chemical changes in different components, such as starch gelatinization and degradation, protein denaturation and polymerization, Maillard reaction, and caramelization. Single-screw and twin-screw extruders are the most common types used for food production. Different variables, such as screw configuration, screw speed, and barrel temperature, can be manipulated to obtain extrudates with different textural characteristics, colors, and expansion rates.

At the end of the extruder barrel, the material is forced to pass through a die with specially designed opening. The opening area of the die can significantly affect the pressure in the extruder barrel. When the material exits the die, expansion occurs as a result of moisture flash-off. The resultant cell structure is critical for the texture of the derived extrudate. A cutter can be installed next to the die to cut the extrudate to a targeted length. The extrudate is usually dried to a low moisture content (<14 %) before storage.

Functions of Different Components in Extrusion

The dry raw material used for extrusion is an important factor determining the quality of the finished product. Flour with a smaller average particle size gives better homogeneity and finer cell structures to the extrudate. For raw material with a larger particle size (e.g., coarse grit), it becomes more challenging to completely gelatinize the starch and denature the protein because the relative surface area is small and the moisture and heat cannot penetrate into the interior of the particles easily. Therefore, raw material with an increased particle size tends to produce extrudate with a decreased expansion rate and an increased density (Garber et al. 1997).

Starch and protein are the key components for the buildup of the extrudate structure. A high starch content generally promotes the expansion and imparts a light structure to the extrudate, provided that the starch is properly gelatinized during extrusion (Kannadhasan et al. 2011). Extra starch can be added to raw material with a low starch content (e.g., bean flour) to promote the expansion. Normal rice, tapioca, and maize starches are preferred for this purpose because of their good expansion rates and bland flavor. Amylose content of starch has a remarkable influence on the expansion, and it has been observed that starch with 50 % amylose generally displays the optimum expansion (Chinnaswamy 1993). Contrary to starch, protein tends to reduce the expansion, and thus, raw material with a greater protein content often produces extrudate with a harder texture (Allen et al. 2007).

Feed moisture content plays a vital role in the extrusion process because it affects starch gelatinization and protein denaturation and thus the overall quality of the extrudate (Gomez et al. 1988; Lin et al. 2000). Lipids in the raw material play a role of lubricant, decreasing starch gelatinization and protein denaturation (Lin et al. 1997). Consequently, a larger lipid content of the raw material results in a denser extrudate. Incorporation of dietary fibers in general limits the expansion (Yanniotis et al. 2007). Sugars in the raw material affect the taste and impart browning color to the product as a result of the Maillard reaction and caramelization. Also, a low concentration (<2 %) of sugar or salt present in the feeding material increases the expansion rate of the extrudate, whereas a higher concentration exhibits a decreasing effect (Chinnaswamy and Hanna 1988; Hsieh et al. 1990).

Quality and Storage

One attractive property of extruded products is their long shelf life, which can be over 12 months if they are properly packaged and stored to prevent the absorption of moisture from the environment and to limit the exposure to oxygen and light. Due to the low moisture content, microbial spoilage and starch retrogradation are not major concerns. Enzymes that can potentially cause deteriorations in the quality, such as lipoxygenase, are denatured in the extrusion cooking. But lipids with good oxidative stability are still preferred for a long shelf life of the products.

Conclusions and Future Directions

Formulation, processing, and storage of the most popular cereal-based foods, including bread, cookies/biscuits, cakes, pasta, noodles, and extruded products, are reviewed in this chapter. Functions of various components in the formulas of different products are comprehensively discussed. These fundamental understandings are important to achieve high-quality, great-tasting, and storage-stable cereal-based foods. It is critical to note that the formulas of these products are often tailored and

the processing methods are usually modified to manufacture a diverse variety of products to meet the demands from consumers in different parts of the world.

Because cereal products are a very important part of human diets, more research attention will be paid to improving their quality and nutritional value to benefit human health. Future trends of research and product development will include the following: (1) boost dietary fiber content by incorporating different forms of dietary fiber, such as resistant starch and β -glucan; (2) lower the calorie density by using sugar and fat substitutes and functional ingredients; (3) develop palatable gluten-free products for consumers suffering from celiac disease; (4) increase the nutritional value of protein by incorporating proteins from other botanical sources, such as soybean, pea, and alfalfa; (5) expand the use of whole-grain and multigrain flours in different products; (6) increase antioxidant content by adding antioxidants from natural sources, such as fruit, vegetable, and tea; and (7) reduce salt content by using salt substitutes.

Cross-References

- ▶ [Chemical Composition of Bakery Products](#)
- ▶ [Chemical Properties and Applications of Food Additives: Flavor, Sweeteners, Food Colors, and Texturizers](#)
- ▶ [Chemical Properties and Applications of Food Additives: Preservatives, Dietary Ingredients, and Processing Aids](#)
- ▶ [General Properties of Major Food Components](#)
- ▶ [Overview of Food Chemistry](#)

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Abstract

For many years the egg has been subjected to negative publicity generally related to the cholesterol content which also resulted in decreased consumption. This was a negative attribute even though eggs offered many positive effects in the consumer's diet. Comprehensive research has now shown that dietary cholesterol does not significantly influence serum cholesterol. Eggs are now being recognized as a highly nutritious food with unique components which offer potential nutraceuticals with specific health benefits. With these recognized benefits, egg consumption has increased substantially in recent years. Much of this higher consumption has resulted from the increased use of eggs as an ingredient in a variety of further processed egg products. The polyfunctional property of eggs continues to make them the preferred ingredient in many food formulations. Also, eggs are considered a healthy food that does not increase serum cholesterol which fits well into high-protein low-carbohydrate diets. The egg is considered as nature's most perfect food containing excellent source of protein of high biological value, high ratio of unsaturated fatty acids to saturated fatty acids, and excellent source of minerals and all the vitamins. Vitamin C and lower concentration of calcium are the only nutrients lacking in eggs. The yolk provides all of the fat and contains half of the protein, most of the calcium, phosphorus, iron, zinc, and vitamins B₆, B₁₂, A, and folic acid, and half of the riboflavin and thiamine. Egg white contains about half of the protein and riboflavin.

Introduction

Unlike mammals, the embryos of birds are not fed by the mother during their development and therefore lack the ability to eliminate metabolic waste. Consequently, the egg yolk provides vital nutrients (protein, lipids, vitamins, and minerals) that are extremely well metabolized by the chicken embryo. Egg yolk is also a very attractive source of nutrients for humans. Its coefficient of digestive use is comparable to that of milk, and the biological value of proteins in the egg is even superior to that of milk proteins (Bourgeois-Adragna 1994). Because of its versatility, hen egg yolk is a multifunctional ingredient widely used in many food products. It possesses emulsifying, gelling, coloring, aromatic, and antioxidant properties. Each constituent of the yolk possesses unique physical and chemical characteristics responsible for its own functional properties. Environmental conditions and preservative treatment can influence and modulate these functional properties. Due to its

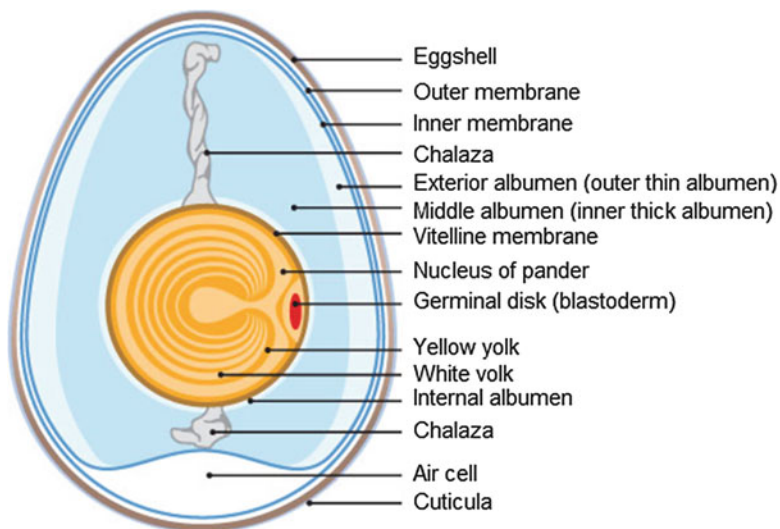


Fig. 1 Schematic drawing of egg

original role as an embryonic chamber, the yolk contains many constituents essential for life. Thus, the yolk is a major source of active factors usable in medical, pharmaceutical, cosmetic, nutraceutical, and biotechnological industries.

The Structure of the Egg

The parts of the egg are shown schematically in Fig. 1. It is generally accepted that a hen can produce an egg in about 2 weeks. This is true, except for the very small core of the yolk. At the time of hatching, the ovary of a female chick possesses many small ova – the number has been estimated to be over 3,000. The yolk of the egg is formed in three stages: (1) the part formed during embryonic development of the female chick, (2) the normal slow development of the ovum from the time of hatching of the chick to a point in sexual maturity some 10 days before ovulation, and (3) the accelerated growth period during the last 10 days before ovulation, after which it is released into the oviduct, a part of the female reproductive system. In the oviduct, the egg white is formed from the layers of secretions of the anterior section of the hen's oviduct to surround the yolk; finally, the shell membrane and shell are deposited to complete the egg formation process.

The yolk is formed during the final 10–12 days prior to the laying of the egg. Its structure consists of the latebra, germinal disk, and concentric layers of light and dark surrounded by the vitelline membrane. The yolk comprises 30–33 % of the total egg weight. At the time of ovulation, the yolk sac, or follicular membrane, releases the fully developed yolk into the open upper end of the oviduct.

The white, or albumen, of the egg is formed in a matter of a few hours and represents approximately 60 % of the total egg weight. The white occurs in four layers (see Fig. 1) in most chicken eggs. These are the chalaziferous or inner continuous with the chalaza. The next outer layer is the inner thin white (layer 3) surrounded by the outer thick white (layer 2). The outer layer of white is the outer thin layer (layer 1). The percentage of the total white found in each of the four layers varies widely, depending on the strain of the layering hen, age of the hen, and age of the egg.

The next layers of the egg are the inner and outer shell membranes. These relatively thin keratin-like membranes act as one of the egg's chief defenses against bacterial invasion. The inner membrane is thinner than the outer membrane, but together they are only 0.01–0.02 mm thick.

The outer shell largely consists of calcium carbonate (94 %), with other components including magnesium carbonate (1 %), calcium phosphate (1 %), and organic matter that are mostly protein (4 %). The shell color of the colored eggs is due to pigments (ooporphins) deposited on the shell surface. The shell is formed in a distinct pattern with pores for gas exchange. Even though the pores are partially sealed by keratin, they allow carbon dioxide and moisture to escape from the egg. Under some conditions, the pores also permit bacterial penetration as far as the shell membranes.

The last structural part of the egg is the air cell. This develops as a separation of the two shell membranes, usually at the large end of the egg, due to the shrinking of egg contents during cooling. The air cell continues to increase in size as moisture and carbon dioxide are lost throughout the existence of the intact egg (Romanoff and Romanoff 1949).

The process of egg formation is a complex series of hormone-controlled reactions, and the study of these reactions requires a thorough knowledge of reproductive physiology. The brief description given here is sufficient for a person interested in eggs as a human food or for commerce.

Physical Properties of the Egg

The viscosity of egg albumen is dependent on the age, mixing treatment, temperature, and rate of shear (Romanoff and Romanoff 1949; Tung et al. 1970). Albumen is pseudoplastic in nature at 32 °C between shear rates of 8.1 and 147/s. At a constant shear rate, the albumen viscosity decreases with time and approaches equilibrium within a few minutes. The egg yolk is a pseudoplastic non-Newtonian fluid. The shear stress (dynes/cm²)–shear rate (/s) relationship is linear. The particulate matter, or granules, in the egg yolk must be responsible for this nonlinearity, since the plasma (yolk without the granules) is essentially a Newtonian fluid.

The pH of albumen from a newly laid egg is between 7.6 and 8.5. During the storage of shell eggs, the pH of albumen increases at a temperature-dependent rate to a maximum value of about 9.7. After 3 days of storage at 3 °C, the pH of albumen

was 9.18. After 21 days of storage, the albumen had a pH close to 9.4, regardless of storage temperatures between 3 °C and 35 °C. The increase in pH is the result of the loss of carbon dioxide from the eggshell through the pores in the shell. The pH level is dependent on the equilibrium between dissolved carbon dioxide, bicarbonate ions, carbonate ions, and protein. The pH of freshly laid yolk is generally about 6, but during storage the pH gradually increases to between 6.4 and 6.9. At storage temperatures of 2 °C and 37 °C, the yolk reached a pH value of 6.4, in about 50 days and 18 days, respectively.

The Chemical Composition of the Egg

Egg Protein

The protein value of the whole egg protein is considered to be 100 and is used as standard for measuring nutritional quality of other food proteins. The addition of two eggs in the diet provides 12 g of protein, which will meet 30 % of the recommended dietary allowance in the United States. Protein is the major component of egg white; there are more than 40 different kinds of proteins that make up 11 % of its entire composition. Due to their functional and pharmacological properties, egg proteins are desirable ingredients in many baked foods. It follows that egg proteins are desirable in the drug industry. Egg white consists of a solution of proteins, containing the major proteins such as ovalbumin, ovotransferrin, ovomucoid, ovomucin, and lysozyme which account for >83 % of the total egg white proteins. Other minor proteins are also found at low concentration and account for <17 % of the egg white proteins. Egg white proteins are predominantly globular proteins having an acidic pI, the exception being lysozyme and avidin. Ovalbumin is the major protein and constitutes up to 54 % of the total egg white proteins. It typically serves as the major source of amino acids for the developing embryo. Ovotransferrin and ovomucoid constitute about 12 % and 11 % proteins, respectively (Sugino et al. 1997). Ovotransferrin is implicated in the transfer of iron to target cells and could therefore be used as a nutritional ingredient in iron-enriched foods. Other proteins include ovomucin, ovoglobulin, ovomacroglobulin, ovoglycoprotein, flavoprotein, ovoidin, avidin, and cystatin. Egg white also contains enzymes, such as lysozyme, phosphatase, and catalase. Among these enzymes, lysozymes constitute about 3.5 %. They are widely used in the food industry due to their antibacterial properties.

The egg proteins distributed in the yolk exist as lipoproteins, of which there are low density and high density. Low-density lipoprotein (LDL) is the major protein and accounts for up to 65 % of the total yolk proteins. The high-density lipoprotein exists as a complex with phosphoprotein known as “phosvitin.” About 80 % of phosphorus in eggs is contained in phosvitin, which is derived from vitellogenin formed in the liver (Sugino et al. 1997). Other yolk proteins include livetin, a water-soluble, non-lipid glycoprotein, and riboflavin-binding protein, a hydrophilic phosphoglycoprotein.

Egg Lipids

The lipids in eggs have attracted attention both at scientific and consumer levels due to the link between high dietary fat consumption and coronary heart diseases. The fat in the egg is exclusively in the yolk and comprises 5.5–6 g in an average 60 g egg. Almost all lipids are present in lipoprotein complexes within the yolk. Trace levels of lipids have been observed in the whites. Yolk lipids are made mainly of triacylglycerol, phospholipid, and free cholesterol. Triacylglycerol and phospholipids are the major components of yolk lipids, comprising up to 65 % and 32 %, respectively. Fatty acids may be of different chain lengths, degrees of saturation, and configurations. Because of the varying degrees of unsaturation in fatty acid and differing effects on health and well-being, these fatty acids are of interest to researchers. Consequently, the most significant characteristic of dietary lipids is the content of different types of fatty acids. Fatty acids are classified into three families, saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids (PUFA).

Egg Minerals

Minerals are contained within the egg yolk. The egg yolk contains 1 % minerals, with phosphorus as the most abundant mineral component. More than 60 % of the total phosphorus in egg yolks is contained in phospholipids. The major inorganic components of egg white are sulfur, potassium, sodium, and chloride. Phosphorus, calcium, and magnesium are next in importance. Table shows the content of major minerals in eggs (Table 1).

Table 1 Mineral content of edible egg portion and their approximate proportion in egg white and yolk

Nutrient	Whole egg (mg)	White (%)	Yolk (%)
Phosphorus	89	5	95
Chlorine	87.1	70	30
Sulfur	82	70	30
Sodium	63	90	10
Potassium	60	80	20
Calcium	25	8	92
Magnesium	5	80.0	20.0
Iron	0.72	5	95
Zinc	0.55	n/a	95
Iodine	0.024	5	95
Manganese	0.012	10	90
Copper	0.007	35	65

Based on 59 g shell weight, with 50 g total liquid whole egg, 33.4 g white, and 16.6 g yolk

Adapted from Cherian (2006), Table 153.4, p. 153–5

Abbreviation: *n/a* not applicable

Table 2 Vitamin content of egg edible portion and their approximate proportion in egg white and yolk

Nutrient	Whole egg (mg or µg)	White (%)	Yolk (%)
Vitamin A (IU)	317	n/a	100
Vitamin D (IU)	24.5	n/a	100
Vitamin E (mg)	0.70	n/a	100
Vitamin B ₁₂ (µg)	0.50	15.0	85
Biotin (µg)	9.98	25	75
Choline (mg)	215.1	0.2	99.8
Folic acid (µg)	23	4	96
Inositol (mg)	5.39	25	75
Niacin (mg)	0.037	80	20
Pantothenic acid (mg)	0.063	5	95
Pyridoxine (mg)	0.07	5	95
Riboflavin (mg)	0.25	60	40
Thiamine (mg)	0.03	7	93

Based on 59 g shell weight, with 50 g total liquid whole egg, 33.4 g white, and 16.6 g yolk

Adapted from Cherian (2006), Table 153.3, p. 153–4

Abbreviation: *n/a* not applicable

Egg Vitamins

The chicken egg is considered a good source of most vitamins, except vitamin C. As shown in the table, vitamins A, D, and E are located exclusively in the yolk. Choline, folic acid, and pantothenic acid are located mainly in the yolk. Niacin appears to be located mainly in the white. Comparing the two, it seems that of the components of the edible egg, the egg yolk contains the more significant percentage of vitamins of an egg. Eggs contain both fat-soluble and water-soluble vitamins (Table 2). Most fat-soluble vitamins are concentrated in the yolk. Although several factors, such as age, strain of bird, and age of bird, are involved, diet is the most important factor for regulating egg vitamin content. Transfer efficiency of a vitamin depends on the vitamin level in the diet, feed intake, rate of egg production, and egg weight. The transfer efficiency may vary between vitamins. For example, vitamin A has a transfer efficiency between 60 % and 80 %; riboflavin, pantothenic acid, and biotin have transfer efficiencies between 15 % and 25 %; and vitamin K, thiamine, and folacin have 5–10 % transfer efficiencies (Naber and Squires 1993).

Egg Shell

The egg shell is a complex compound composed of 95 % minerals, of which calcium is more than 98 %. Other inorganic components include phosphorus, magnesium, and trace amounts of iron and sulfur comprising less than 0.05 %. Egg shell powder is considered to be a good source of highly bioactive calcium and could be used as an ingredient for human consumption. Carbohydrates in egg shell

are composed of glycosaminoglycans that are anionic polysaccharides consisting of hyaluronic acid (48 %) and galactosaminoglycan (52 %) (Nakano et al. 2001). These carbohydrates have wide application in the cosmetics, pharmaceutical, and food industry.

Egg Shell Membrane

The egg shell membrane is composed of collagen-like proteins (collagen type I and V), in a ratio of 100 of type I to 1 of type V. Coarse fibers (2.5 μm in diameter) contain more type I collagen, while type V collagen predominates in the fine fibers (0.6 μm in diameter) and is largely located in the inner membrane.

The egg shell membrane contains several bacteriolytic enzymes, such as lysozyme and *N*-acetylglucosaminidase as well as other membrane proteins that have been through to have beneficial effects in treating injuries. The peptides derived from the membrane were shown to stimulate skin fibroblasts in vitro (Suguro et al. 2000). The egg shell membrane proteins are currently utilized as a cosmetic ingredient for their emollient properties.

Vitelline Membrane

The vitelline membrane, or yolk membrane, surrounds the yolk and prevents the egg yolk from mixing with the albumen. The membrane is the final barrier to microorganisms invading into the yolk. Along with being a physical barrier, it has the essential role in embryogenesis of allowing small molecules to cross the membrane. The dry weight of the membrane is 5–10 mg per egg depending on the size of eggs.

The vitelline membrane consists of the outer layer, continuous membrane, and inner layer. The outer layer contains ovomucin and lysozyme that are also found in egg white. Other compositions of the outer membrane are lectin, vitelline membrane outer protein I and II. The inner layer contains membrane glycoprotein I (27 kDa) and II (240 kDa), both of which play a structural role in the inner layer.

Proteins of the Albumen

Albumen may be regarded as a protein system consisting of ovomucin fibers in an aqueous solution of numerous globular proteins. The albumin proteins and their characteristics are presented in Table 3. The major proteins listed in the table are regarded as ovalbumin, conalbumin (ovotransferrin), ovomucoid, lysozyme, globulins, and ovomucin. The protein compositions of the thin and thick layers of albumen differ primarily in their ovomucin content. The ovomucin content in thick white is about four times than that of thin white. The principal protein fractions of albumen can be separated and purified by the stepwise addition of

Table 3 Proteins in egg albumen

Protein	% of albumen proteins	Molecular weight (kDa)	Characteristics
Major proteins			
Ovalbumin	54	45	Heat-stable polypeptide containing phosphorus and carbohydrate
Ovotransferrin	12	76	Metal-binding transport protein
Ovomucoid	11	28	Trypsin inhibitor
G2 globulin	4	30–45	–
G3 globulin	4	–	–
Ovomucin	3.5	5,500–8,300	Maintaining structure and viscosity of egg white
Lysozyme	3.4	14.3	Damage cell wall bacteria
Minor proteins			
Ovoinhibitor	1.5	49	Serine protease inhibitor
Ovoglycoprotein	1	24.4	Sialoprotein
Ovoflavoprotein	0.8	32	Riboflavin-binding protein
	0.5	769	Strongly antigenic protein
Ovomacroglobulin			
Cystatin	0.05	12.7	Thiol protease inhibitor
Avidin	0.05	68.3	Biotin-binding protein

Adapted from Etches (2008), Table 7.1, p. 293

ammonium sulfate and ion-exchange techniques using carboxymethyl cellulose and dimethylamino ethyl cellulose. Several electrophoresis methods have been used to characterize albumen protein fractions, including ovalbumins A1 and A2, globulins G2 and G3, ovoglobulins, conalbumin, and lysozymes.

Major Protein of Albumen

Ovalbumin

Ovalbumin, the predominant protein in albumen, is classified as a phosphoglycoprotein. This is because carbohydrate and phosphate moieties are attached to the polypeptide. A hen's ovalbumin sequence contains 385 amino acids. The N-terminal amino acid is acetylated glycine and the C-terminal amino acid is proline. The molecular weight of the polypeptide is 43,669 Da. Ovalbumin contains two phosphate residues on serines 68 and 344 that can be removed by phosphatases.

Purified ovalbumin is made up of three components. These are A1, A2, and A3, all of which differ in phosphorus content. Ovalbumins A1, A2, and A3 containing two, one, and no phosphate groups per molecule, respectively, are present in albumen fraction in relative portions of about 85:12:3. The molecule contains a carbohydrate chain attached at asparagine 292. Ovalbumin is the only albumen

protein to contain free sulfhydryl groups. Each ovalbumin molecule contains four sulfhydryl groups, three of which are reactive to *p*-chloromercuribenzoate in the native protein and the fourth in the denatured protein.

Ovalbumin in solution is readily denatured and coagulated by exposure to new surface (e.g., shaking) but is resistant to thermal denaturation. Heating of albumen at pH 9 to 62 °C for 3.5 min altered only 3–5 % of the ovalbumin, whereas heating albumen at pH 7 changed negligible amounts of this protein. During the storage of eggs, a proportion of the ovalbumin is transformed into a more heat-stable protein referred to as *S*-ovalbumin. The content of *S*-ovalbumin increases from 5 % in fresh eggs to 81 % in eggs cold stored for 6 months. At a heating rate of 10 °C/min at pH 9.0, the denaturation temperature of ovalbumin is 84.5 °C compared with 92.5 °C for *S*-ovalbumin (Donovan and Mapes 1976). It has been proposed that probably a thiol–disulfide exchange is involved in the conversion of ovalbumin to *S*-ovalbumin on storage (Smith 1964). Also, the greater stability, compactness, and hydrophobicity of the *S*-form contrast with that of ovalbumin (Nakamura and Ishimaru 1981).

Ovotransferrin

Ovotransferrin is a monomeric glycoprotein consisting of single polypeptide chain of 686 amino acids. The molecular weight of ovotransferrin is about 78 kDa; this constitutes 13 % of total proteins in egg white. This protein consists of two lobes, each containing a specific binding site for iron, although ovotransferrin does not contain iron in the egg. Copper, zinc, or aluminum may also bind to this site. It is the most abundant heat-sensitive egg white protein, and the complexation of iron or aluminum significantly increases its heat stability (Lin et al. 1994). The molecule does not contain iron in the egg, due to the low concentration of free iron, so that ovotransferrin has been known as a bacterial inhibitor. The denaturation temperature of ovotransferrin is the lowest among egg white proteins and forms aggregates by heating at 60 °C, like most heat-labile proteins in egg white (Matsudomi et al. 1991). However, iron-bound ovotransferrin, the holo-form, is relatively stable to chemical and thermal denaturation (Azari and Feeney 1958).

Ovotransferrin from egg albumen inhibits gram-negative bacteria by depriving bacteria of the iron source that is essential for their growth and survival (Lock and Board 1992). Ovotransferrin belongs to the family of transferrins. Transferrins are a metal-binding transport protein family in an *in vivo* preference for iron and widely distributed in physiological fluids. The antimicrobial activity of transferrins can result from a direct effect on the membranes: interaction of the cationic ovotransferrin with the anionic outer membrane of gram-negative bacteria (Valenti et al. 1986).

Ovomucin

Ovomucin is a macromolecule and heavily glycosylated glycoprotein, consisting of peptide-rich α -subunit and a carbohydrate-rich β -subunit. Ovomucin is a minor egg white glycoprotein (3.5 % w/w) with a molecular mass of approximately 165 kDa. It contains O-linked carbohydrate moieties that, upon formation of extensive hydrogen bonds with water, can give rise to a characteristic gel-like structure. Ovomucin

serves physical functions such as maintaining the structure and viscosity of egg white albumen, thus serving to prevent the spread of microorganisms (Ibrahim 1997), and possessing good foaming and emulsifying properties. Ovomucin plays a role in the decrease of the viscosity of thick white during storage of the egg.

Ovomucin has been shown to possess virus hemagglutination inhibition activity (Tsuge et al. 1996), antitumor activity (Oguro et al. 2001), immunomodulation activity (Tanizaki et al. 1997), and cholesterol uptake inhibition activity (Nagaoka et al. 2002).

Ovomucoid

Ovomucoid is a highly glycosylated protein (20–25 % carbohydrates, w/w) of 28 kDa. It constitutes 11 % of egg white proteins. It is a highly glycosylated protein consisting of 186 amino acids. It is also known to exhibit trypsin inhibitor activity passed from the albumen to the embryo during incubation. The molecule consists of three structurally independent tandem homologous domains (Domains Gal d 1.1, 1.2, and 1.3) possesses nine entities. Gal d 1.3 was reported as the immunodominant fraction (Rupa and Mine 2006). A large proportion of the weight of the ovomucoid molecule is carbohydrate, which is attached to asparagine in the sequences Asn-X-thr/ser in up to five places on the sequences. Ovomuroid is a serine proteinase inhibitor. Ovomuroid consists of three domains defined by the amino acid sequences 1–68, 69–130, and 131–186, each domain being cross-linked by three disulfide bridges (Kato et al. 1987). The carbohydrate moiety consists of three oligosaccharide units bound to protein through asparagine residues (Montgomery and Wu 1963). The polypeptide chain is composed of 26 % α -helix, 46 % β -structure, 10 % β -turns, and 18 % random coil (Watanabe et al. 1981). Ovomuroid is highly resistant to heat owing to its high cystine content. Under acidic conditions, ovomucoid can resist to heat treatments up to 100 °C, but it is rapidly denatured at 80 °C and pH 9.0 in the presence of lysozyme (Matsuda et al. 1982).

Lysozyme

Lysozymes can be found in egg albumen, the shell, and the vitelline membrane, belonging to a class of enzyme that lyses the cell walls of gram-positive bacteria. They, also known as muramidase or *N*-acetylmuramic hydrolase, are a relatively small secretory enzyme that catalyzes the hydrolysis of specific polysaccharides contained in cell walls of bacteria. The main activity of the lysozymes is to catalyze the hydrolysis of beta 1,4 bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine. These bonds stabilize the glycans in the cell walls of gram-positive bacteria. Since lysozyme has four disulfide bounds, this small protein molecule is unusually compact and heat stable. The thermal denaturation temperature of lysozyme is around 75 °C but depends on pH and medium conditions. However, it is rapidly inactivated by thiol compounds. In egg white, lysozyme is much more heat sensitive than when present alone (Donovan et al. 1975). Egg albumen is the most plentiful source of this enzyme to form biologically important complexes with other albumen proteins, especially ovomucin. Egg yolk inhibits lysozyme activity as a

result of electrostatic-complex formation with the yolk granules. Lysozyme from hen's egg white is a polypeptide of 129 amino acid residues having a molecular weight of 14.3 kDa. It is an elementary protein with the isoelectric point (pI) of 10–11 and is a strongly basic protein in egg white, well known for bacteriostatic, bacteriolytic, and bactericidal activity particularly against gram-negative bacteria. The protein represents only 3.4 % of the egg protein's total content. It is a good example for naturally occurring enzymes used in the food industry as preservative to maintain product quality and reduce the incidence of spoilage.

Penalbumin

The molecular weight of penalbumin is 61.6 kDa larger than ovalbumin (47.1 kDa). This protein has several features related to ovalbumin. In terms of the composition of penalbumin, it has more carbohydrate and lacks phosphate. The amino acid compositions are significantly different, but the differences could be explained if penalbumin is an extended form of ovalbumin.

Ovoglobulins

The composition of ovoglobulin contains 13.6 % of hexose, 13.8 % of hexosamine, and 3 % of sialic acid. Hexose occurs as mannose and galactose in the ratio 2:1, hexosamine as glucosamine, and sialic acid as *N*-acetylneuraminic acid. It has a minimum molecular weight, calculated from the tryptophan content, of 24,400 Da. The term ovoglobulin refers to ovoglobulins G2 and G3, each constituting about 4 % of egg albumen proteins. Ovoglobulins G2 and G3 are similar in many properties, including their molecular weight (49 kDa). In chickens, ovoglobulin G2 shows polymorphism.

Ovoglobulins are also of interest for commercial applications of albumen because they denature rapidly and may therefore have more effect on the initial foaming of the albumen than the more plentiful albumen proteins.

Minor Proteins of Albumen

Ovoinhibitors

Ovoinhibitor is a glycoprotein in egg white, composed of 447 amino acids with a molecular weight of 48 kDa. The sequence of ovoinhibitors contains seven Kazal-like domains involving 21 disulfide bonds. Like the ovomucoid, this protein is a proteinase inhibitor. It inhibits the activities of trypsin, chymotrypsin, and some proteinases of microbial origin. The oxidation of methionine residues of ovoinhibitor results in a loss of its inhibitory activity against chymotrypsin and elastase (Schechter et al. 1977). The domain I is a potent inhibitor of trypsin but is devoid of inhibitory activity against chymotrypsin, elastase, or proteinase K. Domains I to IV contain an arginine at the P1 position and are thought to inhibit trypsin-like enzymes. Domain V has a phenylalanine at P1, which is consistent with anti-chymotrypsin activity, and domains VI and VII possess a methionine at P1, making them likely to inhibit chymotrypsin and elastase.

The ovoinhibitor has been found to prevent the development of rotavirus-induced gastroenteritis in a mouse model and to inhibit the formation of active oxygen species by human polymorphonuclear leukocytes, which are associated with inflammatory diseases, mutagenicity, and carcinogenicity.

Ovomacroglobulin

The ovomacroglobulin is the largest globular protein in eggs, featuring a wide spectrum of immunological cross-reactivity among different avian species and inhibiting a wide range of proteases by physically engulfing the enzyme. This protein, also known as ovostatin, is composed of four subunits, each having a molecular weight of 175 kDa, with pairs of the subunits joined by disulfide bonds. Ovomacroglobulin inhibits hemagglutination, possesses anti-collagenase activity, and has inhibitory activity against diverse proteolytic enzymes representing serine, thiol, and metal protease (Li-Chan and Nakai 1989). It has demonstrated broad-spectrum inhibitory activity against various types of proteases, including serine proteases, cysteine proteases, thiol proteases, and metalloproteases (Molla et al. 1987). Enzyme inhibitory action by this protein is quite different from that of the low molecular weight inhibitors, such as ovomucoid. The hen's ovomacroglobulin differs from macroglobulins in the blood in that it does not contain a thiol ester. The protein denatures and precipitates on heating to 62–64 °C, pH 7.6. It undergoes a thermal transition at about 60 °C.

Cystatin

A member of a “superfamily” of cystatins, egg white cystatin belongs to the type 2 cystatin, which has about 115 amino acids and two disulfide bonds, but no carbohydrates. Secreted cystatin has a theoretical molecular weight of 13,147 Da. The major isoelectric forms differing by the occurrence of phosphorylation on serine 8 have been identified. The non-phosphorylated and phosphorylated forms display a pI of 6.5 and 5.6, respectively. Cystatin inhibits most of the cysteine proteases with 1:1 stoichiometry, including ficin; papain; cathepsins B, H, and L; and papaya peptidase. Cystatin is stable to heat but the effects of high temperatures are unusual. For example, heating at 100 °C for 3 min decreased the activity against ficin by 64 %, but further heating did not cause a further decrease.

Egg white cystatin has been shown to possess antibacterial activity, preventing the growth of group A streptococcus (Bjock et al. 1989), *Salmonella typhimurium* (Nakai 2000), and the periodontitis-causing *Porphyromonas gingivalis* (Travis et al. 1997).

Cystatin, a naturally occurring protease inhibitor, has fewer side effects than other synthetic protease inhibitors used in medical treatments. This protein can also induce the synthesis of various cytokines, resulting in upregulated nitric oxide release in vitro using mouse peritoneal macrophages (Verdot et al. 1996). It has also been shown in vivo, greatly reducing parasite numbers in a mouse model of visceral leishmaniasis (Korant et al. 1985).

Increased levels of cysteine proteases and the concomitant decrease of cystatin have been observed in various cancers; cystatin has been shown to inhibit tumor

invasion in Ras-transformed breast epithelial cells (Premzl et al. 2001). This would suggest that chicken cystatin may have a role in cancer therapy.

Riboflavin-Binding Protein

Ovoflavoprotein, also referred to as flavoprotein or riboflavin-binding protein, is a phosphoglycoprotein that is responsible for binding most of the riboflavin (vitamin B₂) in egg white. Flavoprotein is considered to have the highest Se content (1,800 ng/g) among egg white proteins. Ovoflavoprotein could serve as a useful food ingredient from egg white as it is abundant as a low-cost egg processing by-product. Ovoflavoprotein binds riboflavin at pH above 4.3 with an association constant of 7.9×10^8 M. It is composed of 219 amino acids (Hamazume et al. 1984) with a molecular weight of 32 kDa. The carbohydrate content is about 15 %, consisting of mannose, galactose, glucosamine, and sialic acid.

Chicken egg white riboflavin-binding proteins are the prototypes of a family that includes other riboflavin- and folate-binding proteins. One unusual characteristic of these molecules is their high degree of cross-linking by disulfide bridges. Another is in the case of avian proteins, where there are stretches of highly phosphorylated polypeptide chain. Each mole of riboflavin-binding apoprotein (apo-RBP) binds one mole of riboflavin with high-affinity constant (1.4 nM), causing loss of the characteristic riboflavin fluorescence.

Avidin

The avidin protein constitutes a maximum of 0.05 % of the total protein content of egg white. Avidin is an alkaline (pI 10.5), highly stable, tetrameric glycoprotein that is best known for its biotin-binding properties. Each of the four monomers binds one molecule of biotin and the avidin–biotin interaction, with dissociation constant of 10^{-15} M, as the strongest non-covalent interaction reported between protein and ligand. Each avidin chain, composed of 128 amino acid residues, is arranged in an eight-stranded antiparallel β -barrel, whose inner region defines the D-biotin binding site. A fairly rigid binding site is readily accessible in the apoprotein structure, making it sterically complementary to the shape and polarity of biotin.

Both chicken egg white avidin and its bacterial relative streptavidin are widely used as tools in a number of affinity-based separations, diagnostic assays, and a variety of other applications. Other applications include the potential of avidin as an insecticide and antimicrobial agent. Due to its high proportion of tryptophan residues, avidin is unstable under oxidizing conditions in strong light.

Thiamin-Binding Protein

Thiamin-binding protein can be isolated from the hen's egg albumen by using affinity chromatography. In terms of function, the protein binds thiamine (vitamin B₁) in a 1:1 ratio and is similar with avidin in that it is a vitamin scavenger. In terms of structure, the protein has a molecular weight of 38 kDa and does not contain carbohydrate. It is not usual in that it forms a stoichiometric complex with the albumen riboflavin-binding protein. An identical protein is present in egg yolk.

Vitamin B₂-Binding Protein

Vitamin B₂-binding protein has a molecular weight of 98 kDa. The vitamin-binding ability of this protein was heat labile in 2 h at 80 °C, but the complex was stable for 6 h at this temperature. Due to this difference, this albumen protein is distinguished from a B₁₂-binding protein in egg yolk.

Ovogloboprotein

Ovogloboprotein is a protein of the lipocalin family present in egg white. It represents about 1 % of the egg white protein. It is an acidic glycoprotein (pI 3.9) with a theoretical molecular weight of 20.3 kDa and a sugar content of 30 %. Despite the information just previously given, very little more is known about this protein. Currently, ovogloboprotein is mainly used as a chiral selector to separate drug enantiomers by high-performance liquid chromatography or capillary electrophoresis.

Enzymes in the Albumen

In addition to lysozyme, albumen contains many enzymes. These enzymes include those with phosphatase, catalase, and glycosidase activity (Sugino et al. 1997). An aminopeptidase has been isolated from albumen. It acts with broad specificity, hydrolyzing aliphatic, aromatic, and basic aminoacyl-2-naphthylamides, di- to hexapeptides, with a preference for methionine at the NH₂ end, and basic or bulky hydrophobic residues at the penultimate position (Skrtić and Vitale 1994). The enzyme is a hydrophilic, acidic glycoprotein with molecular weight of ~180 kDa and optimal activity at pH 7.0–7.5 and 50 °C. Amastatin, bestatin, and *o*-phenanthroline were found to be strong inhibitors, while Co²⁺ activated the enzyme (Skrtić and Vitale 1994). A dimeric glycoprotein containing one molecule of FAD per 80 kDa subunit was isolated from chicken egg white and found to have sulfhydryl oxidase activity on a range of low molecular weight thiols, generating hydrogen peroxide in aerobic solution.

Lipids of the Egg Yolk

Lipids are the main components (32–36 %) of the egg yolk solids. The composition of yolk lipid is generally about 65 % triglyceride, 28–30 % phospholipids, and 4–5 % cholesterol. However, the composition of yolk lipids can be affected by various factors including hen's age, genotype, and changes in the diet of the hens.

Fatty Acids

The predominant saturated fatty acids in eggs are palmitic (C16:0) and stearic (C18:0). The content of these two fatty acids in chicken eggs may range from 22 % to 26 % and 8–10 %, respectively. In addition to these two fatty acids, there are also other minor amounts of C14 and C20. The total saturated fatty acids may constitute up to 30–35 % of the total fatty acids in egg yolks.

Monounsaturated fatty acids (MUFA) in eggs are C16:1 and C18:1, which constitutes to 42–46 % of total fatty acids. Oleic acid (C18:1) is the major monounsaturated fatty acid in chicken eggs. Oleic acid has been reported to be hypolipidemic, reducing both cholesterol and triacylglycerol without decreasing high-density lipoprotein cholesterol in human patients (Mattson and Grundy 1985). Dietary lipids affect monounsaturated fatty acids in egg content. Addition of high oleic acid sunflower seeds in feed has been reported to increase the content of oleic acid in eggs up to 3.2 g, compared with 1.9 g in regular egg (Cherian and Sim 1993). Recent studies reported a significant decrease in egg oleic acid when diets contained conjugated linoleic acids (CLA) (Aydin et al. 2001; Cherian et al. 2002).

In comparison of polyunsaturated and monounsaturated fatty acids, the polyunsaturated fatty acid contents were significantly higher for eggs laid by 39-week-old hens compared with older hens, while monounsaturated fatty acid contents were significantly higher for eggs laid by 93-week-old hens. The contents of long-chain (20 and 22) omega-6 and omega-3 polyunsaturated fatty acids (PUFA) were 20 % and 25 %, respectively, higher in egg yolks from 21-year-old hens than 57-week-old hens. Egg size did not significantly affect yolk lipid or fatty acid concentration. However, lipid levels were lower while linoleic acid level was higher in yolks of eggs from hens older than 47 weeks of age than in those produced by younger birds. The unsaturated to saturated fatty acid ratio for yolk produced at 27 and 39 weeks of age was lower than that for yolk produced at 51 weeks.

There are two families of PUFA in egg, namely, n-6 and n-3 fatty acids. The predominant n-6 PUFA in egg lipids is C18:2n-6 (linoleic acid). Other n-6 fatty acids in eggs may include C20:4n-6, C22:4n-6, and C22:5n-6. The content of long-chain n-6 PUFA (LCPUFA) (>20-carbon) may vary from 1 % to 2 % and is reflected by the type of laying hen diet (Cherian and Sim 1991). The content of n-3 fatty acids in eggs is made of α -linoleic acids (18:3n-3) and docosahexaenoic acids (DHA, 22:6n-3). Among these, DHA is the major n-3 fatty acid in the egg. The α -linoleic content in regular eggs is under 1 % of the total lipids; DHA may constitute between 1 % and 3 %. The content of n-3 PUFA is a reflection of dietary fat. Addition of flax, fish oil, and marine algae in laying hen diet leads to significant increases in α -linoleic acid and DHA in eggs (Cherian and Sim 1991).

Sim (1998) described the development of “designer egg” rich in omega-3 PUFA such as α -linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which have been associated with beneficial effects for human health. The beneficial effects include reduction of triglyceride level, lowering of blood pressure, decrease in platelet aggregation, and a decrease in tumor growth. Many studies have investigated the incorporation of different feed ingredients such as fish oil, vegetable oils (including flaxseed or linseed), soy oil, canola oil, and microalgae into the diet of hens, in order to optimize the omega-3 to omega-6 and PUFA to saturated fatty acid ratios of eggs for human health.

Products enriched with PUFA are prone to oxidation and the enrichment with antioxidants is necessary in order to prevent the risk of oxidative damage. Grune et al. (2001) suggested supplementation of feed with least 80 IU vitamin E/kg to prevent increase in cytotoxic aldehydic lipid peroxidation during production and

storage of omega-3 PUFA-enriched eggs. Dietary vitamin E resulted in a decrease of PUFA, SFA, and total lipids in fresh yolk lipids, whereas MUFA did not change. Also, dietary E supplement slowed down the process of oxidation of egg yolk fatty acid during storage.

Several studies have also investigated the effect of dietary conjugated linoleic acid (CLA) on the composition of egg yolk lipids. The levels of CLA incorporated into the lipid of the egg yolk were proportional to the levels of CLA in the diet, although more CLA was incorporated in the triacylglycerol than were phospholipid components. The incorporation rate of differing CLA isomers in different classes of lipids was significantly different. Furthermore, the inclusion of CLA in the diet also influenced the metabolism of polyunsaturated fatty acids. The amount of arachidonic acid was decreased by CLA added to linoleic acid and linolenic acid-rich diet, but EPA and DHA were increased in the linolenic-rich diet, indicating that synthesis or deposition of long-chain n-3 fatty acids was accelerated after CLA feeding. Despite this, increases in saturated fatty acids in yolk and decreases in MUFA and PUFA by dietary CLA have also been reported (Watkins et al. 2003). Feeding conjugated linoleic acid-enriched diets resulted in gradually increasing deposition of CLA isomers in egg yolk lipids, while feeding CLA led to accumulation of isomer in polar and neutral lipids of the egg yolk that migrated into the egg albumen (Watkins et al. 2003).

Aydin et al. (2001) reported that olive oil prevented CLA-induced increases in 16:0 and 18:0 and decrease in 18:1 (ω -9) in yolk and also prevented CLA-induced abnormal changes in the pH of albumen and yolks. Hur et al. (2003) indicated that lipid oxidation of the egg yolk during cold storage could be inhibited by dietary CLA due not only to the change in fatty acid composition but also to the high concentration of CLA in egg yolk. Szymczyk and Pisulewski (2005) reported that dietary vitamin E increased the rate of laying and egg production per hen and may also exert alleviating effects on fatty acid composition of CLA-enriched eggs.

Phospholipids

The major components of egg yolk phospholipids (PL) are phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which may make up ~81 % and 12 % of egg yolk lecithin; lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), and sphingomyelin are also known components of yolk PL. The major fatty acids in egg PC are palmitic, oleic, stearic, and linoleic acids, represented 32 %, 26 %, 16 %, and 13 %, respectively; arachidonic and docosahexaenoic acids (4.8 % and 4 %, respectively) are also present in significant amounts.

Yolk phospholipid content, expressed in relation to weight of egg oil or whole egg, was reported to be positively related to hen's age. Egg from hens receiving low-dose chitosan treatment contained a 1.8-fold increase in yolk phospholipid level (Vrzhesinskaia et al. 2005).

Kivini et al. (2004) studied the influence of oil-supplemented feeds (containing 15 % vegetable-based or fish oils) on the concentration of the phospholipid content

and their composition in hen eggs. Also, the total phospholipid contents and proportions of PC, PE, and sphingomyelin were similar for all feeding groups. The supplemented feeds had a significant ($p < 0.05$) effect on the fatty acid composition of phosphatidylcholines. Furthermore, supplements decreased the proportion of saturated fatty acids in total fat, but not in the phospholipids.

Shimizu et al. (2001) investigated effects of feeding dietary fish oil to hens on the fatty acid composition of eggs. A variation of fatty acid composition in egg yolks was found in the acyl groups of PC and PE rather than in TG. The results showed that the supplementation of the diet of hens with fish oil altered essential fatty acid composition. In particular, increasing DHA and decreasing arachidonic acid in egg yolk phospholipids did this.

Nakane et al. (2001) reported growth factor-like lipids in hen egg yolk and white, which were associated with high amounts of lysophosphatidic acid (acyl LPA) and small amounts of the lysoplasmanic acid (alkyl LPA). The levels of acyl LPA in hen egg yolk (44.23 nmol/g tissue) and white (8.81 nmol/g tissue) were on the same order as or higher than the levels of acyl LPA containing predominantly saturated fatty acids as the acyl moiety and egg white acyl LPA containing primarily PUFA.

Many studies have been conducted on methods for extraction and separation of phospholipids or lecithins from egg, as well as preparation of lysolecithin by the enzymatic action of phospholipase A2 (PLA2), including immobilized PLA2. In addition to providing sources of purified phospholipids for basic research, these methods have been established to meet the demand to produce purified egg lecithin for pharmaceuticals, nutraceutical, and food applications. Examples of beneficial properties of yolk phospholipids, with potential industrial applications as nutraceuticals and functional food ingredients, include anti-oxidative activity (Sugino et al. 1997) and inhibition of cholesterol absorption.

Fat-Soluble Vitamins

Most egg vitamins, especially the fat-soluble vitamins, are contained in the yolk. Hen egg is considered a source of most vitamins necessary for human nutrition, except vitamin C. One egg may supply almost 12 % vitamin A, more than 6 % of vitamin D, 9 % riboflavin, and 8 % pantothenic acid of the recommended daily allowance in the United States. Only fish contains more vitamin D than eggs.

The antitumor activity of carotenoids and retinoids plays an important role in scavenging peroxide radicals. Modified eggs enriched in α -tocopherol, β -carotene, and retinol are obtained by supplementing these substances in the hen's feed and are highly regarded nutritionally.

Pigments in Yolk

The color of the yolk is an important factor of the consumer acceptability of commercial eggs. The natural pigments in egg yolk are carotenoids that are

conjugated isoprene derivatives. Among carotenoids, lutein and zeaxanthin are incorporated to a larger extent than *b*-carotene and astaxanthin. A large proportion of the yolk pigments is transported through the blood from the intestine by lipoproteins, which are normally deposited in the yolk. The functions of pigments are not known clearly, but the health of the chick after hatching may be improved by these carotenoids.

Proteins of the Egg Yolk

Yolk lipoprotein precursors such as very low-density lipoproteins (VLDL) and vitellogenin are synthesized in the laying hen's liver and are transported in the blood to the oocyte. VLDL consist of apoVLDL II and apolipoprotein-B (Burley et al. 1984). ApoVLDL II is the only apoprotein from blood lipoproteins to be transferred to the yolk without any modification and is called apovitellenin I (Dugaiczuk et al. 1981). The source of yolk low-density lipoproteins (LDL) is VLDL. During the transfer from the blood to yolk, apolipoprotein-B is cleaved into several fragments, referred as apovitellenins I–VI (Burley et al. 1993). Vitellogenin consists of three species designated vitellogenins I, II, and III (Wang and Williams 1980; Wang et al. 1983). Vitellogenin is cleaved into the yolk granule proteins lipovitellin I and II and the phosphoprotein phosvitin. Amino acid analysis indicated the presence of a highly phosphorylated phosvitin in vitellogenins I and II and small phosvettes derived from vitellogenin III (Wallace and Morgan 1986). Schmidt et al. (1956) separated the granules by subjecting yolk to a centrifugal force of 20,000 g and granules consisted of 11–13 % of the solids in yolk and contained both lipoprotein and phosphoprotein, and most of the iron and calcium of the yolk. This was confirmed in a more detailed investigation by Burley and Cook (1961), who reported that the granules represent about 19–23 % of the yolk solids on a dry weight basis and consisted of 70 % high-density lipoproteins (HDL), 16 % phosvitin, and 12 % LDL. These authors concluded that LDL is the structural constituents of the granules. Granules are mainly constituted by HDL and phosvitin, and HDL–phosvitin complex is the basic unit of granules linked by phosphocalcic bridges between the phosphate groups of their phosphoseryl residues (Causeret et al. 1991). Plasma forms the aqueous phase where yolk particles are in suspension. It comprises 77–81 % of yolk dry matter and is composed of 85 % LDL and 15 % livetins (Burley and Cook 1961).

Lipovitellin Apoproteins

Yolk proteins in the water phase consist of lipoproteins (30 %) and soluble proteins (8 %). High-density lipoprotein consists of α - and β -lipovitellins, which differ in amino acid composition as well as bound phosphorus and carbohydrates. The proportion of α - and β -lipovitellins in yolk granules appears to be genetically based. The protein content of lipovitellin is about 80 % while lipid content is

about 20 %, including phospholipids (60 % of the lipid, primarily a lecithin), triacylglycerols (40 %), and small amounts of cholesterol, sphingomyelin, and other lipids. Both lipovitellins are glycoconjugates with mannose, galactose, glucosamine, and sialic acid, but α -lipovitellin contains much higher sialic acid content than does β -lipovitellin, explaining its relatively acidic nature (Juneja and Kim 1997). The apoprotein form of lipovitellins, sometimes referred to as vitelline, is present in a dimeric form linked through hydrophobic interactions; delipidation of lipovitellin has been reported to result in loss of solubility (Juneja and Kim 1997).

Yamamoto and Omori (1994) studied anti-oxidative activity of egg yolk lipoproteins and apoproteins in a linoleic acid emulsion system. High-density lipoprotein was a more effective antioxidant than LDL, and apo-HDL was more effective than apo-LDL. The lipid moiety of HDL also had an anti-oxidative effect on linoleic acid directly or in emulsion and possibly enhanced the anti-oxidative activity of the lipoproteins.

Kassaify et al. (2005) conducted in vitro experiments using confluent Caco-2 cell monolayers to investigate adhesion elimination, adhesion prevention, and antimicrobial properties of various extracted granule and plasma fractions against *Salmonella enteritidis*, *S. typhimurium*, and *Escherichia coli* O157:H7. The result revealed that the granule component HDL was the yolk fraction with protective effect against the foodborne pathogens, and this protective activity was confirmed to remain intact despite peptic and tryptic enzymatic digestion. Thus, the granule component has an anti-adhesive effect but no antimicrobial effect.

The low-density lipoprotein of the egg yolk is the most abundant non-water phase of the egg yolk. It is about 60 % of the dry weight of egg yolk. The lipoproteins can be separated by high-speed centrifuging, gel chromatography, and ion-exchange chromatography. Yolk low-density lipoprotein from egg yolks contains about 12 % of protein, the rest being neutral and phospholipid.

Apovitellenins

The only apoprotein from blood lipoproteins to be transferred to yolk in large amount without any modifications is apoVLDL II, called apovitellenin I in the yolk. Apovitellenin I is a small protein of low molecular weight that lacks histidine with a small homodimer with disulfide-linked subunits of 9 kDa. Apovitellenin II is also isolated from egg yolk low-density lipoprotein. The protein's molecular weight is 20 kDa with polysaccharide residues of glucosamine, hexose, and sialic acid. The functions of both apovitellenins are not clearly understood, but their properties appear to be an essential part of the lipoprotein structure. Apovitellenins III and IV with molecular weight more than 60 kDa are isolated from the total apoprotein mixture of egg yolk low-density lipoprotein by gel and hydrophobic chromatography.

Livetin

Livetin is water-soluble protein that accounts for 30 % of the plasma proteins and is composed of α -livetin (serum albumin), β -livetin (α 2-glycoprotein), and γ -livetins

[γ -globulin immunoglobulin Y (IgY)] (Sugino et al. 1997). The mean molecular weights of α -, β -, and γ -livetins are reported to be 80,000, 45,000, and 170,000 Da, respectively. The relative proportion of the three livetins in the yolk is 2:5:3, respectively.

α -Livetin

Egg yolk α -livetins and chicken serum albumin are identical. It has a molecular weight of 70 kDa and isoelectric point of 4.3 and 5.7. Chicken serum albumin (α -livetins) has been implicated as the causative allergen of the bird egg syndrome. Chicken serum albumin is partially heat-labile inhalant. IgE reactivity to chicken serum albumin was reduced by nearly 90 % by heating for 30 min at 90 °C.

β -Livetin

β -livetins has been identified as a 45 kDa α 2-glycoprotein. Chemically, its composition includes 14.3 % nitrogen and 7 % hexose. Unfortunately, not much information is available about this protein and the existing data available is ambiguous.

γ -Livetin (IgY)

The γ -livetins in yolk are transported from the blood serum of hens. Of the three immunoglobulins (IgM, IgA, and IgG) found in the serum, the laying hens transfer IgG to yolk at concentration of ~25 mg/ml, whereas IgM and IgA are transferred to egg white at concentrations of 0.15 and 0.7 mg/ml, respectively. Morrison et al. (2002) identified several regions within the antibody molecule important for its uptake into the egg yolk. Intact Fc and hinge regions, but not the Fc-associated carbohydrate, are required for transport.

The basic structure of all immunoglobulin molecules is a unit consisting of two identical light polypeptide chains and two identical heavy polypeptide chains. These chains are linked together by disulfide bonds. Typically avian plasma contains IgY plus IgA and IgM, which are evolutionary different from five distinct classes of mammalian immunoglobulins: IgG, IgA, IgM, IgD, and IgE. Although IgY antibody is functionally equivalent to mammalian IgG, they have profound structural differences.

The γ -globulins or γ -livetins in yolk are referred to as immunoglobulin Y (IgY) to distinguish them from mammalian IgG. Although IgY is derived from hen serum IgG, it differs in many chemical and structural features from mammalian IgG (Kovacs-Nolan and Mine 2004). Both IgG and IgY contain Asn-linked oligosaccharides, despite the composition of the oligosaccharides being different. Like IgG, yolk IgY contains two heavy chains (H) and two light chains (L), but the molecular weight of the H chains of the IgY is greater than those of mammalian IgG, yielding an overall molecular weight of 180 kDa compared to 150–160 kDa for mammalian IgG. Furthermore, IgY H chain lacks hinge region and processes four constant regions and one variable domain, whereas the IgG H chain contains a hinge region between the first two of three constant domains, which lead to flexibility of the Fab fragments. The average molecular weights of IgY, heavy-chain, and Fab fragments are 167, 65, and 45 kDa, respectively. Peptic digestion degrades IgY into Fab

Table 4 Comparison of chicken IgY and mammalian IgG

Character	IgY	IgG
Molecular weight	180 kDa	150 kDa
Isoelectric point	>acidic	<acidic
Heat stability	>sensitive	<sensitive
pH stability	>sensitive	<sensitive
F _c receptor-binding activity	Low	High
Protein A/protein G binding	No	Yes
Interference with mammalian IgG	No	Yes
Interference with rheumatoid factor	No	Yes
Complement activation	No	Yes

fragments, in contrast to disulfide-linked F(ab')₂ fragments generated from IgG. IgY is relatively heat stable even after heating to 65 °C for 30 min. It remained stable over the pH 5–11 range, but the antigen-binding activity was rapidly lost at pH 2–3 or lower, probably because of conformational changes. Table 4 provides a comparison between avian IgY and mammalian IgG.

The separation of IgY from the egg yolk involves various chemical reactions and a simple water extraction process. The water-soluble fractions (WSF) of egg yolks can be obtained by using the water dilution method based on the aggregation of yolk lipoproteins at low ionic strengths. Centrifugation or filtration is subsequently used to fractionate the WSF from water-insoluble lipid components of egg yolk. Acidic conditions change the integrity of the egg yolk granules and lead to increases in the lipid-binding ability. Therefore, lowering pH results in not only increasing the recovery of IgY but also decreasing the amount of LDL in the supernatant. The WSF was almost devoid of lipids at mild acidic conditions and the highest yield of IgY was obtained between pH 5.0 and 5.2. To obtain a great purity of the IgY fraction collected from the WSF, a variety of methods have been used. These purification steps involved isolation of IgY from other water-soluble proteins by further concentration using specific salts or acids. Further purification by gel permeation chromatography, ultracentrifugation, and ultrafiltration resulted in a 95 % pure IgY diagnostic agent.

Microbial foodborne diseases are responsible for serious health problems in humans and animals due to pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria* spp., *Campylobacter* spp., enteropathogenic *E. coli*, viruses, and parasites. IgY studies have demonstrated that specific IgY against *E. coli* O157:H7 and *Salmonella* is able to inhibit the growth of pathogens, eventually resulting in bacterial death. This research offers many advantages over traditional antibiotics and provides the basis of a highly effective means of producing inexpensive antibodies in egg yolks as functional food and nutraceutical ingredients for the prophylactic treatment of humans and animals against enteric diseases. Among these, oral passive immunotherapy may be of value due to the advantages of reduced cost, ease of administration, and potential to treat localized conditions in

the gastrointestinal tract (GIT). Chicken egg yolk immunoglobulin (IgY) is ideal for passive immunotherapy, as it may be readily obtained in large quantities from egg yolk, presenting a more cost-effective, convenient, and hygienic alternative to mammalian antibodies. IgY antibody has been proved to neutralize disease-causing pathogens, i.e., *Rotavirus*, *E. coli* O157:H7, *Salmonella enteritis*, *Clostridium perfringens*, and toxic gluten for celiac disease (Gujral et al. 2012).

Using chicken as an antibody producer brings a number of advantages over conventional mammalian antibody and recombinant antibody production and serves as an alternative to antibody sources (Box 1). Combined with the egg industry's capacity to produce thousands of eggs per day and an existing technology for the efficient fractionation and purification of IgY, it is conceivable that kilogram quantities of antibodies could be produced on a daily basis. Thus, IgY has been widely used as a passive immunization therapy to treat enteric infections in humans and animals. Another application is the use of IgY as an immunological tool in the field of diagnostics as well as biomedical research. In this presentation, we summarize published data on properties and applications of IgY in immune-power eggs for prophylactic, therapeutic, and diagnostic uses and suggest directions for its future use.

Box 1: Advantages of IgY

- Maintenance of a large flock of laying hens is inexpensive and practical because large-scale feeding of hens and the collection of eggs are less labor intensive and well integrated.
- Eggs as the source of IgY can be collected from laying hens by the noninvasive method, which is compatible with animal protection regulations, as compared to mammal's sera from which IgG is separated.
- Also, immunization of hens (vaccination) has long been applied to prevent hens from infectious diseases, indicating that immunization of hens is much more systematized to be effective than doing it for animals.
- A laying hen produces an average of 285 eggs in a year with a yolk of approximately 15 g, whereas an immunized rabbit provides about 40 ml of sera. One gram of egg yolk contains about 10 mg of IgY, whereas 1 ml of rabbit serum yields about 35 mg of IgG. An immunized hen produces about 43 g of antibodies per year.
- As egg yolk is known as a perfect food package, the isolation of IgY from the yolk is much easier than that of IgG from animal blood sera. For separation of IgY, a large-scale method is now applicable by automatic separation of the egg yolk with a machine.
- The immune response of chickens could be maintained for a long period of more than 20 weeks with two injections.
- The conventional method inevitably sacrifices animals which have produced the specific IgG in their circulating blood. On the other hand, the method of using hens is sufficient only to collect the eggs laid by super immunized hens.

Phosvitin

Phosvitin is a phosphoglycoprotein that contains about 10 % phosphorus, with α - and β -phosvitin containing about 2–9 % phosphorus, respectively. It is therefore one of the most highly phosphorylated proteins occurring in nature. About 80 % of protein-bound phosphorus in egg yolk is located in phosvitin. Serine residues are predominant in the protein, many of which are phosphorylated and occur consecutively in the primary sequence of the molecule. In addition to phosphorus, the phosvitin molecule contains 2.5 % hexose, 1 % hexosamine, and 2 % sialic acid. However, unlike many of the other yolk proteins, it does not contain any lipid.

Phosvitin is constituted by 217 amino acid residues that comprise a core region of 99 amino acids, grouped in runs of maximally 14 Ser residues interspersed by Arg, Lys, and Asp (Byrne et al. 1984; Van Het Schip et al. 1987). The relative abundance of phosphoserine groups in the phosvitin amino acid sequence confers to the protein a large central hydrophilic portion surrounded by two small hydrophobic parts at the N-terminal and C-terminal. Owing to its polyanionic character ($pI = 4$), phosvitin possesses a very strong metal-chelating property (Castellani et al. 2004). The major site of phosvitin binding to carbohydrate is the Asn residue at position 169, and the carbohydrate moiety is a branched oligosaccharide, consisting of mannose, galactose, *N*-acetylneuraminic, and *N*-acetylglucosamine acid, and linked to protein by *N*-glycosidic bond (Shainkin and Perlmann 1971). Fourier transform infrared spectroscopy showed that the secondary structure of phosvitin is composed of 0 % α -helix, 50 % β -sheet, 7 % β -turns, and 43 % random coil (Losso et al. 1993). However, factors such as pH can affect its secondary structure and shift the percentages of β -sheets to α -helices and random coils (Renugopalakrishnam et al. 1985; Prescott et al. 1986).

Due to its structure, phosvitin is resistant to heat treatments. No precipitate is observed after heating phosvitin solutions with a range of different pH (4–7) for several hours at 100 °C (Mecham and Olcott 1949). Since native phosvitin has a very stable conformation, once iron is bound it is not easily released. After a heat treatment at 90 °C for 60 min, no decrease in the iron-binding capacity could be detected (Castellani et al. 2004). The unique chemical characteristic of phosvitin conferred by its high proportion of ionizable phosphorylated serine residues is accompanied by properties such as high water solubility and resistance to heat denaturation (Anton et al. 2000) and proteolytic attack (Juneja and Kim 1997). Because of the phosphate groups, phosvitin is one of the strongest naturally occurring metal-binding biomolecule. Under low ionic strength and acidic conditions, phosvitin forms soluble complexes with Ca^{2+} , Mg^{2+} , Mn^{2+} , Co^{2+} , Fe^{2+} , and Fe^{3+} . Heating to 90 °C and high pressure up to 600 MPa did not lead to a loss of Fe-binding capacity (Castellani et al. 2004). Nielsen et al. (2000) reported that the addition of ascorbic acid and ascorbic acid 6-palmitate gave rise to an increase in the amount of free iron Fe (II) in egg yolk dispersions, possibly owing to reaction with phosvitin–Fe (III), which subsequently propagated lipid oxidation. The iron-chelating activity of phosvitin has been associated with protection against oxidative damage. Katayama et al. (2006) reported that oligophosphopeptides from hen egg

yolk phosvitin have novel anti-oxidative activity against oxidative stress in intestinal epithelial cells and that both phosphorus and peptide structure have key roles in the activity. The protective effects of phosphopeptide structure against H_2O_2 -induced oxidative stress were almost the same as that of glutathione, and egg phosvitin phosphopeptide with high content of phosphorus exhibited higher protective activity than those without phosphorus. Yet, phosphoserine did not show any significant anti-oxidative stress activity.

Novel hen egg phosvitin phosphopeptide with molecular masses of 1–3 kDa was prepared; phosvitin phosphopeptide with 35 % retention was effective for enhancing calcium-binding capacity and inhibiting the formation of insoluble calcium phosphate. Jiang and Mine (2001) reported that 1–3 kDa fragments of these peptides derived from partially dephosphorylated phosvitin by tryptic digestion showed a higher ability than did commercial casein phosphopeptides to solubilize calcium in a calcium phosphate precipitate, while Feng and Mine (2006) reported that phosvitin phosphopeptide from partially dephosphorylated phosvitin increased iron uptake in a Caco-2 cell monolayer model. Choi et al. (2004) demonstrated high Ca solubilization in the presence of phosvitin or its tryptic peptides when incubated under conditions simulated those of the ileum, while Choi et al. (2005) found that phosvitin peptides improved bioavailability of Ca and thus increased incorporation of Ca into the bones of rats.

Choi et al. (2004) reported that phosvitin and its peptides exhibited antibacterial and DNA leakage effects against *E. coli* under thermal stress at 50 °C and suggested that phosvitin peptides disrupt the bacterial cells by chelating with metals in the outer cell membranes. Antibacterial activity was dramatically reduced by treatment with α -chymotrypsin, although the chelating effect remained.

Chemical Composition of Egg Products

Egg products can be defined as processed forms of chicken eggs for commercial, foodservice, and customers. Egg products include refrigerated liquid, frozen yolk, and specialty egg components. Most egg products can be added with desirable flavors, enhanced with nutrients and functional properties. Convenience foods such as cake and pudding mixes, pasta, ice cream, mayonnaise, candies, and bakery goods are based on egg products. Egg products are widely used by commercial bakers, food processing manufacturers, and the foodservice industry due to their convenience, minimal storage requirements, ease of portion control, product safety, long-term stability, and nutritional uniformity.

Liquid Egg Products

Shell eggs from chicken farms are collected and delivered to the egg-breaking plant. Before breaking, eggs are washed in warm water, spray-rinsed with a sanitizing agent, and dried with cool air. Eggs are broken by automatic breaking

machine. The egg yolk and white are separated, if necessary, and the liquid eggs can be shipped in a container refrigerated at 4 °C to bakeries or other outlets for immediate use or to other plants for further processing.

Liquid egg products allow a consumer to have the nutrition from eggs without the hassle of having to crack some shells. One manufacturer that specializes in liquid egg products includes Burnbrae Farms. Naturegg Omega Plus Liquid Eggs are said to be lower in cholesterol and fat than regular eggs, including more omega-3 polyunsaturates. One hundred milliliters of Naturegg Omega Plus Liquid Eggs contains 40 % of the daily requirement of omega-3 polyunsaturated fatty acids, making them a healthy alternative as a low fat source of protein compared to natural eggs. The product is made with 100 % real egg whites and contains 125 mg of DHA omega-3, 125 mg of EPA omega-3, and 0.5 mg of lutein. While omega-3 polyunsaturated fatty acids have been promoted as being “heart-healthy,” lutein is considered an antioxidant that is important for eye health. It also boasts no fat or cholesterol, reinforcing the heart-healthy image the manufacturer is trying to convey. By being a source of vitamin B₁₂, vitamin D, vitamin E, folate, and protein as well as the ability to be frozen for up to 3 months before the expiry date, Burnbrae Farms has produced quite the consumer product. The manufacturer also suggests that Naturegg Omega Plus Liquid Eggs are actually healthier than consuming Omega Plus Shell Eggs. The reason for this is that the Liquid Eggs product contains 90 % of longer chain fatty acids, while the Omega-3 shell eggs only provide 25 %. This would suggest that consuming Naturegg Omega Plus Liquid Eggs is similar to eating a serving of salmon or other cold-water fish.

Frozen Egg Products

Frozen egg products include separated whites and yolks, whole eggs, blends of whole eggs and yolks, or whole eggs and milk and these same blends with sugar, corn syrup, or salt added. Salt and carbohydrates are sometimes added to yolks and whole eggs in order to prevent gelation during freezing. In terms of preservation, frozen egg products should be kept frozen or refrigerated until used. When it is time for use, they should be thawed under refrigeration or under cold running water in unopened containers. After defrosting, they should be refrigerated and used within 3 days.

Specialty Egg White Products

Egg white products are free of fat and cholesterol and even come as a liquid product that can be poured from a container. They can be used as a substitute for eggs and therefore be prepared scrambled or as omelets, egg patties, and quiche, or they could even be used as a protein drink. Indeed, there are products online where egg whites come separated and in containers with pumps. There is a growing trend in the use of egg whites in bodybuilding. Because there is no fat and cholesterol, and

very little in terms of carbohydrates, egg white as a source of protein is viable. Many protein powder shakes have a high amount of fat and other components, whereas egg whites have zero fat and a very small amount of carbohydrates. A benefit of specialty liquid egg white powder would be the average consumer would not have to buy a dozen eggs, crack them, separate them, and throw away waste to get egg whites. Prepackaged egg whites would save time and energy. There are many major commercial producers that produce egg white products. Major nutrient and supplement and health stores both online and in stores also have egg white products. Aside from specialty egg white products, many cooking recipes use egg whites. Egg yolk is popular in cooking, more so than egg whites and often egg whites are leftover. However, if one were to substitute egg whites instead of using a traditional egg, one would decrease calorie intake along with removing the cholesterol and fat intake that would have occurred due to the egg yolk components. Dieting this way, by substituting egg whites for the entire egg, is also possible. There are many uses for egg whites that can be easily found online. Egg white smoothies and egg white salads are common. Allergies to eggs may be due to the egg yolk and not the egg white. It may be possible for people with egg allergies to substitute egg whites into their recipes that call for the use of eggs and not have allergic reaction. Egg white as a meat alternative is also available due to its capability as a protein source. Because the fat of the egg is located in the yolk, a liquid egg white product that is fat and cholesterol free while providing an excellent source of protein appears to be a viable product.

Egg Yolk Products

By being the component that houses most of the fat in an egg, egg yolks have found many uses in culinary pursuits, especially as an emulsifying agent. Egg yolk products allow manufacturers to provide restaurants and bakeries with an easy source of egg yolks without the worry of what to do with the separated egg whites. They can be used in mayonnaise, salad dressing, and cream-style sauces. Bakery products derive their beautiful color from an egg yolk wash, and the lecithin can prevent moisture loss and tenderize a crumb. They have also found a place in ice cream and frozen custard by imparting richness and flavor. These applications of egg yolk, mainly egg lecithin (phospholipid), are used as an emulsifier and binder and also as an additive to improve or to hold the freshness of foods and act as a flavor-active ingredient.

Egg lecithin is widely used as a component in body care products and cosmetics. The customer prefers more natural ingredients which egg lecithin represents in an excellent combination with a cell membrane phospholipids and a carrier function for vitamins and pigments. Egg lecithin acts as an emulsifier to form a layer on the scalp and on the hair by enhancing the fluidity of the hairstyle, whereas the egg yolk protein stabilizes the foam. The application range of egg lecithin in cosmetic products includes emulsifier, stabilizer function in creams and lotions, skin nutrient as metabolic active and membrane-forming lipid, maintenance in moisture

regulation of the skin, improvement of skin permeation, protection of hair and skin against deformation by making the skin become smooth, and pigment incorporation in creams, lotions, or sticks for the skin colorization. A hydrogenated egg lecithin can form liposomes that are already used as a carrier of other active ingredients in moisturizers, sunscreens, tanning agents, vitamins, and fragrances.

Egg Specialty Products

There are many specialty egg products in the market. Examples of such include pickled eggs, fermented eggs, balut eggs, and powdered eggs. Pickled eggs come in jars or containers and are cured in vinegar or brine in order for the eggs to be preserved for months. In the past, pickled eggs were common in bars where one would enjoy beers and pickled eggs. The hard-boiled eggs have their shells removed and then are pickled in the pickling solution. Fermented eggs, or century eggs, are cured in a special mixture that covers the outside of the egg for up to months in order to break down flavorless compounds into smaller flavorful ones. It comes from a Chinese background. The yolk becomes a dark green or gray color and has a sulfurous odor, while the egg white becomes brown and has a salty flavor. Balut eggs are embryos that have been developed and then boiled alive so that it can be eaten in the shell. They are also known as 14-day eggs due to the act of allowing the embryo to develop for around 14 days. All of the inside of the egg can be eaten. This product is already available in many major commercial chain stores to be bought and eaten. Powdered egg is common due to its price, nature, and shelf life. Dehydrated egg is useful in that it can be stored without refrigeration and also it is very common in camping purposes. The dehydrated egg can be rehydrated in order for culinary purposes; rehydrated egg can be used to make scrambled eggs and omelets. There are many other kinds of ways eggs can be prepared in restaurants. For example, there are tea eggs, smoked eggs, soy eggs, and many more. As listed above, there are several different kinds of egg products already available to the public, aside from separated forms of egg whites and egg yolks.

Conclusion and Future Directions

Eggs are a most reliable food and encompass all the nutrient elements essential for developing new life of chickens. Hen eggs are considered to be a storage container and safe house for nutrients and growth-promoting factors needed for the continuing life cycle of chickens. In particular, the nature of albumen, egg white, is best simply understood if its main function is thought of as to keep microorganisms away from the egg yolk, where it was shown that most of the essential nutrients and vitamins are. This is apparently achieved by the bactericidal activity, which is the removal of nutrients and the possible inhibition of proteolytic enzymes that are likely produced by microorganisms.

In terms of what is possible in the future, a potential avenue for future projects and research is the fact that albumen successfully resists microorganisms over a long period of time without metabolizing. In contrast with the immune systems of animals, it is a passive system that actively produces new antimicrobial agents whenever they are needed. It is probable that not all of the antimicrobial systems in eggs have been uncovered; this means that albumen remains a useful subject for antimicrobial protein research and undiscovered potential.

An important feature of egg yolk is that it preserves essential nutrients against oxidation and against the enzyme activities of the yolk itself. However it is not understood why this is and it would be of value to know, at the molecular level, details about how this is achieved. Also, it would be of interest to know if the livetins are quite independent in unbroken yolk or are associated with the lipoproteins and thus help to maintain their integrity. Furthermore, egg yolk contains fascinating physiologically and immunologically functional carbohydrates and proteins of which biotechnology industries develop the processing technology and explore new applications in nutraceutical and other fields. For example, chicken egg yolk IgY antibodies are convenient and inexpensive to produce at a large scale. The antibody has been known as a replacement of antibiotic agents to prevent food pathogens and antibiotic-resistant diseases. The IgY antibody can be also considered a safe natural health product, nutraceutical and function food supplements, or veterinary biologics. Another example of egg lipid is omega-3 fatty acids in egg yolks. Omega-3 fatty acids must become incorporated into foods rather than be used solely as dietary supplements. The development of various omega-3-rich foodstuffs would allow increased dietary intakes of the nutrient. Among the foodstuffs, omega-3 fatty acid-enrich egg products may attract health-conscious population. This would necessitate the development of research for the nutritional evaluation of the various egg products and the education of the public.

In the near future, innovative egg products such as ultra-pasteurized liquid egg, free-flowing frozen egg pellets, and modified atmosphere packaging for hard-cooked eggs are expected to become available.

Cross-References

- ▶ [Bioactive Substances of Animal Origin](#)
- ▶ [Chemical Composition of Fat and Oil Products](#)
- ▶ [General Properties of Major Food Components](#)
- ▶ [General Properties of Minor Food Components](#)

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Abstract

Fats and oils are an important dietary component and contribute to the nutritional and sensory quality of foods. This chapter focuses on the chemical composition of fats and oils and how these compositions affect the functional properties of fats and oils in foods. The focus will remain on the most economically and commercially important fats and oils that are widely used as or in food products.

Introduction

Edible fats and oils are lipids that are removed and purified from oilseeds, nuts, oil-bearing fruits, fatty animal tissues or animal milk, or other plants, fungi, or algae. Fats and oils are an important food commodity; worldwide, the production of the major vegetable oils (Table 1) exceeds 170 million metric tons. According to the Food and Agriculture Organization (2014), worldwide production of butter and ghee is 9.6 million metric tons, tallow (rendered beef or mutton fat) production is around 6.6 million metric tons, and lard (rendered pig fat) production is around 5.8 million metric tons. In most cases, oils and fats in the plant or animal serve as a storage form of energy. The major components of most commercially important fats and oils are triacylglycerols, which are composed of three fatty acids esterified to a glycerol backbone. Fats and oils often contain small amounts of other lipids as minor components, such as mono- and diacylglycerols, sterols, tocopherols, terpenes, hydrocarbons, and carotenoids. Fats and oils are commonly distinguished

Table 1 Worldwide production of vegetable oils and top producers

Vegetable oil	Million metric tons produced	Top producing countries
Palm oil	58.43	Indonesia, Malaysia, Nigeria
Soybean oil	44.66	USA, China, Brazil, Argentina
Rapeseed oil/canola	25.76	EU-27, China, Canada, India
Sunflower oil	15.67	Russia, Ukraine, Argentina
Palm kernel oil	6.86	Malaysia, Indonesia, Nigeria
Peanut oil (groundnut)	5.67	India, China, Nigeria, Myanmar
Cottonseed oil	5.11	China, India, Pakistan
Coconut oil	3.54	Philippines, Indonesia, India
Olive oil	3.28	Spain, Greece, Italy
Corn oil	2.35	USA, EU-27, China

Sources: FEDIOL (2014), Food and Agricultural Organization of the United Nations (FAO) (2014), and USDA (2013)

by their physical state at room temperature; thus, fats, such as lard, are solid at room temperature, while oils are liquid at room temperature. However, on the nutrition label of US food products, lipids are labeled “total fat,” “saturated fat,” “*trans* fat,” etc., regardless of their actual physical state.

Fats and oils are a unique and complex category of food components; not only are they consumed in pure form, as in butter and olive oil, they are also used as a cooking medium, as in frying, and they are present either naturally or as an added ingredient in almost all foods. Fats and oils are the most calorie-dense food component, providing 9 Kcal/g. Two fatty acids, linoleic acid and linolenic acid, are essential nutrients, i.e., humans are unable to synthesize these fatty acids and thus must obtain them from food or supplements. Most vegetable oils are an important source of vitamin E, and fats and oils assist in the absorption of the fat-soluble vitamins A, D, E, and K. Fats and oils also serve as a carrier of many flavors in foods, since many flavor components are fat soluble. In addition, solid fats, such as butter, margarine, and shortenings, provide a multitude of textural properties important for many foods, including tenderizing, aeration, and creaming, and provide smooth, cooling sensations when they melt upon eating.

Lipids: Definitions and Classification

Lipids are a complex and heterogeneous group of substances found in nature, with a wide variety of structures and functions. Lipids are generally defined as substances that are insoluble in water and soluble in organic solvents such as diethyl ether, benzene, chloroform, hexane, petroleum ether, or methanol. Lipids are either hydrophobic (water hating) or amphiphilic, i.e., a portion of the molecule is hydrophobic and another portion is hydrophilic. As such, they usually contain long chains or rings of hydrocarbons and relatively few oxygen or other polar elements.

The building blocks for most biological lipids are derived from either ketoacyl condensation (fatty acid biosynthesis) or isoprene condensation pathways (Fig. 1) (Fahy et al. 2009). In a relatively recent endeavor to classify all lipids, they were divided into eight categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, and polyketides from ketoacyl condensation and sterol lipids and prenol lipids from isoprene condensation. Examples of important food lipids include fatty acids from the fatty acyl category, triacylglycerols from the glycerolipid category, glycerophospholipids, and cholesterol from the sterol lipids category. Other important food lipids include the fat-soluble vitamins A, D, E, and K. Vitamins A, E, and K are prenol lipids, while vitamin D is a sterol lipid.

Historically, more basic classification systems have been applied. In one, lipids are classified as either derived, simple, or complex (Christie 1973). Derived lipids, such as fatty acids, fatty alcohols, sterols, and carotenoids, come from one of the two pathways mentioned above and are building blocks for simple or complex lipids. Simple lipids can be hydrolyzed into two constituents; for example, triacylglycerols are hydrolyzed to fatty acids and glycerol, and wax esters

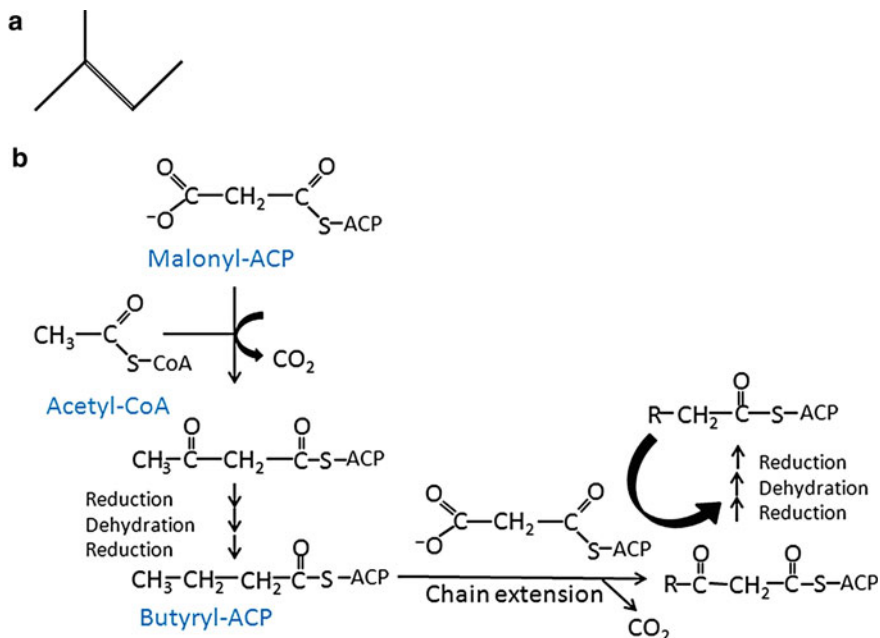


Fig. 1 (a) Isoprene, the five-carbon building block for lipids in the prenyl lipid category. (b) Overview of fatty acid biosynthesis. Fatty acids increase in chain length through the addition of two-carbon units in a cycle of condensation-reduction-dehydration-reduction reactions until the growing acyl chain, which is attached to acyl carrier protein (ACP), reaches a chain length of (typically) 16 to 18 carbons (Adapted from Somerville et al. 2000)

hydrolyze into a fatty acid and fatty alcohol. Complex lipids are composed of three or more constituents. Phospholipids, for example, are complex lipids because upon hydrolysis they will form glycerol, two fatty acids, and a phosphorylated head group. Lipids may also be classified as either neutral (i.e., nonpolar and containing no charge), such as triacylglycerols, or polar, such as phospholipids.

Fatty Acids

Fatty acids are carboxylic acids with linear hydrocarbon chains and are the main building block for fats and oils (Fig. 2). Plants and animals share the same pathway for fatty acid biosynthesis. They are synthesized by inserting two carbons at a time to the growing acyl chain (Fig. 1); thus, most natural fatty acids have an even number of carbons typically ranging in length from 4 to 24 carbons. The aliphatic chain may either be saturated or have one (monounsaturated) or more (polyunsaturated) double bonds introduced by enzymes called desaturases. Most desaturase enzymes are location and isomer specific; thus the double bonds in natural fatty acids are usually the *cis* isomer and are methylene interrupted (a methyl group in between each double bond). However, the double bonds may be converted to the

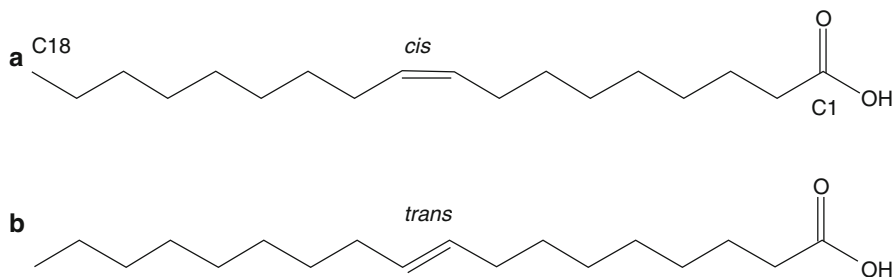


Fig. 2 *Cis* and *trans* unsaturated fatty acid structures. (a) Oleic acid (*cis*-9-octadecenoic). (b) Elaidic acid (*trans*-9-octadecenoic)

trans form via hydrogenation, which can be performed by bacteria in, for example, the stomachs of ruminant animals, or through chemical hydrogenation by vegetable oil processors.

Fatty Acid Nomenclature

There are several systems for naming fatty acids. Systematic names were developed by the International Union of Pure and Applied Chemistry (IUPAC 1978). Under this naming system, the carboxyl carbon is named carbon 1 (C-1). For saturated fatty acids, an “oic” is added to the end of the systematic name of the parent alkane group (e.g., $\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$ is named “octadecanoic acid”). For unsaturated fatty acids, the location and geometry of the double bonds precedes the hydrocarbon name, and the number of double bonds is indicated in the parent alkene name (e.g., $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$ is *cis*-9-octadecenoic acid, and $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$ is named *cis*-9, *cis*-12-octadecadienoic acid). Note that the position of the double bond is identified by the lowest carbon number in the bond. Examples of the systematic naming of some common fatty acids are given in Table 2.

In practice, systematic names can prove cumbersome, so there are several abbreviated naming systems that are more commonly used. First, the most common fatty acids have a trivial name (see Table 2). These names may either be a shortened version of the systematic name, for example, butyric acid, or they may just be historical names or derived from the major source of the fatty acid at that time. For example, the names for oleic acid and palmitic acid are derived from olive oil and palm oil, respectively, where these fatty acids are major components. Most food and nutritional scientists, especially those working with fatty acids and lipids, will commit the names of at least the five most common fatty acids (highlighted in Table 2) to memory.

Abbreviated systems have also been developed which can give information on the carbon chain length, the number and position of the double bonds, and in some cases, the geometric isomer of the double bonds. These abbreviated systems show two numbers separated by a colon to indicate, respectively, the number of carbons, followed by the number of double bonds. For example, linolenic acid is 18:3.

Table 2 Systematic, common, and shorthand nomenclature for fatty acids. The fatty acids in this table are frequently encountered in nature. The five most common fatty acids in commercial fats and oils are highlighted

Systematic Name	Common Name	Shorthand
Butanoic	Butyric	4:0
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
Eicosanoic	Arachidic	20:0
Docosanoic	Behenic	22:0
Tetracosanoic	Lignoceric	24:0
c-9-Hexadecenoic	Palmitoleic	16:1 n-7
c-9-Octadecenoic	Oleic	18:1 n-9
c-9,c-12-Octadecadienoic	Linoleic	18:2 n-6
c-9,c-12,c-15-Octadecatetraenoic	Linolenic	18:3 n-3
c-6,c-9,c-12,c-15-Octadecatetraenoic	Stearidonic	18:4 n-3
c-5,c-8,c-11,c-14-Eicosatetraenoic	Arachadonic	20:4 n-6
c-5,c-8,c-11,c-14,c-17 Eicosapentaenoic	Eicosapentaenoic (EPA)	20:5 n-3
c-4,c-7,c-10,c-13,c-16,c-19 Docosaheptaenoic	Docosaheptaenoic (DHA)	22:6 n-3

The position of the double bond relative to the first, or carboxyl carbon, may be indicated by a delta symbol, i.e., Δx . For example, linolenic acid is abbreviated 18:3 $\Delta 9$. Alternatively, all of the double bond locations relative to the carboxyl carbon may also be listed (18:3 $\Delta 9, 12, 15$). The “n” or “ ω ” system for naming fatty acids lists the position of the double bond relative to the *methyl* end of the carbon chain, instead of the carboxyl end, and is indicated by either an n-x or ωx (omega-x). Thus, for linolenic acid, since the double bond closest to the methyl- (ω -) end of the chain is three carbons away on C15, it is abbreviated under this system as 18:3 n-3 or 18:3 $\omega 3$. Since double bonds in most natural fatty acids are in the *cis* configuration, if the geometry of the double bond is not indicated, as with the systems above, it is assumed that all of the double bonds are in the *cis* configuration. The most specific abbreviated system will give both the location (relative to the carboxyl carbon) and the geometry of the double bond (*cis* or *trans*, abbreviated *c* or *t*). Thus, linolenic acid would be abbreviated 18:3 *c*9,*c*12,*c*15.

Physical Properties of Fatty Acids

The melting points of fatty acids increase with increasing length of the carbon chain and decrease depending on the number, location, and configuration of double bonds. Fatty acids will typically stack together through hydrophobic interactions between the acyl chains, so the longer the chain length, the more hydrophobic interactions and the increased energy needed to disrupt the associations. Short-chain saturated fatty acids ($\leq C8$) are liquid at room temperature, while longer-chain saturated fatty acids are solid at room temperature (Table 3).

Table 3 Melting points of saturated and unsaturated fatty acids. Compiled from Larsson and Quinn (1994) and Gunstone et al. (1994)

Fatty acid	#Carbons	Double bond geometry and location	Melting point (°C)
Butyric	4	–	–8.0
Caproic	6	–	–3.4
Caprylic	8	–	16.7
Capric	10	–	31.6
Lauric	12	–	44.2
Myristic	14	–	54.4
Palmitic	16	–	62.9
Palmitoleic	16	<i>c</i> 9	0.5
Stearic	18	–	69.6
Oleic	18	<i>c</i> 9	16.0
Petroselinic	18	<i>c</i> 6	33.0
<i>cis</i> -Vaccenic	18	<i>c</i> 11	14.5–15.5
Elaidic	18	<i>t</i> 9	45.0
Linoleic	18	<i>c</i> 9, <i>c</i> 12	–5.0
Linolenic	18	<i>c</i> 9, <i>c</i> 12, <i>c</i> 15	–11.0

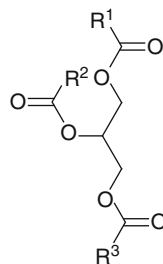
Very short-chain fatty acids, such as butyric acid, are volatile at room temperature and have unpleasant odors, leading to off-flavors and odors of oils where these fatty acids have hydrolyzed from triacylglycerols (hydrolytic rancidity), but in some cases these odors are considered desirable, or at least typical, for example, in cheese products. Hydrolytic rancidity is a problem in any system where lipases (enzymes that catalyze fatty acid hydrolysis from an acylglycerol) or water is present.

The melting points of fatty acids decrease with increased desaturation. The kink in acyl chains formed by *cis* double bonds hinders the ability of unsaturated fatty acids to stack together effectively. Unsaturated fatty acids with *trans* double bonds are linear, like saturated fatty acids; thus the melting point is not affected as much as those with *cis* double bonds. The location of the double bond also has an effect on the melting point, as shown for the various positional and geometric isomers of oleic acid in Table 3.

Triacylglycerols

Triacylglycerols are the major component of fats and oils. Historically, triacylglycerols were called triglycerides, and this name is still commonly used. Triacylglycerols are composed of glycerol (1,2,3-trihydroxypropane) with a fatty acid esterified at each hydroxyl via the C-1 carboxyl group (Fig. 3). Glycerol is a symmetrical (achiral) molecule, but if different fatty acids are esterified at both the top and bottom hydroxyl position, it becomes a chiral molecule that can exist as two enantiomers. Thus, stereospecific numbering (*sn*) is used to distinguish enantiomers. The Fischer projection of glycerol is shown with the hydroxyl group on the top and bottom to the right and the middle hydroxyl group to the left. The carbons are

Fig. 3 General structure of a triacylglycerol. R^1 , R^2 , and R^3 represent fatty acids



numbered 1 to 3 from top to bottom, and when naming the triacylglycerols, the prefix *sn-* is used to distinguish enantiomers if they are known, *rac-* if there are an equal mixture of enantiomers, and *x-* if the stereochemistry is unknown. For example, one of the predominant triacylglycerols in soybean oil is 1-linoleoyl-2-linoleoyl-3-oleoyl-*sn*-glycerol, which may also be abbreviated as LLO.

Given that commercially important fats and oils are composed of four or five common fatty acids as well as up to 15 less common fatty acids, there are numerous possible combinations to make up a wide variety of triacylglycerols. However, the arrangement of fatty acids on glycerol is not completely random. In most plants, the *acyltransferase* enzymes responsible for the transfer of fatty acids and the *desaturase* enzymes that introduce double bonds into fatty acids are somewhat specific in terms of degree of unsaturation and the chain length of the acyl groups that they act on. As a result, in most plant triacylglycerols, the *sn-2* position will most likely have unsaturated fatty acids, while saturated fatty acids, when present, are more likely to reside on the *sn-1* or *sn-3* position. The expression of these and other enzymes involved in plant fatty acid and lipid biosynthesis is different in every species and even subspecies and is also affected by the environment, which contributes to the wide variety of oil fatty acid compositions that can be found in nature.

The types of fatty acids and their location on the triacylglycerol molecule affect their physical and chemical properties. An example that many people can relate to is cocoa butter, the fat in chocolate. Cocoa butter has three predominant triacylglycerols: 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol (POS), 1,3-distearoyl-2-oleoyl-*sn*-glycerol (SOS), and 1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol (POP). The high similarity of these three triacylglycerols and their symmetrical nature (saturated-monounsaturated-saturated) is responsible for its unique melting properties in the range from 32 °C to 35 °C, near the temperature inside the mouth (Shukla 1995).

Minor Oil Components

Mono- and Diacylglycerols

Monoacylglycerols and diacylglycerols, which have one or two fatty acids, respectively, esterified to glycerol, are minor oil constituents in most natural fats and oils. As natural components of fats and oils, they are usually formed by degradation of triacylglycerols, either through the action of lipase enzymes or hydrolysis by

moisture during processing or storage. For refined oils, it is desirable to remove monoacylglycerols and diacylglycerols, because they have a lower smoke point and may act as emulsifiers which can affect the performance and shelf life of the oils. Typical refined vegetable oils have mono- and diacylglycerol concentrations of 0.2–0.4 % and <0.5 %, respectively; however, palm oil has higher contents, ranging from 0.5 % to 3 % and 3–7 %, respectively (Gupta 2008).

Monoacylglycerols and diacylglycerols are used extensively by the food industry as emulsifiers, but these are typically synthetic mono- and diacylglycerols rather than those found naturally in oils. The fatty acid portion interacts with oils or fats in foods, while the free hydroxyl group or groups interact with the water or hydrophilic phase, thus, holding the oil and water parts together. Mono- and diacylglycerols are produced at three concentration levels of *alpha*-monoacylglycerols (*alpha* meaning the fatty acid is either on the *sn*-1 or *sn*-3 position, while *beta* means the fatty acid is on the *sn*-2 position): 40–46 %, 52 % minimum, and distilled (90 % minimum) monoacylglycerols (O'Brien 1998). The starting oil triacylglycerols can be altered to affect the fatty acid composition and, thus, the physical and functional properties of mono- and diacylglycerols (O'Brien 1998).

Free Fatty Acids

As with mono- and diacylglycerols, free fatty acids are fatty acids that are present in oils as a minor component and as a product of hydrolysis by lipase enzymes or by water reacting with triacylglycerols. These may form during processing, especially when oilseeds are being crushed prior to oil extraction. Lipase present in unpasteurized milk may also catalyze hydrolysis. Free fatty acid content of unrefined (crude) oils may range anywhere from 0.5 % to 10 %, depending on the oil source. During oil refining, the free fatty acid content is generally reduced to <0.5 % (Gupta 2008).

Phospholipids

Phospholipids (glycerophospholipids) are lipids that contain a phosphoric acid residue. Most phospholipids are glycerolipids made up of a diacylglycerol with the acyl groups at the *sn*-1 and *sn*-2 position and a phosphate group at the *sn*-3 position (Fig. 4). If there is nothing attached to the phosphate group, it is called phosphatidic acid. However, the phosphate group is usually attached by a phosphoether bond to another organic head group, such as choline (phosphatidylcholine), ethanolamine (phosphatidylethanolamine), serine (phosphatidylserine), glycerol (phosphatidylglycerol), or inositol (phosphatidylinositol). Sphingomyelins are phospholipids found in animals that, unlike glycerophospholipids, have a ceramide backbone instead of glycerol.

Phospholipids are ubiquitous in nature; they are an important constituent of cell membranes and are essential in many metabolic processes. However, they are not commonly used as storage lipids; thus they do not make up a great percentage of the lipids in most fats and oils, but they do comprise a large percentage of the lipids in eggs and milk. In most vegetable oils, <3 % of the lipids are phospholipids. They are often removed from crude oils by degumming, prior to oil refining, because they negatively impact the appearance, quality, and performance of the oils.

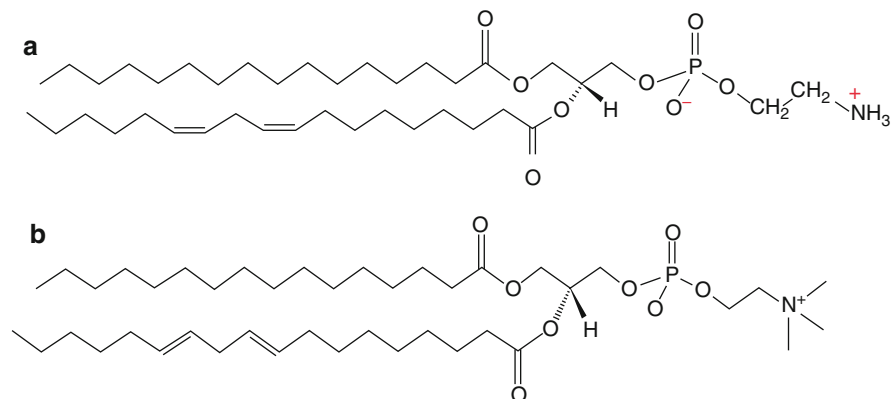


Fig. 4 Two common phospholipids: (a) Phosphatidylethanolamine. (b) Phosphatidylcholine

Fig. 5 Structure of a wax ester, which is an ester of a fatty acid and fatty alcohol



Phospholipids are nature's emulsifier. The phospholipids in eggs, often called lecithin, are largely responsible for the emulsifying properties of egg yolks. As with mono- and diacylglycerols, the amphiphilic structure of phospholipids allows them to bring together oils and lipids. Most of the commercial lecithin used in foods is currently obtained from the degumming of soybean oil or other vegetable oils; thus lecithin is a valuable coproduct of vegetable oil processing. Lecithin is one of the few natural emulsifiers used in foods, besides natural proteins and some polysaccharides.

Waxes

Waxes are mixtures of compounds with a high (>40 °C) melting point, including long-chain (>C₂₀) alkanes, fatty acids, alcohols, wax esters, and other high-melting lipids. Wax esters are composed of long-chain alcohols esterified to long-chain fatty acids (Fig. 5).

Unsaponifiables

Unsaponifiables is a term used to describe the minor oil constituents that remain intact after complete hydrolysis of esterified fatty acids, for example, from triacylglycerols, steryl esters, and wax esters, by the chemical reaction known as saponification (Fig. 6). This reaction, as one might guess from the name, is also used to make soap. After the saponification process, the fatty soaps and glycerol will partition into water, while the unsaponifiables, which are still lipid soluble, will partition into an organic solvent. The typical unsaponifiable components in fats and oils consist of sterols, tocopherols, natural colorants such as chlorophyll and carotenoids, and hydrocarbons.

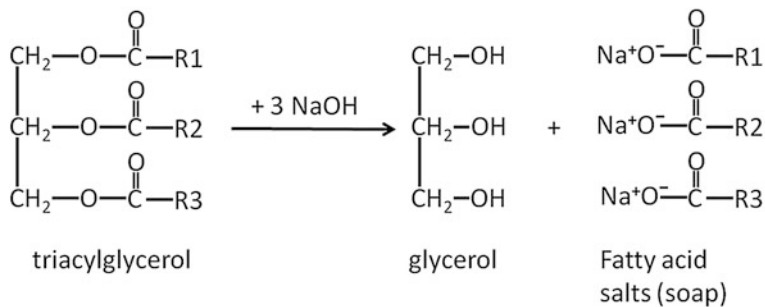
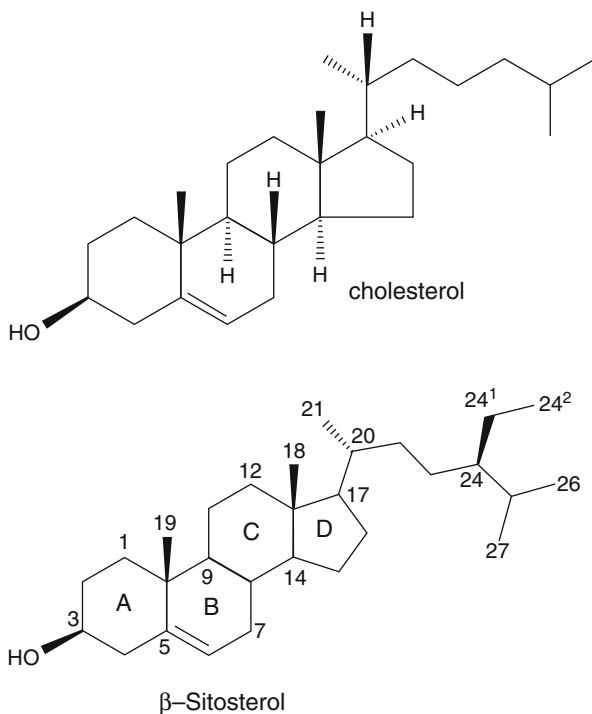


Fig. 6 Saponification reaction. This reaction is used to make soaps, as well as to isolate the unsaponifiables in oils, and also to determine the saponification number, which is defined as the amount of alkali (in mg) needed to saponify 1 g fat or oil. Saponification number can also be used to estimate the average chain length of the fatty acids in a fat or oil

Fig. 7 Chemical structure of cholesterol and the most common plant sterol, β -sitosterol



Sterols

Sterols are ubiquitous compounds in plant and animals; they are critical components of cellular membranes and have other cellular functions. Sterols are triterpene alcohols, derived from the isoprene condensation pathway. The major sterol in animal fats is cholesterol (Fig. 7). Plants can make cholesterol as well,

but most plants only make cholesterol in negligible amounts. Instead, plants synthesize a variety of sterols known as plant sterols or phytosterols (Moreau et al. 2002). Plant sterols and cholesterol in fats and oils exist either as free sterols or acylated sterols, where the hydroxyl group is esterified to a fatty acid. However, plant sterols from plant tissues other than the storage lipids are also often found as conjugates of acylated sterol glycosides or as esters to ferulic or p -coumaric acid. Phytosterols (free and acylated) are present in most commercial vegetable oils, such as soybean, corn, sunflower, and canola, at concentrations ranging from 2 to 10 mg/g (Piironen et al. 2000). Cholesterol content in animal fats varies, ranging from 3 to 4 mg/g in lard, 0.8–1.4 mg/g in beef tallow, and 2–4 mg/g in milk fat (Padley et al. 1994).

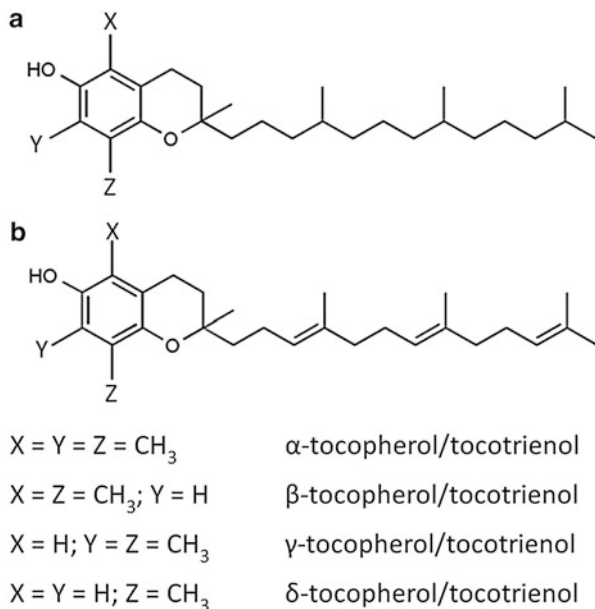
Phytosterols are often added to foods such as oils, margarines, and butter-flavored spreads. They inhibit the intestinal absorption of dietary cholesterol and reabsorption of biliary cholesterol, and it has been shown that consumption of approximately 2 g/day of phytosterols as part of a low-fat diet can reduce total serum and low-density lipoprotein (LDL) cholesterol by ~10 % (Wu et al. 2009). The US Food and Drug Administration (FDA 2006) and the European Food Safety Authority (EFSA 2004) allow a health claim for foods with added phytosterols or phytosterol esters. Currently, the two main sources for large-scale isolation of phytosterols are deodorizer distillates from the refining of vegetable oils and tall oil, a by-product of the wood pulp industry (Fernandes and Cabral 2007).

Tocopherols and Tocotrienols

Tocopherols and tocotrienols are a group of eight structurally similar compounds that have vitamin E activity. Structurally they are composed of a chromanol group, with a phenolic and heterocyclic ring, attached to a 4',-8',-12'-trimethyltridecyl (phytyl) tail (Fig. 8). Tocotrienols have the same chromanol head group as tocopherols but are distinguished by a tri-unsaturated phytyl tail. There are four tocopherol and tocotrienol homologues, α -, β -, γ -, and δ -, which differ in the number and position of methyl groups in the phenolic ring. α -Tocopherol is the most active vitamin E tocopherol, while the other forms have lower activity. Most vegetable oils are good sources of tocopherols, but the ratio of the four tocopherols differs depending on the plant source (Table 4). Tocotrienols are not present in significant quantities in most common refined vegetable oils, except for palm oil and rice bran oil.

Vitamin E tocopherols are scavengers of free radicals; by donating a hydrogen electron to free radicals, it prevents the radicals from reacting with and causing damage to unsaturated lipids as well as proteins and nucleic acids (Burton 1994). Vitamin E is necessary for the protection of cells from damage from free radicals and is also important for reproduction, normal immune function, and protection of the nervous system, neuromuscular system, and normal activities of the retina (Sokol 1996). Thus, they are one of the most important lipophilic antioxidants in plant cells and vegetable oils as well as in human plasma and cells (Burton 1994; Kamal-Eldin and Appelqvist 1996).

Fig. 8 Structures of (a) tocopherols and (b) tocotrienols



Alpha tocopherol has the highest vitamin E activity. Mixed tocopherols, a coproduct of vegetable oil refining, are often added to food products as natural antioxidants to prevent lipid oxidation.

Carotenoids and Other Pigments

Carotenoids and chlorophyll are the two pigment types that are most often found in crude oils, but they are typically destroyed or removed during refining. Chlorophyll is a green pigment involved in plant photosynthesis which may confer a yellow to yellow-green color to oils. Carotenoids are found in plants and algae and are also involved in plant photosynthesis. They are derived from the isoprene condensation pathway and consist of eight isoprene units (40 carbons). They have conjugated double bonds, which absorb light and are responsible for their yellow to red colors. There are over 600 carotenoids in nature, divided into carotenes (no oxygen) and xanthophylls (containing oxygen) (Fig. 9). Several carotenes, including beta-carotene, alpha-carotene, gamma-carotene, and beta-cryptoxanthin, have provitamin A activity, meaning that they can be converted by the body to retinol, a form of vitamin A. In addition, some carotenoids may act as antioxidants. The most common carotenoids found in vegetable oils include the carotenes, alpha- and beta-carotene and lycopene, as well as the xanthophylls lutein and zeaxanthin. Gossypol is a toxic yellow pigment that is produced naturally in cotton and is found in crude cottonseed oil. Refining cottonseed oil reduces the concentration of gossypol to a low level that it is safe enough for consumption.

Table 4 Typical fatty acid composition of commercially important vegetable oils and fats. Derived from CODEX standards (FAO 2013a) unless otherwise stated

Oil or fat	Percentage of total fatty acids										
	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	Others ^a	
Cocoa butter ^b	ND	ND	ND	0.02–0.16	23.6–30.5	30.2–36.5	33.2–38.2	2.2–4.8	0.3		
Coconut oil	4.6–10.0	5.0–8.0	45.1–53.2	16.8–21.0	7.5–10.2	2.0–4.0	5.0–10.0	1.0–2.5	ND–0.2		
Corn oil	ND	ND	ND–0.3	ND–0.3	8.6–16.5	ND–3.3	20.0–42.2	34.0–65.6	ND–2.0		
Cottonseed oil	ND	ND	ND–0.2	0.6–1.0	21.4–26.4	2.1–3.3	14.7–21.7	46.7–58.2	ND–0.4		
Low-erucic acid rapeseed or canola oil	ND	ND	ND	ND–0.2	2.5–7.0	0.8–3.0	51.0–70.0	15.0–30.0	5.0–14.0	20:0 (0.2–1.2), 20:1 (0.1–4.3)	
Olive oil	ND	ND	ND	<0.1	7.5–20.0	0.5–5.0	55.0–83.0	3.5–21.0	<1.5		
Palm oil	ND	ND	ND–0.5	0.5–2.0	39.3–47.5	3.5–6.0	36.0–44.0	9.0–12.0	ND–0.5		
Palm kernel oil	2.4–6.2	2.6–5.0	45.0–55.0	14.0–18	6.5–10.0	1.0–3.0	12.0–19.0	1.0–3.5	ND–0.2		
Palm olein	ND	ND	0.1–0.5	0.5–1.5	38.0–43.5	3.5–5.0	39.8–46.0	10.0–13.5	ND–0.6		
Palm stearin	ND	ND	0.1–0.5	1.0–2.0	48.0–74.0	3.9–6.0	15.5–36.0	3.0–10.0	ND–0.5	20:0 (ND–1.0)	
Peanut oil	ND	ND	ND–0.1	ND–0.1	8.0–14.0	1.0–4.5	35.0–69.0	12.0–43.0	ND–0.3	20:0 (1–2), 20:1 (0.7–1.7), 22:0 (1.5–4.5), 24:0 (0.5–2.5)	
Soybean oil	ND	ND	ND–0.1	ND–0.2	8.0–13.5	2.0–5.4	17–30	48–59	4.5–11.0		
Sunflowerseed oil	ND	ND	ND–0.1	ND–0.2	5.0–7.6	2.7–6.5	14.0–39.4	48.3–74.0	ND–0.3	22:0 (0.3–1.5)	
Sunflowerseed oil Mid-oleic	ND	ND	ND	ND–1.0	4.0–5.5	2.1–5.0	43.1–71.8	18.7–45.3	ND–0.5	22:0 (0.6–1.1)	
Sunflowerseed oil High oleic	ND	ND	ND	ND–0.1	2.6–5.0	2.9–6.2	75–90.7	2.1–17	ND–0.3	22:0 (0.5–1.6)	

^aOnly fatty acids that may exceed 1 % are listed (fatty acid is listed first, followed by typical percentage in parentheses)^bFrom Gunstone et al. 1994

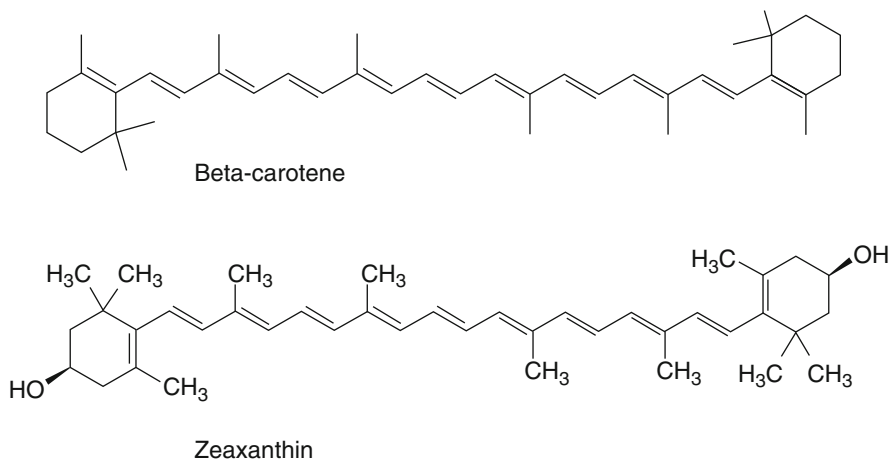


Fig. 9 Examples of carotenoid structures: a non-oxygenated carotene (beta-carotene) and an oxygenated xanthophyll (zeaxanthin)

Oil and Fat Processing

The type of processing that an oil or fat undergoes is dependent on the characteristics of the crude oil or fat and the desired final characteristics. The purpose of oil processing is to remove impurities and some of the minor components, colors, and flavors that affect oil end quality. Commercially processed oils that undergo refining, bleaching, and deodorization are referred to as RBD. RBD oils are often also degummed prior to refining, as described below. The RBD processing may also reduce some desirable components, such as tocopherols and tocotrienols and phytosterols, but conditions are usually optimized to retain as much of these components as possible. Further processing, such as hydrogenation or interesterification, may also be used in order to alter the physical and chemical properties of the oil.

Extraction

The first step in processing fats and oils is to separate them from their original animal or plant source. Animal fats are typically removed from animal tissues by a rendering process, where the animal tissues are heated, which denatures proteins and melts the fats, whereby they are physically separated from the rest of the animal tissues. Vegetable oils are extracted either by pressing processes or by extraction with solvents such as hexane. Vegetable oils such as olive oil are still removed by traditional pressing methods. In addition, nut oils and specialty oils such as cold-pressed canola oil are also extracted by physically crushing the nuts or seeds and

pressing out the oil. However, most commercial vegetable oils are extracted with hexane, which is later removed from the oil and recycled through the extraction process. After the extraction process, the oils are considered “crude” oils. These oils still retain many of the minor lipid components discussed above, along with small amounts of protein, moisture, and in some cases, volatile flavor compounds. In some cases, the crude oils are of acceptable organoleptic quality in their crude state and may only require filtration or centrifugation to remove suspended solids. Extra-virgin olive oil and specialty nut or seed oils (walnut, almond, sesame) are examples of oils that are often left in their crude state where the color and flavor components are desirable. However, in many cases, bland, colorless, and oxidatively stable oils are desired for use as salad and cooking and frying oils and in processed foods. Therefore, most commercial fats and oils are further processed in order to remove minor lipids, flavor compounds, and colors. Prior to processing, crude oils need to be filtered to remove suspended impurities such as dirt or plant tissues (Gupta 2008).

Degumming

Degumming is the process where phospholipids are removed from crude vegetable oils that have high phospholipid content. Phospholipids can have a negative impact on the flavor stability and functionality of oils, for example, causing foaming in frying oils (Gupta 2008). Under this process, crude oils are treated with water, or with a mixture of acid and water, to hydrate the phospholipids. The hydrated phospholipids form a gum-like substance, which agglomerates and can then be removed by centrifugation. The phospholipids may be sold as food additives such as soy lecithin or may be used in animal feed as a source of both fatty acids, as well as nutrients such as choline from phosphatidylcholine.

Refining

The refining process is used to remove minor oil components such as free fatty acids and phospholipids as well as mono- and diacylglycerols (Gupta 2008). Oils can either be alkali refined or physically refined. In alkali refining, oils are pretreated with phosphoric acid or citric acid to chelate metals and hydrate phospholipids. The acidulated oil is then reacted with a sodium hydroxide solution, which reacts with free fatty acids to form soaps and which neutralizes the acid. The oil is centrifuged to remove the gums and soaps and then washed with water to remove additional soap and impurities. In physical refining, oil is treated with acid as above, to remove trace metals and phospholipids. The oil is then bleached and deodorized (see below).

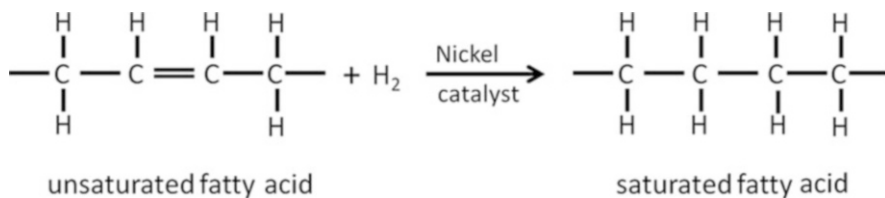


Fig. 10 Hydrogenation is the reaction where hydrogen is added across a double bond to turn unsaturated fatty acids into saturated fatty acids. A catalyst such as nickel is used

Bleaching

In bleaching, oils are treated with an adsorbent material to remove color compounds such as chlorophyll and carotenoids, as well as any remaining phospholipids and trace metals (Gupta 2008).

Deodorization

In the deodorization step, oils are purged with steam while under a vacuum in order to remove undesirable flavors, colors, and odors.

Winterization

In winterization, oils are cooled to crystallize triacylglycerols that have a high melting point, which are then removed by filtration. This prevents oils from becoming cloudy at low temperatures. Similar techniques, along with solvent fractionation, may be used to produce high and low melting point fractions of fats such as palm oil (Gupta 2008; Berger and Idris 2005).

Hydrogenation

Hydrogenation is a process that is used to reduce the levels of polyunsaturated fatty acids in oils. This is done to render them more oxidatively stable and/or to alter the physical properties by raising the melting point. As indicated by the name, hydrogen is added across double bonds, catalyzed by a solid nickel catalyst, in order to convert the double bonds to single bonds (Fig. 10). Vegetable oils may either be partially hydrogenated, so that only some of the double bonds are removed, or fully hydrogenated to make the oil essentially a completely saturated fat. Depending on the extent of hydrogenation, the melting point and physical properties of partially hydrogenated oils can be customized to some extent, making partially hydrogenated oils that range in properties from liquid to semiliquid to hard solids at room temperature.

If oils are only partially hydrogenated, the unreacted double bonds may shift around on the carbon chain, or they may isomerize from the natural *cis* form to *trans* isomers. Thus, partially hydrogenated oils may have increased saturates as well as increased *cis* and *trans* isomers of unsaturated fatty acids.

Interesterification

Interesterification is another method of changing the physical properties of oil triacylglycerols. In this process, oils with different types of fatty acids and triacylglycerols are blended together. Then through either chemical or enzymatic processes, the fatty acids are interchanged among the triacylglycerols, effectively creating new triacylglycerols, which may alter their physical properties. Interesterification is used for the production of special fats, such as medium-chain triacylglycerol oils or oils with similar properties to partially hydrogenated oils, but without the production of *trans* fatty acids.

Nutritional Properties of Fats and Oils

Fat is an important macronutrient in the human diet. It is an important source of energy, providing 9 kcal/g, while proteins and carbohydrates each supply 4 kcal/g. Fats and oils are also a source of two essential fatty acids, linoleic acid (18:2 n-6) and linolenic acid (18:3 n-3). These fatty acids cannot be synthesized by the body; thus, they must be consumed in the diet. They are used to synthesize eicosanoids, which are signaling compounds used in the body to regulate inflammation and immunity. US nutritional guidelines suggest that 20–35 % of calories should come from fat while providing a recommended “adequate intake” (AI) for n-6 fatty acids, including linoleic acid, of 10–17 g/day and for n-3 fatty acids, including linolenic acid, of 1.1–1.6 g/day (USDA and USDHHS 2010). Consumption of fats and oils also helps with the absorption of fat-soluble vitamins and vitamin precursors, such as carotenoids.

A lot of research has been devoted to the role of fats in the diet and of the role of specific fatty acids or types of fatty acids in normal health as well as in disease. Consumption of high amounts of saturated and, in particular, *trans* fatty acids is associated with a risk of high LDL cholesterol and cardiovascular disease, which is the leading cause of death for both men and women in the USA. It should be noted that these studies mainly focused on *trans* fatty acids from hydrogenated oils and not on the naturally occurring *trans* fatty acids found in some meat and dairy fats. Replacing *trans* and saturated fatty acids in the diet with monounsaturated or polyunsaturated fatty acids has been shown to lower the risk for cardiovascular disease (Vannice and Rasmussen 2014). Since 2006, the Food and Drug Administration (FDA) has required *trans* fatty acid content to be listed on packaged food labels (FDA 2008); then in 2013, the FDA issued a preliminary determination that partially hydrogenated oils are not generally recognized as safe (GRAS) for use in

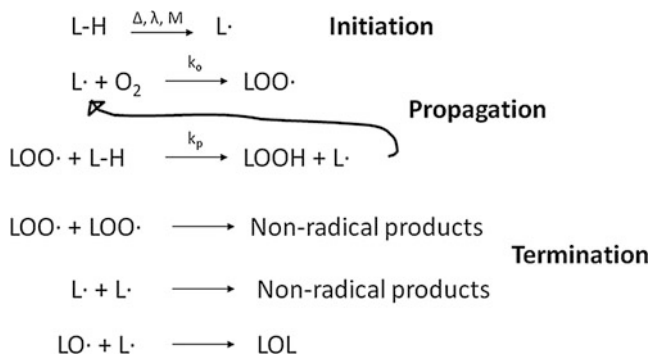


Fig. 11 Free radical-catalyzed oxidation (autoxidation). *L* lipid

foods (FDA 2013). Such a ruling would effectively mean that partially hydrogenated oils could no longer be used in food products in the USA.

In recent years, a lot of attention has also been given to omega-3 (n-3) fatty acids, especially long-chain omega-3 fatty acids found in cold-water fish oils. Consumption of fish oil has been shown to reduce plasma triacylglycerol levels (Balk et al. 2006), to lower blood pressure, and to be associated with lower instances of cardiovascular disease and cardiac death (Mozaffarian and Wu 2011). Two long-chain omega-3 fatty acids, in particular eicosapentaenoic acid and docosahexaenoic acid, are believed to be responsible for this effect (Superko et al. 2014). Omega-3 fatty acids have also been investigated for other effects such as lowering blood pressure and stroke risk, but at this time the evidence is not conclusive that omega-3 consumption is beneficial for these risks (Kris-Etherton et al. 2002).

Oxidation of Fats and Oils

Lipid oxidation is a series of reactions between lipids and oxygen that results in the degradation of lipids, the destruction of nutrients and bioactive lipids, and the development of off-flavors and odors in oils and in lipid-containing foods (Fig. 11; Frankel 2005). As shown in Fig. 11, lipid oxidation is initiated when a free radical abstracts hydrogen from a lipid. The hydrogens allylic to a double bond in unsaturated fatty acids require less energy for abstraction; therefore more highly unsaturated fatty acids or lipids will oxidize at a faster rate because they are more susceptible during both the initiation and the propagation stage. Co-oxidation of proteins and starch can also occur, leading to further degradation of nutritional quality and texture of complex food products. In addition, lipid oxidation can occur in bulk oils and in foods ranging from high-fat to whole-grain food products that have no added oils. Thus, lipid oxidation is one of the leading causes of reduced shelf life of foods and has a major economic impact on food manufacturers.

Composition of Major Fats and Oils

Oils and Fats of Plant Origin

Oil or fat has been extracted, characterized, and consumed from thousands of plant tissue, nuts, and oilseeds. The general term for these oils is “vegetable oils.” Many oils are produced and consumed only in the regions where the plant is sourced naturally. In other cases, the oils may be produced for commercial sale worldwide, but production is low compared to most commodity vegetable oils. These oils are typically called “specialty oils.” Information on “specialty oils” can be found in the references by Gunstone et al. (1994, 2007) as well as by Moreau and Kamal-Eldin (2009). The composition and properties of the most commercially important oils worldwide are briefly described below.

Palm Oil and Palm Kernel Oil

Most commodity oils are produced from seeds or nuts, but palm oil is extracted from the mesocarp (inner flesh) tissue of the oil palm fruit (*Elaeis guineensis*). Palm kernel oil is extracted from the inner seed (kernel) of the oil palm fruit, which is 46–57 % oil by weight. More palm oil is produced than any other vegetable oil, and it is also the most efficiently produced oil; approximate yields of palm oil are about 4,000 kg per hectare, which is about ten-fold higher than soybean oil yields. Malaysia, Indonesia, and Nigeria are the world’s largest producers of palm and palm kernel oil. The fatty acid profiles for palm oil and palm kernel oil are substantially different (Table 4) and thus have different physical properties and applications.

Palm oil is actually a solid (technically a semisolid) due its high content of saturated fatty acids, so it is actually a fat. It can be used in any number of applications that require a solid fat, such as in margarines and shortenings used in baking and confectionary applications. Palm oil and palm oil blends are also used for deep fat frying, because it is very resistant to oxidation due to its high content of saturated fatty acids. Palm kernel oil has high content of lauric acid, which is valued for use in lotions and cosmetics. In foods, it is often used in confectionary applications. In order to further modify their physical properties for various applications, palm oil and palm kernel oil can be fractionated by crystallization (similar to winterization) to yield products such as palm stearin, palm olein, palm superolein, palm kernel olein, and palm kernel stearin, which have different fatty acid compositions, which alters the physical properties and functionality (Berger and Idris 2005).

Crude palm oil is red in color due to high levels of carotenoids (500–700 mg/Kg), especially alpha- and beta-carotene. The carotenoids are destroyed or removed by processing and refining, but a product called “red palm oil” is minimally refined in order to retain its provitamin A beta-carotene content (Choo et al. 1993). Unlike most commercial fats and oils, palm oil also has substantial quantities of tocotrienols. However, palm kernel oil is very low in both tocopherols and tocotrienols.

Soybean Oil

Soybean oil is extracted from the soybean legume (*Glycine max*). It is the second most highly produced oil in the world, after palm oil. The USA, China, Brazil, and Argentina are the world's largest producers of soybean oil. Soybean oil is high in polyunsaturated fatty acids, namely, linoleic and linolenic acids. It is widely used as a salad and cooking oil, is blended with oils or fats for margarines and shortenings, and can be found in a wide variety of processed food products. It is also used for frying, but because of its susceptibility to oxidation, it typically needs to be stabilized by the addition of antioxidants or by blending with more stable oils for frying and in food applications that require a long shelf life. In order to address the oxidative stability issues for frying, several soybean oil varieties with altered fatty acid compositions have been developed, either using traditional breeding practices or genetic modification. These include low-linolenic acid soybean oil, ultralow-linolenic acid soybean oil, and high-oleic soybean oil. These trait-modified oils are still produced in low quantities, but because they have better oxidative stability than regular soybean oil, one or more of these oils may eventually replace all or most traditional soybean oils.

Low-Erucic Acid Rapeseed Oil/Canola Oil

Low-erucic acid rapeseed oil is extracted from the seeds of plants in the Cruciferae family, namely, *Brassica napus* L., *B. juncea* L., and *B. rapa* L. Rapeseed has been cultivated for centuries, but its popularity as a crop and oil in Europe, Canada, and the USA has increased rapidly in the last 25 years (Gupta and Pratap 2007). Traditional rapeseed varieties were high in erucic acid (C22:1 n-9). In the 1960s, research indicated that high erucic acid may be harmful, particularly to heart tissue (Borg 1975), which raised concern over the safety of rapeseed oil consumption. In addition, the seed meal from rapeseed oil production was high in glucosinolates, which cause problems with digestion and thyroid activity when the seed meal is used in animal feed. Thus, canola and most other modern, low-erucic acid rapeseed oils were developed from naturally low-erucic acid/low-glucosinolate varieties. Canola oil and other low-erucic acid vegetable oils have high levels of oleic acid instead of erucic acid. According to FAO standards, all low-erucic acid rapeseed oils must have less than 2 % erucic acid. Varieties containing erucic acid are still produced and consumed in some parts of the world.

Sunflowerseed Oil

Sunflowerseed oil is extracted from seeds of the sunflower (*Helianthus annuus* L.). Russia and Ukraine are by far the top producers of sunflower, followed by EU countries, Argentina, China, and the USA. Traditional sunflowerseed oil is very high in polyunsaturated fatty acids, especially 18:2 n-9,12 (linoleic acid). However, through traditional breeding efforts, cultivars that produce either mid-oleic acid (~65 % oleic acid/ 25 % linoleic acid), high oleic acid (~80 %)/low linoleic acid (10 %), and even high oleic acid (72 %)/high stearic acid (18 %) have been developed (National Sunflower Association 2010). In the USA, over 85 % of the sunflower grown is now the mid-oleic variety, known commercially as NuSun[®],

because it has a longer shelf life and is more stable for frying applications. Due to its popularity for frying and other food applications, most of the US production of sunflowerseed oil is sold domestically. Traditional sunflowerseed is produced in small volumes in the USA only for confectionary purposes (sunflowerseed snacks) or as bird feed but is still widely grown in Russia, Ukraine, and the EU.

Peanut Oil

Peanut oil is known outside of the USA as groundnut oil or arachis oil and is extracted from seeds of peanuts (groundnuts, *Arachis hypogaea* L.). The largest producers of peanut oil are in Africa, India, Asia, and the USA (Gunstone et al. 1994).

Cottonseed Oil

Cottonseed oil is a coproduct of cotton production. Relative to many other vegetable oils, except for palm oil, cottonseed oil is high in palmitic acid (16:0). It is used for frying and in margarines and shortenings.

Corn Oil

Corn oil is a coproduct of corn wet milling. In corn wet milling, the corn is degermed, and corn oil is then extracted from corn germ. The largest corn oil producers are the USA, EU countries, and China. Corn oil is used in frying, as a salad and cooking oil, and in margarines.

Coconut Oil

Coconut oil is also sometimes referred to as copra oil, and it is extracted from copra, which is the white solid endosperm, or meat, of the nut from the coconut palm (*Cocos nucifera*) (Gunstone et al. 1994). Coconut oil is semisolid, so it is really a fat. Its fatty acid composition is similar to palm kernel oil, and it is used in similar applications.

Cocoa Butter

Cocoa butter is not produced at the volumes of the other oils in Table 1; however, it is one of the most expensive and highly valued fats produced in the world. It is produced from the dried seeds from the fruit of the cocoa tree (*Theobroma cacao*). This tree only grows close to the equator; thus the largest producers are in Africa, Asia, and South America. Cocoa butter is mainly used in the production of chocolate and other confectionary items. As mentioned previously, it has a few major triacylglycerols that, due to their unique nature, provide a smooth melting profile in the mouth.

Olive Oil

Olive oil is extracted from the mesocarp tissue of olives, the fruit of the olive tree (*Olea europaea* L.). It is grown and produced throughout Spain, Italy, Greece, as well as Turkey, Tunisia, and other North African and Middle Eastern countries bordering the Mediterranean (Firestone). Olive oil is often consumed in its “virgin”

Table 5 Typical fatty acid composition of selected animal fats (Adapted from Haas 2005)

Fatty acid	Beef tallow	Lard	Poultry fat
10:0	0–0.1	0.1	
12:0	0.1	0.1	
14:0	2.7–4.8	1.4–1.7	1.3
16:0	20.9–28.9	23.1–28.3	23.2
16:1	2.3–9.1	1.8–3.3	6.5
18:0	7.0–26.5	11.7–24.0	6.4
18:1	30.4–48.0	29.7–45.3	41.6
18:2	0.6–1.8	8.1–12.6	18.9
18:3	0–0.7.3	0.7–1.2	1.3
Others^a	20:1 (0.3–1.7) <i>trans</i> fatty acids, unspecified (1.3–6.6)	20:1 (0.8–1.3), <i>trans</i> fatty acids, unspecified (1.1–1.4)	None listed

^aOnly fatty acids that may exceed 1 % are listed (fatty acid is listed first, followed by typical percentage in parentheses)

form, which is the more highly valued oil used as salad and table oil, due to desirable flavors retained in this oil. According to CODEX (FAO 2013b) and the International Olive Council: “Virgin olive oils are those oils obtained from the fruit of the olive tree solely by mechanical or other physical means (i.e., no solvents used in extraction) under conditions that do not lead to alterations of the oil and which have not undergone any treatment other than washing, decanting, centrifugation and filtration” (IOC, <http://www.internationaloliveoil.org/estaticos/view/83-designations-and-definitions-of-olive-oils>). Virgin olive oils are further classified as “extra-virgin,” “virgin,” or “ordinary virgin,” depending on the content of free fatty acids along with other chemical and organoleptic standards developed by the International Olive Council (<http://www.internationaloliveoil.org/estaticos/view/222-standards>) and implemented through the FAO Codex (2013b). Oils that do not meet the standards set for the definitions above have to be refined and are referred to as “refined olive oil.” Olive oils have a high content of oleic acid (Table 4) and low amounts of saturates and polyunsaturates. However, because of the high content of free fatty acids in virgin olive oils, they are not highly used in processed or fried foods.

Oils and Fats of Animal Origin

The major animal fats produced for use in foods include the depot fat from pigs (lard), cattle (beef tallow), and sheep (mutton tallow), while poultry fat is mainly used in animal feed. Animal fats also include milk fats from cows, sheep, goats, and other animals. The fat from cow’s milk is described thoroughly in the next section “[Butter, Butter Oil, and Ghee](#).” Depot fats include subcutaneous fat (under the skin), intramuscular fats, and fats surrounding organs (Haas 2005). The composition of beef tallow, lard, and poultry fat is shown in Table 5. Fatty acid composition is variable depending on the species, breed, diet, and other factors. Animal fats tend

to have higher contents of saturated fatty acids compared to most vegetable oils. In addition, small amounts of uneven-chain fatty acids, *trans* fatty acids, and conjugated double bonds may be found in animal fats, due to microbial action during digestion.

Butter, Butter Oil, and Ghee

Milk fat is mainly triacylglycerols (97–98 %) along with some free acids, monoacylglycerols and diacylglycerols, cholesterol (0.2–0.4 %), phospholipids (0.2–1.0 %), traces of carotenoids, squalene, and fat-soluble vitamins. Milk fat is consumed in the form of milk such as butter, ghee, butter oil, or fat-rich dairy products and is an important source of dietary lipids. Butter and ghee making originated in India between 2000 BC and 1400 BC and milk, honey, and butter were used in ceremonial feasts. In many Indian households, butter for manufacture of ghee is made by the direct churning of milk. In the middle of the nineteenth century, by gravity creaming process, cream was separated which was used in the production of butter. With advancements such as the centrifugal cream separator and the butter churn, rapid development of butter making at a large scale took place. Fat-rich dairy products like ghee (clarified milk fat), butter oils, butterfat-vegetable oil blend (e.g., margarine), and fractionated butterfats are nowadays produced (McDowall 1953; De 1991). Butter has saturated and high cholesterol content which are associated with vascular and heart disease that reduces the popularity of butter. Margarine developed from cheap substitute vegetable oil (polyunsaturated) has become quite popular. Butter oil describes the fat recovered from butter or fat obtained directly from cream by de-emulsification followed by direct centrifugation. The terms milk fat, anhydrous milk fat, dry butter fat, and dehydrated butter fat are used synonymously with butter oil (McDowall 1953; De 1991). Ghee is almost completely anhydrous milk fat which can be produced by clarification of milk fat at high temperature and there are no similar products in other countries. Ghee has a long shelf life at room temperature (25–40 °C). It is a rich source of energy, fat-soluble vitamins (A, D, E, K), and essential fatty acids. A major portion of ghee is used for culinary purposes and the remainder used in confectionary items and consumed on religious ceremonies (Ganguli and Jain 1972).

Butter

Butter is a solid emulsion of fat globules and liquid as well as crystallized fat, water, and air. It consists of fat (80–82 %) and an aqueous phase (18–20 %) which contains salts and solids-not-fat (lactose, protein, minerals, vitamins). The moisture content in butter should not be more than 16 %. Its color varied from nearly white to pale yellow to deep yellow. Butter is a semisolid, soft mass which melts easily at room temperature but poor spreadability at refrigeration temperature. Butter is made from cream obtained by centrifugation separation of milk. The cream, so obtained having 35–45 % fat, is churn in butter churn followed by gathering the fat into a compact mass and then working it. During the churning process, oil-in-water (in cream) phase is inverted into water-in-oil emulsion (in butter) (Hunziker 1940).

In milk, fat is in emulsion state and exists in the form of globules shaping having 0.2–22 μm size. The fat globules are covered by a thin membrane, known as the milk fat globule membrane. The membrane is composed of surface-active agents like phospholipids and lipoproteins (Mulder and Walstra 1974). This membrane provides the entity of fat globules, prevents it from coming closer and joining together, and keeps it dispersed in milk serum. Agitation in churn ruptures the membranes and results in coalescence to form large fat granules and separate out. The separated milk fat is known as butter, while the watery portion is called as buttermilk. Various types of butter like creamery butter, salted butter, unsalted butter, pasteurized cream butter, ripened cream butter (*makkhan*), unripened cream butter, sweet cream butter, and sour cream butter are found in the market (De and Mathur 1968; Solanky 1990; De 1991).

The butter making process, whether by batch or continuous methods, consists of preparation of cream, destabilization and breakdown of the oil-in-water (o/w) emulsion, aggregation and concentration of the fat particles, formation of a stable water-in-oil (w/o) emulsion, packaging, and storage.

The pasteurized cream is aged to 5–10 °C for 10–12 h (generally overnight) that transferred to the butter churn. The efficiency of churning or churnability is measured in terms of the time required to produce butter granules and by the loss of fat in the buttermilk that depend on the temperature of cream or churning. The churning of cream is usually carried out at a temperature of 11–15 °C (in winter) or 9–11 °C (in summer) (Thakar 1990; De 1991). In ripened cream butter, fermentation (ripening) of cream is carried out using *Streptococcus lactis* and/or *Streptococcus cremoris* together with aroma (diacetyl) producers such as *Streptococcus diacetilactis*, *Leuconostoc citrovorum*, and *Leuconostoc dextranicum*. The starter cultures are added in cream at 0.5–2.0 % which is incubated at 20–22 °C for 15–16 h. The typical flavor of butter from ripened cream is mainly the effect of 2,3-butanedione (diacetyl) and to a smaller extent of acetic and propionic acids. The normal diacetyl content of ripened cream butter is on average 2.5 ppm (De 1991). There is no diacetyl in sweet cream butter. However, the ripening of cream is time consuming and expensive. Alternatively, the mixing of starter distillate or synthetic flavor compounds in sweet cream during the working process imparts a characteristic flavor to butter but somewhat a harsh unnatural aroma and lacks the pleasing, mellow, uniformly blended aroma of ripened cream butter (De 1991). To maintain the uniformity of yellow color in butter throughout the year for consumer satisfaction, yellow color is sometimes added (varies from 0 to 250 ml or more per kg of butter fat) in cream prior to the churning process. Generally either annatto or β -carotene is used to color butter. Annatto is obtained from the seeds of the annatto plant (*Bixa orellana* L.), whereas β -carotene is extracted from carrots and other β -carotene-rich vegetable matter. The coloring substances are extracted by dissolving the annatto seeds in various oils like sesame, castor, and groundnut (De 1991; Satyanarayana et al. 2003).

The churning of cream consists of violent agitation until the fat globules adhere, forming larger and larger masses, a relatively complete separation of fat and serum occurs. Air is beaten into the cream and is dispersed into small bubbles during churning. Fat globule membrane substances (e.g., phospholipids/lecithin) and some

of the liquid fat are spread over the air-water interface and become attached to the bubbles. The air bubbles keep moving and collide with each other and their surface area diminishes resulting in the adhering fat globules to be driven toward one another. The liquid fat then acts as a sticking agent and the fat globules are clumped together. In this way, small fat clumps are formed that precipitate in the churning process, resulting in still larger clumps (Walstra et al. 1999). After producing butter granules of approximately the size of a peanut, the buttermilk should be drained from the churn (Walker-Tisdale and Robinson 1919; McKay and Larsen 1939). Washing the butter (with pasteurized chilled water) enables the removal of free buttermilk from the butter granules thereby reducing the curd content of the butter and it helps to control the temperature of the granules for the subsequent working process. Unwashed butter may contain 1.1–1.5 % of curd compared to 0.6–1.0 % found normally in washed butter (Thakar 1990). The quantity of salt to be added is based on an estimate of the amount in finished butter expected from the churn. Generally salt content in finished products should not be more than 3 %. After the addition of salt, working is continued until the free moisture is absorbed by the butter and the churn is dry and final moisture content in butter should not be more than 16 %. On completion of the working stage, the churn is unloaded and the butter is taken in the trolley for packaging of the butter and subsequently stored at refrigeration temperature.

Butter Oil

Butter oil refers to the fat concentrate obtained mainly from butter or cream by the removal of practically the entire water and solids-not-fat content. The terms milk fat, anhydrous milk fat, dry butter fat, and dehydrated butter fat are used synonymously with butter oil. Anhydrous milk fat should have a minimum 99.8 % fat and should not contain more than 0.1 % fat (Spreer 1998). Butter oil (anhydrous milk fat) is mainly used for cooking, baking, and frying. Butter oil which is popular in continental countries differs from ghee (in India and Pakistan) or samna (in Egypt) in color, granularity, and flavor resulting from the different methods of manufacture (Hagrass et al. 1983; Singh 2014). Butter oil is darker in color and less granular in appearance and has a bland/flat flavor than that of ghee/samna. Butter oil can be made from both butter and cream with different methods, viz., evaporation of molten butter under vacuum, decantation of molten butter, centrifugal separation of butterfat followed by vacuum drying, and direct from cream by de-emulsification and centrifugation. Butter oil should be cooled and crystallized under careful control, so as to form a large number of fine crystals. The desired result may be obtained by rapidly supercooling to 13–18 °C and stirring the mass during forced crystallization (by adding 5–15 % of finely crystalline fat from a previous batch); this yields a smooth homogeneous mass which does not separate into solid and liquid layers on standing (De 1991; Singh 2014).

Ghee

Ghee is a concentrate of butter fat with over 99 % milk fat and less than 0.3 % moisture. It has a shelf life of 6–8 months at ambient tropical temperatures. Ghee is

manufactured by direct heating of cream or butter churned from fresh or ripened cream or *dahi* obtained by fermentation of milk with bacteria native to milk or selected starter cultures (Srinivasan 1976). The different methods of ghee manufacture include *desi* or indigenous or traditional method, creamery butter method, direct cream method, and pre-stratification method which are briefly discussed at below.

The traditional (*desi*) method in which fermentation (by lactic acid bacteria) of the primary raw material, i.e., milk, is carried out followed by concentrating the milk fat by mechanical process and then heating of the fat concentrate at a specified range of temperatures to remove moisture and induce the interaction of milk fat with fermented residues of nonfat milk solids (Pandya and Sharma 2002). Creamery butter method is the usual industrial practice to produce ghee. Cream is separated from milk by a cream separator. The cream is pasteurized, cooled, aged, and converted into butter. In order to further improve the flavor of the final product, the cream is sometimes ripened using a lactose fermenting starter culture and the churning is carried out in the usual manner. Butter is then clarified at temperatures ranging from 110 °C to 140 °C. The ghee residue is removed either by filtration or through a ghee clarifier. In direct cream method, ghee is obtained by directly heat clarification of the cream. In pre-stratification method, butterfat is heated to around 80 °C and left undisturbed. The lower layer is made up of the serum portion while upper layer is largely fat and the curd particles generally form an intermediate stratum. The upper layer is subsequently used for clarification and the yield of ghee also increases by about 8 % by this method (Rangappa and Achaya 1974; Singh 2014).

Chemical Composition

Among the other constituents, milk fats play a significant role in milk and dairy products. Fats provide energy, perform a number of vital functions in the body, and have nonnutritional functions like their use in frying and baked goods and their contribution to the flavor and palatability of foods. Moreover, it readily takes up off-flavors if stored near substances with a strong odor. Milk fats are mainly composed of triacylglycerols which represent about 97–98 % of the total lipid, whereas the remaining 2–3 % include phospholipids, cholesterol and cholesterol esters, diacylglycerols, monoacylglycerols, and free fatty acids (Bitman and Wood 1990). Milk fats also contain ether lipids, fat-soluble vitamins, hydrocarbons, and flavor compounds (keto and hydroxy acids) as trace amounts (Parodi 2003). The various components of milk lipids are presented in Table 6.

Milk lipids have about 500 separate fatty acids. The fatty acids are esterified in three positions resulting in various positional isomers which affect different physical (e.g., melting point) and chemical properties (e.g., rate of hydrolysis) (Kurtz 1974). Milk phospholipids, associated mainly with the fat globule membrane, do not contain short-chain fatty acids, but they contain higher concentrations of long-chain and polyunsaturated fatty acids than triacylglycerols (Parodi 2003; Hettinga 2005). Milk fat contains considerable amounts of short-chain fatty acids that are important in contributing flavor particularly to butter and ghee (Walstra 1983).

Table 6 Various components of milk lipids (Bracco et al. 1972; Christie 1995; compiled from various sources)

Composition of milk lipids	
Lipid class	Amount (wt.% of total lipid)
Triacylglycerol	97.5
Diacylglycerol	0.36
Monoacylglycerol	0.027
Cholesterol ester	Trace
Cholesterol	0.31
Free fatty acids	0.027
Phospholipids	0.6
Composition of lipids from milk fat globule membrane	
Lipid component	Wt. % of membrane lipids
Carotenoids (pigment)	0.45
Squalene	0.61
Cholesterol ester	0.79
Triglycerides	53.4
Free fatty acids	6.3
Cholesterol	5.2
Diglycerides	8.1
Monoglycerides	4.7
Phospholipids	20.4
Fatty acid composition of milk fat	
Fatty acid	Mole % of milk fat
Butyric	3.3–3.6
Caproic	1.5–1.6
Caprylic	1.1–1.3
Capric	1.9–3.0
Lauric	2.0–3.1
Myristic	8.7–9.5
Palmitic	26.3–30.4
Stearic	10.1–14.6
Oleic	28.7–29.8
Linoleic	2.4–2.5
Linolenic	0.8–2.5

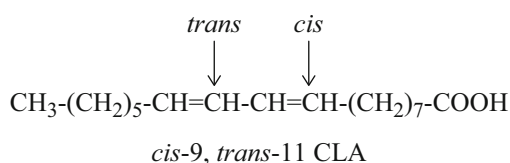
Milk fats are solid or semisolid at room temperature due to a high proportion of palmitic and stearic acids (Mortensen 1983). Conjugated linoleic acid (CLA) is a collective term for a series of conjugated dienoic positional and geometrical isomers of linoleic acid (C_{18:2}). CLA is found in relative abundance in milk (Lawson et al. 2001). Conjugated linoleic acid is formed as an intermediate product in the ruminal digestion that leads to shift the normal position of two double bonds in linoleic acid. The various possible positions are 8/10, 9/11, 10/12, or 11/13 giving a range CLA types. The *cis*-9, *trans*-11 CLA is the principal dietary form that accounts for 73–94 % of total CLA in milk and dairy products of ruminant origin (MacDonald 2000; Bell and Kennelly 2001; Kelly 2001; Parodi 2003). The total

Table 7 Composition of butter and butter oil

Table butter		Butter oil ^a	
Constituents	Percentage	Constituent	Percentage
Fat (minimum)	80.0	Butterfat	99.5–99.8
Moisture (maximum)	16.0	Moisture	0.1–0.3
Salt (maximum)	3.0	Acidity (oleic)	0.2–0.5
Curd (maximum)	1.0	Peroxide value	0.0–0.1

^aMcDowall 1953

CLA content in sour cream varies from 0.46 % to 0.75 %, in butter 0.47–1.19 %, in cow ghee 0.60–2.10 %, and in buffalo ghee 0.50–1.90 % (Aneja and Murthy 1990; Bhavbhuti 2009). The chemical formula of CLA is shown below.



Chemical Aspects of Butter and Butter Oil

Butter is mainly the concentrated form of milk fat and fewer amounts of protein and milk sugar are present. Fat-soluble minor constituents of milk, the yellow pigment β -carotene, and its transformation product vitamins A and D are present in butter. The curd consists of the nonfatty constituents of the buttermilk such as casein, albumin, and milk sugar and a small amount of minerals are also found in butter and the amount does not exceed 1.0 %. Butter prepared from ripened cream has a diacetyl content of, on average, 2.5 ppm (Sharma 1990). The composition of table (creamery butter) and butter oil in general is mentioned in Table 7.

Flavor Compounds in Butter and Butter Oil

The initial quality of cream and its processing conditions such as heating and ripening ultimately affect the flavor quality of butter and butter oil. The processed cream has different odorants such as diacetyl (buttery), 2-pentanone (carrot-like), dimethyl trisulfide (cabbage-like), acetic acid (acidic), furfural (caramel-like), and butanoic acid (cheese-like) (Pionneir and Hugelshofer 2006). The aroma of cream is contributed by the fat globule membrane and aqueous phase of milk (Badings and Neeter 1980), while butter aroma is primarily derived from the volatile compounds present in the fat fraction. Sweet cream butter, sour cream butter, and butter oil have odor-active compounds and some of them are mentioned in Table 8 and the chemical structures of a few potent odor-active compounds of butter and butter oil are in Fig. 12 (Mallia et al. 2008).

Chemical Aspects of Ghee

Chemically ghee is a complex lipid of (mixed) glycerides, free fatty acids, phospholipids, fat-soluble vitamins, tocopherol, carbonyls, sterols, sterol esters,

Table 8 Odor-active compounds in sweet cream butter (SwCB), sour cream butter (SoCB), and butter oil(BO) as determined by gas chromatography-olfactometry (Modified from Mallia et al. 2008)

Compound	Concentration ($\mu\text{g}/\text{kg}$ butter)			Odor quality
	SwCB	SoCB	BO	
(E)-2-Nonenal	10	nq	6.75	Green, fatty, tallow
(Z)-2-Nonenal	nq	nq	0.2	Green, fatty
(Z)-4-Heptenal	nq	nq	0.3	Green, fatty, cream, biscuit-like
(Z)-6-Dodeceno- γ -lactone	–	260	nq	Peach
1-Hexen-3-one	0.004	–	nq	Vegetable-like, metallic
1-Octen-3-one	0.58	nq	1.1	Mushroom-like
2,3-Butanedione	6.6	620	nq	Buttery
2-Methylbutanal	4.9	–	–	Chocolate, fruity
3-Methyl-1H-indole (skatole)	12.6	nq	nq	Mothball, fecal
3-Methylbutanal	11.9	–	–	Chocolate
Butanoic acid	192	4,480	nq	Buttery, sweaty, cheesy, rancid
Dimethyl sulfide	20	–	–	Corn-like, fresh pumpkin
Dimethyl trisulfide	17.4	–	–	Garlic, sulfury
Hexanal	29	nq	nq	Green, fatty
Hexanoic acid	732	1,840	nq	Pungent, musty, cheesy, acrid
Nonanal	43	–	–	Waxy, fatty, floral
γ -Dodecalactone	441	–	–	Peach
δ -Decalactone	1,193	5,000	nq	Coconut-like, peach
δ -Hexalactone	47.9	–	–	Creamy, chocolate, sweet aromatic
δ -Octalactone	72.8	–	nq	Coconut-like, peach

nq compound detected but not quantified

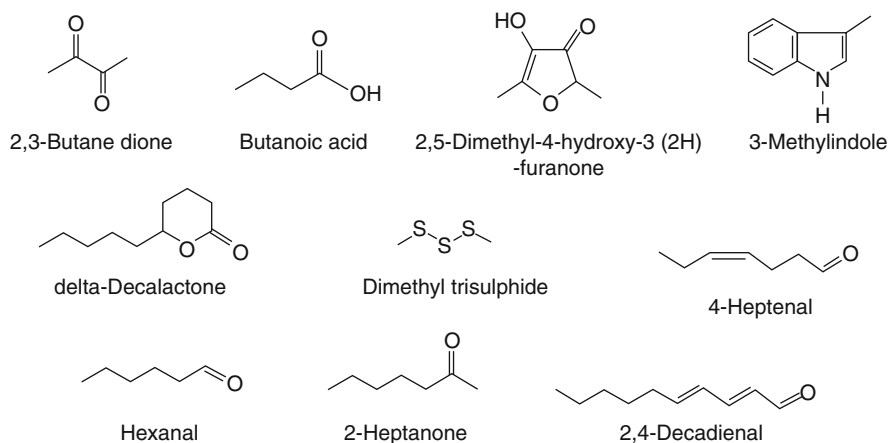
**Fig. 12** Chemical structure of some potent odor-active compounds of butter (Source: Mallia et al. 2008)

Table 9 Composition of ghee

Component	Cow ghee	Buffalo ghee
Moisture (%)	0.3–0.5	0.3–0.5
Milk fat (%)	99.0–99.5	99.0–99.5
Triglycerides		
Short chain (%)	37.6	45.3
Long chain (%)	62.4	54.7
Trisaturated (%)	39.0	40.7
High melting (%)	4.9	8.7
Partial glycerides		
Diglycerides (%)	4.3	4.5
Monoglycerides (%)	0.7	0.6
Phospholipid (%)	0.03	0.03
Cholesterol (mg %)	302–362	209–312
Lanosterol (mg %)	9.32	8.27
Lutein (µg/g)	4.2	3.1
Squalene (µg/g)	59.2	62.4
Carotene (µg/g)	7.2	0.0
Vitamin A (µg/g)	9.2	9.5
Vitamin E (µg/g)	30.5	26.4
Ubiquinone (µg/g)	5.0	6.5
Free fatty acids (% oleic acid)	<2.8	<2.8
Salts of Ca, P, Fe, Cu	Traces	Traces
Charred casein	Traces	Traces

hydrocarbons, carotenoids (only in ghee derived from cow milk), small amounts of charred casein, and traces of calcium, phosphorus, and iron. Glycerides constitute about 98 % of the total material. Of the ~2 % remaining constituents of ghee, sterols (mostly cholesterol) comprise 0.5 % (Ganguli and Jain 1972; Sharma 1980, 1981). The composition of ghee is shown in Table 9 and the fatty acid composition of ghee is shown in Table 10.

Ghee residue is obtained on the cloth strainer after the ghee is filtered. Ghee residue, a charred light to dark brownish solid mass obtained as a useful by-product of the ghee industry, contains considerable amounts of heat-denatured milk proteins and caramelized lactose and varying amounts of entrapped fat, besides some minerals, phospholipids, and water (Sharma 1980). The ghee residues have moisture at 4–13 %, fat 33–65 %, protein 18–42 %, lactose 0.5–15 %, and ash 2–5 %. The lipids of ghee residues contain less of butyric acid and total saturated fatty acids than that of ghee. The fatty acid composition of phospholipids shows no fatty acid lower than 12 carbon atoms (Singh 2014). Ghee residues are rich in phospholipids and also contain α -tocopherol, vitamin A, amino acids, proteins with free sulfhydryl groups, and protein-carbohydrate interaction products that show strong antioxidant properties (Santha and Narayanan 1978; Singh et al. 1979; Lal et al. 1984). The active sulfhydryl groups and H₂S give a cooked flavor. Due to the presence of lactones (3,992.9 µg/g), carbonyls (43.4 µM/g), and free fatty acids (627.5 µM/g), it

Table 10 Fatty acid composition of cow and buffalo ghee (mole %)

Fatty acid	Cow	Buffalo
Saturated		
Butyric	8.8–9.6	11.4
Caproic	3.5	3.1
Caprylic	1.8–2.2	1.0
Capric	3.0–3.1	1.6
Lauric	3.6–3.8	2.6
Myristic	9.5–9.9	10.6
Palmitic	23.4–26.1	30.3
Stearic	9.1–9.2	10.5
High saturated	0.8–1.0	0.7
Lower unsaturated	1.8	1.0
Hexadecenoic	2.8–3.6	3.6
Oleic	24.7–26.2	21.6
Values of some fat constants		
Reichert value	27–32	32
Polenske value	1.8–2.5	1.6
Iodine value	35–36	30
Butyro refractometer reading at 40 °C	40–45	40–45

Compiled from various sources

is used to enhance the flavor and quality of bland products such as vegetable fats and butter oil (Srinivasan and Anantakrishnan 1964; Santha and Narayanan 1979; Sharma 1980; Wadhwa et al. 1991; Aneja et al 2002; Singh 2014).

Ghee Flavor

The chemistry of ghee flavor is very complex as more than 100 flavor compounds were identified using GLC (Wadhwa and Jain 1990). Ghee has a pleasant, nutty, slightly cooked, or caramelized aroma. It is also described as having a lack of oiliness or of blandness and sweetly rather than sharply acidic. The flavor of ghee is generated during fermentation and/or heating. The constituents responsible for the butter flavor like indole, dimethyl sulfide, and skatole can be passed into ghee upon heating in their original or modified forms. Ghee is prepared from ripened cream in which lactic acid serves as a precursor for flavoring substances (Singh 2014). Carbonyls and lactones play a major role in imparting typical ghee flavor in addition to free fatty acids and reducing substances. Free fatty acids C₆–C₁₂, although present in very low concentration (0.4–1.0 mg/g) and account only for 5–10 % of total fatty acids, contribute significantly to ghee flavor (Pandya and Sharma 2002). From flavoring compounds in ghee, about 50 % are carbonyls (Wadhwa and Jain 1990). The total carbonyls, volatile carbonyls, and headspace carbonyls in ghee vary from 7.2 to 8.86 µM/g, 0.26–0.33 µM/g, and 0.027–0.035 µM/g, respectively, whereas free fatty acids and lactones are 53.6 µM/g and 30.3 µg/g, respectively (Pandya and Sharma 2002; Aneja et al. 2002).

Flavor Defects in Butter and Ghee/Butter Oil

Fresh butter having a clean buttery flavor is liked by most consumers. However, due to faults that occur starting from the handling of raw materials to production, various defects are likely to occur in the butter. The flavor defects are most likely due to the off-flavors in cream (e.g., feed, weed, cowy, barny, bitter, stale, yeasty, metallic, etc.), faulty methods in manufacture (e.g., flat, high acid and sour, cooked, oily), and post-defects after manufacture (e.g., surface taint, cheesy, putrid, rancid, oxidized flavors) (Bhavbhuti 2009).

The ghee/butter oil contains relatively low moisture which has a better keeping quality than that of butter. However, it undergoes various deterioration which spoils its delicate flavor and produces some toxic compounds. The ghee has undergone hydrolytic and/or oxidative deterioration. The hydrolytic rancidity is the secondary pathway in which lipolytic enzymes in the presence of moisture hydrolyze the triglycerides of milk fat and liberate free fatty acids and glycerol (Bhavbhuti 2009). The release of short-chain fatty acids is responsible for various off-flavor defects in ghee/butter oil. In addition to this, molds like *Penicillium* and *Aspergillus* or certain bacteria (e.g., *Micrococcus*, *Bacillus*) produced enzymes which hydrolyze the triglycerides and produce free fatty acids. These free fatty acids are degraded through β -oxidation. In β -oxidation, the carbon atom at a β position with respect to the carboxyl group is activated that undergoes a series of reactions to produce ketone from keto acids. The ketones are responsible for various off-flavors and this is known as ketonic rancidity. The oxidative rancidity is the major route by which ghee undergoes deterioration. This is referred to as autoxidation because the rate of oxidation increases as the reaction proceeds under usual processing and storage conditions. The hydroperoxides formed in the autoxidation of unsaturated fatty acids are unstable and readily decompose. The main products of hydroperoxide decomposition are saturated and unsaturated aldehydes. In addition to aldehydes, other secondary products of lipid oxidation such as unsaturated ketones, saturated and unsaturated alcohols, saturated and unsaturated hydrocarbons, and semi-aldehydes have been observed in the decomposition of hydroperoxides of the oxidized lipid system. These all compounds are responsible for various flavor defects in ghee and/or butter oil (Bhavbhuti 2009).

In addition to the above defect, heating ghee to around 120 °C for around 40 min increases the brown color, which eventually becomes deep brown, and the ghee finally gives a fishy flavor, because of the interaction between the amino group from the phosphatidylethanolamine and phosphatidylserine with the aldehyde groups. Moreover, such a fishy flavor defect is also found in butter when it is stored for a longer time.

Fish Oil

Fish oils are largely a coproduct of the production of fish meal (Ackman 2005), which is widely used as an animal feed and in aquaculture. They are produced by grinding and cooking fish, followed by pressing to remove the oil. At one time, extracted fish oil was partially hydrogenated and, apparently, widely used in margarines and

shortenings in the United Kingdom and Scandinavia (Kochhar and Matsui 1983). Due to concerns raised about the health effects of the *trans* fatty acids in hydrogenated oils and the increasing interest in the health benefits of long-chain n-3 polyunsaturated fatty acids found, most reliably, in cold-water fish, pure (unhydrogenated) fish oils have gained popularity, particularly in the form of supplements. However, the development and testing of food products incorporating fish oils is an active area in both research and product development, particularly in order to capture the health benefits of fish oils. Numerous types of fatty acids are found in fish oil (see ► [Chap. 14, “Chemical Composition of Fish and Fishery Products”](#)). However, according to Ackman et al. (1988), the fatty acids 14:0, 16:0, 16:1, 18:1, 20:1, 22:1, 20:5n-3, and 22:6n-3 constitute approximately 90 % of the oils in most fish of temperate and northern latitudes. The two fatty acids of major interest for their health and nutrition effects are eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6n-3).

Conclusion and Future Directions

The worldwide production of fats and oils, especially vegetable oils, has increased remarkably in the last 100 years. Fats and oils are one of the four important macronutrients, besides water, carbohydrates, and proteins. Fats and oils in foods provide calories, are good sources for vitamin E, and assist in the absorption of other fat-soluble vitamins from foods. In addition to their nutritional properties, fats and oils carry flavors in foods and provide textural properties that enhance the sensory experience and enjoyment of foods. Research over the past 40 years has led to further understanding of the role that specific fatty acids and lipids play in numerous physiological processes, including weight loss, exercise performance, neurological function, and disease formation, progression, and prevention. The relationship between dietary and supplementary lipids and health is expected to guide the future of fat and oil research.

Cross-References

- [Chemical Composition of Fish and Fishery Products](#)
- [Chemical Composition of Meat and Meat Products](#)

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Abstract

Aquatic organisms are considered a low-fat and protein-rich source, with other nutritional components that are positively attributed to health. Some micronutrients are in general more abundant in aquatic animals and plants than mammalian meats or terrestrial vegetables. Since ancient times, fish and shellfish have been used as food, but only in the second period of the twentieth century has aquatic food gained enormous relevance. Chemical composition of fish varies greatly among species and from an individual fish to another,

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depending on age, sex, environment, and season. Fish is the only protein source that contains all the essential amino acids. Lipids and proteins are the major components of fish food; meanwhile carbohydrates are usually detected at very low levels (<0.5 %). Fish is an excellent source of valuable micronutrients, vitamins, and minerals; vitamin content in fish is compared to that in mammals with the exception of vitamins A, D, and B₁₂. Fish enzymes obtained from fish offal and discards from the fishery industry are relevant owing to their applications to other food industries besides fisheries. Finally, a huge number of nutraceuticals from fish are being researched with enormous great application to human health. Hence, there is a growing demand for food from the aquatic, either marine or freshwater environment, owing to their nutritional components with health benefits to humans and animals.

Introduction

The aquatic environment contains a great amount of diverse species adapted to habitats with special conditions, hence offering an enormous potential of chemicals with many applications on food industry. Fish and mollusks are considered the best source of food, since they are very numerous in the aquatic environment and are composed of a great diversity of species. Marine biomass and freshwater environment offer a relevant source of proteins owing to their content in essential amino acids (Erkan et al. 2010), thus helping to solve the problem of food to the growing human population. Fish enzymes constitute an enormous potential owing to their unique properties that could be applied on different food industries (De Vecchi and Coppes Petricorena 1996). Laboratories from several countries are developing commercial processes to isolate enzymes from fish offal that result from fishery industry: Iceland, Norway, Canada, Great Britain, Japan, Denmark, and the USA. Utilization of the discard from fishery industry could serve to minimize contamination and instead to generate biochemical products in food processes.

At the beginning of the research on aquatic food, the interest on fish food was focalized on lipids, mainly fish oils, since they constitute the main source of very-long-chain polyunsaturated fatty acids, PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); however, the initial synthesis of these PUFAs is being done by algae which are the base of the food chain. Vitamins and minerals are the most relevant micronutrients from fish and shellfish; fish meat is a good source of vitamin B complex, and fat fish and shellfish are excellent source of fat-soluble vitamins A, D, and E (Dias et al. 2003). Hydrosoluble vitamins are distributed along the whole fish, with niacin, thiamine, riboflavin, pyridoxine, and B₁₂ being the most representative in aquatic organisms (Holland et al. 1993).

Marine Biotechnology explores aquatic organisms in order to develop new pharmaceutical products, chemicals, enzymes, bio-peptides, and toxins. Oceans occupy 70 % of the earth's surface and contain diverse life forms, offering an excellent source of new products used as nutraceuticals from fish food with

beneficial effects on health. The objective of the present contribution is to review the chemical composition of aquatic organisms with the purpose of their application to food industry and human health.

Proteins in Fish

The importance of fish as source of high-quality, balanced, and easily digestible protein, vitamins, and polyunsaturated fatty acids is well understood now. Fish are the most important source of animal protein and have been widely accepted as a good source of protein and other elements for the maintenance of a healthy body. The consumption of fish and fish products is recommended as a way of preventing cardiovascular and other diseases and has greatly increased over recent decades in many European countries (Cahu et al. 2004). Besides, fish are good source of immense antimicrobial peptides in defending against dreadful human pathogens (Ravichandran et al. 2011). They have significant role in nutrition, income, employment, and foreign exchange earning of the country. Fish and shellfish are the primary sources of animal protein and valuable in the diet because they provide a good quantity (usually 70 % or more) of protein of high biological value, particularly sulfur-containing amino acids. Next to meat, fish is the only protein source that contains all the essential amino acids in right proportion, hence called “complete protein.” Consumption of fish provides important nutrients to a large number of people in the world and makes a very significant contribution to nutrition (Pawar and Sonawane 2013).

About 50–60 % of fish weight is constituted by muscle, with proteins being the main constituents (16–21 %), followed by lipids (0.5–2.3 %), ash (1.2–1.5 %), and water (52–82 %); carbohydrate content is low, around 0.5 %. According to Love (1997), both lipid and water contents are constant; hence when lipid increases, water decreases or vice versa. Postmortem pH of fish muscle varies, depending on species and stress level, from 6.8–7.0 (cod and flounder) to 6.0–6.2 (mackerel and bluefish) (Kelleher et al. 2004). Fish has two types of muscles: light and dark, with low percentage of dark one in white fish such as cod and haddock, where only a small strip of dark or red muscle is just under the skin on both sides of the body. On the other hand, in fatty fish, such as herring and mackerel, the proportion of dark muscle is high, with more vitamins and fats; however, light muscle is more abundant and contains about 18–23 % proteins.

There are three main groups of proteins in fish, myofibrillar, sarcoplasmic, and stroma proteins, which constitute the 70–80 %, 20–30 %, and 3 % of total muscle proteins, respectively. Myofibrillar proteins, high-salt-soluble proteins, correspond to 65–75 % (w/w) of the total protein in fish and shellfish muscle (Venugopal 2009) and consist mainly of myosin (which account for 65–78 %) and actin, tropomyosin, m-protein, alpha-actinin, beta-actinin, c protein, and troponins I and C (Vareltzis 2000). Besides, paramyosin, a protein found in invertebrates, is not found in vertebrate myofibrils, and there are differences concerning the level of paramyosin from one shellfish myofibril to another (Vercruyssen et al. 2005). Postmortem

muscle shortening, postmortem pH, and proteolysis cause myofibrillar breakdown; hence the texture of fish flesh is modified (Coppes Petricorena et al. 2002; Coppes Petricorena 2011).

Sarcoplasmic (water-soluble or low-salt buffer-soluble) proteins constitute 20–30 % approximately of total muscle protein. Most of the sarcoplasmic proteins are considered to be enzymes, since the majority of components are myoglobins and hydrolases, oxidoreductases, transferases, phosphorylase, PFK (phosphofructokinase), and transglutaminase. However, this group of proteins is used to be contaminated by membrane proteins, which are not strictly soluble. Besides, muscles of fish and lower vertebrates differ from land animals owing to a great amount of protein ligands aquatic animals have. The level of each protein can vary significantly among species, as, for example, some mollusks contain no hemoglobin. Besides, there are compositional differences between fish- and mammalian-derived myoglobin, the first containing cysteine, while in mammals Mg is mainly lacking (Belitz et al. 2004). Muscle proteins, in general, contain approximately 3 % stroma proteins which consist mainly of collagen and elastin. In some fish, such as shark, ray, and skate, stroma proteins can account for 10 % (w/w) of total muscle proteins (Venugopal 2009).

In farmed fish species, protein content ranges from 10 % to 25 % with an average of 17 g in 100 g, which accounts for the 80–90 % of the energy produced per 100 g of lean species (Nunes et al. 2006). The protein found in seafood is of good quality due to its high digestibility and the specific amounts and relative proportion of essential amino acids (Nunes et al. 2011). The amount of protein is similar in pelagic and demersal fish. Fish is the most important source of animal protein of the people in developing countries, since approximately 53 % of the world's catch comes from these countries, and most of the catch is consumed within such countries. In the Southeast Asian region, people receive 60–70 % of their protein from fish. Protein content varies from fish to fish, either being marine or freshwater; hence a protein content variation can be expected in various fish products. The amount of connective tissue in fish and shellfish muscle is quite low, and it softens and dissolves more readily when heated compared to the connective tissue of land animals, making seafood meat easy to chew. Almost all species are well balanced with respect to their essential amino acids. The predominant amino acids are lysine and leucine, and within the nonessential, aspartic and glutamic acids are the most abundant. Very often the amount of essential amino acids is greater than that in the standard protein (32–100 g protein) and values regularly referred to the literature for the chemical score, biological value, and protein efficiency ratio. Protein digestibility and corrected amino acids are also good indicators of the quality of fish proteins (Nunes et al. 2011).

Functional Food and By-Products from Fish Proteins

Worldwide demand for functional food that is inexpensive, nutritionally well balanced, and with pleasing sensory composition is well met from fish and fish

products. Methods, by which the solubility of myofibrillar and other fish muscle proteins can be maximized, remain an area in which food chemists and technologists have been relentlessly working to meet the ever-increasing demand for high-quality proteins. Solubility of proteins is considered the most important factor and an excellent index of their functionality. Knowledge of protein solubility can provide important information on the potential utilization of proteins and their functionality, especially in foams, emulsions, and gels. Earlier studies showed that myosin and other myofibrillar proteins, which are extracted at relatively higher ionic strength, can be extracted at low or high pH as well. Myosin, the protein responsible for most of the functional properties of muscle foods, is the major protein extracted from fish meat at extreme pH (Gehring et al. 2011).

Fish protein hydrolysates have primarily been used for the production of low-value animal feed, aquaculture (fish and shellfish) feeds, and flavors and ingredients for food supplementation (Venugopal 2009). Besides, fermented fish sauces and pastes are used as staples or condiments in Southeast Asia (Joshi and Coppes Petricorena 2013). However a growing body of scientific evidence demonstrated that many marine-derived protein hydrolysates and peptides, including macroalgae, fish, and shellfish processing waste by-products, may play a role in the prevention and management of certain chronic diseases, such as cardiovascular disease, diabetes, cancer, and obesity-related chronic conditions, and thus can be used as functional food ingredients (Harnedy and Fitzgerald 2013).

Fish Bioactive Peptides

Proteins from fish and shellfish as well as macroalgal represent a vast resource for the mining of novel biofunctional peptides with specific or multifunctional activity. To date, numerous bioactive peptides have been characterized from these protein-rich marine sources. Furthermore, given their high structural diversity, proteins produced from macroalgae, fish, and shellfish contain a range of as yet undiscovered novel bioactive peptides encrypted within their primary structure (Harnedy and Fitzgerald 2013).

Fish processing by-products like food proteins can be easily utilized for producing bioactive peptides which have potential as active ingredients for preparations of various functional foods or nutraceuticals and pharmaceutical products. Thus, the relevance of aquatic organisms as a source of novel bioactive compounds is growing quickly. Very different kinds of substances have been searched from marine organisms because they must cope with a very exigent, competitive, and aggressive surrounding, so much different from the terrestrial environment. Thus, a source of functional materials, like polyunsaturated fatty acids (PUFAs), polysaccharides, minerals, vitamins, antioxidants, enzymes, and bioactive peptides, is being isolated from aquatic organisms (Kim et al. 2008). Marine-derived bioactive peptides have been shown to possess many physiological functions: antihypertensive or angiotensin-I-converting enzyme (ACE) inhibition, antioxidant, anticoagulant, and antimicrobial activities (Kim and Wijesekara 2010).

All over the world, high blood pressure is one of the relevant independent risk factors for cardiovascular diseases. Angiotensin-I-converting enzyme (ACE) plays a critical role in the regulation of blood pressure and is a multifunctional enzyme, since it catalyzes the degradation of bradykinin, a blood pressure-lowering nonapeptide, as well as neuropeptides that may interact with the cardiovascular system (enkephalins, neurotensin). Thus, inhibition of ACE is considered to be a useful therapeutic approach in the treatment of hypertension. Till present, many ACE-inhibitory peptides have been isolated from marine organisms: Alaska pollock, bigeye tuna muscle, shrimp, sea cucumber, sea bream, yellowfin sole, and oyster, among many. Thus, for example, to produce natural and effective antihypertensive products from oyster through enzymatic hydrolysis is possible; such oyster hydrolysate can be utilized as a nutraceutical or functional food, and the purified ACE-inhibitor peptides have the potential of an antihypertension drug (Xie et al. 2014).

A considerable number of studies have demonstrated that peptides derived from various marine protein hydrolysates act as potential antioxidants and have been isolated from jumbo squid (Mendis et al. 2005), oyster (Qian et al. 2008), blue mussel (Jung et al. 2005), hoki (Kim et al. 2007), cod (Slizyte et al. 2009), Alaska pollock (Cho et al. 2008), and macroalgae (Sheih et al. 2009), among many others. It has been shown that fish antioxidant potency is mostly due to the presence of hydrophobic amino acids in the peptide (Mendis et al. 2005); as an example, the bioactive antioxidant peptide isolated from oyster (*Crassostrea gigas*), Leu-Lys-Gln-Glu-Leu-Glu-Asp-Leu-Leu-Glu-Lys-Gln-Glu, showed a higher activity against polyunsaturated fatty acid peroxidation than α -tocopherol (Qian et al. 2008). Several studies have also indicated that peptides derived from marine fish proteins have greater antioxidant properties than α -tocopherol in different oxidative systems. Although the exact mechanism of fish peptides as antioxidants is not clearly known, it seems that some aromatic amino acids and histidine would play a vital role for the observed antioxidant activity.

Bioactive peptides from fish have also exhibited anticoagulant and antimicrobial activities. Meanwhile marine-derived antimicrobial peptides are well described from many invertebrates (spider crab, American lobster, green sea urchin); the anticoagulant marine bioactive peptides have rarely been reported, although they have been isolated from echiuroid worm, starfish, and blue mussel, and also fish anticoagulant proteins from blood ark shell and yellowfin sole (Kim and Wijesekara 2010).

The potential pharmaceutical from marine-derived proteins and bioactive peptides to support reducing or regulating the diet-related chronic malfunctions is a hopeful application to human health. Fish processing by-products like food proteins can be easily utilized to produce bioactive peptides which, besides, may have application as active ingredients for preparation of various functional foods, as well as nutraceutical or pharmaceutical products. However, till present, most of the biological activities of fish bioactive peptides have been observed in vitro or in mouse model systems. Further studies and clinical trials with these fish-derived proteins are needed to be carried out in the near future.

Proteins from fish frames can be extracted by enzymatic hydrolysis, rather than being discarded as waste, and utilized for production of amino acids, bioactive peptides, etc. (Venugopal and Shahidi 1996). Besides, an interesting production of collagen and gelatin can be obtained from the fish skin waste, being a good source used in food, cosmetic, and biomedical industries. Both proteins are two different forms of the same macromolecule in which gelatin is a partially hydrolyzed form of collagen, and heat denaturation of collagen easily converts it into gelatin. Compared to fish muscle proteins, collagen and gelatin are two unique and more significant forms of proteins because of their amino acid content, being more than 80 % nonpolar amino acids such as glycine, alanine, valine, and proline (Byun and Kim 2001). Gelatin extracted enzymatically from fish skin has better biological activities as antioxidant and antihypertensive agent. The unique repeating sequence of glycine-proline-alanine in the structure of gelatin compared to the peptides derived from fish muscle proteins gives gelatin antioxidant property (Byun et al. 2005). Hence, fish offal from the industry is an excellent source for obtaining natural compounds with applications to food, pharmaceutical, and cosmetic industries.

Amino Acids from Fish

As stated above, fish and shellfish proteins are of relevant high biological value, particularly for being a source that contains all the essential amino acids in right proportion; for that reason fish is considered to be constituted by complete protein. Consumption of fish provides important nutrients to a large number of people in the world and makes a very significant contribution to human nutrition (Pawar and Sonawane 2013). Fish contains well-balanced amino acid composition consisting of eight essential and eight nonessential amino acids. Due to the rich amino acid content, fish is being utilized as fish meal, fish sauce, fertilizer, animal feed, and fish silage (Hamid et al. 2002).

Amino acids can be produced by hydrolyzing proteins. Chemical (acid or alkali) and biological (enzymatic) methods are most commonly used to hydrolyze proteins, and also, microwave-induced hydrolysis of proteins has been reported. The hydrolysis of protein consists of liberating amino acids which then are recovered without degrading their properties. However, some factors affect the hydrolysis of proteins: temperature, time, hydrolysis agent, and additives, all of them affecting the quality and yield (Ghaly et al. 2013).

The eight amino acids generally regarded as essential for humans are lysine, methionine, threonine, tryptophan, isoleucine, leucine, phenylalanine, and valine. Certain amino acids, necessary for growth and maintenance must be included in the human diet, for example, aspartic acid, glycine, and glutamic acid; all the three play a key role in the process of wound healing – the dietary lack of niacin (B₃ vitamin) and protein, especially proteins containing the essential amino acids lysine and tryptophan, causes pellagra. Also, the way of cooking may be a determinant factor of the content of essential amino acids in consumed fish products (Erkan et al. 2010). Aspartic acid, glutamic acid, and lysine were reported as the major

amino acids in fish (Ozden 2005), and similar results were determined for horse mackerel either raw or cooked (Erkan et al. 2010). Hence cooking conditions in general increased the content of essential, semi-essential, and other amino acids compared to raw fish species, with some differences not very significant, whether being fried, grilled, or steamed.

Changes in fatty acid and amino acid concentrations were found to be useful as an index of freshness and decomposition of marinated fish in storage (Ozden 2005). High amount of nonpolar amino acids with aliphatic side chains and amino acids with a sulfur group and those with aromatic ring on the side chains, which together formed 37.4–52 % of the total amino acids, is a good indicator of stability of protein in these fish. Amino acids with aromatic side chains are also highly hydrophobic. Nonpolar amino acids are hydrophobic, that is, they have an aversion to water and like to cluster, while SH-containing side chains are hydrophilic. Changes in the plasma amino acid pool reflect the nutritional state of fish; hence they can help to understand the complex amino acid metabolism and to evaluate the quality of the diet (Iodarche et al. 2011).

Protein from carp, a freshwater fish, is characterized by a high content of exogenous amino acids, irrespective of the area of breeding or the production intensity level. The dominant amino acids are histidine, methionine, and cysteine, phenylalanine and tyrosine, and lysine and threonine, and the amino acid valine is the one that limits the nutritional value of carp protein (Skibniewska et al. 2013). Also, significant differences in quantitative amino acid composition of different edible parts (fillet, gonads, hepatopancreas) of two groups of hybrids from carp were determined, finding dependence on specific tissues; only three amino acids, leucine, methionine, and threonine, were the same in all tissues analyzed, and muscle proteins of these two fishes were characterized by Lys, Leu, Asp, and Glu (Butchova et al. 2008).

Zuraini et al (2006), analyzing amino acid composition of various fishes of genre *Channa*, freshwater fishes from Malaysia, found that glutamic acid, aspartic acid, and lysine were the most abundant amino acids. People from Sudan, living along River Nile, eat freshwater fish for subsistence and supplemental income. Thus, five freshwater fish from Nile were studied for their amino acid composition, in order to determine the stability of fish meat, finding a high content of nonpolar amino acids, hence here again making sure the good quality of those species as well as the high content of essential amino acids which constitute near 46 % of the total amino acid content (Elagba-Mohamed et al. 2010). Besides, Mohammed and Alim (2012) analyzed the amino acid content of other four commercial species of Nile fish, and only one species had the highest percentage of essential amino acids (72 %); hence, knowing the amino acid content of commercial fish should be one of the main elements used by a consumer for choosing the type of fish to eat. Also, freshwater fish in Nigeria constitute 70 % of the total fish supply available and two of the most consumed fishes, African catfish and tilapia, demonstrated to have all the essential amino acids relevant to human nutrition for Nigerian population (Osibona et al. 2009). Females and males of Red Sea fish from Saudi Arabia were analyzed for their amino acid content, finding that females were characterized by a

high content of essential amino acids; meanwhile most amino acids from male fish were nonessential, being maximum during spawning season (Qari et al. 2013).

Amino acid composition of whole fish body was determined for marine fishes, Atlantic halibut, yellowtail flounder, and Japanese flounder, finding no significant differences among species for the amino acids studied (Kim and Lall 2000). Amino acid profile of red and white muscle of a tuna fish from Arabian Sea was found to be the same, with the exception of three amino acids, histidine, lysine, and arginine, showing that red and white meat samples satisfy the ideal protein features except for sulfur amino acids which are found to be 2.5 % and for lysine 2.6 % in red meat (Remya and Vineeth 2013).

Dietary fish protein rich in arginine, glycine, and taurine may have various biological functions in muscle repair, such as an improved resolution of inflammation, owing to its ability to decrease the production of major proinflammatory cytokines (TNF- α , IL-6) and to limit the accumulation of proinflammatory macrophages at the site of injury (Dort et al. 2012). Of these amino acids, taurine is a β -amino acid and does not take part in the structure of proteins, being the free amino acid most abundant in biological fluids. The clinical utility of taurine in relation to cardiovascular health has been demonstrated. The taurine content of fish purchased in supermarkets was in the order plaice > cod > mackerel > farmed salmon. Spot sample tests on 14 other fish species showed a wide range in taurine contents. Albacore tuna and ray wing had the highest contents while some of the deep water species had virtually none. Wild salmon had a taurine content of 60 mg/100 g fresh weight. Taurine contents were relatively high in flatfish and ray and relatively low in silver pomfret, yellow croaker, and baby croaker. Therefore, consumers eating 150–200 g portions of fish per day would fall short of the level of taurine supplement (1.5 g/day). The added taurine was well retained in processed tuna cubes and did not adversely affect the sensory acceptability of the samples (Gormley et al. 2007).

Amino Acid Production from Fish Waste and Their Utilization

The largest market of world fishery comes from China and approximately 40 % of ocean marine products are processed there; besides 40–45 % waste is produced from fish industry, meaning that a large amount of biomass is discarded, containing a lot of protein and bioactive matter. Such biomass can be hydrolyzed into high-value industrial raw material: amino acid, unsaturated fatty acid, oil, polysaccharide, etc. (Yoshida et al. 2003a). Thus, Zhu et al. (2008) investigated the hydrolysis technology and reaction kinetics for amino acid production from fish proteins in subcritical water reactor, obtaining promising results.

Amino acids have wide nutritional value, taste, medicinal action, and chemical properties, and they are used as food additives in pharmaceutical applications, feed, and food supplements. There are two larger consumers of amino acids: the flavoring industry, which uses monosodium glutamate, alanine, aspartate, and arginine to improve flavor, and the animal feed industry, which uses lysine, methionine, threonine, tryptophan, and others to improve the nutritional quality of animal feed.

Several pharmaceutical industries use amino acids for application in protein purification and formulations and production of antibiotics such as jadomycin from fish. Thus, fish are important sources of all amino acids, essential and nonessential, which can be obtained by hydrolyzing proteins, using acid or alkali or enzymatic hydrolysis, or in a subcritical water reactor, hence being a relevant and promising challenge for improving both animal health and human health.

Fish Enzymes

Enzymes from fish have been considered relevant during the last 20 years owing to the great number of industrial applications they have, mainly enzymes obtained from fish offal discarded by fishery industry. For food application, an ideal enzyme must possess unique properties such as thermal stability, optimum temperature, pH stability, being sensitive to inhibitors, and catalytic specificity. Thus, a great number of fish enzymes have been identified from fish offal, being adequately used in food and other industrial sectors (Coppes Petricorena and Haard 2004). Additional applications to the fishery industry have emerged, to improve traditional uses of fish, production of other products than fish oils rich in PUFAs, and use of fish and shellfish enzymes to improve feed for fish culture, or as alternative processes in the production of seafood: selective extraction of skin and fish scales or production of caviar.

Enzymes obtained from fish are a good opportunity to food industry in general, owing to their unique properties that are unusual when applied to food processing, not only of aquatic origin but from other sources. In general, industrial enzymes of relevance to food industry are obtained from waste products from animals, plants, or microorganisms known as safe. In recent past years, a considerable number of fish enzymes have been commercially available for food and other applications. Aquatic organisms constitute more than 50 % of the world's total biodiversity, and marine organisms in particular are 90 % of all the aquatic species in the planet. Owing to the area water occupies in the earth (70 %), as well as the total volume of water (95 %), aquatic organisms comprehend a wide taxonomic variety which includes Arthropoda (lobsters, crayfish, shrimps), Mollusca (i.e., sea urchin), and Chordata (teleosts and cartilaginous fishes and marine mammals). Besides, in natural conditions, a given species shows genetic variation (stocks) by natural selection, in which today less than 1 % comes from the aquatic environment. Almost all aquatic species are ectothermic and meanwhile deep ocean temperature is mostly near 4 °C; temperature at sea level varies from less than 0 °C in the saline gradients at the poles to 103 °C in hydrothermal vents. There are aquatic organisms that also live at 4,000 m depth, and some fish from the Antarctica at 7,000 m depth, where they must cope with high pressure and dark environment. There are organisms that live in waters rich in nutrients as well as waters with poor nutrients, also aquatic organisms that can live in water with high saline content as Dead Sea or in intertidal waters, where they must have aquatic and terrestrial behavior. Thus, the aquatic environment offers a great variation of fish enzymes adapted to the many variable conditions these organisms must cope with.

Applications of Fish Enzymes in Food Industry

Since the beginning nearly 50 years ago, most research on fish enzymes has been to study their adaptation to a cold environment as well as to physical variations of the aquatic environment. A huge amount of scientific research through many years has shown the relevant properties of fish enzymes: molecular activity relatively high and physiological efficiency and optimum temperature and stability relatively low, when compared to the homologous enzymes from endothermic organisms. Activation energies of enzymes from fish are lower than those of terrestrial endothermic animals (Coppes Petricorena and Somero 1990). All these properties, mainly thermal stability of fish enzymes, allowed the scientific research to begin the study of their application in food industry, not only in the fishery products but also in other foods. The seafood technologist is thus concerned with the influence of adaptation and intraspecific factors on the comparative biochemistry of fish tissues, mainly muscle tissues.

Crude or partly purified preparations of hydrolytic enzymes from fish are applied as biotechnological tools in the production of fishery products such as caviar and descaled skin on fillets and in the maturation of various fish delicacy products (Gildberg et al. 2000). The hydrolytic enzymes from fish sauce and fish silage play a relevant role in the solubilization and degradation of tissue proteins, leading to the production of amino acids, most of them essential amino acids (Joshi and Coppes Petricorena 2013). Seafoods and/or their processing wastes can serve as a viable source of enzymes, with potential industrial applications either on fish, shellfish, or their by-products. The most studied enzymes from fish to be applied to food industry are (1) protein-degrading enzymes, (2) lipid-degrading enzymes, (3) carbohydrate-degrading enzymes, and (4) miscellaneous enzymes; these later are lipoxxygenase, myosin ATPases, polyphenol oxidases, and transglutaminases from fish species. Fish proteases (Group 1) have been the most studied enzymes since their applications today are not only on fish products but also they are used as processing aids on baked foods, fermented beverages; milk, dairy foods such as cheese, eggs, and their products, and meat and their products for the production of protein hydrolysate and flavor compounds. Digestive proteases from marine invertebrates have shown to have applications in various industries such as food industry, leather industry, and detergent industry (De Vecchi and Coppes Petricorena 1996). The second group, fish lipases and phospholipases, has also application on dairy, detergent, oleochemical, paper, and food industries, apart from their use in the production of biofuels. The third group is composed mainly of alginate lyases and chitinase, the last one used as protection against fungal pathogens, since chitin is the primary constituent of the fungal cell wall. The oligosaccharides produced by fish chitinases act as antioxidants scavenging different free radicals responsible for a number of chronic diseases (Hathwar et al. 2011).

Several other applications of fish enzymes on fish processing have been determined: skin and scale removal, production of caviar and spermary extracts, preparation of carotenoprotein complexes from crustacean waste, supplementation of squid and herring fermentation with Greenland cod trypsin, prevention of

copper-induced off-flavors in raw milk, preparation of shrimp flavor extract with turbot protease, extraction of enzymes from shrimp and scallop processing waste and enzymes from squid processing waste, use of fish enzymes to improve feed quality, and use of fish enzymes as biosensor.

Thus, several fish enzymes have been used in the fishery industry; as an example, cod pepsin and collagenase from the pyloric caeca of crayfish have been used as a soft way of removing fish skin, the membranes of fish eggs, and other products. Cod trypsin, a cold-adapted proteolytic enzyme, is commercially available today as a fermentation aid. The time of fermentation for elaboration of fish sauce used to last 2 years. Today in only 4 months, the process is concluded, using the great variety of proteases and peptidases from the pyloric caeca of squid; this process is being used for more than 15 years. As an example, lysozyme from an Arctic mollusk has a molecular activity so much higher than the homologous enzymes from chicken, particularly at refrigerated temperatures: at 4 °C it shows around 55 % of its activity at 37 °C. Thus, this invertebrate enzyme has been obtained from mollusk offal and has a great potential to be applied in the preservation of refrigerated foods.

The adaptation to extreme conditions of fish enzymes makes food from fish to have higher quality than terrestrial counterpart. Fish and shellfish processing by-products can serve as potential sources of fish enzymes, with viscera being the most important source for obtaining them. Fish enzymes have a great number of potential applications, allowing an integrate utilization of fish industry with fish processing wastes. Isolation, characterization, and application of fish enzymes have led to the development of improved value of seafood products, and other food products (like accelerated cheese ripening), utilization of processing waste materials for valuable by-products, and basic biochemical applications, diagnosis of the nutritive value of fish feed, and improvement of the nutritional value of fish feed ingredients. All these applications of enzymes obtained from fish offal could allow decreasing pollution/environmental problems associated with their discard, hence resulting in valuable added products such as enzymes and pigments from aquatic resources. Table 1 summarizes some applications of fish digestive enzymes in fishery industry and others.

Lipid and Fatty Acid Contents in Fish

Fish lipids consist mainly of saturated fatty acids (SFA), with palmitic acid (16:0) as the most relevant, monounsaturated fatty acids (MUFA) with oleic acid (18:1 n-9) as predominant, and polyunsaturated fatty acids (PUFAs), which are represented by eicosapentaenoic acid (EPA, 10:5 n-3), docosahexaenoic acid (DHA, 22:6 n-3), and omega-3 fatty acids in the linolenic series, and their content varies from one species to another; in general, fatty fish contain more long-chain omega-3 PUFAs than leaner species, and SFA content is usually constant in every aquatic fish. However, PUFAs are the predominant group of fatty acids in most fish. Exceptions exist like in black scabbard fish, with MUFA content higher than that of PUFA (Nunes et al. 2011).

Table 1 Uses of fish digestive enzymes to improve seafood quality and applications to other areas of food industries

Problem	Application
Poor production of caviar	Enzymatic removal of connective tissue
Difficulty to remove the skin from fish fillet and squid	Enzymes used as “biological knives”
Elimination of skin and scales in fillets	Enzymes remove selectively scales without spoiling the skin
The liver and swim bladder of cod have melanochromes in the membranes	Removing membranes by fish enzymes improves the value of the product
Fermentation of herring has variable quantities of endogen enzymes	Extracts from cod pyloric ceca are used to accelerate and make fermentation homogeneous
Fermentation process to produce fish sauce takes 1–2 years	Pyloric cecum extracts from squid accelerate fermentation yielding a high-quality product in less time (4 months)
Milk junket for a continuous yield of cheese requires rennet activation at low temperature	Psychrophilic pepsin from cod has been immobilized
Oxidative flavor of milk when exposed to light	Psychrophilic trypsin from cod avoids such problem and is then destroyed by pasteurization
Lysozyme from chicken can be only activated by gram-negative bacteria	Lysozymes from Arctic mollusk and other marine organisms are active against gram-positive and gram-negative bacteria
Commercial protease generates bitter taste in cheddar cheese	Squid peptidases can be used to accelerate cheese maturation
Trypsin is not effective to hydrolyze native proteins	Trypsin from fish without stomach is used to inactivate enzymes without heat treatment
A low yield of carotene proteins is obtained from discard of crustacean shells	Psychrophilic trypsin from cod can extract intact carotene proteins from crustacean shells

Coppes-Petricorena and Haard (2004)

Fish lipid content variation depends, besides the species, on the season, geographic regions (variables such as water temperature, depth, and salinity), age, gender, typical maturity, and nutrition as well as whether the species is being cultured or living in the wild. Considering dependence on the season, for example, the lipid content can vary from 4 % to more than 30 % in mackerel and from 2 % to 25 % in herring, and also species living in temperate waters have more lipid content in their flesh than leaner tropical fish. The total lipid content of farmed fish and shellfish depends on the diet since lipids are a valuable source of energy; hence most farmed fish are usually fed high-lipid diet to maximize growth rates, and thus their flesh tends to contain more lipids than wild fish of the same species, sometimes twice as much (Yesilayer and Genc 2013). Comparison among 20 different species of freshwater and marine fish showed that most of the marine species had lower fat content than freshwater fish (Praparsi et al. 1999). However, many studies have shown that freshwater fish generally contain lower proportion of n-3 PUFA than marine fish, and data demonstrated in the literature that fatty acid composition of lipids from tissue and eggs reflects the fatty acid content of the lipid in the diet

supplied to the brood stock; the fatty acid composition of tissue and egg lipids in farmed fish can really differ from wild fish composition (Hussain 2011).

In general, fish have less fat than red meats, ranging from 0.2 % to almost 30 %, and contrary to terrestrial animals which deposit their lipids in the adipose tissue, fish have lipids in the liver, muscle, and perivisceral and subcutaneous tissues. Distribution of lipids in fish decreased from head to tail; distribution of lipids in dark muscle is several times higher than in white muscles. Thus, long-term migratory fish species (e.g., herring, tuna, mackerel) have more dark muscle, hence more lipids when compared to slow floating species since lipid content depends on the necessity of the species based on their functions. Thus, the flesh of fast-moving, migratory species (many pelagic fishes such as tuna, herring, mackerel, sprat, anchovy, and sardine) contains more dark muscle tissue and more fat than species such as cod, which are generally slower-moving species composed mainly of white meat; however their dark muscle contributes about 10 %. Lipid content of the skin of fatty fish accounts for more than 50 %; meanwhile in lean species, it averages from 0.2 % to 3.9 % only. Considering other organs, lipids contribute to 0.3–1.5 % in roe from cod and 70 % of the total lipid in the liver. On the other hand, fatty fish store its fat in muscle tissue, with their meats pigmented with yellow, gray, and pink colors and their adipose cells are located in the subcutaneous tissue, in the belly flap muscle, and in the muscle that moves fins and tail. Thus, the fat deposits are typically throughout the muscle structure.

Taking into account the fat content, fish products are classified into four categories as (1) lean (less than 2 % fat), (2) low fat (2–4 % fat), (3) medium fat (4–8 % fat), and (4) high fat (more than 8 % fat), being in the first category are hake, cod, haddock, and shellfish and in the second are sole, halibut, and flounder. The third category is represented by wild salmon and finally the high-fat fish by herring, mackerel, sablefish, and farmed salmon. Other species with medium fat content are trout, sea bass, or sea bream.

Cholesterol is another lipid relevant to health and is the main sterol in marine species, accounting for more than 90 % of all sterols, but in some shellfish species, such percentage can lower to 25 %. According to Nunes et al. (2011), cholesterol levels are not significant in most seafood products, fish and shellfish being between 24 and 85 mg/100 g. Cephalopods have higher content of cholesterol near 140 mg/100 g tissue in the European squid; however, these invertebrates have high amounts of taurine, helping in reducing cholesterol absorption (Militante and Lombardin 2004), because taurine enhances cholesterol degradation and excretion of bile acid.

Micronutrients in Fish

The role of fish as source of micronutrients, mainly *vitamins and minerals*, has not been paid much attention. However, recent research comparing small fish with big ones suggest that as small fish are consumed as a whole, they play a critical role in micronutrient intakes since bones, heads, and viscera concentrate most of them.

According to Kawarazuka and Béné (2013), small fish also offer other nutritional advantages: they can be processed and stored for a long period, they are more affordable for the poor as they can be purchased in small quantities, and they can also be more evenly divided among household members. Fishery industries in developed and developing countries catch bigger fish since what people consume is their flesh. Nutrient content of the species and the local processing methods and eating patterns determined the relevance of fish to micronutrient intakes. Various studies have indicated the actual nutrient content of the edible part by reflecting the local methods used to clean and prepare the fish for the meal (leaving or cutting off the head, removing a part of the viscera) and correcting the calculation for plate waste after meals (Roos et al. 2007a). The micronutrients that will be reviewed here are vitamins and minerals from fish.

Vitamins from Fish

Vitamin content in fish varies with the species, age, season, sexual maturation, and geographic area. The information about vitamin content in fish is not consistent however scarce, with few scientific articles if compared to the number of publications concerning proteins or lipids from fish. In general, fish flesh is a good source of vitamin B, and in fat species in particular, also vitamins A and D. Vitamin content in fish could be considered similar to the vitamin content in mammals, with the exception of vitamins A and D which are in higher amount in the flesh of fatty fish and in the liver of lean species like cod. Fish and shellfish are the best known sources of vitamins A, D, and E, although they also can provide significant amounts of vitamin B (Dias et al. 2003). Considerable amount of vitamin A, as retinol, is found in shark and oysters, as well as in oily fishes such as small sardines, herring, and horse mackerel. These species provide, each 100 g of flesh, 10–15 % of the RDA (recommended dietary allowance) for retinol. Pelagic fish with high fat content provides excellent dietetic sources of vitamin D₃ (cholecalciferol).

In fish, higher amounts of data values are found for fat-soluble (58 %) than for water-soluble (42 %) vitamins. Crustaceans showed, in comparison to finfish and mollusks, the highest amount of compiled vitamin data (3 %). The reason of such small amount of existing data on vitamins in fish is thought to reflect either the low interest in vitamins, the higher cost of research on them, or the limited capacity of laboratories to analyze vitamins (Rittenschober et al. 2013).

Seafood is rich in fat-soluble vitamins with varying concentrations depending on the lipid content, and when comparing to land animals, seafood has higher amounts of these vitamins (Venugopal and Shahidi 1996). Vitamins A, D, and E are more concentrated in the liver, viscera, and eyes of fish and shellfish, although also present, but in less amount, in their flesh and meat (Roos et al. 2007a). Cholecalciferol (vitamin D₃) is the most frequently occurring fat-soluble vitamin reaching 15 % of all data on (pro)vitamins, but compiled data belong mainly to finfish. β -Carotene is the most frequent representative of vitamin A group according to published data, chiefly referring to fish as a whole or to edible portions, eyes, and viscera.

Water-soluble vitamins are found mainly in the flesh of fish and shellfish, with most values obtained for thiamine, riboflavin, and niacin, 6 %, 7 %, and 5 % of the whole vitamin data, respectively; for folate and pantothenic acid, 3 % was found. B₁₂ has been found chiefly in mollusks in a considerable amount (18 %).

Fat-Soluble Vitamins from Fish

Fat-soluble vitamins have been studied more than water-soluble vitamins, owing to the former's solubility in lipids of the organism; hence, fat-soluble vitamins are being retained longer in the organism's body than the water-soluble ones which are easily excreted. Vitamins A, D, and E are the fat-soluble vitamins most commonly determined in fish, owing to their high concentration not only in the liver but in the flesh as well. Aminullah-Bhuiyan et al. (1993) determined vitamins A, D, and E in fresh and smoked flesh from mackerel, without getting differences in their content; hence 100 g of fresh fillet contains 170 µg of vitamin A, 5.81 µg of vitamin D₃, and 1,300 µg of α-tocopherol.

Vitamin A. Vitamin A deficiency is a serious problem in the world since several vital functions relevant to the human body are affected. This vitamin is essential to maintain the health of the outer cover of the eye. Xerophthalmia, an inflammation of the eye driving to blindness, can be prevented with adequate quantities of vitamin A. Other epithelial tissues depend on vitamin A for a good health: normally, epithelial cells secrete mucus to prevent infection, but when vitamin A is not present, they secrete, instead, a protein, keratin, which turns the epithelial cells dry and hard causing eventually their death. Besides, vitamin A is known to prevent nocturnal blindness or the inability of the eye to be able to change from light to darkness. Also, vitamin A plays an important role in growing, since its deficiency causes keratinization of lingual cells or deterioration of epithelial tissue of the intestine, provoking lack of appetite, resulting in cease of growing.

Thus, intake of vitamin A could solve such health problems, fish being relevant concerning the content of this vitamin which is concentrated mainly in the liver. Fish liver oil is an excellent source of vitamin A. Fat species, as herring and horse mackerel, have a high amount of vitamin A in their flesh compared to lean species that can only accumulate vitamin A in their liver, rather than in their flesh. However, as these lean fish cannot concentrate fat in their meat, their liver is the organ that concentrates higher vitamin A content than meat, independent of the species, being even higher than mammals (Aro et al. 2005). However, liver vitamin A content varies a lot, depending on several factors like age and size of the fish, sex, stage of sexual maturation, anatomical structure, temperature of water, availability of nutrients, etc.

Major sources of vitamin A as provitamin A (β-carotene) have been orange, yellow, and dark-green vegetables, which are often used in food-based interventions in order to increase vitamin A intake. Comparison among fish, taking into account their size, shows that small fish are also very rich in vitamin A, as has been demonstrated by Roos et al. (2007a, b) when studying many species of freshwater fish from Cambodia and Bangladesh. Thus, vitamin A contents from some species from Bangladesh, as *mola* and *chanda*, are as high as 2,500 and 1,500 µg retinol

activity equivalents (RAE/100 g raw edible parts), respectively (Roos et al. 2007a); also similar results were obtained with small indigenous species from Cambodia, with a vitamin A content higher than 1,500 µg RAE/100 g raw edible parts (Roos et al. 2007b).

In rural areas in Asia, fish often exceed vegetables with regard to both the amount and frequency of consumption. Children from Cambodia consume more fish than vegetables during all three seasons (an average of 65.2 g/raw fish, cleaned parts) and 19 g/day for vegetables; of the surveyed households, 54.6 % consume fish 7 days/week versus the 47.9 % that consumed vegetables. In Bangladesh, field data show that daily consumption of small fish contributes 40 % of the total daily requirement of vitamin A at the household level (Roos et al. 2007a). Consuming, for example, 5 g fat/person/meal is enough to ensure adequate bioefficacy of vitamin A (Michaelsen et al. 2009). Fish rich in vitamin A, cooked with some vegetables and some vegetable oils, are therefore an ideal combination to enhance vitamin A intake and bioefficacy.

Kawarazuka and Béné (2013) present two problems concerning vitamin A in fish. On one hand, vitamin A content is species specific and may be therefore very different among fish species belonging to very close taxonomic groups. Taking into account the environment where different fish live, there is no relationship with the habitat: species living in the same habitat may have totally different vitamin A contents. Hirao et al. (1959, in: Kawarazuka and Béné 2013) examined vitamin A content in 157 species of fish finding that nearly 85 % of the species contained little vitamin A (less than 60 µg RAE/100 g flesh), whereas a few others were extremely rich (more than 18,000 µg RAE/100 g flesh). However, measurements of these species could be advisable to take again, owing to the advance in technologies for determination of vitamin concentrations. The second problem is that vitamin A in freshwater fish exists mainly in the form of 3,4-dehydroretinol. Thus, Kongsback et al. (2008) examined the efficacy of the intake of the local small fish *mola* in the daily diet, during 9 weeks (6 days/week) in improving vitamin A status of children measured through marginal serum retinol concentration in blood. The authors did not find significant effect in the group of children fed with fish curry, suggesting that vitamin A from *mola*, of which 80 % is 3,4-dehydroretinol, was not converted to retinol or was converted in an insufficient rate. Further studies are needed to determine the effect of 3,4-dehydroretinol in humans in order to confirm or refute the above data.

On the other hand, owing to the advance of technology which allows to determine smaller quantities and each time more precise and with less limit of error, vitamin concentrations show differences compared to previous determinations taken years ago. Not only the type of measurement of the sample is relevant but also the preparation of sample previous to determination.

Vitamin D. Significant amounts of vitamin D are found naturally only in a few foodstuffs. This vitamin is essential to the normal development of bones and teeth. Adequate absorption of calcium and phosphorous, as well as their movement, deposition, and excretion, depends on the adequate level of vitamin D which, besides, has an important role in proliferation of colon cells (Kallay et al. 2002) and in the normal function of the central nervous system (Garcion et al. 2002).

The fat-soluble vitamin D mainly occurs in nature as ergocalciferol (vitamin D₂) or cholecalciferol (vitamin D₃). Ergosterol, the provitamin of D₂, and ergocalciferol are found in plants, particularly in yeast and fungi, and cholecalciferol is mainly derived from animal sources. Although the chemical structure of both forms of vitamin D is similar (they only differ on the side chain at C17 position), the general assumption that both forms have the same nutritional value is considered wrong (Ostermeyer and Schmidt 2006). Vitamin D₃ is much more potent than vitamin D₂ (Barthel and Scharla 2003) Ergo- and cholecalciferol are biologically inert compounds. Their activities are due to their metabolites. Vitamin D is converted mainly in the liver to 25-hydroxyvitamin D, which is further hydroxylated in the kidneys to the much more metabolically active form 1,25-dihydroxyvitamin D, also called vitamin D hormone. The major biological function of vitamin D is to regulate the absorption and utilization of calcium and phosphorus, which are essential for normal growth and development of healthy bones and teeth. Animal food products are the main dietary source for naturally occurring vitamin D₃ (Schmid and Walther 2013).

Fish and fish products are commonly regarded as the most important natural food sources of vitamin D, fish liver and oils being very rich sources of vitamin D₃. National databases from various countries like Switzerland, Germany, France, Denmark, the USA, and Canada show values in the range of 0 and 300 µg/Kg. It was driven by the early observation that the amount of vitamin D in a teaspoon of cod liver oil was sufficient to prevent rickets in infants. Fish liver oil is the source with the highest amounts of vitamin D₃. Takeuchi et al. (1984) reported the vitamin D₃ content of eight different fish products, with the values ranging between 5 and 356 µg/kg. The highest amounts were found in fresh eel and a Japanese fish product named shiokara. Eighteen different fish were analyzed by Kobayashi et al. (1995) who found vitamin D₃ content ranging from 18 to 350 µg/kg, and fish liver had a vitamin D₃ content of 1,200 µg/kg. Contrary to the general belief, there was no significant correlation between fat and vitamin D content, and vitamin D content of frozen fish and fish products ranged between <2(shrimp) and 196 µg/kg (roe of vendace) (Mattila et al. 1997). Vitamin D concentrations were also found for other fish, between 6 and 453 µg/kg (Lu et al. 2007; Bilodeau et al. 2011).

Bendik et al. (2013), analyzing several values obtained for fish by different authors, observed a huge variation in the vitamin D₃ content per 100 g wet weight in fresh fish. Fish obtain their requirements of vitamin D₃ through their diet (Holick 2003); hence vitamin D₃ levels in the zooplankton, the primary food source of fish, or seasonal changes in the zooplankton reservoirs in the different habitats might be the reasons for the observed fluctuation in the fish product (Bendik et al. 2013). Mattila et al. (1997) did not find correlation between vitamin D₃ content and the sex or age of fish, and interestingly, they did not detect any relationship between the tissue fat content and vitamin D levels. However, Takeuchi et al. (1986) determined significant differences in vitamin D₃ content between muscle and skin tissues, and even a much more difference between muscle and liver tissues; besides, 25-hydroxyvitamin D₃ compound was also detected in fish but at low concentrations (Mattila et al. 1997; Bilodeau et al. 2011; Bendik et al. 2013).

Vitamin D synthesis depends on light from the sun, and light is not available in natural habitats where fish live; it is suggested that vitamin D in fish tissues comes from their food intake. Phytoplankton and zooplankton, both containing vitamins D₂ and D₃, are the first step in the food chain (Mattila et al. 1997). However, according to Holick (2003), the only vitamin D determined in fish was vitamin D₃; hence whether fish accumulate only vitamin D₃ or whether fish can convert vitamin D₂ to vitamin D₃ is unclear, and the physiological function of vitamin D in fish is uncertain (Bendik et al. 2013).

Ostermeyer and Schmidt (2006) measured the contents of vitamin D and provitamin D in the edible part of the most commonly consumed freshwater and marine fish species in Germany, developing a highly sensitive HPLC method. The species were divided into lean and fat fish, this latter group into three: low, moderate, and high fat content. Vitamin D content in muscle tissues of marine, freshwater, and farmed fish was determined. The vitamin and provitamin D₃ differed greatly among species, with provitamin in every case higher than vitamin D₃. Vitamin D₃ contents ranged between 2 and 212 ng/g, and the provitamin D₃ amounts ranged from 35 to 9,565 ng/g wet weight. The authors found that vitamin and provitamin D concentrations in the farmed salmon were lower than in the wild salmon; besides, the results for the vitamin D₃ content in the fish under study were the same order of magnitude as those reported by other groups (Mattila et al. 1997; Dias et al. 2003). Recent determination of vitamin D levels, in various species of fish and shellfish, was carried out by HPLC with ultraviolet detection and mass spectrometry, and the values obtained agreed with the literature and tabulated database results for most species; however, much lower average vitamin D₃ concentrations were found for oysters (0.05 µg/100 g raw) and clams (0.18 µg/100 g cooked) compared to other reports for these products (Byrdwell et al. 2013).

Vitamin D content in fish depends on the species. Fatty fishes like horse mackerel or herring have a higher concentration of vitamin D than lean species like flounder or marine trout. Aro et al. (2005) determined vitamin D in salty herring, observing no changes in its concentration. Meanwhile vitamin A was reduced considerably; of all the substances analyzed, the authors concluded that PUFAs and vitamin D were the only substances maintained without alteration during the whole study under salty conditions. Tissue distribution of vitamin D is similar to vitamin A, fish oils being the most rich. In fish flesh, both vitamins have similar distribution; they are in small quantities or quasi-absent in lean species. On the other hand, flesh from fatty fish is an excellent source of both vitamins. Canning does not affect concentrations of vitamin D, which does not decrease during storing contrary to what happens with vitamin A; hence vitamin D is more stable than vitamin A.

Vitamins E and K. Vitamin E is found in significant amount in marine fish. Salmon and some shellfish have up to 15 % of RDA of vitamin E, in 100 g of flesh (Holland et al. 1993). Vitamin E functions as a potent antioxidant protecting PUFAs and LDL cholesterol, from oxidation by free radicals, and has also incidence in anti-inflammatory processes. Fish flesh seems to be considerably rich in

antihemorrhagic vitamin K; however, to generalize this statement, more research is necessary to be carried out.

Vitamin E comprises in nature four tocopherols (α -, β -, γ -, and δ -tocopherol) and four tocotrienols (α -, β -, γ -, and δ -tocotrienol); all of them can be provided by seafood and aquaculture products. The tocopherol vitamers, mainly α -tocopherol, are the most important (Yoshida et al. 2003). RRR- α -tocopherol (chiral with three stereocenters) is considered to have the highest biological activity. No conversion of α -tocopherol into other naturally occurring tocopherols has been found in some cultured fish species (Schulz 1986); hence vitamer contents of vitamin E in fish correlate with diet levels particularly in the liver, existing in a direct relationship experimentally observed, since α -tocopherol increases exponentially in response to increased dietary concentration; thus, vitamin E content in fish depends on diet, since fish are unable to synthesize tocopherols (Hamre 2011).

Although in general fish contain higher concentrations of vitamin E than meat or poultry, fish products have low to moderate levels of tocopherols (Afonso et al. 2014). Fujisawa et al. (2010) suggest that such differences could be due to biochemical adaptation of marine organisms to cold-water environments, since many seafood species live in cold waters, and could be related to the high production of unsaturated fatty acids of species living at low temperatures.

Tocopherol content varies in fish species and tissues, with dark muscle having higher amounts of vitamin E than light muscle; considering fat and lean fish, tocopherols (similar to what happens with vitamins A and D) are concentrated in the muscle tissue and liver, respectively (Afonso et al. 2014). Although α -tocopherol is the main tocopherol in fish and fish products, minor amounts of β -, γ -, and δ -tocopherols occur in some alga species (Nakamura et al. 1994). A new vitamin E form was discovered in salmon roe termed “marine-derived tocopherol” (MDT) which has a similar structure as the α -tocopherol (Gotoh et al. 2011). This new vitamer is distributed in a wide range of marine products, from phytoplankton to fish (Fujisawa et al. 2010), and has a relative biological activity higher than that of β -tocopherol, which is considered to have the highest relative vitamin E activity next to α -tocopherol (Gotoh et al. 2011).

During processing and frozen storage of aquatic products, as lipid peroxidation progresses, tocopherol concentrations decrease; hence fish oils that contain high levels of PUFAs are more prone to autoxidation which lowers tocopherol content. These facts lead to sensory rejection of fishery products when tocopherol levels are low. Thus, tocopherol content of seafood and its products is relevant to the quality not only for the nutritional value but also for preservation of fish and fish products (Afonso et al. 2014).

Tocopherol content in fish species ranges from 0.1 mg/100 g in some wild fish species to 3–4 mg/100 g in aquaculture species. Lean fish species have a small level of tocopherols in muscle with concentrations from 0.3 mg/100 g for cod to 0.5 mg/100 g for hake. However, higher levels of tocopherol were reported for a lean species such as black scabbard fish (*Aphanopus carbo*) (Afonso et al. 2008), and for eel, a fatty fish species, a content of 2.4 mg/100 g was reported (Dias et al. 2003).

Water-Soluble Vitamins from Fish

Meanwhile fat-soluble vitamins are common in the liver of lean fish or in the flesh of fatty fish; water-soluble vitamins can be found throughout the whole fish. The most relevant water-soluble vitamins found are thiamine, riboflavin, pyridoxamine, niacin, folic acid, pantothenic acid, vitamins B₁₂ and B₆, and vitamin C. Vitamins from B complex are generally found in the same groups of food, and their functions are related with energy production in the cells.

Vitamin B₁ (thiamine) functions as a coenzyme, helping in the energy generation inside the cell, and its deficiency does not allow such production. Fish flesh has a vitamin B₁ content of approximately 100 µg/100 g flesh, and shellfish, a very rich source of thiamine, has around 0.25 mg/100 mg, oyster being an especially good source, containing about 1 mg/100 g. *Niacin*, like thiamine, forms part of a coenzyme essential in the production of energy and, in fish muscle, ranges from 0.9 mg to 3.1 mg/100 g flesh. Adult women need about 1.0 mg/day, while men need 1.4 mg/day. Symptoms of its deficiency include diarrhea, dementia, and dermatitis. The concentration of riboflavin in fish is quite variable, since red meat contains more riboflavin than white muscle. This vitamin plays an important role in fatty acid degradation and amino acids that are going to be used for energy. The amount of riboflavin in many fish species is comparable to that found in terrestrial animals (50–980 µg/100 g muscle).

Vitamin B₆ (pyridoxine) is involved in a number of body functions: conversion of one amino acid to another, degradation of amino acids used for energy production, transformation of *linoleic* acid into *arachidonic* acid, synthesis of various substances like hemoglobin, and keeping glucose level in blood. Whole fish is a very good source of pyridoxine with values around 100–1,200 µg/100 g of fish flesh. However, in fish sauce, B₆ values range from 40,000 to 50,000 µg/100 g (Joshi and Coppes Petricorena 2013).

Vitamin B₁₂ (cobalamin) is found in significant quantities in fish, mainly in fatty fish and shellfish, with a higher content in red meat of herring and also in white meat of flounder. Vitamin B₁₂ is synthesized only by certain bacteria and is concentrated mainly in the bodies of higher predatory organisms in the natural food chain system. The major dietary sources of vitamin B₁₂ are animal foods, like meat, milk, egg, fish, and shellfish, but plant foods do not contain this vitamin. Anchovies, clams, herring, oysters, pilchard, and sardines are particularly rich sources of vitamin B₁₂ containing 25–40 µg/100 g meat. Oyster, mussels, and short-necked clam have a B₁₂ content of 46.3, 15.71, and 87.0 µg/100 g, respectively. Certain fishes like salmon, sardine, trout, and tuna have a B₁₂ content that varies from 3.8 to 8.9 µg/100 g; however, mollusks and clams have an average of 98 µg/100 g of B₁₂ (Watanabe et al. 2001). Meats of larger-sized fish, like yellowfin tuna, contain vitamin B₁₂, either in white meat which is a popular food for people in various countries or in dark meat which is commonly used as a source of pet food for dogs and cats but not as food for humans; however, vitamin B₁₂ content was so much higher in dark muscle of yellowfin tuna (52.9 + 8.9 µg/100) than white muscle (5.8 µg/100 g) (Nishioka et al. 2007). Dark muscle of skipjack contains a substantial

amount of vitamin B₁₂ (159 µg/100 g) compared to light muscle (10 µg/100 g, dorsal portion, and 8 µg/100 g, ventral portion) (Nishioka et al. 2006). Recommended dietary intake of vitamin B₁₂ for adults is 3 µg/100 g, a very small amount, so seafood can generally be considered a very good source. B₁₂ content varies from 0 in shark to 1.9 µg/100 g in Pacific herring.

Other water-soluble vitamins, folic acid and vitamin C, are not found in any significant amount in edible portions of fish. The flesh of Atlantic salmon, European hake, and sardine has a folic acid concentration of 10, 27, and 24 µg/100 g, respectively (Nunes et al. 2011). Aminullah-Bhuiyan et al. (1993) determined several water-soluble vitamins in fresh and smoked mackerel, with no significant differences in both samples. Thus, vitamin C content was 4.7 mg/100 g fillet, losing very low percentage (4 %). For the same species, niacin and riboflavin contents were 5.4 and 0.38 mg/100 g fillet of mackerel.

It is important to take into account the technology used for vitamin determination through the years. Thus, it would be very valuable to unify the criteria of measurements in order to be able to compare different data obtained to date. Thus, relevant future research could be to determine the vitamin content of different fish species and tissues and fish products, like fish sauces and pastes, using updated advanced technology in order to corroborate the first data obtained for fish years ago when the technology was not so advanced as today; however it was a research of deep scientific value.

Minerals in Fish

Marine foods are also very rich sources of mineral components since aquatic animals require minerals for their normal life processes. Thus, fish absorb mineral not only from their diets but also from the surrounding water via their gills and skin. Calcium, magnesium, sodium, and potassium are important for human nutrition. Certain elements such as zinc, copper, iron, and manganese are indispensable for the maintenance of normal growth and reproduction (Roy and Lall 2006). The major elements K, Ca, Na, and Mg, essential to cellular metabolism, are very common and generally found in high concentrations in biological tissues. Fish are relevant for their mineral content; hence research on the content of these micronutrients in aquatic organisms has been of great value to human health, and many data are joined till present. Taking into account the same species, like sea bass as an example, to compare results concerning mineral content in fish tissues obtained by several authors is not an easy task.

Mineral content in the dorsal and ventral muscles and liver of wild and farmed sea bass showed variable distribution, being similar in both the muscles and liver of wild fish but different in the farmed fish (Mnari-Bhourri et al. 2010): K is more concentrated in the muscle than liver, ranging from 4,425 to 19,180 mg/kg, much higher than the reported values for the same species (Erkan and Ozden 2007) and for other fish (Pervin et al. 2012). Na is concentrated in the liver of wild fish while farmed sea bass has a concentrated Na in dorsal muscle. Recently,

Pervin et al. (2012) studying the same minerals found that concentration of sodium was higher in *Lates calcarifer* (580 mg/100 g) than that measured in sea bass (Erkan and Ozden 2007; Abdullah et al. 2011), followed by potassium, calcium, magnesium, and iron. Other minerals were magnesium, calcium, zinc, iron, manganese, and copper, with statistically significant differences in concentrations of Zn (dorsal and ventral muscle), Cu (ventral muscle, liver), and Mn (liver, dorsal and ventral muscles) between wild and farmed fish with higher concentrations in farmed sea bass. Higher concentrations may be due to the release of minerals from fish diet and fish excreta (Mnari-Bhourri et al. 2010).

Calcium is necessary to maintain an optimal bone development; hence fish are a good source of this mineral. Mnari-Bhourri et al. (2010) determined a calcium concentration from 231 mg/100 g to 265 mg/100 g in sea bass, higher than what was found for *L. calcarifer* (140 mg/100 g) (Pervin et al. 2012). An adequate intake of magnesium has some useful roles in a human body due to the fact that it regulates enzyme systems, helps to maintain bone health, is required for energy metabolism, and acts as a part of the protein-making machinery in all cells of soft tissues. Mg content varies from fish to fish, 120 mg/100 g in bhetki fish and from 88 mg/100 g in the liver to 162 mg/100 g in dorsal muscle of wild sea bass (Pervin et al. 2012).

Iron, zinc, copper, and manganese concentrations are generally higher in the liver than in muscle tissues, either being wild or farmed fish, as is the case in sea bass. Several studies have considered fish as a major source of Fe for children and adults. Iron content (581.4 mg/kg) in the liver of wild sea bass was higher than in the muscle from 0 to 18.42 mg/kg in wild and farmed sea bass, respectively (Mnari-Bhourri et al. 2010), values lower than the ones obtained by Alasalvar et al. (2002) and Erkan and Ozden (2007) for wild and farmed sea bass. Iron content of *L. calcarifer* (9 mg/100 g) (Pervin et al. 2012) was similar to the results obtained for other fishes as bass, cod, salmon, and halibut resulting in good source of iron, containing 4.2, 9.4, 8.6, and 9.5 mg/100 g, respectively (Gehring et al. 2011).

Variation in the mineral concentrations of marine foods is closely related to seasonal and biological differences (species, size, dark/white muscle, age, sex, and sexual maturity), area of catch, processing method, food source, and environmental conditions (water chemistry, temperature, and contaminants) (Alasalvar et al. 2002). Within an individual organism, the concentrations may vary, from tissue to tissue and with age (Erkan and Ozden 2007). Thus, mineral content obtained for the same species by different authors varies; hence comparing values obtained by different studies is not recommended owing to the different conditions the fish was living in at the moment of the catch.

Zinc is another relevant mineral for human health, and fish is a good source of this mineral as well. Alasalvar et al. (2002) reported low values for wild and farmed sea bass, 45.1 and 43.6 mg/kg, respectively, and Erkan and Ozden (2007) compared farmed sea bass (2.83 mg/kg) to the ones obtained by Mnari-Bhourri et al. (2010); however, the liver concentrates higher amounts of zinc than the muscle, around 131.8 mg/kg for wild and 97.2 mg/kg for farmed sea bass. Cu concentrations of different fish tissues also vary, as those reported for sea bass, a fish very well studied concerning its chemical composition; thus, Mnari-Bhourri et al. (2010) obtained a

Cu concentration that ranged from 7.02 to 33.97 mg/kg in wild sea bass and from 3.73 to 76.22 mg/kg in the farmed ones, higher values if compared to the ones obtained by Alasalvar et al. (2002) of 2.96 mg/kg for wild and 3.87 mg/kg for farmed sea bass. Daily intake of small amounts of Mn is needed for growth and good health in humans; otherwise deficiency can cause nervous system problems. Concerning sea bass, Mn content is higher in the liver than in muscles, and in both tissues, various authors obtained different results, ranging from 9.01 to 16.58 mg/kg in wild sea bass and from 11.45 to 18.41 mg/kg in farmed fish, being the highest in the liver in both; for the same species, lower values were obtained by Alasalvar et al. (2002) (7.25 and 6.53 µg/g) and Erkan and Ozden (2007) (0.547 mg/kg). Mn belongs to the essential elements which display higher concentrations in the liver than in muscle tissues due to its function as a cofactor for the activation of a number of enzymes (Sures et al. 1999). Trace and macromineral composition showed variations through the years, for sole, striped red mullet, and whiting, and their macromineral levels were found sufficient for recommended daily allowance (Ozden et al. 2010). The different concentrations of minerals in fish muscles could have been as a result of the rate in which they are available in the water body and the ability of the fish to absorb these inorganic elements from their diets and the water bodies where they live.

Tuzen (2009) determined trace elements in 10 fish species from the Black Sea, finding an average of 36.2–145 µg/g for iron, 0.65–2.78 µg/g for copper, 2.76–9.10 µg/g for manganese, 38.8–93–4 µg/g for zinc, 0.19–0.85 µg/g for selenium, and 0.63–1,74 µg/g for chromium. The levels of trace minerals and heavy metals in canned fishes (mackerel, tuna, salmon, sardines, and herring) from Georgia and Alabama (USA) were also determined and assessed for its quality by comparing element levels in samples purchased with permissible limits stipulated by various agencies and organizations (Ikem and Egiebor 2005). The analytical data obtained from this study shows that there are no health risks from consumption of canned fish analyzed when data are compared with the US EPA classified health criteria for lead, chromium, copper, and zinc as carcinogens in canned fish. The authors advise low-risk groups (adolescents and adults) and high-risk groups (pregnant mothers and children) to consume fish in moderation since large consumption pattern especially for tunas may result in increased health risks. Globally, further reduction in the levels of environmental contaminants emanating from power plants and other industrial emissions and effluent discharges is highly needed to reduce contaminant inputs into the aquatic environment. More research and assessments of seafood quality are needed in many countries to provide more data and help safeguard human health.

Minerals as Contaminants in Fish

Many scientists have studied many minerals considering them as possible as contaminants in fish and fish products, since although good for health, at high concentrations could be toxic and some pathologies could appear. Thus, Sivaperumal et al. (2007) analyzed 67 different fish, shellfish (mollusks and crustaceans), and fish products in India, without finding dangerous concentration for

health, hence advising that such species are safe for human consumption. However, the most worrying metals are mainly cadmium and lead, which concentrate in fish flesh, specifically for freshwater fish, owing to contaminants from industries (Begum et al. 2005).

Copat et al. (2012) determined cadmium, lead, mercury, and chromium in European anchovy, European pilchard, and red mullet inhabiting the Sicilian areas of the Mediterranean Sea, finding that although some metal concentrations exceed the limits set by the European regulation, the estimated weekly intake was below the provisional tolerable weekly intake established by the European Food and Safety Authority, and the target hazard quotient values indicated that there is no carcinogenic risk for humans. A similar statement was concluded for analysis of metals in 12 fish species from the Aegean and Mediterranean Seas (Turkmen et al. 2008). Recently, Rivas et al. (2014) determined mineral and metal content in the muscle of three commercial fishes of the genus *Trachurus* from Spain, determining metal concentrations below the proposed limit values for human consumption; thus, trace minerals and metal content were acceptable for humans from a nutritional and toxicity point of view. Heavy metal contents in marine organisms from Antarctica, a pristine environment, are higher in comparison to those of other oceans, as has been demonstrated by Beltcheva et al. (2011).

It is relevant to point out that globally, levels of environmental contaminants emanating from power plants and other industrial emissions and effluent discharges are highly needed to be reduced in order to stop contaminating the aquatic environment. More research and assessments of seafood quality are needed in many countries to provide additional data and help safeguard the health of humans and animals that depend on this aquatic source of food.

Marine Nutraceuticals

The father of medicine, Hippocrates, in 480 BC advised “let food be your medicine and medicine your food.” Epidemiological studies demonstrate with conviction that diet factors may modify carcinogenesis during initiation, promotion, and progress of a human cancer. Marine biotechnology explores oceans to develop new pharmaceutical products, chemical compounds, enzymes, and other products and industrial processes. For 1,000 years, human being has taken advantage of nature to produce medicine to treat infection, inflammation, pain, and many diseases. Today, in some regions of the world, natural medicine continues being the only available treatment. Today, more than 50 % of chemicals come from natural sources or are produced by synthesis using natural products as patterns or starting material. The recent acceptance of alternative medicine by WHO (World Health Organization) recognizes the history as well as the importance of natural products for treating diseases. However, owing to the intense scientific research carried out for many years, chemical substances have been isolated from food, most of them having pharmacological activity. Hence, the term “nutraceutical,” a contraction form that joins both words, nutrition and pharmaceutical, coined by DeFelice in 1989, is

defined as: “any substance that can be considered a food or part of a food, that provides medical benefits on health, including prevention and treatment of diseases” (De Felice 1994).

Oceans constitute 70 % of the earth’s surface, with very diverse and ancient forms of life. Since 1950, the potential compounds of aquatic origin exceed 15,000, and each year hundreds of new compounds are discovered. Marine organisms are very heterogeneous being adapted to extremely different and fluctuating environmental conditions, through their own structural compounds or by production of secondary metabolites which show many biological properties which let fish compounds have health benefits. Most of the marine-derived biologically active compounds remain unexploited, but recently marine research has increased owing to the interesting applicability of identified marine-derived compounds for the industry of nutraceuticals. Hence, some of the natural compounds fish have are introduced here in order to show their applications for improving human health.

The diverse aquatic organisms include macroalgae, microalgae, bacteria, cyanobacteria, fish species, and shellfish, all of them producing compounds to cope with the changing aquatic environment to overcome the surrounding stresses. Besides, due to anticancer, anticoagulant, anti-inflammatory, antihypertensive, and anti-lipogenic properties of many aquatic chemicals, those compounds are being used in various industrial applications. Some are at the stage of research, others at clinical trials, and some others being sold by the industry. Thus, aquatic food is an excellent source of a great amount of bioactive compounds with beneficial effects on health and participates in a reduction of risk of a disease. Examples are fish enzymes, omega-3 fatty acids, proteins, bio-peptides, essential amino acids, vitamins, minerals, carotenoids, carotenoproteins, enzymes, chitin, chitosan, glucosamine, and many other specialized products.

The biological and medical applications of aquatic nutraceuticals are enormous. Most of aquatic natural products have been isolated from sponges, coelenterates, echinoderms, tunicates, and bryozoans as well as a wide variety of marine microorganisms. Sponges were the first marine organisms studied from which two medicaments were extracted for the treatment of leukemia (Bergmann and Burker 1955). Afterward, polysaccharides, polyphenols, polyunsaturated fatty acids, and carotenoids have been extracted from aquatic organisms, being considered as health beneficial owing to their characteristic biological properties as antioxidant, anti-inflammatory, anticancer, antiangiogenesis, antimicrobial, anticoagulant, prebiotic, and probiotic activities. For example, recently, proteases from discard of shrimp industry, found in sauce from shrimp hydrolysate, could be applied to tenderize meat; astaxanthin is a carotenoid that was first described in aquatic crustaceans (lobsters, crawfish, and shrimps) and is being utilized as a pigment in the shrimp culture and also as antioxidant in the diet, since it avoids LDL oxidation, stimulates production of antibodies, protects from hypertension and heart attack, prevents macular degeneration, and is used to treat neurodegenerative disorders as Alzheimer and Parkinson diseases; phycobilins from cyanobacteria and red algae give color to food and also are used as cosmetics; marine algae are very well known

since they are the only marine organisms from which polysaccharides can be extracted, like carrageenan, alginate, fucoidan, and agar-agar, all of them very important in the food industry; proteins, amino acids, enzymes, and lipids are not present here since they were discussed at the beginning of this chapter.

Aquatic resources provide a source of nutraceuticals and ingredients for functional foods, with a wide variety of chemical compounds with beneficial effects on human and animal health. Marine algae have natural pigments, like the carotenoids and phycobilins, and high protein and carbohydrate content and are also very rich in polyunsaturated fatty acids. Fish are very rich in digestive enzymes with a great application in food industry, as well as health. On the other hand, several species of fish with high omega-3 fatty acid content are normally recommended by cardiologists for cardiovascular disease prevention. Today, nutraceuticals of aquatic origin are positively confirmed as advisable to be applied in food industry, as well as to be used as natural products to improve a good health, and also in the cosmetic industry.

Conclusions and Future Directions

Fish and shellfish, owing to their content of proteins, with the essential amino acids, lipids with high-value PUFAs (EPA and DHA); enzymes, mainly digestive enzymes from fish waste; and micronutrients as vitamins and minerals, are highly nutritive and fundamental in a balanced diet. However, more research must be carried out concerning mineral and vitamin content in fish and fish products, since normally research is focalized on proteins and polyunsaturated fatty acids. Aquatic organisms offer also a rich source of nutraceuticals which belong to a great variety of biochemical compounds with beneficial effects on human and animal health. Hence the extraction of these substances from the diverse aquatic life forms, as well as the fish offal generated by the fishery industry, can be applied to food and medicine, allowing human health to be improved.

Cross-References

- ▶ [Bioactive Substances of Animal Origin](#)
- ▶ [Chemical Composition of Meat and Meat Products](#)

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Abstract

This chapter describes the safety assessment of food from genetically modified organisms (GMOs) in terms of its chemical composition. An analysis of the chemical composition of a GMO relative to that of a defined comparator is the principal approach used to assess the food safety and nutritious value of the modified entity. The comparison is relative to the chemical composition of the raw plant/animal/microorganism, and when found equivalent, it is assumed that the processed food would be as safe and nutritious as the products from the counterparts. In exceptional cases, a comparative analysis of the products is indicated. Where differences are observed (intended or unintended), the focus would be on an assessment of possible risk. Examples of products from different types of crops are given, such as stacked traits, abiotic and biotic stress tolerance, and enhanced nutritional value, with a brief explanation of how the food safety assessment would be undertaken in each case. The focus in this chapter is on the chemical compositions of crops as these form the center of international research activities, and to a lesser extent on animals and microorganisms.

Introduction

The chapter outlines the importance of assessing the chemical composition of food products from genetically modified organisms (GMOs). A number of questions may be asked regarding the chemical composition: What are the chemical components of importance? Do they change during the modification process? If they do change, will the food still be safe and nutritious for human and animal consumption? To answer such questions, the approaches to safety assessment advocated by international organizations are explained in some detail.

Genetic modification (genetic engineering, modern biotechnology) is a technique used to alter genetic material of living organisms in order to make them capable of producing new substances or performing new functions. The techniques used are (a) *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (r-DNA) and direct injection of nucleic acid into cells or organelles, or (b) fusion of cells beyond the taxonomic family that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection (CPB, Cartagena Protocol on Biosafety to the Convention on Biological Diversity, Article 3 (i) (a) (b)). r-DNA is described as genetic deoxyribonucleic acid (DNA) made by recombining fragments of DNA from different organisms. The result of genetic modification is a

genetically modified organism (GMO), an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. The term “transgenic” is also used the same way.

The most important examples of genetically modified (GM) crops grown during 2013 are soybeans (48 %), maize (33 %), cotton (14 %), and canola (5 %) and small amounts of other crops amounting to a total global adoption rate of 175.3 million hectares by 27 countries (James 2013). These contain genes that confer herbicide tolerance (HT) (57 %), insect resistance (IR) (16 %), or stacked traits (HTx/IR) (27 %) (James 2013). Other important commercialized crops with low areas planted include sugar beet, alfalfa, and papaya, tomato, and sweet pepper. Insect-resistant brinjal has been approved for commercialization in Bangladesh, but not in the Philippines. Major traits in the pipeline are water efficiency, salinity tolerance, drought tolerance, gossypol-free cotton, nitrogen use efficiency, virus resistance, non-browning apples, and golden rice (increased levels of provitamin A) (James 2013). Ongoing research is being conducted at various stages of approval, for example, with cassava, sweet potato, sugar beet, banana, and wheat (James 2013). Genetically modified microorganisms (GMMs) as sources of food ingredients have not received much world attention compared with the crop plants, although they are commonly used to produce food additives such as vitamins, flavorants, and enzymes. Genetically modified animals, for example, Atlantic salmon, have only recently been receiving the headlines of world news with the pending approval of Atlantic salmon by Canada and the USA. Since much has already been published on GM crops, the focus in this chapter is primarily on food from these commodities although safety assessment of GMMs and GM animals is described briefly.

First, brief information is provided about various international organizations that are involved in food safety assessment. The roles played by international bodies such as Codex Alimentarius Commission (CAC) and the Organization for Economic Cooperation and Development (OECD) and their published guidelines are significant. However, a number of other organizations, such as the European Food Safety Authority (EFSA), International Life Sciences Institute (ILSI), World Trade Organization (WTO), and the Cartagena Protocol on Biosafety, are referred to in the text.

Second, a short introduction to chemical components of crops, the evolution of agriculture, specifically plant breeding, and future developments in plant breeding techniques set the scene.

Third, the differences between the concepts of safety and risk are described as a basic consideration in chemical compositional analysis. This is illustrated by the landscape of safety of food and the concepts of “GRAS” (generally recognized as safe) and “history of safe use,” which are pertinent to the approach followed internationally to assess the safety of the composition of food derived from GMOs.

Fourth, the approach by the international body CAC and the development of the approach are detailed for GM crops with agronomic and nutritionally enhanced traits, as well as briefly for GM microorganisms and GM animals.

International Organizations Providing Guidance on Safety and Risk Assessment of Food Derived from Genetically Modified Organisms

Food and Agriculture Organization, World Health Organization, and Codex Alimentarius Commission

Codex Alimentarius Commission (CAC), an international body under the auspices of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), sets the standards on food safety and quality, based on “sound science” to facilitate trade. Codex principles and safety requirements for food from GMOs (CAC 2009) serve to guide countries in risk analysis (risk assessment, risk management, and risk communication).

Codex, for short, adopted four standards for foods derived from modern biotechnology (see descriptions in section “[Introduction](#)”): “Principles for the risk analysis of foods derived from modern biotechnology” (CAC 2009, pp. 1–5), “Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants” (CAC 2009, pp. 7–33), “Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA micro-organisms” (CAC 2009, pp. 35–55), and “Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA animals”(CAC 2009, pp. 57–76). These guidelines include sections on allergenicity, r-DNA plants modified for nutritional or health benefits, and assessment in situations of low-level presence of r-DNA plant material in food.

Organization for Economic Cooperation and Development

The OECD is an intergovernmental organization representing 34 industrialized countries. Its purpose is to coordinate and harmonize policies, to discuss issues of mutual concern, and to work together to respond to international problems. OECD was one of the international bodies that contributed to the development of the principles and safety requirements of food from GMOs. Most of the work is carried out by specialized committees and working groups composed of member country delegates. It has a number of safety guidelines for biotechnology for application by industry, agriculture, and the environment. Those that are important to food and feed safety are the consensus documents prepared by the OECD Task Force for the Safety of Novel Foods and Feeds ([OECD Consensus documents for the safety of novel foods and feed online](#)). A number of consensus documents are in progress such as one on food from animals (meat, milk, eggs). In addition to these documents, several other OECD publications are important to risk assessments of GMOs. The OECD has played a big role in the development of the approach that is followed in the safety and risk assessment of food from GMOs. The approach is discussed in detail in this chapter.

World Trade Organization

The WTO has food safety as a fundamental requirement in international trade with agricultural products. The establishment of the WTO was the outcome of the Uruguay Round of trade negotiations, following the Marrakesh Agreement Establishing the World Trade Organization in 1995. Two WTO trade agreements are important for trading in food products derived from GMOs, namely, the Sanitary and Phytosanitary Agreement (SPS Agreement) and the Technical Barrier to Trade Agreement (TBT Agreement). The SPS Agreement aims to (a) recognize the sovereign right of members to provide the level of health protection they deem appropriate and (b) ensure that SPS measures do not represent unnecessary, arbitrary or scientifically unjustifiable, or disguised restrictions on international trade.

The measures of the SPS Agreement place strict requirements on health protection based on scientific principles. WHO recognizes measures applied by countries that are based on international standards such as Codex. However, members are permitted to adopt measures that are more stringent than international ones, provided that they are founded on scientific risk assessment, applied consistently, and are not more trade restrictive than is necessary (Article 3 and Article 5). TBT requirements cover technical measures and standards such as labeling requirements and aim to ensure that technical regulations do not create unnecessary obstacles to international trade (WTO 1995).

Trade disputes may arise between countries, for instance, because of differences in the interpretation of the approach to risk assessment and the significance of possible unintended effects. A dispute between the USA, Canada, and Argentina as complainants and the European Communities over the “de facto” moratorium on approval of GMOs, which lasted from 1998 to 2004, illustrates the role of the WTO. The USA, Canada, and Argentina sought legal recourse at the WTO under a WTO SPS law based on unjustified and illegal denial of access to European markets (EC Biotech Products case), which resulted in financial losses to US farmers. The WTO found in favor of the complainants in 2006 and based its final decision on the failure of the defendant to conduct “adequate” risk assessments (SPS Article 5.1; Annex A (4)) by not taking the risk assessment techniques (protocols) of relevant international organizations into account (WTO 2006).

Other International Bodies of Interest

European Food Safety Authority

The EFSA was established in 2002 as a centralized risk assessment body for Europe with one of its key tasks being to embrace scientific advice on all aspects of food safety. The EFSA is currently recognized as a leading role player in preparing “opinions” on, for example, GM food safety. However, its positions are often disputed in the European Parliament (Kuiper et al. 2013).

Each GMO risk assessment is carried out by EFSA's GMO Panel, which is made up of 21 independent scientific experts. The safety of each GM product is assessed case by case. EFSA takes on "self-tasking" projects such as development of risk assessment guidelines that provide for being in the forefront of new scientific developments and to develop GM risk assessment approaches incorporating, for example, statistics, allergenicity, animal feeding trials, and post-market environmental monitoring. It is open to scientific contributions from third parties.

International Life Sciences Institute

The ILSI is a nonprofit organization whose mission is to provide science that improves human health and well-being and safeguards the environment. It achieves this mission by fostering collaboration among experts from academia, government, and industry on conducting, gathering, summarizing, and disseminating science. It publishes on nutrition, toxicology, and GMO food safety.

Cartagena Protocol on Biosafety to the Convention on Biological Diversity

This is an international agreement of the United Nations that aims to ensure the safe handling, transport, and use of living modified organisms (LMOs), resulting from modern biotechnology that may have adverse effects on biological diversity, taking into account risks to human health. It was adopted on 29 January 2000 and entered into force on 11 September 2003. Risk assessment requirements for food from GMOs are included in Annex 3 of the Protocol. LMOs are defined as any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. In everyday usage, LMOs are usually considered to be the same as GMOs, but definitions and interpretations of the term GMO vary widely.

Chemical Composition of Food Plants and Their Modification Through Plant Breeding

Chemical Composition of Food Plants

Plants synthesize thousands of substances, including organic compounds that provide the macronutrients in food (carbohydrates, proteins, and lipids), micronutrients (vitamins and minerals that are not synthesized, but absorbed from the environment), antinutrients (e.g., phytate that reduces bioavailability of Zn, Fe, Ca, and Mg), and allergens and toxicants (e.g., cyanogenetic glycosides, glycoalkaloids). The focus of chemical analysis of food products from GMOs would be on these substances. As a wider range of GM crops becomes available, often with improved nutritional value, the need for a more thorough understanding of biochemistry and composition of the conventional crops from which they are derived becomes important. Cassava is one such crop that is scheduled for

development of improved nutritive qualities and reduced toxicants (Ganjewala et al. 2010; Siritunga and Sayre 2004).

Plants have a certain metabolic plasticity that makes it possible to respond to environmental stimuli. This can result in major shifts in metabolic activity. Cassava, a plant that is known for the presence of toxicants, mainly cyanogenetic glycosides (CG), is an interesting example of environmental adaptation. Of all the toxicants present in food, the CG toxicants are of great interest, being produced in more than 2,600 plant species, which include crop plants such as barley, sorghum, and cassava (Ganjewala et al. 2010). CG toxicants may have various functions: chemical defense, plant–insect interactions, nitrogen–storage compounds, and phagostimulants. The metabolic pathway of CG anabolism and catabolism has been researched, and much is already known about the toxicants of this crop (Ganjewala et al. 2010). See also ► [Chap. 23, “Plant-Associated Natural Food Toxins.”](#)

Other groups of phytochemicals are of interest including the terpenes, plant steroids, and a number of alkaloids, polyphenols, carotenoids, glucosinolates, dietary fiber, and lectins, all of which have a place in the biochemistry of plants but may also have a positive or negative effect on human health. Of particular interest in food safety assessment is documented scientific knowledge of toxicants and antinutrients present in crop plants, for example, toxic amino acids, lectins, proteinase inhibitors, antigenic proteins, alkaloids, saponins, and condensed tannins (Duffus and Duffus 1991). See also ► [Chap. 23, “Plant-Associated Natural Food Toxins.”](#) Several biochemical pathways are important in phytochemical metabolism (shikimate pathway, isoprenoid pathway, polyketide pathway, glucosinolate biosynthesis), of which the shikimate pathway is noteworthy in the genetic modification of plants that tolerate the application of the herbicide glyphosate (section [“Herbicide Tolerant Crops”](#)).

Knowledge of crops and methods of preparation in order to produce safe and nutritious food has developed through trial and error by selection and preservation of plant variants with desirable traits and human preference for taste and color (Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods . . . 2004). Human beings are aware of antinutrients and toxicants from traditionally gained knowledge and experience in cultivation and food preparation. However, preparation of the crops into various forms of products may not always reduce the levels of toxicants.

Plant Breeding

The domestication of wild plant species started thousands of years ago. Plant breeding resulted in the evolution of agriculture. Today almost every crop plant is related to a wild species that occurred naturally in its center of origin. For instance, maize apparently originated from a Mexican maize-like plant called teosinte. Farmers selected for and maintained as landraces a vast array of traits, such as

improved yield, insect resistance, drought tolerance, improved nutrient values and palatability, and decreased toxicity. Meyer et al. (2012) came to the conclusion from their study of patterns and processes in crop domestication that the most common changes are in secondary metabolites (e.g., less bitter or toxic compounds and pigment changes) occurring in 66 % of domesticated crops. A few of the examples in more recent times are the elimination of the toxicant erucic acid and glucosinolates from rapeseed to produce canola (Canadian oil) and breeding “kiwi fruit” from the wild and inedible Chinese gooseberry. These changes happened through new mutations and natural hybridization. The science of genetics and scientific tools contributed to improve breeding practices such as hybrid embryo rescue, the application of colchicine (a chemical employed to induce polyploidy), ionizing irradiation, the application of mutagenic chemicals, and natural somaclonal variation (in cell culture) (Prakash 2001). Gene transfer techniques to develop GM crops introduced another phase in plant breeding evolution. In 1978, the first GMO was produced at the commercial level by the creation of an *Escherichia coli* bacterial strain producing the human protein insulin. In 1994, the first genetically modified seeds were planted in the United States for commercial use. That was a tomato with a long shelf life (FLAVR SAVR™ by Calgene, Inc).

Recent molecular techniques have shown that the methods used in traditional (non-transgenic) plant breeding are associated with genetic changes such as mutations, deletions, insertions, and rearrangements. These occur in addition to the movement of mobile genetic elements such as transposons (jumping genes), which are responsible for most of the genome plasticity (Weber et al. 2012). Plant breeders traditionally eliminate observed off-types during the evaluation process. Despite the dynamic nature of genomes, and the effect of traditional techniques on them, only a few safety concerns from traditional plant breeding have been recorded over years.

Several recent articles have shown that traditional breeding such as mutation breeding, natural variation, and environmental effects often causes more inherent variability than genetic modification (Ricroch et al. 2011 and references therein). As a result, arguments for GM crops to be subject to less stringent requirements or exempted from regulation (Herman et al. 2009) and policy reform (Durham et al. 2011) are heard frequently.

A number of the new plant breeding techniques (NPBT) that go beyond the conventional concept of genetic modification have been described by Lusser et al. (2012). These are *cis*-genesis/intragenesis, oligonucleotide-directed mutagenesis (ODM), reverse breeding, RNA-dependent DNA methylation, site-directed nuclease (e.g., zinc finger nucleases), agro-infiltration, and grafting on GM rootstock. The questions are whether all these techniques can be considered transgenic, whether DNA is inserted, whether the DNA is foreign, whether it is indistinguishable from conventional breeding, and whether the unintended effects are the same as with traditional or conventional breeding. These discussions are ongoing and depend on a country's legislation and definition of a GMO.

Safety and Risk Assessment of Food from Genetically Modified Crops: Chemical Composition Analysis

Risk, Risk Assessment, Uncertainty, and Food Safety

The explanations in this section serve as introduction to the safety assessment and risk assessment of food from GMOs. An understanding of the historical development of knowledge of food safety and of the nutritional value of food is also important when defining the way forward with safety and risk assessment of food from GMOs.

The term “safety” (and hence food safety) needs explanation, as it is often confused with “risk” as meaning an absence of risk. According to Wolt (2008, p. 2), the concept of “food safety is not absolute, since it is a judgment, it is value laden. . . [that is] understood within the context of society, culture, politics, and economics.” Even with a concept of safety, risk is not negated because there is always a degree of risk. The OECD describes safety vaguely as “reasonable certainty” of “no harm” (OECD 1993, p. 17). Concepts of “harm,” in turn, could differ among societies and regulatory bodies because of the same value-laden issues affect the concept of safety. However, a given level of risk cannot be deemed uniformly acceptably safe because of different norms of acceptability of risk in different societies (Wolt 2008).

The way people assess the safety of any new food such as a GMO product is often based on a comparison with existing knowledge. Therefore, there is a need for a better description of what we commonly consider safe food. The description “generally recognized as safe” (GRAS) is applied to food or food additives that either have a long history of use or by virtue of the nature of the scientific information available about the nature of the substances, their customary or projected conditions of use, and the information generally available to scientists about the substances (United States Food and Drug Administration (US FDA): GRAS online).

The concept of GRAS led to the use of the principle of “history of safe use,” which is of cardinal importance in the approach to the safety assessment of food products from GMOs in comparison with their conventional counterpart (CAC 2009). The concept remains hard to define. Constable et al. (2007, p. 2513) state that it “relates to an existing body of information which describes the safety profile of a food, rather than a precise checklist of criteria” and should be regarded as a “working concept.” They propose a food safety profile consisting of the “period over which the traditional food has been consumed, the way it has been prepared and used and at what intake levels, its composition and the results of animal studies and observations from human exposure” (Constable et al. 2007, p. 2513). With a profile of the food in mind that includes the “best available scientific knowledge” (CAC 2009, p. 11), a judgment can be made of the safety of the conventional counterpart.

“Risk” refers to the probability of an adverse (health, environmental) effect (leading to harm or undesired consequence) and the severity of that effect,

consequential to a hazard(s) or threat(s). In scientific terms, zero risk is nonexistent (CAC 2014, pp. 116–117). Some uncertainty is always present in a risk and forms an inherent and integral element of scientific analysis and risk assessment.

The assessment of a risk is described by CAC (CAC 2014, pp. 116–117) as a scientifically founded process based on four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization. These are also the steps followed in the risk assessment of food from GMOs.

The Approach to Safety and Risk Assessment of Food from GMOs

Codex guides the safety and risk assessment of food from GMOs in a precautionary way by proposing the steps to be taken in the assessment. The purpose of this chapter is not to explain these steps in detail, but to focus on chemical composition as a critical part in the assessment. However, a short description of risk assessment is in order to give perspective on the place of the compositional analysis. The pre-risk assessment phase includes gathering information to provide a description of the host and donor organism as well as the modification process and the final modified organism. The risk assessment phase includes the four steps mentioned in section “[Risk, Risk Assessment, Uncertainty, and Food Safety](#)”. The conclusion is made relative to the safety of the counterpart with a history of safe use.

Hazard Identification and Hazard Characterization

The step in risk assessment called hazard identification is described as the identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods (CAC 2014, pp. 116–117). The step called hazard characterization is described as the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical, and physical agents that may be present in food. For chemical agents, a dose-response assessment should be performed. For biological or physical agents, a dose-response assessment should be performed if the data are obtainable (CAC 2014, pp. 116–117).

Comparative Assessment

“Substantial equivalence” forms the core of the comparative risk/safety analysis but was previously mistaken as the endpoint of the assessment and left a great deal of scope for individual and national interpretations. It was replaced with a comparative risk/safety assessment, but the basic concept behind it remained untouched (Kuiper et al. 2001; Kok and Kuiper 2003). The comparative safety assessment of GM crops focuses on determining similarities and differences and/or lack of equivalences between composition, phenotype, and agronomic characteristics of the GMO and the conventional counterpart. Together with the molecular

characterization, comparative analysis is regarded as the starting point of an assessment (Kok and Kuiper 2003).

An analysis is based on relative safety, and not risk, because of the comparison with the counterpart with “a history of safe use.” Should there be differences between the GMO and its comparator, these are investigated further to characterize risks to human and animal health. A statistically significant difference between the GM plant and the comparator may not be a safety concern or of biological significance. The equivalency determination has been developed to capture such differences for interpretation in a more meaningful way (EFSA 2011b).

The compositional comparison approach is regarded as a more sensitive method than traditional toxicological testing because of many difficulties with testing whole food (CAC 2009, p. 9) (see also section “[Toxicological Studies with Whole GM Plants](#)”).

The Comparator: History of Safe Use

Defining the comparator for the comparative safety assessment is critical. The comparator in the safety assessment for plants containing single events is normally a non-GM genotype with a genetic background as close as possible to the GM plant (Constable et al. 2007; CAC 2009; EFSA 2011a, b), described as a near-isogenic line in sexually propagated crops and as an isogenic line in vegetatively propagated crops. The recommended term “conventional counterpart” is used to refer to the comparator in these cases (EFSA 2011a, b). Such a comparator should have a “history of safe use” in order to make a statement on the safety of the GM food. The extent to which non-GM comparators are genetically related to the GM plant varies, depending on the breeding scheme used in the production of the GM plant and its comparator(s) (EFSA 2011a, b). There may also be exceptional cases in which the nearest non-genotype may not have “a history of safe use,” as with cassava. Processed products from the nearest isoline (or the isoline) could be an option to consider (Jansen van Rijssen et al. 2013).

GM plants containing single events are gradually being replaced by products from increasingly complex breeding schemes, for example, by combining (stacking) events through conventional breeding, by stacking events through techniques other than conventional breeding, or by intentional introduction of major compositional changes (see section “[Stacked traits of increasing complexity](#)”). An event refers to the unique DNA recombination event that took place in a single cell through insertion of a particular genetic construct and was then used to generate entire transgenic organisms. There are a number of options for comparators when events are stacked. In stacking events by conventional breeding, the choice could be the conventional counterpart or, if not available, a “negative segregant.” Plants that are negative segregants (or null segregant) lack the transgenic event and can be produced by self-fertilization of hemizygous GM plants or from crosses between hemizygous GM plants and non-GM plants (EFSA 2011a, p. 9). Another option for a comparator would be a previously assessed GM parent(s) of the stacked GM plant with no unintended effects being present in all instances (EFSA 2011a).

In the GM plants containing events stacked by retransformation, co-transformation, or a transformation cassette containing multiple genes, the conventional counterpart or, if not possible, the comparator could be from a negative segregant. The requirements for comparators in all situations should be considered case by case (EFSA 2011a).

Deciding on an appropriate comparator for a nutritionally enhanced GM crop may not be straightforward. Where the enhanced GM crop is a direct replacement for the comparator, the closely related or near-isogenic variety would be appropriate to consider the nutritional impact. Where a comparator cannot be identified within the same crop, a specific food component (e.g., a fatty acid profile) from another food could be considered (ILSI 2004, p. 40). More information on nutritionally enhanced food is provided in section “[Nutritionally Enhanced GM Crops](#).”

Composition Analyses

The comparative compositional assessment is an integral part of the identification of hazards associated with a GM food. Compositional analysis of conventionally produced food is seldom required except for labeling processed food, and so GM food is in fact subject to much more intense scrutiny for safety than conventional food.

Consensus on the chemical components to be considered is important among nations to harmonize the assessment of these GM products. Consensus documents are produced by the OECD Task Force for the Safety of Novel Foods and Feeds; these provide information on the chemical components, examined crop by crop, that are important to consider in a safety and risk assessment.

In addition to information on the production, harvesting, and processing of crops, consensus documents contain data on these components: proximates (including moisture and total ash), macro- and micronutrients, antinutrients, toxicants, allergens, and other plant metabolites. Lists of key components are identified as characteristic of the crops that are consumed by human and animals. Not all components are included in the analysis but only those that are characteristic of the crop and are of critical importance regarding risks to human and animal health. By selecting and targeting characteristic components, the requirements of a risk hypothesis for GMO crops are realized ([OECD Biotrack online](#)).

It is important that chemical analysis of the components should be conducted by accredited analytical laboratories and according to internationally approved methods. Information on the laboratory, a certificate of good laboratory practices (GLP), and a quality assurance statement should accompany applications for registration by the regulatory authority.

Nutritional Assessment of Products from Genetically Modified Crops with Agronomic Traits

If the GM plant has been assessed as not compositionally different from the comparator, except for the introduced trait(s), further studies to illustrate nutritional equivalence are not required. Where differences and/or no equivalency has been observed, further studies could be considered, for instance, by performing

wholesomeness studies with livestock species and laboratory animals (rodents) depending on the GM crop under assessment. Results from wholesomeness studies are often included in dossiers, which are then regarded as complementary information to the results from the chemical compositional analysis. It is unlikely that such studies would reveal unintended minor compositional changes that have gone undetected by the targeted analysis because of lack of sensitivity (ILSI 2004, p. 41).

Wholesomeness studies should not be confused with toxicological studies, as their purposes differ. There are challenges to including these studies for GM crop products that are not normally consumed by animals, such as macadamia nuts, which cause transient paralysis when fed to dogs (ILSI 2004, p. 41). Wholesomeness studies as described here do not provide information on nutrient availability for which additional studies are designed. The requirements for wholesomeness have been elaborated on by ILSI in two “Best Practices” publications (ILSI 2003, 2007).

Nutritionally enhanced crops are discussed further under section “[Nutritionally Enhanced GM Crops](#).”

Natural Variation, Differences, and Equivalences

Natural variation of component concentrations occurs because of genetic variation, environmental effects (soil, temperature, altitude, rainfall), and farming practices. The differences between the components of the test substance (GM crop) and those of the comparator are considered against the background of the range of values found in the edible varieties of the crop under different environmental conditions. The test of difference is to verify whether the GM plant is different from its comparator. A test for equivalency verifies whether the GM plant is equivalent or not to non-GM reference varieties with a history of safe use, apart from the introduced trait (EFSA 2011b). The GM crop, its comparator, and a number of conventional non-GM varieties or hybrids are normally included in the same field trials, which are executed at about three to eight localities representative of different environmental conditions, depending on the country’s requirements. The results from such trials can be compared with information in recognized databases and publications such as the OECD consensus documents and the ILSI Crop Composition Database ([ILSI-CCDB online](#)). The influence of crop genetics and interaction with environmental conditions on the chemical composition can be identified in this way (Harrigan et al. 2007). Data from the ILSI-CCDB are valuable because a wide range of geographies, years, and conventional varieties of soybean, cotton, and maize are presented in a searchable format (Ridley et al. 2004). The ILSI-CCDB contains only the chemical composition data from conventional maize, soybean, and cotton (but not other crops) collected from controlled field trials at multiple worldwide locations. Currently data from GM crops are not included.

Data on the composition of GM and conventional crops that have accumulated over more than 20 years were analyzed for the effects of multiple germplasms, diverse geographical areas (environment), and multiple seasonal planting (years) on the stability of the inserted gene(s) and the compositional profiles of GM maize and GM soybean varieties. The conclusions from the analysis (including meta-analysis and exploratory data analysis) were that the environment and genotypes had

pronounced effects on variability, much more so than genetic modification (Zhou et al. 2011; Harrigan and Harrison 2012). The results from such studies are informative in many respects, in particular when the food fortification of products from traditional crops is considered, since compositional variability would affect the levels of food fortification required.

Processing of Raw Crop Material

Different possible scenarios exist for assessing risks associated with processed and preserved food products from GMOs. Where the modification is intended for improved agronomic traits such as insect resistance and herbicide tolerance and no differences are observed in composition concentrations between the GM crop and its comparator, it is assumed that the processed or preserved food products would also not differ. The assessment of food and feed products is considered case-by-case for genetic modifications where the major metabolic pathways are targeted, as in nutritionally enhanced and functional GM food or food with reduced levels of toxicants. It would also be advisable to consider assessment of the processed food case-by-case from stacked event GMOs where there is interaction between the stacked traits (EFSA 2011a, b).

Intended Effects

The risk is characterized by an assessment of intended and possible unintended differences between the GM crop plant and the conventional counterparts (CAC 2009).

The major GM crops on the market contain genes that express proteins that are detected in specific parts of the plant. The concentration of these proteins in the different parts of the plant is determined based on dry weight and the exposure calculated according to the dietary intake information of the population of a country. These novel proteins are considered for possible toxicity and allergenicity in a tiered approach.

Toxicity of the Novel Proteins: Assessment of the toxicity of the novel protein consists of a study of the bioinformatics structure–activity relationship to determine sequence homology with known toxicants. An acute toxicity study with rodents is often included with the purpose to determine acute effects of a single dose of the protein. This is usually orally administered in a large concentration of protein (2,000 mg/kg) and observations made for 14 days (OECD Guidelines for the testing of chemicals online). Tests are often conducted with surrogate proteins produced at high levels in bacteria (e.g., *E. coli*). The surrogate protein has to be validated for its integrity by a number of biochemical assays. Subsequent testing for chronic and subchronic toxicity is described in section “[Toxicological Studies with Whole GM Plants](#).”

Allergenicity of Novel Proteins: Knowledge of the structure and the biochemical and physicochemical properties of the novel protein provides important information in the assessment for allergenicity. Starting with bioinformatics structure–activity relationship, investigations are made to determine sequence homology with known allergens. These are followed by in vitro studies for allergens including studies to

determine protein stability. This is the ability of the protein to retain its original native three-dimensional structure after treatments (digestibility and thermal stability studies) and resistance to degradation by proteases. The conclusion on allergenicity is made on a weight of evidence basis.

Should the results from the hazard assessment and the hazard characterization not indicate a safety concern, then no further testing is required.

Unintended Compositional Differences

Early in the development of the GM plant, detailed agronomic assessment is specified to identify unintended effects at whole-plant level (morphology, phenotype, and agronomic performance). Plant breeders traditionally eliminate observed off-types during the evaluation process. Targeted compositional analyses of GM crops focus on the possible changes at plant metabolic level (biochemical phenotype) (Chassy et al. 2005).

All methods of crop improvement, whether involving conventional or genetically modified crops, may cause unintended compositional changes, as described by an advisory group for the US National Academy of Sciences (Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health 2004). Unintended effects are expected, as the comparator isoline and the near isoline could never be an absolute fit, for a number of reasons. The US group contended that it was unlikely that all methods of GM, non-GM, and conventional breeding would have equal probability of unintended effects. They identified induced mutagenesis as the most genetically disruptive technique and thus the most likely to display unintended phenotypic changes. This was followed by biolistic transfer and then by *Agrobacterium* transfer of r-DNA from distantly related species. *Agrobacterium* transfer of r-DNA from closely related species was ranked less likely to cause unintended changes than any of the above methods, including conventional pollen-based crossing of distantly related species and/or embryo rescue. *Agrobacterium* transfer of r-DNA is conducted by *Agrobacterium tumefaciens*, a ubiquitous soil bacterium which is used to insert genetic information into plant cells by means of a small segment of DNA known as the T-DNA from a plasmid. This is done by cloning a desired gene sequence into the T-DNA that will be inserted into the host DNA. Biolistics or bioballistics is a technique whereby a gene gun or a biolistic particle delivery system is used to inject cells with genetic information. The payload is an elemental particle of a heavy metal coated with a plasmid DNA.

Unintended effects have been reported from non-GM crop varieties at the point of commercialization, although this is rare (Cellini et al. 2004). Trace amounts were reported of the unintended metabolite *cis*-15-octadecadienoic acid, an isomer of linoleic acid that is not usually present in non-hydrogenated soybean oil, but is present in hydrogenated soybean oil and other food sources (Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health 2004). A number of examples of increased levels of toxicants have been reported, such as psoralens in celery (furanocoumarins), apparently owing to environmental factors or genetics (Diawara et al. 1993).

Chassy (2010) is of the opinion that it is unlikely that completely new toxicants will be formed as a result of genetic modification. The observed changes to date have been only in the levels of existing toxicants, including precursors or catabolic products. Nontargeted techniques, which include genomics, transcriptomic profiling, proteomics, and metabolomics (“omics”), are types of methodologies that have opened up possibilities for in-depth comparative studies to gain a better understanding of the genomic and environmental effects on the composition of crop plants. The use of metabolomics has been advocated as an approach to expand the range of metabolites that can be measured for potential unintended effects (Davies 2010). At this stage, interpretation of the vast amount of information is a challenge, and methodologies still need to be standardized and validated, and are qualitative and not fully reproducible. The need for such studies is still being considered. The current results from “omics” studies with single traits confirm the hypothesis that GM techniques are less disruptive to the genome than non-GM methods, according to the analysis of Ricroch et al. (2011). Nontargeted techniques should not be considered in the absence of targeted approaches. Harrigan and Chassy (2012, p. 335) suggest that “targeted assessments could easily facilitate a partnership with “omics” researchers conducting semi-targeted profiling on pathways associated with toxic metabolites.” Harrigan and Chassy (2012, p. 334), however, challenge the likelihood that metabolic profiling would provide “immediately interpretable data in safety assessments that would provide added value to, or otherwise enhance, rigorously quantitative assessments of known nutrients and anti-nutrients that comprise foodstuffs.”

Toxicological Studies with Whole GM Plants

Toxicology feeding studies with laboratory animals (rat, mouse, and dog) fed on GM plant products are required on a case-by-case basis. Subchronic toxicology studies are designed to observe effects over a 90-day period and chronic toxicity feeding studies over the lifetime of the laboratory animal. Chronic feeding studies are often designed to include parameters for observations on carcinogenicity of chemicals. The strain of the specific animal species is important in these studies because of the genetic makeup of the animal strain ([OECD Guidelines for the testing of chemicals online](#)). OECD guidelines for testing toxicity of chemicals are useful study materials for considering toxicological evaluations should such studies be considered. However, interpretation of toxicological data from animal studies requires multidisciplinary skills and experience in laboratory animal evaluation.

Note should be taken of the disputed value of subchronic and chronic toxicity and carcinogenicity evaluation in mammalian feeding studies. Strong views are expressed that the need for these studies should be determined on a case-by-case basis when substantial differences are observed in the comparison studies (Bartolomaeus et al. 2013). In 2013 the European Commission (EC) voted in favor of a regulation requiring applicants to carry out an obligatory 90-day oral toxicity study for each GMO submitted for regulatory approval. At the request of the European Commission, EFSA has recently published guidelines for routine

conducting of chronic toxicity and carcinogenicity studies with rodents based on the OECD guidelines for chemicals (EFSA 2013a). These resulted in reactions from various groups (Bartolomaeus et al. 2013; Kuiper et al. 2013). The arguments against any animal feeding studies other than case-by-case are based on the comparative robustness of the agronomic, phenotypic, and compositional analysis, the wealth of data available for commercialized GM crops, and predictions of safety based on crop genetics (Bartolomaeus et al. 2013). Kuiper et al. (2013) argue against routine toxicology studies because there is no added value to assessing safety of food from GMOs due to the limitations of feeding studies. These studies have “low power” of detecting adverse effects as a concentration of a toxic substance produced as a consequence of the genetic modification of at least 4,000 mg/kg in the animal diet (assuming median toxicity potential) would be required in order to induce a toxic effect. Another argument against these studies is the increased use of animals in experimentation as a matter of animal welfare (Kuiper et al. 2013).

In addition, an argument against including chronic and other toxicological studies with whole-plant material is that retrospective assessment of data from chronic toxicity studies with rodents and dogs (Betton et al. 1994; Box and Spielmann 2005) has demonstrated that in many cases the lowest and most conservative safety endpoint (no-observed-effect level, NOEL) came from subchronic studies (Munro et al. 1996).

Endogenous Allergens

The case-by-case assessment of endogenous allergens is included in the Codex requirements for compositional assessments (CAC 2009). The OECD consensus documents include a section on these allergens (OECD Biotrack online). These are considered in the light of the risks to humans. Eight foods are significant because of allergenic incidences in about 3–5 % of the world population (Sicherer and Sampson 2010). These are soybean, peanuts, tree nuts, cow’s milk, fish, crustaceans, eggs, and wheat (Bush and Hefle 1996). Wheat causes intolerance reactions that are not equivalent to allergenicity. The eight foods are labeled as such by many countries. Consumers have learned from experience to avoid them. Allergens can cause effects of varying intensity in allergic consumers (Anderson 1996; Sten et al. 2004). The level of allergenic substances in these food crops varies depending on the environment in which they grow, but the variability has mostly not been documented (Goodman and Tetteh 2011). This results in difficulties in identifying possible increases in significant levels from genetic interventions such as genetic modification. Another issue is the status of detection methods. Specific serum screening to confirm allergenicity may not be possible because of the difficulties in identification of a sufficient number of donors, whereas animal models have not been validated and are thus inconclusive for assessments (Goodman and Tetteh 2011). Much research and development have still to be conducted. It would be for the regulatory authorities to decide what level of risk would be a cautious option, because of all the difficulties encountered with identifying allergenicity in humans.

Antibiotic Resistance Marker Genes

Antibiotic resistance marker (ARM) genes are sometimes included in the transformation technologies. The use is discouraged, although gene transfer from plants and their food products to gut microorganisms or human cells is considered a rare possibility (CAC 2009, p. 18; EFSA 2009).

Some Examples of GM Crops and Their Food Safety Assessment

Insect-Protected GM Crops

About 13 % of potential world crop yield is lost to pests (Slater et al. 2003 p.105). The insect orders, Lepidoptera (butterflies), Diptera (flies and mosquitoes), and Coleoptera (beetles and weevils), are known to cause a lot of crop damage. These are examples of biotic stress. Insect-resistant crops that were developed through modern biotechnology with bacterial insecticidal genes were among the first initiatives to provide protection. In 1987 Marc von Montagu from Ghent in Belgium and fellow scientists were first to develop GM tobacco plants with insect tolerance by expressing genes (*cry* endotoxin genes) from the bacterium *Bacillus thuringiensis*. *B. thuringiensis* (Bt) has been widely used as a biopesticide in conventional practices since 1901. Various different Cry toxins show specific pathogenicity to insects of the mentioned orders but also to some hymenopterans (wasps and bees) and nematodes. Different entomocidal protein protoxins are produced which are activated upon ingestion to produce the Cry toxin; they, in addition to other virulence factors, weaken or kill insects. A toxicity profile for each configuration of Cry toxin is determined by the specificity of the midgut epithelial receptors in the insects. “Specificity” is an important criterion in safety assessment. The details of the modes of action are the subject of ongoing research (Vachon et al. 2012).

A variety of safety studies have been conducted with native Bt protein to show that it does not have characteristics of food allergens or toxins. Cry proteins are not structurally related to food allergens or protein toxins as has been confirmed by bioinformatics. They are unstable in digestive fluid, and acute toxicity studies with rodents confirm their safety when consumed in food derived from GMOs. Immunological studies have confirmed the findings of the allergenicity studies. However, a special case was the investigation of the native Cry9 protein in maize (StarLink™) that has no history of human dietary exposure, was not readily digestible, and was also stable at an elevated temperature of 90 °C. It did not originate from an allergic source and had no amino acid homology with known toxin or allergen in available protein databases. Immunological assays did not confirm allergenicity (University of Californianucbiotech.org online). StarLink maize was approved for use in animal feed but not for human consumption, but after it was found in a number of food products, it was recalled from the market.

Herbicide-Tolerant Crops

Plants can tolerate exposure to the phytotoxic effects of herbicides in several ways: through plant enzymes that detoxify the herbicide, by altering the target site of action, and by physical and physiological barriers to the uptake of herbicides into plant tissues and cells. Only one example of genetic modification is given to illustrate the assessment approach. The synthetic chemical glyphosate is widely used as a broad-spectrum systemic herbicide. Its mode of action is by inhibiting the plant enzyme 5-enolpyruvyl-3-phosphoshikimic acid synthase (EPSPS), which is an enzyme in plants and microorganisms, but not in animals. EPSPS is involved in the biosynthesis of aromatic amino acids, vitamins, and many secondary metabolites. The inhibition of EPSPS results in the disruption of protein synthesis and plant death. Plants have been genetically transformed with a gene expressing an EPSPS protein from the soil bacterium *Agrobacterium*, which is not a human or animal pathogen, but is highly tolerant to glyphosate. With this encoded EPSPS enzyme with less affinity for glyphosate, the plant becomes tolerant to the action of glyphosate. The end products of the shikimate pathway, such as aromatic amino acids, are included in the compositional analysis. The introduced enzyme(s) is/are further assessed for toxicity and allergenicity. The toxicity and recommended maximum residue levels (MRLs) for both glyphosate and its hydrolytic metabolite aminomethylphosphonic acid (AMPA) have been assessed by Codex Alimentarius (Codex Alimentarius online). MRLs are statutory concentration limits based on results from field studies to determine the withdrawal period. The label instructions specify application rates of the chemical by which the farmer is legally bound. The characteristic components of glyphosate-tolerant soybean and maize have been analyzed by various methods. The results from comparative compositional assessment at multiple locations (geographical areas, environments), multiple germplasm, and multiple planting seasons showed that variability in levels of components was attributed mainly to environmental and genotypic effects and not to genetic modification. Extensive variability of particular fatty acids, vitamin E, and isoflavones was obvious even among different non-GM germplasm as mentioned in section “[Natural Variation, Differences, and Equivalences](#)” (Harrigan et al. 2007; Zhou et al. 2011; Harrigan and Harrison 2012). Such analysis of historical data contributes to a better understanding of the variability in component concentrations to which the consumers are exposed.

Nutritionally Enhanced GM Crops

Assessment of crops nutritionally enhanced through genetic modification is conducted in the same way as crops with altered agronomic traits (insect resistance and herbicide tolerance). Unintended effects are considered and researched as before. The intended compositional changes are given specific attention with nutritionally enhanced crops (CAC 2009, pp. 24–27).

An analysis of nutritionally enhanced crops includes examination of metabolites important to the anabolic and catabolic pathways, as well as the possible impact of the genetic modification on the metabolites in related pathways. Morandini and Salamini (2003) have described the complexity when permanent changes in the biochemical pathways may affect other pathways that are essential for producing critical nutrients. Knowledge of the mechanism of action of the introduced trait in the transgenic plant is important.

The possible types of nutritionally improved crops are diverse, for example, increased protein levels, higher levels of a specific amino acid, changes in the fatty acid profiles, or crops with desirable functional or organoleptic properties. The requirements for the assessment are determined case-by-case because of the possibility that enhanced nutritional qualities result from diverse interactions at the level of plant biochemistry.

Assessment of bioavailability and biological efficacy of nutrients under different processing, storage, and transport conditions is important. It is also important to know the anticipated dietary intake and nutritional impact at population level. Post-release monitoring should be considered for a number of reasons (e.g., impact of nutritional enhancement).

Studies with laboratory animals and targeted livestock species could contribute to the assessment (ILSI 2004, 2007). Feeding studies with broiler chickens are commonly used because fast-growing animals are useful for assessing nutritional quality.

Golden Rice

Vitamin A deficiency (VAD) is widespread in the major rice-eating regions of the world. Enhancing the concentration levels of the vitamin A precursor by genetic modification seems to be a feasible solution as beta-carotene does not occur naturally in rice endosperm. Golden Rice 1 was produced using two beta-carotene biosynthesis genes expressing the enzymes: phytoene synthase (*psy*) from daffodil (*Narcissus pseudonarcissus*) and carotene desaturase (*crtl*) from the soil bacterium *Erwinia uredovora*. Golden Rice 1 produced only about 1.6 µg total carotenoids per gram of dry weight grain. Because of this low concentration, Golden Rice 2 was developed. Golden Rice 2 has the combined genes phytoene synthase from maize and the *crtl* gene from the mentioned bacterium. The latter rice variety has a much higher level of 37 µg total carotenoids per gram of dry weight grain (31 µg/g β-carotene) (ILSI 2008).

The safety assessment of Golden Rice 2 involves examination of the history of safe use of the DNA elements incorporated into the construct, characterization of the DNA insert and the inserted proteins, in vitro assays for digestibility, heat tolerance of the proteins, compositional analysis (with conventional rice as comparator), and analysis of the carotenoid pool. Animal feeding studies for wholesomeness are important. Animal feeding studies for toxicity assessment and nontargeted “profiling” compositional analysis conducted case-by-case would be indicated.

For the analysis of the carotenoid pool and other possible metabolites of interest, the biosynthesis of β -carotene in plants is important to follow both for the phenotypic and the compositional analysis of Golden Rice 2. The building unit isopentenyl pyrophosphate (IPP) for the synthesis of terpenoids, including β -carotene, is also a precursor for plant hormones, other secondary metabolites, and defense molecules. IPP occurs in the cytosol and plastids of plants. In plastids, it serves as a substrate for carotenoid biosynthesis, among others, and the major or only product of the pathway leading to cyclic carotenoids. Important compounds in the biosynthetic pathway to β -carotene are geranylgeranyl diphosphate (GGPP), phytoene, and lycopene. By increasing the flux through the β -carotene biosynthesis pathway, the biosynthesis of other metabolites may alter (e.g., vitamins E and K, chlorophyll, gibberellic acid, xanthophylls, and abscisic acid) (ILSI 2008).

High Oleic Acid Soybean

The example of soybean with an enhanced fatty acid profile should be studied in conjunction with ► [Chap. 13, “Chemical Composition of Fat and Oil Products.”](#)

Consumers are aware of the impact of dietary lipids on health such as potential cardiovascular problems presumably caused by the presence of *trans*-fatty acids (TFA) in food. TFA are unsaturated fatty acids that contain at least one nonconjugated double bond in the *trans* configuration and are present in our diet through industrial processes and from ruminant or plant origin. The major process contributing to formation of TFA is hydrogenation of plant oils, which is carried out to increase oxidative stability and prevent oxidative breakdown that leads to rancidity and off flavors. Modification of the endogenous fatty acid profile provides a means to increase oxidative stability while avoiding *trans*-fat generation.

Monounsaturated fatty acids, e.g., oleic acid, are fatty acids that have one double bond in the fatty acid chain with all of the remainder carbon atoms being single bonded, whereas stearic acid is one of the most common saturated fatty acids found in nature with no double bonds in its structure. Monounsaturated fat consumption has been beneficially associated with decreased low-density lipoprotein (LDL) cholesterol and possibly increased high-density lipoprotein (HDL) cholesterol. However, its ability to raise HDL is still debated.

Commodity soybean oil is composed of five major fatty acids: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3), respectively, in the percentage average of 10 %, 4 %, 18 %, 55 %, and 13 %. Oxidative instability results from the relatively high percentage of linoleic acid and linolenic acid which are polyunsaturated fatty acids. The strategy is to reduce the polyunsaturated fatty acid content (linoleic acid concentrations) and increase the concentrations of oleic acid (monounsaturated omega-9 fatty acid) and stearic acid oil. The latter is considered cardiovascular neutral. Monounsaturated fatty acids have a higher melting point than polyunsaturated fatty acids and a lower melting point than saturated fatty acids (no double bonds) and with the improved stability would be suitable for use in nut roasting, as flavor and color carriers, food

processing at high temperatures, and fried products requiring extended shelf life (Clemente and Cahoon 2009).

Another development is GM soybeans with increased levels of the ω -3 fatty acids, such as stearidonic acid (Clemente and Cahoon 2009). This is another approach to nutritional enhancement as this acid is a precursor for a number of important derivatives, not essential to the diet; however, scientific evidence indicates that these fatty acids may be beneficial in reducing cardiac diseases. Stearidonic acid is biosynthesized from alpha-linolenic acid by the enzyme delta-6 desaturase. It is converted into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) omega-3 fatty acids (Clemente and Cahoon 2009). Typically, EPA and DHA omega-3 fatty acids are contained in oily fish, such as salmon, lake trout, tuna, and herring.

In addition to reduction in the levels of polyunsaturated fats, increasing the content of vitamin E (tocopherols) also contributes to oil stability. The greatest degree of oxidative stability is conferred by delta- and gamma-tocopherol. Genetic modification of the enzymes involved in the synthesis of tocopherols shows promise to improve the tocopherol content.

The compositional assessment for GM soybean 305423 illustrates some of the aspects considered for safety and nutritional values (EFSA 2013b).

Soybean 305423 contains soybean *gm-fad2-1* gene fragments that, through RNA interference, downregulate endogenous omega-6 desaturase enzymes resulting in a changed fatty acid profile in seeds by increasing the proportion of oleic acid (C18:1) and decreasing the proportions of linoleic (C18:2) and palmitic acid (C16:0). Soybean 305423 was transformed by biolistic DNA delivery system and in addition to a changed fatty acid profile also expresses resistance to the herbicide group sulfonyleureas. Its intended use includes a variety of food and feed products.

The key constituents included in the compositional analysis of soybean seeds and forage were in accordance with OECD recommendations (OECD Biotrack online). These included proximate analysis and specific fiber fractions, amino acids, fatty acids, vitamin E, antinutrients (i.e., phytic acid, trypsin inhibitor, lectins, stachyose, and raffinose), and secondary metabolites (isoflavones). Specific fatty acids are case-by-case included in a specific food safety assessment because of the nature of the genetic modification. The nutritional assessment includes evaluation of animal feeding studies for chickens, laying hens, pigs, and rats. According to the dietary intake data submitted, full replacement of vegetable oils with oils derived from soybean 305423 would not be of concern because the changes in average intake would be small and without impact on health and nutrition. The conclusion was that the food and feed products from Soybean 305423 were as safe and nutritious as the conventional counterparts.

In general, the safety and nutrient value resulting from intended changes in the profile of fatty acids should be confirmed by several studies as described in this chapter but also assessed in accordance with the dietary intake data for a given country. If not available, then the international grouping of countries according to the World Health Organization (WHO) information (WHO Global Environment Monitoring System GEMS/Food online) should be considered.

Abiotic Stress-Tolerant Crops

Examples of abiotic stresses include drought, flooding, heat, cold, and exposure to high salt content. Water scarcity represents the most severe constraint to agriculture, and drought has been the cause of major famines in the past. Global warming could add to the detrimental conditions. Conventional breeding for drought tolerance, although with some success, is a slow process and limited by the availability of suitable genes. The development of drought-tolerant crops by genetic modification requires identification of the genetics that underlie stress tolerance as drought triggers many physiological responses in plants. These responses are complex and integrated, including leaf wilting, reduction in leaf area, leaf abscising, stimulation of root growth, directing nutrients to underground parts, and closing leaf stomata. Water balance in cells is maintained by accumulating solutes (sugars, amino acids, organic acids, and ions, e.g., potassium). Compatible solutes are synthesized and accumulate in the cytosol such as sugar alcohols, proline, and glycine betaine to enhance tolerance to drought. All these reactions to protect the plant are the results of at least four independent stress-responsive genetic regulator pathways. A better understanding of the effects of abiotic stress on plants and the critical chemical components involved in the process contribute to designing the safety assessment of the food derived from stress-tolerant crops.

Maize MON 87460 was developed to tolerate drought conditions (EFSA 2012b) through *Agrobacterium*-mediated transformation of cold-shock protein B and is the first GM abiotic stress-tolerant crop to achieve commercial introduction. Compositional and phenotypic differences between the GM plant and the conventional counterpart are to be expected when grown under water-limited and other stressful conditions. The applicants selected locations in the USA and in Chile to demonstrate the compositional profile under water conditions typical of the local agronomic practices and under well-watered and water-limited conditions. They included a large number of economically important varieties (18) in the study to allow for interpretation of observed differences within the natural variability. Greenhouse trials were included to demonstrate exposure to drought, salt, heat, and cold. A total of 77 endpoints in line with the OECD recommendation and additional stress-related biochemical maize components were analyzed. These stress-related compounds were free proline, choline, glycine betaine, fructose, glucose, glycerol, mannitol, sorbitol, and sucrose, and the plant hormones were abscisic acid and salicylic acid. No biologically significant differences were observed. The two expressed proteins tested negatively for toxicity and allergenicity, and a toxicological assessment of the whole GM plant for toxicity was not considered necessary because there was no biologically relevant difference in the composition, agronomic, and phenotypic analysis. Allergenicity of the whole plant was not considered, because the compositional analysis of stress-related compounds in grains did not show significant changes that would suggest alterations in expression of stress-related allergenic proteins, nor did the bioinformatics analysis of the DNA sequence at the insertion sites show evidence of significant changes in the overall allergenicity of the GM maize. The wholesomeness study with chickens showed no difference in biological relevance.

Composition of Food from GM Microorganisms

EFSA has developed guidelines for the assessment of genetically modified microorganisms (GMMs) including Archaea, Bacteria, and Eukarya (filamentous fungi, yeasts, protozoa, microalgae) and only the final products from fermentation of GMMs. The information in this chapter is taken primarily from the EFSA guidance document (EFSA 2011c). This document covers the requirements according to various European directives. The principles and strategies for risk assessment in general are according to Codex (CAC 2009, pp. 35–55), which requires an assessment on a case-by-case basis, identifying and evaluating potential adverse effects and applying the comparative approach by considering closely related microorganisms or their products with a history of safe use. Categorization of the GMMs and their products, because of the diversity of GMMs, is proposed to optimize the risk assessment:

Category 1: Chemically defined purified compounds and their mixtures in which both GMMs and newly introduced genes have been removed (e.g., amino acids, vitamins).

Category 2: Complex products in which GMMs and newly introduced genes are no longer present (e.g., cell extracts, most enzyme preparations).

Category 3: Products derived from GMMs in which GMMs capable of multiplication or of transferring genes are not present but in which newly introduced genes are still present (e.g., heat-inactivated starter cultures).

Category 4: Products consisting of or containing GMMs capable of multiplication or of transferring genes (e.g., live starter cultures for fermented foods and feed).

All relevant information on the donor organism(s), genetic modification, host organism, and the GMM is important. The compositional approach requires that a comparator with a history of safe use should be considered. Codex (2009) defines it as a “microorganism/strain with a known history of safe use in producing and/or processing in food and related to the r-DNA strain; or food produced using the traditional food production microorganisms or which there is experience of establishing safety based on common use in food production.” The concept of “qualified presumption of safety” (QPS) has been developed, which has a similar meaning to GRAS. Justification for the use of a specific microbial strain is always necessary. Depending on the category and use, qualitative and, when appropriate, quantitative composition of the GMM and/or its product should be provided. Intended and unintended changes are analyzed according to the most appropriate methodology. Toxicological and allergenicity assessments are indicated case-by-case for the expressed proteins and the food products from the GMM. A nutritional assessment is relevant only to products from Category 3 and 4 and in some cases Category 2 (EFSA 2011c).

As an example, a genetically modified wine yeast, *Saccharomyces cerevisiae* ML01, which is a modified malolactic yeast, for use in winemaking and of wines derived from the use of this yeast was approved by the Canadian health authorities

in 2006. The purpose was to remove malic acid from wines without the use of starter cultures. Starter cultures introduce biogenic amines with undesirable health effects into wines. The assessment of the wine from GM yeast included a number of issues that illustrate the case-by-case assessment approach. A comparative analysis constituted the basis of the assessment. The chemical composition of Merlot wine produced from GM yeast was compared with two Merlot wines produced by different methods. With the exception of ethyl lactate, the concentrations of the other components were of similar magnitude. However, higher levels of ethyl lactate in wines from ML10 were not unexpected since the bacterial malolactic fermentation was incomplete. The presence of protein products from the genes that encode for the two novel enzymes was considered. The concentration of a chemical toxic substance ethyl carbamate in ML01 fermented wine compared favorably with other alcoholic fermented wines and was within the limits set by the Canadian guidelines ([Health Canada online](#)).

Composition of Food from GM Animals

Reference should be made to ► [Chap. 16, “Chemical Composition of Meat and Meat Products.”](#)

A distinction should be made between cloned and GM animals. Both terms relate to biotechnology techniques. Cloning is a reproduction technique for multiplication of genetically “identical” animals. For the purpose of this chapter, a GM (r-DNA or transgenic) animal is defined as an animal in which genetic material has been altered in a heritable way through techniques of genetic modification (EFSA 2012a, CAC 2009 p.59). GM animals produced through genetic modification could be (a) via germ-line modification to develop animals with enhanced performance for agronomic and/or economic purposes (e.g., more efficient/increased production of food/feed of animal origin, enhanced nutritional value, lower emissions to the environment, improved health of animals) or (b) by somatic cell modification for medical research (e.g., production of vaccines) or to produce, for example, substances in milk, egg, or blood. Animals fed on feed or food produced by genetic modification are not considered GM animals. Codex Alimentarius guidelines (CAC 2009, pp. 57–76) and the EFSA document on food and feed from GM animals (EFSA 2012a) are relevant for the assessment of food and feed from GM animals. An OECD consensus guideline for composition and nutritional comparison has not yet been prepared.

The comparative approach to risk assessment for food and feed from GM animals follows, in general, that for GM plants. Similarities and differences between the GM animal-derived food/feed and its non-GM comparator(s) are identified. The outcome of this analysis would further structure the safety and risk assessment.

In animals the concept of “history of safe use” to describe the comparator and the health and welfare status of the food and feed producing non-GM animal is important. This would entail a comprehensive comparative analysis of the

phenotypic characteristics, including health and physiological parameters for the organism in question. The requirements for comparators for animal species would obviously differ among husbandry animals, fish, crustaceans, and mollusks. The European guideline excludes insects and other invertebrates except for honey bees that are used in agricultural practices. The safety assessment protocol should include instructions for controlling animal hygiene procedures and quality control during the production phase for various animal species (e.g., lactating cows, laying hens).

With the criteria for a comparator in mind, the focus turns to the food and feed safety assessment and nutritional impact. A comparison is conducted between the GM animals and the non-GM animals, and intended and unintended differences are identified and characterized. Where no comparator animal is possible, a comprehensive safety and nutritional assessment is conducted with the GM animal. The following steps, after qualitative and/or quantitative evaluation to characterize the hazard, are to estimate the likely exposure of humans and animals and then to characterize the risk which includes uncertainties, probability of occurrence, and severity of known or potential adverse health effects in a given population.

Analysis of the key components consists of identifying those components that are relevant to animal tissues, organs, fluids, and/or derived products, taking into account the uses of the food and feed and the nature of the genetic modification. The potential toxicity, bioactivity, and allergenicity of the gene products (expressed proteins) and the metabolites of GM-derived food and feed should be included in the safety assessment. Analysis is carried out on the unprocessed animal material which is the entry point of material into the food chain for production and processing. The effect of processing on the characteristics of the food/feed is also important. Samples are taken at different stages in the productive life of the animal. Materials to be used in the analysis (EFSA 2012a) are:

- (a) Animal body:
 - Mammal tissues: meat (muscle), body fat, blood, organs (kidney, liver, spleen), and residue (bone meal)
 - Bird tissues: meat (breast and thigh muscle), abdominal fat, blood, organs (kidney, liver, spleen), and residue body (bone meal)
 - Aquaculture: tissues (edible fraction e.g., fillet) and residues, e.g., fish meal
- (b) Samples for food/feed produced by GM animals:
 - Mammals (milk), birds (eggs), aquaculture (eggs), and insects (honey from bees)

The proposed range of chemical components, selected case-by-case, depending on the type of GM-derived food to be assessed, includes macro- and micronutrients (proteins, carbohydrates, lipids/fats, vitamins, minerals), bioactive compounds (hormones, growth factors), key allergens, and antinutritional and toxic compounds. In animal feeds, the compounds for analysis depend on the target species. More detailed information may be required such as a profiling of fatty acids where the genetic modification focuses on a changed or improved fatty acid profile.

Atlantic Salmon

Safety assessment of GM Atlantic salmon follows the steps described above for GM animals. An example of germ-line modification is the first transgenic fish, AquAdvantage salmon. This GM fish is in the regulatory approval process of the US FDA. It is a fast-growing fish that reaches maturity within 6 months, compared with the 3 years of the non-GM-farmed Atlantic salmon. It was developed by microinjecting an r-DNA construct, containing a protein-coding sequence from a Chinook salmon growth hormone gene, into the fertilized eggs of triploid monosex females of wild Atlantic salmon (*Salmo salar*). The studies included comparison with diploid male and female as well as triploid female salmon of the same lineage.

These issues were considered by the US FDA (US FDA AquAdvantage Salmon online):

- (a) To conduct an analysis of the composition, it was confirmed that AquAdvantage salmon was an Atlantic salmon. This was done empirically by laboratory testing and referencing to the U.S. FDA Regulatory Fish Encyclopedia.
- (b) The direct hazard was identified as relating to the introduced construct and changes in endogenous substances resulting from the growth hormone expression. Several studies were conducted to investigate levels of hormones in salmon tissues: insulin growth factor (IGF1), estradiol, testosterone, 11-ketotestosterone and thyroxin (T4), and triiodothyronine (T3) as growth hormone enhances the conversion of T4 to T3. The only difference was an increase in IGF1 concentration, but an exposure assessment confirmed that there was no additional risk to human beings. Potential allergenicity was considered for the newly expressed protein and endogenous allergens. The conclusion was that no new allergenicity risk was envisaged.
- (c) Indirect food consumption hazards were investigated for changes in concentration levels of key nutrients compared with those of the comparator groups. The studies included several groups of non-GM salmon with different genetic background as well as the “near isoline” or the sponsor control. The fish were screened visually for general health status and for traits relevant to commercial marketability (skin, fin conditions, color, marking, body morphology, genotype, and ploidy analysis). Ploidy refers to the number of sets of chromosomes in the nucleus of a cell. Polyploidy (triploid and tetraploid chromosomes) occurs in some animals such as salmon. The compositional analysis included proximates, mineral, vitamin, amino acid, and fatty acid content. Statistical analysis showed all levels of components were similar between the GM salmon and the control groups except for vitamin B₆ which was higher in the GM salmon. A margin of exposure was regarded as increased vitamin B₆ levels, but this was not considered to be a food consumption hazard. Total fat content, fatty acids, and levels of omega-3 and omega-6 fatty acids were acceptable within the range of the controls, and the ratio between omega-3 and omega-6 fatty acids was comparable with the controls. Allergenicity from endogenous

allergens in the GM Atlantic salmon raised concern because of insufficient data and information to draw conclusions for the diploid salmon though not in the triploid salmon. This was regarded as an “uncertainty” in the risk assessment, and the recommendation was that another study on the allergenicity of the diploid fish should be submitted.

Livestock

Hernandez-Gifford and Gifford (2013) reported on a number of successful transgenic livestock, namely, pigs, sheep, goats, and cattle. The purpose of the genetic modifications were increased growth rate with less body fat, increased polyunsaturated fatty acids, increased milk production, altered milk fat composition, wool growth, and disease resistance. No international consensus has as yet been reached on the individual chemical food components that are recommended for regulatory purposes by governments.

Mention should be made of milk containing the recombinant bovine somatotropin (r-BST) protein with the purpose to increase milk production in animals. Although they are not GM animals, the safety of milk from cows treated with this drug has been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1998 (WHO 1998) and concluded to be safe. The history of the development of regulation in Europe provides the explanation for the rejection of the use in livestock in Europe (Brinckman 2000).

Stacked Traits of Increasing Complexity

Stacking or pyramiding genes by genetic engineering in a single plant which then contains at least two novel traits is a process that is fast gaining importance. Apart from the convenience of a number of traits being combined in a single crop, this is a strategy to reduce or prevent possible weed or insect resistance to specific agricultural remedies.

Stacking is a process carried out either by (a) conventional plant breeding, whereby individual GM plant lines, each expressing one or more traits, are cross fertilized, resulting in a new generation of plants expressing the traits of both the parent lines, or (b) genetic modification by direct introduction of multiple novel genes simultaneously or consecutively into a single plant line. Various methods are described, namely, retransformation, co-transformation, and transformation cassettes containing multiple genes (EFSA 2011a).

First, a risk assessment of the GM plant containing the events independently would be required. Second, consideration would be necessary to ensure that the combination of events (stacked events) is stably inserted and that there are no interactions, synergistic or antagonistic, between the stacked events that may raise safety concerns.

Requirements for compositional assessment vary. While some countries require compositional assessment to be conducted in all new applications for registration of the GMO crops, others, such as Australia, (Australia and New Zealand Food Standards (FSANZ) Food derived from GM plants containing stacked genes online) do not require separate regulatory approval for stacked events in (a) but only assessment as in (b) above. The justification for the first approach is that conventional stacking of GM traits is unlikely to pose new or additional food safety risks. The motivation is that phenotypic assessment, which is standard practice in all plant breeding, is of cardinal importance.

The papers prepared by ILSI concerning genetically engineered stacks are of significance (Weber et al. 2012; Steiner et al. 2013). Studies on genomic plasticity resulted in the conclusion by scientists that the dynamic nature of the genome is a given background and that natural and conventional stacking of genes has occurred over hundreds of years. Major disruption of genomic plasticity is observed normally in plant breeding practices, resulting in the elimination of off-types.

Conclusion and Future Direction

The chemical composition of food from genetically modified organisms is one of the most important considerations for safety and nutritional assessment of products from genetic modification. The cautious approach proposed by international bodies, and in particular Codex Alimentarius and the OECD, has paved the way for many new products that are considered safe. With new technologies in the pipeline, the role of international organizations and the active participation of governments and scientists in the development of approaches for safety and risk assessment are important to ensure safe and nutritious food.

Cross-References

- ▶ [Chemical Composition of Fat and Oil Products](#)
- ▶ [Chemical Composition of Meat and Meat Products](#)
- ▶ [Plant-Associated Natural Food Toxins](#)

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Abstract

Meat is the flesh of certain animal species that is used as food by humans and includes many tissues and edible parts although the main tissue is the muscle. This chapter describes the muscle composition and the conversion of muscle into meat, the meat components (nitrogen compounds and enzymes, lipids, carbohydrates, vitamins, minerals, and water), their influence in meat quality, and the factors that affect meat composition. Mechanically recovered meat composition is also outlined, due to it is a raw material used in meat product manufacture. The effects of curing and the chemical characteristics of the different varieties of meat products are also detailed, including enhanced meat, comminuted and reformed fresh meat products, cooked-cured meat products (emulsion-based and products from whole pieces), dry fermented sausages, dry-cured meat products, frozen meat and meat products, dried meats, and meat extracts. Finally, chemical aspects of the most recent type of meat products developed, healthier and functional meat products, have also been included (low-sodium, low-fat and lipid-modified and nitrite-reduced meat products, meat and meat products with bioactive compounds).

Introduction

Definition

Meat is generally defined as the flesh of certain animals used as food that is often widened to several edible tissues (mainly muscular tissue, but also associated adipose and connective tissues) and edible organs (Lawrie 1998). According to European Union legislation, meat means the edible parts of animals, including blood and viscera. These animals are domestic ungulates (bovine, porcine, ovine, and caprine animals) and domestic solipeds, poultry (domestic and not domestic birds, excluding ratites), lagomorphs (rabbits, hares, rodents), small and large wild game (land mammals and birds), and farmed game ratites (European Commission 2004). However, many other animal species are eaten in the world: antelopes, elephants, giraffes, etc. in Africa, marsupials (kangaroo) in Australia, dogs and felines in several Asian countries, and marine mammals in the Arctic region or Japan (Lawrie 1998). This chapter is mainly focused on meat from animal's carcass; it consists of muscle, fat, and bone, the muscle being the most abundant part. Fat deposits are also consumed together with muscle and are included in the broader definition of meat (Kauffman 2012).

Muscle Composition and Conversion of Muscle into Meat

Although meat is usually used as a wide term that includes many tissues and edible parts of animals, the main edible tissue is the muscle, and many aspects of meat composition and its properties are determined by the characteristics of muscles.

Muscle cells, called muscle fibers, are long, narrow, very specialized cells covered by the cell membrane (sarcolemma), whose cytoplasm (sarcoplasm) contains the organelles and the structures responsible of muscle contraction, the myofibrils. Myofibrils are cylindrical structures made up of repeated units known as sarcomeres that cause the striated appearance of muscle when meat is observed by electron microscopy. Striations result from the complex organization of the myofibrillar proteins, responsible for the muscle contraction. The striations originate from alternating dense bands (A-bands) and less dense I-bands; the latter are divided in two parts by dark lines known as Z-lines. The space between two Z-lines is a sarcomere (Huff Lonergan et al. 2010).

Muscle fibers also contain proteins in the sarcoplasm (sarcoplasmic proteins) and enzymes in different cell structures or organelles (Lawrie 1998; Devine and Chrystall 2000; Huff Lonergan et al. 2010). Connective tissue structures define the organization of muscle. Muscle fibers are individually surrounded by a thin connective tissue sheath (the endomysium) and are bundled into groups or fascicles by connective tissue septa, the perimysium, associated to blood vessels and nerves; the muscle is completely surrounded by the epimysium (Lawrie 1998; Devine and Chrystall 2000). The types and characteristics of muscle fiber and connective tissue proteins are described in the next sections.

According to their color and metabolism, muscle fibers can be classified in three types: red, white, and intermediate. Red fibers have a high content of myoglobin and mitochondria, are highly irrigated, and possess oxidative metabolism; they are abundant in red muscles, which are related to locomotion. White fibers contain low concentration of myoglobin and their metabolism is glycolytic; the proportion of white fibers is higher in white muscles, involved in support tasks. Intermediate fibers display intermediate characteristics (Toldrá and Reig 2012).

As muscle is converted to meat, the muscle fibers modify their metabolism in order to adapt to the new conditions (absence of oxygen and nutrient supply, lack of residues removing), and as a consequence, many changes occur. The available energy is gradually depleted and the metabolism displaces from aerobic to anaerobic, less efficient in energy [adenosine triphosphate (ATP)] generation, favoring lactic acid production. Its accumulation results in the decrease of tissue pH from values near neutrality to 5.4–5.8. The ionic strength rises, partially due to the inability of ATP-dependent calcium, potassium, and sodium pumps to function. The fibers become less capable of maintaining reducing conditions. All of these alterations have deep effects on muscle proteins and also on proteolytic enzyme systems (Huff Lonergan et al. 2010). Rigor mortis occurs when the level of ATP declines and its concentration is too low to maintain muscle relaxation. After a

variable period of time, the resolution of rigor mortis happens, with a progressive softening of the muscles due to the action of proteolytic enzymes (Warriss 2000).

Meat Components

Meat is composed of approximately 72–75 % water, 21 % nitrogenous compounds (19 % proteins and 1.5 % nonprotein nitrogen compounds which include nucleotides, peptides, creatine, and creatinine), 2.5–5 % lipids, 1 % non-nitrogenous compounds (vitamins) and carbohydrates (a very small amount of glycogen, transformed into lactic acid during postmortem period), and 1 % ash (potassium, phosphorus, sodium, chlorine, magnesium, calcium, and iron). The most variable compounds are lipids, with values that can vary between 1 % and 15 % (Keeton and Eddy 2004; Kauffman 2012). Meat composition is variable due to the influence of several factors: animal species, breed, sex, feeding, muscle, etc. Their influence is described in section “[Factors that Influence Meat Composition.](#)”

Proteins and Other Nitrogen Compounds

Meat proteins can be divided into three classes: myofibrillar (responsible for contraction-relaxation of muscle and soluble in concentrated salt solutions), sarcoplasmic (metabolic and soluble in water or diluted salt solutions), and connective tissue (support and insoluble) proteins (Tornberg 2005). Location, function, and proportion of main myofibrillar and sarcoplasmic muscle-meat proteins are shown in Table 1.

Myofibrillar Proteins

The most abundant myofibrillar protein is myosin; thick filament is made of hundreds of molecules of this protein. Myosin molecule is an oligomer of 1,500 Å in length and a molecular weight (MW) of about 500,000 and consists of two identical units with a pair of identical heavy chains with a MW of 200,000 each and two pairs of nonidentical light chains of 16,000 and 20,000. These protein chains form a long tail (double-stranded α -helical rod), a collar (a region between two flexible regions), and two globular heads. These heads have enzymatic activity (ATPase): they can hydrolyze ATP during muscle contraction and provide energy for myosin bound to actin to swivel and pull the thin filaments toward the center of the sarcomere (Huff Lonergan et al. 2010). Trypsin digestion of myosin yields the heavy (MW about 340,000) and light (MW 150,000) meromyosin fragments that correspond to the globular head and to the helical rod, respectively. Heavy meromyosin digestion with papain releases two fragments, S-1, with ATPase activity, and S-2, with fibrous structure, without ATPase activity and unable to bind to F-actin (Lawrie 1998; Murray 2003; Kerth 2013a). Myosin possesses high contents of glutamic and aspartic acids and of dibasic amino acids that make it a highly charged molecule with affinity for calcium and magnesium ions (Lawrie 1998).

Table 1 Location, function, and proportion of main myofibrillar and sarcoplasmic muscle-meat proteins (Lawrie 1998; Calkins and Killinger 2003; Keeton and Eddy 2004)

Protein	Location	Function	Proportion (%)
Total protein			19
Myofibrillar			11.5^a
<i>Major contractile proteins</i>			
Myosin	Thick filaments	Contraction	43 ^b
Actin	Thick filaments	Contraction	22
<i>Regulatory proteins</i>			
Tropomyosin	Thin filaments	Regulates contraction	5
Troponin	Thin filaments	Regulates contraction	5
α -Actinin	Z-line	Join thin filaments of different sarcomeres in the Z-line	2
<i>Cytoskeletal proteins</i>			
Connectin or titin	From M-line to Z-line	Protect sarcomere from overstretch	8
Nebulin	Thin filaments	Controls thin filament length	3
C protein	Thick filaments	Inhibits skeletal muscle ATPase	2
M protein	M-line	Transverse portion of the M-line	2
Desmin	Z-line periphery	Transverse link of myofibrils at Z-line	<1
Other myofibrillar proteins			7
Sarcoplasmic			5.5^a
Myoglobin	Sarcoplasm	Meat pigment	0.2 – 36 ^c
Glyceraldehyde phosphate dehydrogenase	Sarcoplasm	Metabolic enzyme	22
Aldolase	Sarcoplasm	Metabolic enzyme	11
Enolase	Sarcoplasm	Metabolic enzyme	9
Lactate dehydrogenase	Sarcoplasm	Metabolic enzyme	7
Pyruvate kinase	Sarcoplasm	Metabolic enzyme	5
Phosphorylase	Sarcoplasm	Metabolic enzyme	4.5

^a% of total protein^b% of myofibrillar protein^c% of sarcoplasmic protein

Actin is the main structural component of the thin filament; each thin filament is composed of two actin filaments turned around themselves, to form a helical strand. Fibrous actin (F-actin) is composed of two chains of globular monomers (G-actin) that consists of 375 amino acids and with a MW of 43,000–47,000; in the presence of Mg^{2+} , G-actin polymerizes noncovalently to form F-actin. Each monomer also possesses a region for binding myosin (Murray 2003; Kerth 2013b).

The propound mechanism for muscle contraction involves the use of the energy from ATP hydrolysis for activating conformational changes in myosin head. The myosin head binds actin and forms an actomyosin complex. The ATPase activity of myosin head hydrolyzes ATP producing adenosine diphosphate (ADP) and inorganic phosphorus (Pi), and myosin head suffers a conformational change when ADP leaves it. That provokes the stroke of muscle contraction, pulling the thin filaments toward the center of the sarcomere. This shortens the myofibril and, at a greater scale, the muscle fiber and the complete muscle. Another ATP molecule is necessary for actomyosin dissociation during relaxation causing the myosin head to tilt back to its initial position (Murray 2003; Kerth 2013b).

In rigor mortis development, the proteins that constitute the thick and thin filaments, myosin and actin, combine irreversibly to form actomyosin due to the lack of available ATP, and the muscle remains shrunk permanently. In rigor mortis resolution, the myofibrils are fragmented by proteolytic enzymes, and the breakdown of the attachments of thin filaments to the Z-line is observed; however, the thin and thick filaments remain joined. Besides, the intramuscular connective tissue weakens due to some cleave of collagen cross-links (Warriss 2000; Huff Lonergan et al. 2010).

Troponin and tropomyosin are two regulatory proteins that play important role in muscle contraction and relaxation and can also be found on the thin filament. Tropomyosin is a long, fibrous protein with two strands with α -helix structure wound around one another and a molecular mass of about 66,000. Tropomyosin covers the myosin binding sites on F-actin when the muscle is in a relaxed state. Troponin consists of three polypeptides or subunits, T, I, and C, with specific functions in muscle contraction: troponin T attaches tropomyosin; troponin I inhibits F-actin-myosin interaction; troponin C is a calcium-binding polypeptide. Both troponin T and I join the three components (Murray 2003; Kerth 2013a).

The myofilaments are maintained in their positions by a cytoskeleton composed of several proteins. Titin, nebulin, and desmin are proteins located in the Z-line region (between either the thick filament and the z-disk or the thin filament and the M-line) that serve as bridges between the thick and thin filaments of adjacent sarcomeres (Toldrá and Reig 2012; Kerth 2013a). Other elements contribute to the structure and functioning of sarcomeres and muscles, the intermediate and the costameric filaments. The intermediate filaments run perpendicular to the length of muscle fiber, keeping the three-dimensional shape of contractile structure. These proteins are, among others, skelemin, desmin, paranemin, and synemin, and their degradation during postmortem aging can play an important role in tenderness development. The costameric filaments keep the transversal structure organization of muscle fibers, interacting directly with the cell wall and probably also with the endomysium layer outside the sarcolemma. Filamin, dystrophin, talin, and vinculin are proteins of this group and seem to have a role in postmortem degradation of muscles and in the development of meat tenderness (Kerth 2013a).

During postmortem aging, the oxidation of myofibrillar proteins occurs, in which some amino acid residues, including histidine, are converted into carbonyl derivatives and can cause the formation of intra- and inter-protein disulfide cross-

links. The reactions affect peptide backbone and functional groups in the side chain of amino acid residues (cysteine, methionine, tryptophan, lysine, threonine, arginine, proline). Several factors influence the rate of postmortem oxidation: antemortem factors (breed, diet, rearing system, antemortem stress), type of muscle, and handling of carcasses. These changes produce protein denaturation and loss of protein functionality, affecting water holding capacity, texture, flavor, and nutritional value.

Potential initiators of protein oxidation include radical (superoxide, hydroperoxyl, hydroxyl) and non-radical (hydrogen peroxide, hydroperoxides) species. Natural muscle tissue components such as unsaturated fatty acids, heme pigments, metals, and oxidative enzymes are potential precursors or can catalyze the formation of reactive oxygen species during the conversion of muscle to meat and meat aging. Acidification and chilling storage enhance oxidation; high H^+ concentrations favor prooxidant potential of myoglobin, and acidity affects protein susceptibility to oxidation due to its effects on protein structure and aggregation. Besides, alteration of cellular compartmentalization, release of free catalytic iron and oxidizing enzymes, and lipid oxidation reactions promote the formation of protein carbonyls (Estevez 2011).

Sarcoplasmic Proteins

Sarcoplasmic proteins are found in the sarcoplasm and include oxidative enzymes, heme pigments (mainly myoglobin), mitochondrial oxidative enzymes, lysosomal enzymes, and nucleoproteins. Sarcoplasmic proteins are good emulsifiers, but less effective than myofibrillar proteins (Keeton and Eddy 2004). Myoglobin (MW 16,000) is the main sarcoplasmic protein and is responsible for the color of meat. It is a globular protein, globin, consisting of 153 amino acids, bound to the iron atom of a prosthetic heme group (iron(II) protoporphyrin-IX complex) by a histidyl residue of the protein. The heme iron atom can exist in the ferrous (+2) or the ferric (+3) state which is determined by the presence of oxidants and reductants in the medium. Myoglobin (Mb) concentration varies depending on the species, breed, sex, age, type of muscle, exercise, and nutrition. Myoglobin supplies oxygen to the muscle and is responsible for color in meat products, binding oxygen, water, or nitric oxide in the sixth coordination site on the iron molecule. The bright-red color of fresh raw meat surface is due to the great affinity of Mb for oxygen that forms oxymyoglobin; this reaction is rapid and reversible. The interior of meat is purple-red in color due to the pigment called deoxy-Mb that persists as long as reductant compounds are available. This pigment is responsible for the color of vacuum-packed meat. When the reductant concentration is depleted, or the oxygen pressure is low, the heme iron is oxidized to the ferric state, unable to bind oxygen, forming the brown pigment metmyoglobin. In raw fresh meat, these pigments are constantly interconverted (Lawrie 1998; Keeton and Eddy 2004).

Connective Tissue Proteins

The main proteins in connective tissues are collagen and elastin. Collagen is the most abundant protein and includes several types of polypeptide chains. Type I

collagen is the major component of epimysium and perimysium, while types III, IV, and V collagen are in the endomysium. Collagen fibers are formed by long tropocollagen molecules. Each tropocollagen molecule consists of three polypeptide chains twisted together into a coiled triple α helix. The polypeptide chains have the repeating sequence [-glycine-proline-hydroxyproline-glycine-(one of the other amino acids). . .]. Proline and hydroxyproline are approximately 25 % and glycine 33 % of the total amino acid residues. The N-terminals, non-helical regions of the α chains, are forming intermolecular cross-links that are important in collagen resistance in living animals and also in meat toughness. Cross-links are covalent bonds in which lysine and hydroxylysine are involved; the enzyme lysyl oxidase catalyzes the oxidative deamination giving aldehyde groups that condense with those from other lysine residues. The cross-links increase with age and augment collagen insolubility and hardness of matured animal meat (Lawrie 1998; Warriss 2000). Under heating (60–65 °C) in moist conditions, collagen swells, its triple helix structure is destroyed to a great extent and transforms into random coils; then, it is called gelatin, becomes soluble, and can retain and immobilize high amounts of water and form gels after rapid cooling to 4 °C (Keeton and Eddy 2004; Belitz et al. 2009).

Elastin can be found in lower amounts and is located in capillaries, ligaments, tendons, and nerves. This protein contains about 40 % glycine, 40 % hydrophobic amino acids (18 % valine), and small amounts of proline and hydroxyproline and suffers cross-linking by lysyl oxidase action. Elastin becomes more insoluble with the increase of animal age (Lawrie 1998; Toldrá and Reig 2012).

Enzymes

Many enzymes can be found in the muscle and most of them are peptidases: calpains, cathepsins, proteasome, tri- and dipeptidylpeptidases, amino- and carboxypeptidases, and dipeptidases. Other enzymes are lipases, glycohydrolase, nucleotidases, etc. Peptidases are important in meat tenderness and texture and flavor development in meat products and can be divided into two groups: endopeptidases, endoproteases, or proteinases (calpains, cathepsins, and proteasome), when they hydrolyze internal peptide bonds, and exopeptidases, when they hydrolyze external peptide bonds at the carboxy or the amino termini of the protein/peptide chain.

Calpains are a group of Ca^{2+} -dependent cysteine endopeptidases (110 KDa) which show maximal activity at neutral pH values (pH 7.5) and become ineffective activity at pH 5.5. The most important isoforms are calpain 1 or μ -calpain and calpain 2 or m -calpain. Calpain 1 requires micromolar calcium concentrations (10–50 μM) for activity and has low stability in postmortem muscle. Calpain 2 needs millimolar Ca^{2+} concentrations (0.3–1.0 mM) for full activity and seems to be stable for a few weeks postmortem, up to 56 days. Their activity is regulated by an endogenous inhibitor, calpastatin, that disappears few days after slaughter by autolysis. Another isoform, p94/calpain 3 isoform, is active even without calcium ions in the medium; its role in the muscle and in postmortem aging is not well known. Calpains can degrade a wide variety of myofibrillar proteins responsible for

the fiber structure (titin, troponins T and I, tropomyosin, C protein, filamin, desmin, and vinculin), but they are inactive against myosin and actin. These proteases are considered as the predominant enzymes that cause postmortem proteolysis and meat tenderization. Calpains are particularly susceptible to inactivation by oxidation, although this might not completely inhibit proteolysis.

Cathepsins are a group of over 15 lysosomal small proteinases (20–40 KDa) that are distinguished by their active sites and substrate specificity. Main cathepsins are cathepsins B and L (cysteine proteinases, very active at pH 6.0), cathepsin H (endo- and exopeptidase, optimal pH at 6.8), and cathepsin D (aspartate proteinase, optimal activity at pH 3.0–5.0). Sometimes, cathepsins are not considered as important in meat tenderization because of their location into lysosomes of muscle cells; however, lysosome membranes can rupture during postmortem period due to the decreasing temperature and pH, and cathepsins B, D, H, and L have activity during postmortem aging and also during meat product manufacture. These proteinases can degrade different myofibrillar proteins (myosin, actin, tropomyosin, troponins, titin, and α -actinin), some collagen cross-links, and mucopolysaccharides of the connective tissue.

Proteasome is a large multicatalytic protease with chymotrypsin- and trypsin-like activities and peptidyl-glutamyl hydrolyzing activities at neutral pH values. It degrades myofibrils affecting M- and Z-lines and could play a role on tenderness in some muscles.

There are several types of exopeptidases, located in the lysosomes or in the cytosol, that release small peptides and amino acids important for meat taste. Tripeptidylpeptidases are enzymes that hydrolyze tripeptides from the amino termini of peptides; dipeptidylpeptidases are able to hydrolyze the amino termini of dipeptides. Aminopeptidases are neutral or basic metalloproteases of high molecular mass that release amino acids from the amino termini of peptides, while carboxypeptidases, located in lysosomes, generate amino acids from the carboxy termini of peptides and proteins (Warriss 2000; Huff Lonergan et al. 2010; Toldrá 2012).

Lipolytic enzymes degrade lipids with different modes of action and substrates. Lipases are situated in lysosomes and in cytosol and are located in the skeletal muscle and in the adipose tissue. In the skeletal muscle, lysosomal acid lipase and acid phospholipase release long-chain free fatty acids from triacylglycerols (at positions 1 or 3) and phospholipids (at position 1), respectively, and are active at acid pH (4.5–5.5). Phospholipase A and lysophospholipase are basic lipases that release fatty acids from phospholipids at positions 1 and 2, respectively, and are more active in oxidative muscles. Acid and neutral esterases hydrolyze short-chain fatty acids from tri-, di-, and monoacylglycerols.

In adipose tissue, the most important lipase is hormone-sensitive lipase that hydrolyzes long chain diacylglycerols and triacylglycerols, with specificity for positions 1 and 3. Other adipose tissue lipases are lipoprotein lipase and monoacylglycerol lipase; this last enzyme produces glycerol and free fatty acids as end products (Toldrá 2012).

Meat also contains lipoxygenase, an enzyme that catalyzes the incorporation of molecular oxygen into PUFA, mainly arachidonic acid; the final product of the

reaction is a conjugated hydroperoxide. It remains active and stable during frozen storage of meat and develops rancidity in frozen chicken (Toldrá 2012).

Nonprotein Nitrogen Compounds and Other Minor Constituents

Free amino acids are present in muscle (0.1–0.3 %) in part due to the action of muscle aminopeptidases and are more abundant in oxidative than in glycolytic muscles. The most predominant amino acids are taurine (0.02–0.1 %), alanine, and glutamic acid (0.01–0.05 %). Their content increases during postmortem storage of meat (Belitz et al. 2009; Toldrá and Reig 2012).

Meat contains in variable amounts three natural dipeptides that develop physiological functions in the muscle (buffers that maintain the muscles in the physiological range, antioxidants, neurotransmitters, etc.): carnosine (β -alanyl-L-histidine), anserine (β -alanyl-L-1-methylhistidine), and balenine (β -alanyl-L-3-methylhistidine). Carnosine and anserine are present in all species (0.01–0.3 %); beef and pork have a higher content of carnosine than anserine, lamb has similar concentrations of both, and poultry is rich in anserine. Balenine is more abundant in whales' muscles, shows minor amounts in pork muscle, and is very low in other animals. In whales this may be an adaptation to the anaerobic metabolism and the improvement of lactic acid concentration during diving (Warriss 2000; Toldrá and Reig 2012).

Minor constituents of meat are amines, guanidine compounds (creatine and creatinine), quaternary ammonium compounds (choline and carnitine), and nucleotides (Belitz et al. 2009).

Lipids

Lipids in meat animals are commonly classified as depot fats and intramuscular lipids. Depot fat is localized as subcutaneous fat, between muscles as intermuscular fat and in the body cavity around kidneys, heart, and pelvic regions (Rhee 1992). Most of the fat in the body are localized in these deposits. The proportion of the total body fat in each fat depot varies between species (Warriss 2000). Triglycerides (triacylglycerols) are the main lipid component (>90 %) of these adipose tissues (Wood et al. 2008). Low quantities of other components such as diglycerides, monoglycerides, free fatty acids, fat-soluble vitamins, and cholesterol esters can be also found in depot fat.

Intramuscular lipids represent a low percentage of total lipids of the body. For example, in mature pigs, about 15 % of the extractable lipids are in intramuscular fat. Muscles contain around 5 % lipids. However, this content is very variable, ranging from 1 % to 15 % (Kauffman 2012). Lipids are necessary to increase flavor, juiciness, tenderness, and visual characteristics of meat. Meat with very low levels of lipids has poor organoleptic evaluation by the consumers. However, high contents of lipids in meat that are visible by the consumers are not appreciated because the meat fat is related to the incidence of some diseases (cardiovascular disease, obesity, and cancer).

Intramuscular lipids are composed of deposit and structural lipids. Although some lipids are found inside of the muscle cells, deposit lipids in muscles are mainly localized in intramuscular adipocytes (Cobos et al. 1994). This depot is commonly called marbling. As in other depot fats, triglycerides are the main component and there are small amounts of other substances such as diglycerides, monoglycerides, free fatty acids, fat-soluble vitamins, and cholesterol esters.

Structural lipids are in membranes of muscle and intramuscular adipocytes, being phospholipids and cholesterol the main constituents. The amount of total phospholipids in the muscle stays relatively unchanged (3.5–6.0 mg/g); however, the proportion of phospholipids in relation to total lipids can change due to the increase in the amount of triglycerides. The relative amount of phospholipids can range from 10 % to 50 %. Depending on the type of meat, age of animals, and other potential factors such as diet, there are large variations in total lipids and phospholipids. The most common phospholipids in muscle tissue are phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin with around 40 %, 15 %, 10 %, and 5 % of total phospholipids, respectively (Willian 2013). Lower proportion of other phospholipids as plasmalogens and other polar lipids as cerebrosides can be found in muscle membranes.

Cholesterol content of meat is around 50–70 mg/100 g and this amount is partly independent of the fat content (Leth and Ertbjerg 2004). Cholesterol content of adipose tissues is around 115 mg/ 100 g tissue (Smith et al. 2004). Cholesterol in meat exists as free cholesterol and as cholesterol ester. Free cholesterol is associated primarily with cellular and subcellular membranes of muscle and intramuscular adipocytes. Cholesterol ester is located with the triglycerides in adipose tissue. Muscle fibers, which are rich in membranes and poor in lipids, have around 75 % of their total cholesterol associated with membranes and the other 25 % associated with triglycerides. Intramuscular adipocytes, which are rich in lipids and poor in membrane content, have small proportion (around 25 %) of cholesterol associated with membranes (Smith et al. 2004).

The fatty acids in meat are mainly found in triglycerides and phospholipids. In depot fats, the fatty acids are found mainly in triglycerides. In muscles, the contribution in the total fatty acids of these compounds depends on the total fat content. The phospholipids contribute between 10 % and 40 % of the total fatty acids in the muscle (Wood et al. 2008).

Most fatty acids in meat contain between 14 and 20 carbon atoms in the molecule. The total content of saturated fatty acids (SFA) is 30–50 %. The main SFA are palmitic acid (C-16:0), stearic acid (C-18:0), and myristic acid (C-14:0). Palmitic acid is the most abundant (20–25 %) followed by stearic acid (5–20 %).

About 35–50 % of fatty acids are monounsaturated, oleic acid (C-18:1) (30–45 %) being the main monounsaturated fatty acid (MUFA) followed by palmitoleic acid (C-16:1) (2–5 %).

A smaller proportion of fatty acids in meat are polyunsaturated (2–30 %). Polyunsaturated fatty acids (PUFA) are of the n-6 and n-3 family. The most important n-6 fatty acids in meat are linoleic acid (C-18:2 n-6) and arachidonic acid (C-20:4 n-6); the n-3 fatty acid present in the largest quantity is α -linolenic

acid (C-18:3 n-3). Double bonds in MUFA and PUFA are mainly of the *cis*-type. *Trans* fatty acids, fatty acids with conjugated double bonds [such as conjugated linoleic acids (CLA)], fatty acids with an odd number of carbon atoms, and fatty acids with branched chains are present in higher proportion in ruminant meat than in nonruminant meats. Ruminant meat (beef, lamb, goat) has more complex fatty acid composition than those of nonruminants due to the activity of microorganisms in rumen. These fatty acids are absorbed in the small intestine and incorporated into meat (Wood et al. 2008). The *trans* fatty acids C-18:1 are present at levels of 2.8–4.7 % in meat from beef and lamb, respectively; however, these fatty acids are not detected in pork meat (Wood et al. 2008). The levels of CLA in meat from lamb (4.3–19.0 mg/g lipid) and beef (1.2–10.0 mg/g lipid) are higher than in pork and chicken (lower than 1 mg/g lipid); it is very interesting due to the positive effects on cancer, cardiovascular disease, diabetes, body composition, immune system and bone health of this fatty acid (Schmid et al. 2006). CLA describes a mixture of positional and geometric isomers of linoleic acid; among them, the most studied isomers are *cis* 9, *trans* 11 and *trans* 10, *cis* 12-CLA.

Carbohydrates

Carbohydrates are present in a relatively small concentration in living muscle tissue, ranged from 0.5 % to 1.5 %. The main carbohydrate is glycogen, a branched polysaccharide composed of α -D-glucose units (up to 50,000) linked by α -1,6 glucosidic and α -1,4 glucosidic bonds, which in living animal functions as an energy store supplying energy for muscle contraction through aerobic glycolysis. After death, no oxygen is available from blood, aerobic pathways stop, and this causes, for a short period of time, a conversion to anaerobic glycolysis, in which glucose is converted into lactate. At 24 h postmortem, glycogen concentration drops to less than 1 %. Other carbohydrates include glucose, other mono- and disaccharides (0.1–0.15 %), and intermediates of glycogen metabolism (Warriss 2000; Keeton and Eddy 2004).

Vitamins

Meat and meat products are good sources of most of the water-soluble vitamins, mainly thiamin (vitamin B₁), riboflavin (vitamin B₂), niacin, and vitamins B₆ and B₁₂. Their concentrations range from a few micrograms (vitamin B₁₂, 0.31–3.1 μ g) to several milligrams (niacin, 3.6–12.6 mg) per 100 g. Liver is rich in folate. Red meats, such as beef and lamb, are specially good sources of vitamin B₁₂. Pork meat and products are one of the best sources of thiamin (0.9–1.2 mg/100 g). The content of vitamin C in meat is variable (0–2.3 mg/100 g); meat is not an important source of this vitamin.

Table 2 Meat composition of various species (value per 100 g) (Data from USDA 2014)

	Unit	Veal	Lamb	Pork	Chicken	Turkey
Proximates						
Water	g	75.91	73.78	72.90	75.46	75.37
Protein	g	20.20	20.75	20.48	21.39	22.64
Fat	g	2.87	4.41	5.41	3.08	1.93
Ash	g	1.08	1.12	1.05	0.96	1.04
Vitamins						
C (ascorbic acid)	mg	0.0	0.0	0.9	2.3	0.0
Thiamin	mg	0.08	0.15	0.88	0.07	0.05
Riboflavin	mg	0.28	0.39	0.23	0.14	0.19
Niacin	mg	7.83	6.54	5.34	8.24	8.10
Pantothenic acid	mg	1.37	0.49	0.81	1.06	0.84
B-6	mg	0.43	0.14	0.50	0.43	0.65
Folate	µg	13	0	9	7	7
B-12	µg	1.40	2.71	0.71	0.37	1.24
A	µg	0	0	2	16	9
D3 (cholecalciferol)	µg			0.6	0.1	0.2
E (alpha-tocopherol)	mg	0.26	0.21	0.17	0.21	0.09
K (phylloquinone)	µg			0.0	1.8	0.0
Minerals						
Calcium	mg	15	8	6	12	11
Iron	mg	0.85	1.64	1.01	0.89	0.86
Magnesium	mg	25	19	25	25	27
Phosphorus	mg	211	202	229	173	190
Potassium	mg	328	171	369	229	235
Sodium	mg	86	46	55	77	118
Zinc	mg	3.23	3.01	2.27	1.54	1.84
Copper	mg	0.113	0.099	0.075	0.053	0.079
Manganese	mg	0.029	0.024	0.029	0.019	0.012
Selenium	µg	8.6	1.5	35.4	15.7	22.6
Cholesterol	mg	83	74	68	70	67

Regarding fat-soluble vitamins, vitamin A is detected in small amounts in meat and meat products (0–40 µg/100 g) with the highest values in meat with high lipid content and in larger amounts in the liver (15,000 µg/100 g), mainly as all-*trans*-retinol, and smaller amounts of 13-*cis*-retinol. Vitamin D and its metabolite 25-hydroxy vitamin D are also present in some meat products, but at very low levels [0.03–0.60 µg/100 g vitamin D3 (cholecalciferol) and 0.4–0.20 µg/100 g 25-hydroxy vitamin D]. Meat is not an important source for the other fat-soluble vitamins, that is, vitamins E and K, that are present at low levels (0.16–0.69 mg and 0.0–6.8 µg per 100 g, respectively) (Leth and Ertbjerg 2004; Lofgren 2005; USDA 2014). The contents of vitamins in meat of various species are shown in Table 2.

Minerals

Meat is a very rich source of some minerals: phosphorus, potassium, magnesium, iron, copper, zinc, and selenium. Iron content is very important as nutrient (mainly in red meat) because it is present in the high bioavailable heme form (Lofgren 2005). Other minerals (calcium and sodium) are present at low levels in meat. Mineral content in meat of various species is shown in Table 2.

Water

Water constitutes 75 % of lean meat in average. In postmortem muscle, water is the major component of sarcoplasm of muscle cell and surrounds the myofibrillar proteins. There are three types of water in meat: bound, immobilized, and free. Bound water (about 4–5 % of water in muscle, 0.3–0.5 % water/g protein) is held tightly by myofibrillar protein charges and it is also referred as constitutional water. Several amino acids can attract and bond water in myofibrillar proteins: glutamic acid and lysine due to charged side groups and glutamine and tyrosine that contain nitrogen and oxygen atoms in side groups with sufficient polarity to attach water. Bound water cannot move among water compartments, remains unfrozen at -40 °C, and can only be eliminated by severe drying.

Immobilized or entrapped water is the largest proportion of water bound in meat; it is retained in the muscle ultrastructure by either steric effects or by attraction to bound water, but not directly bonded to the myofibrillar proteins. It can be removed by conventional heating and converted into ice during freezing.

Free water is held in the meat by weak capillary force and flows from meat unimpeded. It is not readily observed in pre-rigor meat and appears when the entrapped water moves during rigor and post-rigor changes.

Water holding capacity (WHC) is defined as the ability of meat to retain its inherent water during force application and/or processing (grinding, curing, thermal processing, etc.) and also the water added during meat product manufacturing. Myofibrils play a predominant role in the water holding capacity of meat. Losses of water can occur via evaporation, gravitational drip, thawing, or cooking. Water content is important because it affects weight, consumer acceptability, and functional properties of fresh meat and meat products. Low water holding capacity and, consequently, excessive moisture loss result in considerable economic and quality losses.

Several factors contribute to WHC: antemortem factors, such as genetics, animal production practices, carcass chilling, stress, and postmortem items, that include factors related to the transformation of muscle into meat (mainly pH), heating, and addition of other components (sodium chloride, phosphates, etc.).

During postmortem changes, the accumulation of lactic acid, due to anaerobic metabolism, is responsible for the decline of pH muscle from about 7 to values of 5.4–5.7 at 24 h after slaughter. When muscle pH falls to values near the isoelectric point (pI) of the main myofibrillar proteins (about 5.0–5.3), the charged groups of

the proteins (virtually equal number of positive and negative charges) are attracted to each other, reducing the interaction with water and the spaces between myofibrils. In this situation, WHC reaches minimum values. WHC increases with the addition of acids or alkalis due to the increment of negative and positive charges of proteins, respectively, that enhance repulsion.

During postmortem aging, WHC of meat increases without substantial changes in pH value due to the disorganization of myofibrillar structure. Poor antemortem handling can produce meat of poor quality and altered WHC. Stress preslaughter and stress-susceptible genotypes may alter postmortem changes producing pale, soft, and exudative (PSE), with low WHC, and dry, firm, dark (DFD) meats, with very high WHC, two of the major quality problems for meat industry. Sodium chloride is usually added to meat products and, among other effects, alters the water holding capacity of meat. Salt addition modifies the electric charges of myofibrillar protein groups. Chloride anions bind firmly to positively charged protein groups, while sodium cations are weakly bound to the negatively charged groups. At pH values above the isoelectric point, the binding of chloride ions to proteins increases its net negative charge. This displaces the pI toward a lower pH, produces an increase of repulsion between myofibrillar proteins, and opens the structure, resulting in enhanced water retention of meat. WHC of connective tissue proteins is presumably enhanced by ions too. Conversely, at pH values below pI, the positive charges of protein groups are neutralized by chloride ions, reducing net positive charge and WHC.

WHC improves with increasing amounts of added salt from 1.8 % to concentrations higher than 1 M. Maximum swelling of meat proteins is reached at salt concentrations between 0.85 (about 5 %) and 1 M (about 6 %). The water uptake may be caused by the expansion of the grid of myofibrillar filaments as a consequence of the increasing repulsion of negatively charged groups and also by the disruption of forces that determine the arrangement of filaments at the Z- and M-lines and between the myosin heads and actin filaments. Salt concentrations higher than 6 % reduce the swelling effect. This apparent dehydrating effect has been attributed to the precipitation of myosin that would reverse its depolymerization and cause shrinkage. Salt-induced swelling and water expulsion also depend on the postmortem status of the meat as well as the type of muscle and fibers studied and the influence of physical phenomena that depends on the type of salting process applied to the meat (Lawrie 1998; Devine and Chrystall 2000; Warriss 2000; Keeton and Eddy 2004; Apple and Yancey 2013).

Factors That Influence Meat Composition

Species

The chemical composition of meat from different species is similar in the percentages of nitrogenous compounds (21–22 %) and ash (1.0–1.1 %), but some differences can be observed in relation to fat content and amounts of cholesterol and

Table 3 Meat fatty acid composition (% of total fatty acids) of various species (calculated from the data of USDA 2014)

Fatty acid	Veal	Lamb	Pork	Chicken	Turkey
10:0	0.00	0.26	0.20	0.00	0.22
12:0	0.00	0.26	0.20	0.41	0.45
14:0	1.92	2.37	1.43	0.82	0.97
16:0	23.56	23.75	24.34	21.72	21.53
18:0	14.42	19.26	11.86	9.02	10.02
Other SFA	1.45	3.70	0.21	0.41	0.89
Total SFA	41.35	49.60	38.24	32.38	34.08
16:1	4.33	2.11	3.48	4.92	3.19
18:1	39.42	42.22	45.40	31.15	31.33
Other MUFA	0.48	0.26	1.02	0.82	0.89
Total MUFA	44.23	44.59	49.90	36.89	35.41
18:2	10.10	3.43	9.61	22.54	25.17
18:3	0.48	1.85	0.41	0.82	1.26
20:4	3.85	0.53	1.43	3.28	2.30
Other PUFA	0.00	0.00	0.41	4.10	1.78
Total PUFA	14.42	5.80	11.86	30.74	30.51

some vitamins and minerals (Table 2). Other animals such as duck and rabbit deposit little fat in muscles (Kauffman 2012). Rabbit meat is characterized by a lower content of sodium (37–47 mg/100 g edible fraction) and cholesterol than other meats such as pork, beef, and chicken (Dalle Zotte and Szendro 2011).

Important differences between species are related to the fatty acid composition (Table 3). In relation to the meats from main animal species (beef, lamb, pork, and chicken), ruminant meats (beef and lamb) have higher proportion of SFA and lower content of PUFA than pork and chicken. Ruminant meat is more saturated due to the hydrogenation of unsaturated fatty acids by microorganisms in the rumen. Rabbit meat shows higher content of PUFA (32.5 %) than beef, pork, and chicken; the proportion of linoleic acid in rabbit meat is 22 % and the content of α -linolenic acid is 3.3 % (Dalle Zotte and Scendro 2011).

Nowadays, there is a growing interest in meat for alternative species (horse, ostrich, game meat, etc.). These meats have low fat and cholesterol contents and high concentration of iron and PUFA (specially the n-3 fatty acids) (Polawska et al. 2013).

Myoglobin concentration in meat governs its color and is influenced by species. Beef and lamb (“red meats”) contain substantially more myoglobin than pork and poultry meat (“white meats”) (James and James 2010).

Other Factors

Many factors influence the meat composition, being lipids the most variable component. As we described before, the lipid content of the main component of

the meat, the muscle, may range from 1 % to 15 %. This variation is due to many factors such as type of muscles, breed, age (stage of growth), sex, physical exercise, and nutrition. The fatty acid composition of meat is also influenced by these factors. Besides, there are differences in the fatty acid composition between muscles and depot fats. Intramuscular lipids have a higher proportion of PUFA than depot fats. This difference is due to the higher proportion of phospholipids in muscle tissue than in depot fats and phospholipids have a higher concentration of PUFA than triglycerides (Rhee 1992).

Muscles in the carcass differ in fiber type and in nature and concentration of the connective tissue. These differences influence the chemical composition of the muscles. Myoglobin concentration is higher in red fibers (Kauffman 2012). The fatty acid composition of muscles is also affected by the type of fibers. Red oxidative fibers contain more mitochondria and a higher proportion of phospholipids than white fibers and as a result have a higher proportion of PUFA (Wood et al. 2008). The nature of the connective tissue matrix also affects the accumulation of fat in the muscles. Loosely arranged muscles that have parallel connective tissue strands contain more fat than tightly compacted muscles. The latter's connective tissue strands are thicker and more tight structures, this prevents excess lipid accumulation. Anatomical location of muscles is also important because some of them have higher contents of tendon and epimysial sheaths of connective tissue. Due to this, differences in the amounts of stroma proteins comparing to myofibrillar, sarcoplasmic, and granular proteins can be found (Kauffman 2012).

Breed types can influence lipid content and fatty acid composition. Some breeds of pigs, cattle, and sheep contain more intramuscular fat for a given degree of body fatness and age. Duroc is an example of breed that accumulates more intramuscular fat in pigs. In bovine species, Angus deposits more intramuscular fat at a given physiological state of maturity than Hereford and Charolais (Kauffman 2012). Effects of breeds and genetic lines on muscle and adipose tissue fatty acid composition have also been reported for ruminants (beef and lamb) and monogastrics (pigs) (Wood et al. 2008).

Stage of growth is another important factor: in young animals, the accumulation of proteins is higher; when the muscles stop growing, intramuscular lipids may accumulate, decreasing the proportion of other components. Muscles that mature earlier have the structural potential for accumulating more lipids at a given age than muscles that mature a later stage (Kauffman 2012). Meat fatty acid composition can also be influenced by changes in age (Rhee 1992). In relation to meat proteins, older animals show higher proportion of heat-stable or heat-insoluble collagen cross-links, and this makes their meat tougher than that of younger animals (Warriss 2000).

Exercise simulates fiber hypertrophy and also stimulates mobilization of lipids and decreases of the lipid content in muscles. There is little evidence suggesting changes in other chemical components (Kauffman 2012).

The chemical and fatty acid composition can be influenced by sex. The testosterone regulates lipid deposition in muscles. The males have less intramuscular fat than females. Male castrated animals contain more intramuscular fat than

non-castrated animals (Kauffman 2012). Differences in fatty acid composition have also been reported between males and females and between castrated and non-castrated males (Rhee 1992).

Although several factors can influence chemical composition of meat, diet is the most important factor that can modify chemical composition, specially lipid and fatty acid composition. Diets with high caloric content increase lipid accumulation; in submaintenance diets, lipids are mobilized from muscles. In relation to cholesterol and fatty acids, the effect of the modification of the diet is different. It is difficult to modify cholesterol content by dietary manipulation, except when increasing the amount of intramuscular lipids which will cause small increases in cholesterol concentration (Smith et al. 2004); however, the fatty acid composition of meat is very influenced by animal diet. Fat from the diet constitutes the most important factor that modifies the fatty acid composition of meat lipids (Cobos et al. 1994). The consumption of meat fat is related to increased incidence of cardiovascular disease due to their levels of saturated fat. The objective of the meat industry is the reduction of the levels of palmitic acid and the increase of the proportion of MUFA and PUFA, mainly n-3 fatty acids. Modifying the animal diet is possible to obtain healthier balance of fatty acids to the consumer.

The fatty acid composition of meat is sensitive to dietary manipulation, specially in monogastric animals (pigs, poultry and rabbits), since fatty acids are deposited unchanged by digestion. Fatty acid composition of ruminant meat is less influenced by dietary lipid composition. In ruminants, the microorganisms of the rumen modify the fatty acid composition of the diet because they convert unsaturated fatty acids from diet into SFA (Rhee 1992; Cobos et al. 1994; Wood et al. 2008).

Diets supplemented with a source of n-6 (such as sunflower oil or soybean oil) or n-3 fatty acids (such as linseed or fish oil) lead to an increased n-6 or n-3 fatty acid content in meat, respectively. These fatty acids are entirely derived from the diet. These results are stronger in monogastrics than in ruminants. However, the PUFA content of ruminant meat can also be increased, to a certain extent, by feeding diets supplemented with polyunsaturated fats. Despite the hydrogenating effect of the conditions of the rumen on dietary PUFA, small but significant amounts enter the duodenum to be absorbed into the blood and delivered to the tissues as in monogastrics (Wood et al. 2008). In ruminants, diets with high levels of PUFA also increase total CLA. In monogastric animals, the only way to increase CLA in meat is the supplementation with CLA or its precursor *trans*-vaccenic acid in the diet (Schmid et al. 2006). The content of MUFA in meats is increased by incorporation of oils rich in MUFA (such as canola or high-oleic variety of sunflower) into the diets of animals (Rhee 1992).

Changes in the fatty acid composition of meat also have effects on meat quality, for example, fat tissue firmness, color, shelf life, and flavor (Wood et al. 2008). The main problem associated to increased PUFA in meats is the tendency of PUFA to oxidize and reduce meat shelf life due to the rancidity and color deterioration. Meat with high levels of PUFA can rapidly oxidize, *showing* rancidity and color deterioration. A good way to avoid such problems is to use antioxidants products (such as vitamin E) in the diet. Some herbs and spices

(rosemary, sage, clove, etc.) can be efficient food ingredients in improving the shelf life of meats vulnerable to oxidative changes because they contain many natural antioxidants (Zhang et al. 2010).

Grass-based diets have also been shown to enhance CLA isomers, *trans*-vaccenic acid (C-18:1 t11), a precursor to CLA, and n-3 fatty acids in meat from ruminants. Grass-based diets also allow the production of beef meat with higher contents of precursors for vitamin A and E and other antioxidants such as glutathione and superoxide dismutase activity than grain-fed diets (Daley et al. 2010). In monogastric animals, it has also been observed that meat obtained from animals reared in a free-range system is rich in unsaturated fatty acids and antioxidants.

Although the diet is the most important factor that modifies the fatty acid composition of animals, biotechnology is a future way to improve the fatty acid composition of meat lipids. Nowadays, there are transgenic pigs that express a plant gene encoding an n-3 fatty acid desaturase, and they can produce meat with high levels of n-3 PUFA and reduce n-6/n-3 PUFA ratio (Jiménez-Colmenero et al. 2012).

Meat color can be affected by a variety of factors, including postmortem handling, chilling, storage, and packaging. Besides, myoglobin content increases with age, e.g., veal is brownish pink, while beef from 3-year-old steers is bright, cherry red (James and James 2010).

Mechanically Recovered Meat

Mechanically recovered, separated, or deboned meat is the meat obtained by the application of mechanical forces (pressure and/or shear) to bones from pork, beef, sheep, or goat or from poultry carcasses, whose meat has previously been manually removed. The mechanical process of removing meat from bones produces several changes in structure and composition of meat; the process causes cell breakage, protein denaturation, and increases in lipid and heme contents, and the meat has a pasty structure and poorer mechanical properties than manually deboned meat. Mechanically recovered meat (MRM) is mainly used in comminuted meat product formulation because of its texture and low cost compared with other meats although it is also incorporated to nonemulsified meat products in lower proportion. Its proteins show good gelling properties.

The chemical composition of MRM depends on the species (Table 4) and age of animals, the proportion of bone and fat of the raw material, and the type of machine and the operating conditions applied.

MRM is a fine ground, paste-like product due to the great fragmentation of myofibrils, with breaks in the Z or M bands. The shearing process modifies the length of fibrils and results in spherical to oval particles.

Depending on species and anatomical part of the material being deboned, the protein content varies from 11.4 % to 20.6 %. The protein content in MRM is higher than in hand-boned meat mainly due to the incorporation of collagen during extraction.

Table 4 Composition of mechanically recovered meat from various species (value per 100 g) (Data from USDA 2014)

	Unit	Beef	Pork	Poultry
Proximates				
Water	g	59.39	56.87	69.29
Protein	g	14.97	15.03	13.79
Fat	g	23.52	26.54	15.48
Ash	g	2.14	1.56	1.00
Carbohydrate	g	0.00	0.00	0.00
Minerals				
Calcium	mg	485	315	123
Iron	mg	5.67	4.25	1.73
Sodium	mg	57	50	51
Phosphorus	mg	324	200	154

Lipid content of MRM is also higher than that of manually deboned meat because of the inclusion of the skin of abdominal fat, but fat mainly comes from the bone marrow and bone tissue. This fat is rich in PUFA due to the incorporation of phospholipids from the bone marrow; unsaturated fatty acids are considered as beneficial to health, although they are more prone to autoxidation which produces losses in meat sensory quality during storage. Cholesterol content is higher in MRM than in hand-boned meats. It is mainly released from the bone marrow, but can also be affected by the fat content and the presence of skin.

The incorporation of bone particles and powdered bone during mechanical deboning cannot be avoided, and this increases ash and calcium contents to levels much higher than those of hand-boned meat. The calcium content varies depending on the species, bone type, feeding and age of the animals, and also the system used for deboning (pressure force machines increase calcium content due to the incorporation of higher amounts of bone particles).

Iron content is two to three times higher in MRM than in manually deboned meat because the red marrow hemoproteins are incorporated to it during pressing. MRM contains two to three times more hemoglobin, with no change in myoglobin content. This fact modifies meat color (is redder) and makes MRM even more susceptible to lipid oxidation.

MRM is used in the manufacture of Bologna and mortadella sausages, frankfurters, patties, and hamburgers (Field 2004; Viuda-Martos et al. 2012).

Meat Products

Meat products are foods in which meat is the main ingredient, mixed with other components such as fat, water, salt and curing ingredients, spices, etc. Meat has been processed and transformed into various products from ancient times with several objectives: preserve it for long time periods, the total use of carcass, the increase of palatability and variety, and enhance convenience.

Meat Curing

Meat curing, the addition of salt with or without nitrate and/or nitrite, is a very common method for meat preservation and the increase of the variety of meat products.

Salting is, like drying, one of the oldest methods for meat preservation. Sodium chloride inhibits microorganisms and increases meat shelf life and contributes to the development of the characteristic taste and flavor. It also has an important role in water holding capacity of meat (see section “[Water](#)”). NaCl content is very variable in meat products: in sausage, batters are usually about 2 %, while in dry-cured ham is around 5 % or higher. The NaCl content in modern cured meats is much lower than that of produced traditionally. This is due to changes in preferred saltiness and, in the last years, to the desire to reduce sodium levels of meat products for health reasons.

Sodium chloride produces microbial inhibition. It dissociates in water into Na^+ and Cl^- ions that become surrounded by water molecules due to their polarity. Water molecules are immobilized around ions in several layers and are no longer available for chemical or enzymatic reactions and for microorganisms. As a result, water activity is reduced to levels that depend on the amount of NaCl added to the product. In emulsion-type products, 2 % salt reduces water activity to 0.96–0.97. In dry-cured products (dry fermented sausages, dry-cured ham), water activity falls below 0.93. Most bacteria cannot grow in that condition.

Sodium chloride is necessary for the extraction and solubilization of myofibrillar proteins (actin and myosin). This ability is decisive for fat and water binding, emulsion formation, and creation of the meat gel matrix, which produce the development of the structure and texture of meat products.

Salt has a negative effect in meat products: it possesses prooxidant effects that can be observed at typical inclusion levels; this action is particularly important in frozen meat products. The mechanism of action is not clear; it is possible that the impact of salt on the structural integrity of muscle and adipose cells brings catalysts, mainly iron, into closer contact with unsaturated lipids increasing their oxidation.

In addition to salt, potassium or sodium nitrate (KNO_3 , NaNO_3) and potassium or sodium nitrite (KNO_2 , NaNO_2) are usually used in meat curing. Nitrate acts only as a potential reservoir of nitrite, being reduced to nitrite by the action of nitrate reductase produced by bacteria present in raw meat (*Micrococcaceae*), but also by the inherent reducing systems in the meat.

Nitrite has several roles in meat curing: (a) it has the ability to inhibit the growth of a variety of aerobic and anaerobic microorganisms, controlling pathogens such as *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium perfringens*, and specially suppress the outgrowth of *Clostridium botulinum* spores; (b) retards lipid oxidation and rancidity; (c) develops the cured meat flavor; and (d) produces the characteristic reddish-pink cured color after its reduction and the reaction with myoglobin. The mechanisms of lipid antioxidant and flavor development effects are not well known.

Nitrite is converted to nitric oxide (NO) through several pathways. It is reduced to NO by the naturally occurring enzyme systems of muscle and by reducing agents such as ascorbates, sugars, thiols, or NADH. Nitrite, which has strong oxidizing properties, first oxidizes myoglobin to metmyoglobin, and in the reactions, it is transformed into NO. Nitric oxide is very reactive and reacts with metmyoglobin to form nitrosylmyoglobin, of bright-red color, in the presence of reducing compounds that reduces heme Fe^{3+} to Fe^{2+} . Upon heating, this pigment yields pink nitroso-hemochrome due to the denaturation of globin protein, while the NO-porphyrin ring remains intact. When cured meat is exposed to light and oxygen, or is spoiled, nitroso-hemochrome changes to brown, due to the dissociation of NO and myoglobin and oxidation of heme iron (Fe^{3+} to Fe^{2+}) and NO to NO_2 . In the presence of oxygen, NO is oxidized back to nitrite or nitrate.

The use of nitrate and nitrite addition to meat products is regulated in many countries. In the European Union, the amounts vary depending on the type of meat product. In general, the maximum amount that may be added during manufacturing is 150 mg nitrite/kg and 300 mg nitrate/kg, and the maximum residual level is 50–175 mg nitrite/kg and 10–250 mg nitrate/kg, although there are a large number of exceptions. In the USA, the limit in the finished product is no more than 200 ppm sodium nitrite and 500 ppm sodium nitrate.

N-nitrosamines are potent carcinogens that can be formed from nitrite and low molecular weight amines. Only secondary amines form stable *N*-nitrosamines; most amines in meat are primary amines, derived from α -amino acids that are immediately degraded to alcohol and nitrogen. In cured meat products, *N*-nitrosamine formation occurs when these compounds react in an acid environment (such as stomach) or when they are subjected to heating at very high temperatures, above 130 °C (e.g., during frying). Bacon frying, grilling, or frying cured meat products (such as pizza toppings) may result in such conditions that *N*-nitrosamines are formed, but exist only in small amounts and can be avoided by proper culinary treatments. Ascorbate and alpha-tocopherol can inhibit *N*-nitrosamine formation.

There are several compounds that assist to nitrite in curing process. Ascorbic acid, ascorbate, isoascorbic acid, and isoascorbate (erythorbate) are usually added to meat batters (500–550 mg/kg) and help color development but also act as antioxidant compounds, sequester oxygen, and react with nitrite generating flavor compounds. Ascorbate and erythorbate differ only in that ascorbate is biologically active as vitamin C and erythorbate is not. Ascorbate reacts with nitrite and binds the resulting NO. The nitrite is reduced to NO and the ascorbate is oxidized to dehydroascorbate.

Phosphate is added to meat products in several forms; pyrophosphates (with two phosphate groups) and tripolyphosphates (with three phosphate groups) are frequently used due to their alkaline effect that increases WHC and protein swelling and decreases drip and cooking losses, improving yields. Phosphates increase the ionic strength and pH and can act much in the same way that ATP dissociates the actomyosin complex. They also enhance the capacity of sodium chloride to increase myofibril swelling, protein solubility, and water retention. Phosphate can decrease microbial growth, reduce lipid oxidation, and stabilize the color of meats by chelating

free divalent cations (Fe, Cu). Acid phosphates share most of the effects of alkaline phosphates, but decrease WHC. Phosphate usage in meat products is limited by legislation in several countries to 0.5 %, although it is prohibited in others.

Reducing sugars (glucose, fructose, lactose, mannose) also aid color development, although they have other functions such as to provide flavor and Maillard browning, lower water activity, and increase solid content. Other carbohydrates of higher molecular weight (starches, dextrin) stabilize emulsions, participate in gel formation, and increase water retention (Warriss 2000; Belitz et al. 2009; Honikel 2010; Smith 2012).

Smoking of meat and meat products also modifies their composition. Nowadays, smoking process is used to enhance sensory properties, although some of the chemical compounds adsorbed in the surface of meat and meat products act as preservatives. Smoke contains several hundred organic compounds, although some of them are involved in sensory effects. The formation of the typical color comes from the reactions of carbonyl compounds (mainly glycolaldehyde and methylglyoxal) with amino groups of proteins and nonprotein nitrogen components. The smoky flavor is associated with the presence of a mixture of mainly syringol and 4-methylsyringol, but other compounds such as 4-allylsyringol, guaiacol, 4-methylguaiacol, and *trans*-isoeugenol also contribute. However, the wide variety of smoky flavors is likely due to the contribution of many compounds: carbonyls, furans, esters, short-chain carboxylic acids, pyrazines, terpenes, etc.; some of these chemicals interact with meat constituents and generate new flavor components. Phenol compounds are responsible for the antioxidant properties of smoke (Sikorski and Kolakowski 2010).

Enhanced Meat and Comminuted and Reformed Fresh Meat Products

Some meat products are formed by fresh, raw meat and other ingredients and do not suffer heat or drying treatment and, in many cases, not even curing. These products can be classified, according to the structural integrity of the meat used in their manufacture, into whole muscle and comminuted products.

Enhanced meat manufacture involves the addition of an aqueous solution, by pumping or injecting, to fresh whole meat cuts to improve juiciness and tenderness. The solution usually contains salt, phosphates dissolved in water, and sometimes other ingredients may be used: organic acids (lactic and citric acids) and antioxidants for improving shelf life and flavor and flavorings and flavor enhancers (spices, monosodium glutamate) and tenderizers (proteases such as papain, ficin, or bromelain) that hydrolyze muscle fibers and connective tissue.

Fresh comminuted meats include ground beef, pork and poultry, nuggets, restructured patties, steaks, and chops, among others. These products are uncured, typically uncooked (although nowadays they can be found in the market full cooked and ready to use), and unseasoned but may include limited seasoning and/or binders.

Fresh sausages (pork and beef sausages) are uncured ground products, seasoned with salt (usually about 0.5–1.0 %), sweeteners, and spices, usually stuffed into

casings and not smoked or cooked. They do not contain curing salts (nitrate, nitrite) or phosphates. Fresh pork sausage typically has a high fat content (about 50 % fat) and contains antioxidants (butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, rosemary extract). Fresh beef sausages usually have lower fat content (around 30 % fat).

Nugget and restructured products may also be coated with batter or breading. In this case, some ingredients that help with batter adhesion are added: flour, starches, hydrocolloids, proteins, etc. (Sebranek 2003; Maddock 2012).

Restructured meat products, or restructured whole-tissue meats, are manufactured by binding together pieces of meat of different sizes (from whole muscles to small particles) to give the final impression of a large meat cut that can be sliced into fillets or steaks, or cooked as a whole, resembling high-priced cuts such as a ham, a beef roast, or a turkey breast. They can be commercialized as raw (refrigerated or frozen) or cooked products. Restructured meats show lower cooking losses; uniformity of color, texture, and fat distribution; and the possibility of programming their nutritional value. They allow for the use of cheap meat cuts.

Salt and alkaline phosphates are added to meat pieces in appropriate levels and, combined with mechanical action, result in the extraction of myofibrillar proteins to form a surface protein matrix that binds the pieces and the particles of fat. There are two methods of restructuring, cold-set binding and hot-set binding. In hot-set restructuring, a heat set gel is formed from extracted myofibrillar protein which stabilizes particle binding and also retains water and lipids. The surface-binding matrix can also be created by the addition of other binders such as starches and proteins (whey, soy, blood plasma and egg proteins, caseins, gluten, gelatin).

Cold-set techniques include the use of alginate, fibrinogen and thrombin, and transglutaminase to form gels that bind the meat pieces. Transglutaminase is an enzyme that catalyzes an acyl transfer reaction between the γ -carboxylamide group of a peptide-bound residue and a primary amine; in restructured meat, the enzyme catalyzes the formation of chemical cross-links between glutamine and lysine residues of protein chains. It is a fast, cold-gelling meat binder that produces restructured meats with high physical strength and thermal stability. Commercial preparations contain transglutaminase of microbial origin in concentrations of 1 %. It can develop its action at temperature of refrigeration, although its optimum conditions are around 55 °C and pH 6–7; it is used in conjunction with sodium caseinate, sugar, fatty acid ester, and dextrin or with sodium polyphosphate, sodium pyrophosphate, ascorbate, and lactose (Farouk 2010). Table 5 shows the chemical composition of comminuted, enhanced, and reformed meat products.

Cooked-Cured Meat Products

Cooked-cured meat products are products manufactured with the addition of curing agents and submitted to heat treatment. They are classified into two types according to their structure: emulsion-based products and products from whole meat cuts.

Table 5 Comminuted, enhanced, and reformed meat products (value per 100 g) (Data from USDA 2014)

	Unit	Hamburger	Enhanced meat (turkey)	Enhanced meat (pork)	Sausage (turkey)
Proximates					
Water	g	38.45	76.25	76.91	70.42
Protein	g	16.52	20.87	20.39	18.79
Fat	g	12.01	2.39	2.09	8.08
Ash	g	1.52	1.35	1.56	2.24
Carbohydrate	g	31.50	0.00	0.00	0.47
Minerals					
Calcium	mg	62	14	4	19
Iron	mg	3.06	0.64	0.92	1.17
Sodium	mg	331	194	243	593
Phosphorus	mg	134	223	290	177

Heat treatment modifies the structure of meat proteins. Most of the sarcoplasmic proteins aggregate at 40–60 °C, although in some cases the coagulation can prolong up to 90 °C. Myofibrillar protein unfolding starts at about 30 °C, followed by protein-protein associations at 36–40 °C and consequential gelation at 45–50 °C. Collagen denaturation occurs between 53 °C and 63 °C, followed by collagen fiber shrinkage. If collagen fibers are not stabilized by heat-resistant intermolecular bonds, it forms gelatin on further heating (Tornberg 2005).

Emulsion-Based Meat Products

Emulsion-based meat products, or meat batters, are products based in a complex matrix in which fat is emulsified into a viscous fluid mainly composed of solubilized myofibrillar proteins previously extracted from meat and finally stabilized by a heat treatment that produces gel formation. In these products, meat particles are so small that they are not visually distinguishable on the product surface. Examples of these products are frankfurters, wieners, bologna sausage, mortadella, fine liver sausages, and liver pate. Pork and beef are the most common meats used in these products, although the usage of poultry meat is increasing in the last years due to its nutritional characteristics. Mechanically recovered meat is frequently used in the formulations, and, specifically, liver is one of the main raw materials for liver sausages and pate.

An emulsion consists of two immiscible liquids (usually water and fat in food emulsions) in which one of the liquids is dispersed as small droplets (dispersed phase) in the other (continuous phase). Meat emulsions, however, are much more complex systems: the dispersed phase is itself a multiphase media that contains solid fat particles (1–50 µm in size), liquid fat droplets, and air bubbles. The continuous phase is constituted by water and many other compounds, such as proteins, salt, carbohydrates and insoluble proteins, connective tissue, and meat particles.

According to the processing method, there are two types of emulsified meat products: cold emulsions (e.g. frankfurters) and hot emulsions (e.g. pates). In cold emulsions obtaining, the ingredients are raw (uncooked) when they are finely comminuted and the emulsion is formed. In hot emulsions, some raw materials are precooked before emulsification. The resulting viscous batter is portioned or stuffed into casings and submitted to heat treatment.

In meat emulsions, the main function of sodium chloride is to release the myofibrillar proteins and to increase their ability to emulsify fat, specially at pH values near the pI. Both myofibrillar and sarcoplasmic proteins can act as emulsifiers, although myofibrillar proteins are preferably absorbed to the water/fat interface. Myosin is more surface active than actin or actomyosin. Protein hydrophobicity is important for effective formation of the interfacial protein film that surrounds fat globules and stabilization of the protein matrix. Hydrogen bonds and electrostatic attractions appear to have some importance, participating in interfacial film-protein matrix binding.

Salt also contributes to improve the water retention of myofibrillar proteins by the mechanisms described above (section “[Water](#)”); this is a very important characteristic that helps the sensory properties of these products.

Mild warming during grinding and mixing for emulsion formation aids in solubilization and releasing of myofibrillar proteins, but temperatures above 22 °C may cause emulsion breakdown due to protein denaturation before they can act as emulsifiers and orientate in the water-lipid interface.

The system is stabilized by protein denaturation during heat treatment, due to the strong gel produced by myofibrillar proteins; sarcoplasmic proteins produce very weak gels and they do not contribute significantly to stabilization and structure formation. During heating, myofibrillar proteins start to denature, exposing hydrophobic domains. This makes the formation of hydrophobic interactions more probable; protein aggregation is enhanced and immobilizes the fat globules by physical entrapment (Lawrie 1998; Ugalde-Benítez 2012).

Cooked Products from Whole Pieces

Cooked meat products from whole meat cuts are usually manufactured using brine that is injected or infused through soaking, followed by the application of thermal treatment. Cooked ham, one of the most popular products, is the complete ham, or part of the ham, added with brine and cooked at high temperature and finally stored at refrigerated temperature.

Raw material for cooked ham production is selected to avoid PSE and DFD meats; PSE meat generates hams with lower WHC, less color, and softer than hams that suffer normal postmortem changes. DFD hams show higher WHC, but they are more prone to microbial deterioration than normal meat. After, skin and bones (optional) are removed; then, brine is injected into the hams. Brine contains sodium chloride, nitrite (120–150 mg/kg), ascorbate or erythorbate (200–400 mg/kg), sucrose, dextrose, and frequently alkaline phosphates (0.15–0.3 %). Low-quality

hams can also contain nonmeat ingredients such as milk powder, caseinates, soy proteins, starches, and carrageenan, in order to improve water retention. Final salt content is around 2 %.

Brine solubilizes myofibrillar proteins that will be responsible for muscle binding during cooking. The brine is injected into the hams through multineedle systems and after they rest a short period of time. Then, hams are submitted to a massaging/tumbling phase; this is applied to allow the diffusion and uniform distribution of brine components through the entire piece. The mechanical treatments also improve protein extraction, tenderness, and juiciness.

The next stage is the heat treatment that can be considered as pasteurization: the internal temperature is increased up to 72 °C for 30–60 min. This temperature is high enough to coagulate meat proteins, destroy microorganisms, and inactivate enzymes. Globin protein of cured meat pigment (nitrosylmyoglobin) is denatured into the pink pigment, nitrosohemochromogen, that gives the characteristic color of cooked ham. Finally, hams are cooled quickly in order to avoid microbial growth.

Enzymatic reactions, oxidations, and Maillard reactions take place in hams during cooking that contribute to the development of sensory properties. Proteolytic and lipolytic reactions occur during the preheating period while ham temperature is below 50 °C approximately, because enzymes denature at higher temperatures during cooking. Free amino acids and fatty acids contribute to flavor and may act as substrates of further chemical reactions such as Strecker reactions. The spices added also influence the flavor of this product. Many volatile compounds have been identified (aldehydes, ketones, alkanes, alcohols, aromatic hydrocarbons, terpenes, pyrazines, amines, etc.), although none of them has an aroma similar to that of cooked ham; probably its flavor and aroma depend on the combination of several volatiles (Arnau Arboix 2004; Toldrá et al. 2010).

Table 6 shows the chemical composition of some cooked-cured meat products.

Table 6 Cooked-cured meat products (value per 100 g) (Data from USDA 2014)

	Unit	Frankfurter	Sausage Vienna	Pork cured ham cooked
Proximates				
Water	g	56.31	64.90	72.42
Protein	g	10.26	10.50	17.44
Fat	g	25.76	19.40	6.16
Ash	g	3.50	2.60	3.29
Carbohydrate	g	4.17	2.60	0.69
Minerals				
Calcium	mg	99	10	7
Iron	mg	1.09	0.88	1.07
Sodium	mg	1,090	879	1,210
Phosphorus	mg	206	49	278

Dry Fermented Sausages

Dry fermented sausages are meat products manufactured by selecting, chopping, and mincing meat and fat, with or without offal, with the addition of condiments and authorized additives, stuffing in natural or artificial water vapor permeable casings, and subjecting to fermentation, ripening, or drying and sometimes to smoking. The composition of these products is a consequence of formulation, fermentation, and ripening/drying stages. The formula of a typical fermented sausage includes meat, pork fat (except in some countries for religious grounds or some specialty products), salt and curing agents (nitrates and nitrites, carbohydrates, ascorbate), spices, and other condiments. The proportion of meat and fat can change depending on the type of dry sausage. Meat usually comes from pork, but also beef and poultry are frequently used for fermented sausage production. However, there are traditional or new products that incorporate meat from other animals: mutton and lamb, goat, camel, ostrich, horse, buffalo, and game meat.

During fermentation, two main changes happen: the production of lactic acid from carbohydrates via glycolysis, with an important decrease of pH values, and the formation of nitric oxide from nitrate/nitrite. These reactions are carried out by lactic acid bacteria and nitrate- and nitrite-reducing bacteria, respectively, and have great influence in sensory properties. During ripening phase, moisture content decreases and complex biochemical and chemical reactions occur, which finally develop the texture, flavor, and odor of dry fermented sausages and stabilize them (Ordoñez et al. 1999; Toldrá et al. 2007).

Texture development starts during the fermentation and ends in the ripening period due to changes in protein structure. During grinding and mincing process, the added salt promotes the solubilization and extraction of myofibrillar proteins, mainly myosin and actin. Throughout the fermentation, previously solubilized proteins coagulate and form a strong gel that binds meat and fat particles. The coagulation is associated with the release of water, which is eliminated during drying in the ripening phase, that creates a dense structure due to protein denaturation forming the typical texture of dry fermented sausage.

The generation of flavor compounds during fermentation and, particularly, during ripening of dry fermented sausages is a complex process that involves complicated chemical and biochemical reactions, in which, very frequently, the compounds produced in the reactions become the substrate of others. The biochemical reactions are originated by some enzymatic pathways of microorganisms and by the endogenous enzymes of meat. The chemical reactions are mainly nitric oxide reactions and lipid autoxidation.

The relative importance of microbial and endogenous enzymes in the generation of flavor compounds is not clear, although it has been deeply studied in last decades. The most abundant microorganisms in dry fermented sausages, *Micrococcaceae* and lactic acid bacteria, are able to produce proteases (endopeptidases, aminopeptidases, and dipeptidases) and lipases.

Regarding muscular proteases, cathepsins are the most active proteases during ripening; cathepsins B, L, and D are mainly active during mixing and fermentation,

while only cathepsin D seems to remain active during ripening. During this last phase, dipeptidyl aminopeptidases and carboxypeptidases could also collaborate in protein-peptide degradation. Calpains lose their activity at the acid pH values typical of dry fermented sausages and are also affected by curing salts addition (Ordoñez and Hoz 2007).

Among lipolytic muscle enzymes, acid lipase could develop an important activity due to the typical pH of these products (4.8–6.0) and its resistance to inactivation at the low water activity values at the end of ripening, while neutral and basic lipases are strongly inactivated in these conditions. Subcutaneous adipose tissue lipases are less stable than muscular enzymes. Due to the action of lipolytic enzymes on triacylglycerols and phospholipids, free fatty acid amount increases from 1 % to 2 % in raw matter to 4–5 % in 1 month, with higher levels of PUFA probably because of the preferential hydrolysis of the outer fatty acid of triacylglycerol molecule or the preference for the polar lipid fraction (phospholipids). Outer fatty acids (positions sn1 and sn3) are mostly occupied by unsaturated fatty acids, nearly 30 % by oleic and linoleic acids in sn1 position and about 50–60 % of these fatty acids in sn3 (Ordoñez et al. 1999).

Free amino acids and free fatty acids, released as a consequence of protein and lipid breakdown, contribute to the taste of the sausages, but also play an important role as substrates of complex reactions that finally generate numerous aromatic and flavored compounds. Some of the reactions, in which substrates are amino acids, are due to the activity of microbial enzymes such as aminotransferases, but chemical reactions such as Strecker degradation could also occur. Among other compounds, both pathways generate branched aldehydes (3-methylbutanal, 2-methylbutanal, phenylacetaldehyde). By oxidative deamination, free amino acids are transformed into α -ketoacids that after decarboxylation produce aldehydes. The decarboxylation of amino acids produces amines that also generate aldehydes by deamination. Sulfur amino acid degradation produces phenol, indol, and sulfur compounds. Aldehydes are transformed into alcohols by reduction or into acids by oxidation; both alcohols and acids can produce esters. This complex mixture of chemical compounds plays an important role in dry fermented sausage flavor (Ordoñez and Hoz 2007).

Lipid autoxidation is frequently one of the main causes of food deterioration; however, a certain degree of oxidation can generate volatile and nonvolatile compounds that have a beneficial impact in odor and taste of dry fermented sausages. The main substrates of these chemical reactions are PUFA, particularly when they are in free form because, at the initiation phase, it is easy to remove a hydrogen from a methylene carbon as the number of double bonds in the fatty acid increases. Lipid autoxidation in muscle initiates by numerous molecules such as free radicals and iron-oxygen complex. Propagation phase starts with the formation of a peroxy radical which then extracts a hydrogen from a fatty acid and forms hydroperoxides. These primary products of oxidation have no odor or taste, but are very unstable and quickly decompose into a high number of nonvolatile and volatile compounds through very complex reaction pathways. These final products modify the taste and aroma of foods and include a large variety of compounds: aldehydes,

Table 7 Dry fermented sausages and dry-cured meat products (value per 100 g) (Data from USDA 2014)

	Unit	Salami (pork)	Thuringer, cervelat	Chorizo	Bacon	Dry-cured ham
Proximates						
Water	g	34.60	45.18	31.85	44.24	55.93
Protein	g	21.70	17.45	24.10	12.62	27.80
Fat	g	37.00	30.43	38.27	39.69	8.32
Ash	g	5.50	3.63	3.92	2.17	-
Carbohydrate	g	1.20	3.33	1.86	1.28	0.3
Minerals						
Calcium	mg	10	9	8	5	10
Iron	mg	1.52	2.04	1.59	0.41	1.11
Sodium	mg	1,890	1,300	1,235	662	2,695
Phosphorus	mg	229	111	150	144	318

ketones, alkanes, alcohols, esters, furanes, carboxylic acids, etc. The addition of some ingredients and additives influences and controls lipid oxidation in dry fermented sausages; these include nitrite, ascorbate, spices, tocopherol, and, in smoked sausages, antioxidant compounds such as phenolic derivatives (Ordoñez et al. 1999). Table 7 shows the chemical composition of some dry fermented sausages.

Dry-Cured Meat Products

Dry curing of meat products consists of the application of salt alone, or in conjunction with nitrate or nitrite, in dry form. It is one of the most ancient methods of meat preservation.

Dry-cured ham is mainly produced in Mediterranean countries: Serrano and Iberian hams in Spain, Parma ham in Italy, and Bayonne ham in France. They are characterized by dry salting and a long period of maturation (7–24 months). Other varieties are manufactured in the Northern Europe and they are usually smoked and matured for a short period of time (e.g., Westphalia hams in Germany).

The manufacturing process starts with the selection of raw materials that can be refrigerated or frozen/thawed. PSE and DFD hams must be discarded due to the excessive rapid water loss and salt intake of the former and the high risk of undesirable microbial growth of the latter. Green hams are classified based on their weight, because dry salting duration depends on it. The weight usually ranges from 9 to 14 kg. Hoof, bones, and part of the rind and fat can be eliminated. After, the residual blood is eliminated in order to reduce possible microbiological problems.

Salting is developed in two phases. Firstly, the green ham is rubbed with salt alone or with a mixture of salt, nitrate, ascorbate, and sugars. In the second phase, hams are covered in salt individually by piling them in containers for approximately

1 day/kg, although in other cases salt is added in smaller amounts several times during salting period. After it, the excess of salt is eliminated by washing, and hams are submitted to smoking (optional) and post-salting (1–3 months) which allows for the homogenization of salt content. Ripening-drying is the last phase, in which the stabilization and flavor development occur. The length of this period is very variable and ranges from 3 to 36 months. Iberian hams are further ripened for another period in cellars.

During dry-cured ham manufacture, important changes in its chemical composition occur. Hams suffer high dehydration during their manufacture, although it is more intense during ripening due to the ambient conditions; dry matter increases to values higher than 50 %. Salt content in hams is higher than in other meat products and, together with loss of water, is responsible of the microbial stabilization of the product; it is greater than 4.5 %.

Color pigment in hams with added nitrate/nitrite is due to the formation of nitrosomyoglobin. However, in the absence of nitrification, the color compound is due to a Zn protoporphyrin IX complex, formed by protein denaturation or partial degradation of myoglobin which associates to zinc porphyrin through noncovalent binding.

Proteolysis affects both sarcoplasmic and myofibrillar proteins, although it is more intense in the latter. Proteolytic changes in dry-cured ham are due to the action of endogenous enzymes. Sodium chloride reduces the action of many proteolytic enzymes, so it is considered that their action is higher in the first elaboration stages. Calpains have poor stability, so their action is restricted to the initial days of processing. Cathepsin D could contribute to proteolysis during the first 6 months, while cathepsins B, L, and H could act during the complete process. These enzymes generate peptides and free amino acids that, after decarboxylation and deamination phenomena and also chemical reactions (e.g., Strecker degradation), release flavor and aroma compounds. Proteolysis may also contribute to dry ham texture, although the dehydration and the high salt content produce protein denaturation and are responsible of this property. Muscle proteins can also suffer oxidation during dry ham manufacture.

In relation to lipid changes, lipid hydrolysis is favored by the high salt content and low water activity. Muscle phospholipases seem to be the most important enzymes in meat lipolysis. In the adipose tissue, triacylglycerols are mostly hydrolyzed by neutral lipases to mono- and diacylglycerols and free fatty acids up to 6 months of processing. A preferential hydrolysis of PUFA has been observed, and also triacylglycerols containing oleic and linoleic acids are more hydrolyzed than those rich in SFA. Free fatty acids are released at high rates up to 10 months of ham processing; after, autoxidation mainly degrades PUFA and MUFA. SFA are accumulated in high amounts due to their better stability. These reactions produce important amounts of volatile compounds responsible for the final aroma of dry-cured ham (Toldrá and Aristoy 2010; Gou et al. 2012).

Bacon is a meat product with difficult classification, because it is matured (although for a short period of time) and salted by different methods and it does not suffer any thermal treatment during processing. As bacon can be elaborated by dry curing in some of their varieties, it has been included in this section.

In North America, bacon is produced from boneless pork belly, while in Ireland and in the UK it is made from boneless pork loins. The most used procedure for bacon manufacture is the Wiltshire style process. Salt, nitrite, and other ingredients (polyphosphate, ascorbate, sugar) are dissolved in water, and the brine is incorporated to the meat by mechanical injection. After the pieces are stacked in small tanks covered with brine and are held for up to 3 days. Then, the meat pieces are removed, stacked, and matured enough time to dry the surface. Optionally, bacon is smoked.

Some bacon is dry cured by manual rubbing of the lean meat surfaces with the curing ingredients. The pieces are vacuum-packed and stored refrigerated for about 10–14 days, and after, it is ready for slicing. This short maturing period is not enough to allow high proteolytic and lipolytic changes, so the major contributors to flavor and aroma in dry-cured bacon are derived from the curing agents and those generated during cooking prior to consumption (Sheard 2010).

Table 7 shows the chemical composition of two dry-cured meat products (bacon and dry-cured ham).

Frozen Meat and Meat Products

Freezing has great advantages in prolonging the shelf life of meat due to the low temperatures: stops microbial growth and slows down chemical and biochemical reactions. The effect of freezing in the chemical composition of meat depends on the freezing rate, the storage conditions, and the method of thawing.

WHC decreases when meat is frozen, so drip increases during thawing and water, intracellular soluble proteins (including myoglobin), minerals, and vitamins are lost. Drip loss increases with slow freezing because of the high damage to membranes, and also when oscillations in storage temperature occur. Myofibrillar proteins denature in a higher degree than in optimal conditions due to their temporary contact with high salt concentrations in unfrozen zones.

There is no evidence of any effects of freezing and thawing on meat flavor, although it can be altered during frozen storage. This is mainly caused by lipid oxidation that results in undesirable “off flavors” or “rancid flavors” which are great obstacle for long-term frozen storage. The intensity of autoxidation is affected by intrinsic factors (species, content and type of fat) and extrinsic factors that include catalyzers of the reactions (light, heat, oxygen). Mincing and addition of sodium chloride increase lipid oxidation; these factors dramatically shorten the frozen storage length of meat products. Protein oxidation can also occur during frozen storage of meat. The carbonylation of meat proteins seems to be connected to the occurrence of lipid oxidation; it is affected by the type of muscle, freezing temperature, packaging conditions, and mincing. Frozen meat can also show a dark brown color at the surface due to the oxidation of myoglobin to metmyoglobin, specially with slow freezing rates or prolonged storage (Belitz et al. 2009; James and James 2010; Estévez 2011).

Dried Meats

The objective of meat drying is the preservation of meat through the elimination of most of water content of meat and the resulting decrease of water activity. Water loss during drying involves losses of weight and increases the concentration of dry solids (fat, protein, carbohydrate).

During drying, sarcoplasmic and myofibrillar proteins denature, which is related with decreases in water holding capacity and rehydration capacity. At hot-air drying, the activity of meat enzymes is negligible. Aroma compounds are generated due to lipid oxidation (aliphatic hydrocarbons, aldehydes, ketones) and to Maillard reactions (furans, sulfur and nitrogen compounds). Color is also modified, changing from red to light gray (at temperatures below 63 °C) or brown (at temperatures higher than 67 °C), also depending on the chemical characteristics of muscle. All these changes are strongly influenced by drying methods and conditions (Santchurn et al. 2012).

Meat Extracts

Meat extracts are obtained by liquid extraction at high temperatures (90–100 °C) and concentration (in vacuum evaporators at decreasing temperatures, 92–46 °C) of the water-soluble fraction from the meat, bones, and liver. Very frequently, extracts are also a by-product from canning meat production. Their chemical composition changes depending on the raw materials extracted.

Beef extracts contain variable content of organic solids, 56–64 %, and minerals are around 18–24 %, of which sodium chloride is 2.5–5 %. The most abundant organic compounds are amino acids and peptides (15–20 %) and creatinine (5.4–8.2 %). Pigments are also quantitatively important compounds (about 10–20 %). Different amounts of B vitamins have also been detected. The considerable amount of inosine and inosinic acid is another interesting characteristic of meat extract composition due to their properties as meat flavor enhancers, and probably these compounds are the main responsible for the flavor and aroma of this product. The high concentration of amino acids may also contribute to flavor by means of their participation in Maillard reactions during heating in extraction and concentration stages (Pearson 2003; Belitz et al. 2009).

Healthier and Functional Meat Products

Healthier Meat Products

Low-Sodium Meat Products

A direct relationship between excessive intake of sodium and an increased incidence of hypertension has been observed, and meat product consumption contributes about 16–25 % to the total intake of sodium. Several ingredients affect the

sodium content: the sodium salts of polyphosphates, nitrate, nitrite, ascorbate and erythorbate, and glutamic acid. However, the main source of sodium in meat products is salt. Salt levels range from 2 % to 4 %, although dry-cured ham can reach much higher values.

Sodium chloride is an essential ingredient in meat products due to its ability to provide multiple functionalities. Besides, salt is one of the cheapest ingredients available, and all these aspects make very difficult sodium chloride substitution in meat products. There are several approaches in order to reduce sodium content of meat products: the use of salt substitutes, flavor enhancers, and masking agents, the optimization of the physical form of salt, and the application of alternative processing techniques.

Among sodium chloride substitutes, potassium chloride is the most common compound used. Although potassium chloride covers many functions of salt, a complete substitution is not possible for some reasons. At blends over 50:50 sodium chloride/potassium chloride in solution, a significant increase in bitterness and loss of saltiness is perceived. Besides, concerns about the possible vulnerability of certain population groups (such as those with Type I diabetes, chronic renal insufficiency, severe heart failure, etc.) to high potassium intakes have been raised. Mixtures of substitutes with almost half of the sodium have been commercialized and include potassium chloride in proportions 60:40 or 70:30 NaCl:KCl, sometimes added with magnesium sulfate and amino acids (L-lysine). Experimentally, good results using mixtures of NaCl, KCl, and potassium or calcium lactate, calcium citrate, and potassium phosphate, together with curing ingredients, have been obtained.

Commercial flavor enhancers and masking agents include 5'-ribonucleotides (IMP, inosine 5'-monophosphate; GMP, guanosine 5'-monophosphate, AMP, adenosine 5'-monophosphate) and monosodium glutamate. Lysine and succinic acid have also been used for providing salty flavor and some antimicrobial and antioxidant properties. Water binding maintenance can be achieved by the addition of phosphates, starches, carboxymethyl cellulose, and carrageenan.

The modification of the physical structure of sodium chloride, i.e., changes in particle size, may lead to a more rapid dissolution behavior in the mouth, yielding a more pronounced salty taste of the product with lower salt addition. Flake type salt has been shown more functional than granular salt.

Alternative processing techniques include the use of pre-rigor meat, and the application of high pressure technology or high intensity ultrasound also allows the use of lower amounts of salt with similar or better solubilization of myofibrillar proteins and good texture (Desmond 2006; Weiss et al. 2010).

Low-Fat and Lipid-Modified Meat Products

The reduction of fat content in meat products for improving their nutritional properties is difficult because fat has a great influence in their appearance, flavor, and texture. The use of fat replacers allows reductions of about 30–80 % of typical fat content, and they have been used in emulsion-type sausage, patties, and salami. Fat replacers include vegetable oils (olive, corn, soybean, canola), proteins

(soy protein, caseinate), fiber-rich ingredients (oat, citrus, broccoli, spinach, and other vegetable fibers), inulin, fructooligosaccharides, carrageenan, and carboxymethyl cellulose.

The change of fatty acid profile of meat products can be achieved by the inclusion of oils of different origins in animal diets. Long-chain n-3 PUFA (EPA and DHA) can be increased by the use of plant oil, fish, and algae oils. Fish oils are difficult to include due to the generation of “fishy” flavor in meat. Plant and algae oils have good results in lipid composition modification, although a high level of unsaturated lipids increases fat sensitivity for lipid oxidation in meat products, which is more intense in dry-cured products. This problem can be avoided by the inclusion of antioxidants, synthetic (BHA, BHT) or natural (natural herb and spice extracts, α -tocopherol) in animal feeding. Antioxidant can also be included in meat product formula, although the antioxidant effect is lower.

Cholesterol reduction can be achieved by the use of fat replacers of plant origin. A completely different approach is the inclusion of conjugated linoleic acid (CLA). This fatty acid is naturally present in ruminant meat and has a positive effect in cholesterol level reduction, although this reduction is accomplished when CLA concentrations are higher than the natural ones. CLA content can be increased in meat by the supplementation of the animal feed. CLA is also a functional compound with healthy properties that are detailed in section “[Functional Meat Products](#)” (Weiss et al. 2010).

Nitrite Reduction

Nitrite is also an ingredient in meat product manufacturing with multiple and very different functions (preservative, antioxidant, flavor, and color development) but under certain circumstances can react with amines and form nitrosamines. Only combinations of antimicrobials, colorants, flavorers, and antioxidants may be able to substitute nitrite or at least reduce required levels in meat products.

The preservation functions of nitrite could be substituted by the addition of ingredients that have natural high nitrate content, such as unrefined sea salt, turbinado sugar, spices, and vegetable matter. Another option is the addition of naturally occurring antimicrobials, as those contained in spices, herbs, or in their essential oils (terpenes, flavonoids) or those from microbial (nisin) or animal sources (lysozyme). Other useful antimicrobials are potassium sorbate, sodium lactate, sodium diacetate or sodium citrate. However, because of the broad antimicrobial spectrum of activity of nitrite, the replacement by a single antimicrobial is not often possible and the combination of several compounds is necessary.

Some antioxidants can be used in nitrite-reduced meat products. They include chelators [ethylenediaminetetraacetic acid (EDTA), citrate, polyphosphates], free radical stabilizers (BHA, BHT, propyl gallate), and naturally occurring compounds (α -tocopherol, sodium ascorbate, spices, spice extracts from rosemary, sage, or oregano). Related to color development, many colorants have been tested although the colorant of choice is the cooked-cured meat pigment, which is the nitrosylated heme pigment preformed outside the meat matrix and then applied to meat. It is

manufactured from the red blood cells of animals and a nitrosating agent in the presence of a reductant. Due to the lack of a protein matrix that protects the pigment from oxidation, it must be stabilized by microencapsulation using modified starch, cyclodextrins, and gums.

The mechanism involved in the production of the cured meat flavor remains uncertain, so there is no known nitrite substitute that can reproduce it. It has been suggested that nitrite effects in flavor could be related to its antioxidative effects, the retardation of the breakdown of unsaturated fatty acids, and the formation of carbonyl compounds and other secondary products of lipid oxidation. The addition of antioxidants could also, at least in principle, develop the cured meat flavor (Pegg and Shahidi 2006; Weiss et al. 2010).

Functional Meat Products

Several strategies can be used to improve the presence of bioactive compounds in meat and meat products and develop functional meat products.

Dietary Supplementation of Functional Ingredients

Numerous physiological and biological properties (antioxidant, antiobesity, anticarcinogenic, antiatherosclerotic, protection of immune system, etc.) have been attributed to CLA. Although the results of the inclusion of CLA in animal feeding are variable, it seems to improve the body composition through reducing fat deposition and backfat thickness and also modify the fatty acid composition of tissue lipids, increasing the proportion of SFA and reducing lipid oxidation in pigs and poultry.

Vitamin E supplementation in animal diet and meat products can improve the quality of meat and meat products by reducing protein and lipid oxidation and increasing color stability.

Selenium is an essential trace mineral for animals because it is implicated in the regulation of physiological functions. Selenium deficiency in humans is related to the decrease of the immune function that results in the increase of the cancer susceptibility, cardiovascular diseases, diabetes, arthritis, and other pathologies. As selenium deficiency is common in some countries, inorganic and organic selenium has been added to animal diets to enrich its content in meat. Pork meat with 10 times more selenium content than normal pork has been produced, showing good quality properties. Beef and lamb meats have also been supplemented with these mineral at different levels.

Functional Ingredients Addition During Meat Product Processing

Fibers have been added to meat products as an optional way of increasing the intake of dietary fiber and obtaining its beneficial effects (reduction of the risk of colon cancer, diabetes, obesity, and cardiovascular diseases). Addition of fiber from different origins (peach, apple, orange, oat, wheat, pea, chicory root, lupin-kernel, inulin) has been assayed in fresh and cooked meat products and fermented sausage.

Fiber effects on sensory properties of products are variable. The addition of fiber is also useful for low-fat meat products manufactured as fat replacers.

Herbs and spices have been used for centuries in meat product manufacture for improving flavor and aroma. However, they contain many phytochemicals with antioxidant activity, such as flavonoids, tannins, or phenolic acids. Besides, these compounds also have anti-inflammatory and anticancer activities in living cells and antimicrobial properties in meat products. Compounds with these capacities have been detected in clove, rosemary, sage, oregano, garlic, etc.

Probiotic Bacteria

Probiotics are live microorganisms contained in food that, when ingested in sufficient quantities, exert health benefits to the consumer related to gastrointestinal disorders, food allergies, or immune functions. The inclusion of probiotic bacteria in meat products is being studied and marketed in recent years. The most suitable vehicles for probiotic intake are dry fermented sausages that have been added with a number of lactic acid bacteria of the genera *Lactobacillus* and *Bifidobacteria* (Jiménez-Colmenero et al. 2006; Zhang et al. 2010).

Conclusion and Future Directions

Meat and meat products are an important source of nutrients, such as proteins, lipids, minerals, and vitamins, in the context of a healthy diet. Meat composition is complex and is strongly influenced by a wide number of factors that include species, breed, animal feeding, age, and physical exercise. Meat products arose from the need for meat preservation for long periods of time; nowadays, when meat can be preserved at low temperatures in many countries, meat products increase the variety of available foods. Their chemical composition varies depending on the raw materials and the processing methods used in their manufacture (curing, salting, drying, heating, etc.) which determine their quality attributes and sensory properties.

Future trends in meat and meat product processing are related to the advances in healthier and functional products that will achieve the reduction of compounds involved in some pathologies and the addition of prebiotics and probiotics with improved health effects.

Cross-References

- ▶ [Chemical Properties and Applications of Food Additives: Preservatives, Dietary Ingredients, and Processing Aids](#)
- ▶ [General Properties of Major Food Components](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Drying](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Heating and Cooking](#)

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Abstract

Milk is a heterogeneous mixture which can be defined as a complex chemical substance in which fat is emulsified as globules, major milk protein (casein), and some mineral matters in the colloidal state and lactose together with some minerals and soluble whey proteins in the form of true solution. The overview of biosynthesis of milk and its constituents is highlighted. The key constituents of milk (fat, protein, salts, lactose, enzymes, vitamins) and their composition as well as factors affecting the chemical composition of milk are described. The compositions of milk fat globule membrane are also covered. Moreover, various types of milk products like fermented dairy products (e.g., dahi, yogurt, kefir, and cheese), fat-rich dairy products (e.g., cream), concentrated milk, sweetened condensed milk, milk powders, ice cream, heat-desiccated milk products (like khoa, khoa-based sweet), and heat-acid-coagulated milk products (like paneer and chhana) and its chemical compositions are discussed in this chapter.

Introduction

Milk is the fluid secreted by female mammals for the purpose of nourishing their offspring. Milk is considered as nearly complete human food which can be generally consumed without further processing. Although it is very essential for infant feeding, milk and milk products are important in our diet throughout life. Milk may be defined as the whole, fresh, clean, lacteal secretion obtained by complete milking of one or more healthy milch animals, excluding that obtained within 15 days before parturition and 15 days after calving or such periods as may be necessary to render the milk practically colostrum-free and containing the minimum prescribed percentages of milk fat and milk solids-not-fat (SNF). Milk is a complex, nutritious product that contains more than 100 substances that are either in emulsion, suspension, or solution in water. Various factors like genetic factors, stage of lactation, health status of the animal, and environmental factors are responsible for wide variation in milk compositions. The various fermented products (e.g., sour milk, yogurt, curd, dahi, lassi, shrikhand, kefir, kumiss, cheese), concentrated milks (e.g., evaporated milk

and sweetened condensed milk), dried milk, fat-rich dairy products (e.g., cream, butter, ghee), ice cream, heat-desiccated products (e.g., khoa, rabdi, basundi), and heat-acid-coagulated products (e.g., paneer and chhana) are some of the typical milk products having different chemical compositions.

Biosynthesis of Milk

Mammals have the ability to produce milk in the mammary glands for the nutrition of their newborn. The number of mammary glands varies with species from two (e.g., goat, sheep, and human), four (e.g., cow and buffalo) to as high as 14–16 (e.g., pig). Each gland, anatomically and physiologically separate, is emptied via a teat. Milk constituents are synthesized in secretory cells (epithelial cells) from raw materials absorbed from the blood. Milk production requires a lot of nutrients that are brought to the udder by the blood. To produce 1 kg of milk, 400–500 kg of blood must pass through the udder. The lactose, lipids, and most proteins as principal constituents of milk are synthesized in the rough endoplasmic reticulum of the mammary gland in which modification of the constituents occurs (Fox and McSweeney 1998). On the other way around, some of the constituents like water, immunoglobulins, vitamins, salts, some hormones, and serum albumin leak into milk unchanged from blood (Chandan et al. 2008). However, the secretion of milk salts is not well defined but has been reviewed by Holt (1985). Larson (1985) stated that fat (3.7 %), lactose (4.6 %), potassium (0.15 %), calcium (0.13 %), and phosphorus (0.1 %) are several times more abundant in cow milk than in the blood (i.e., 0.06 %, 0.0 %, 0.025 %, 0.01 %, and 0.01 %, respectively, in the blood). However, the concentration of immunoglobulins (2.6 %), serum albumin (2.6 %), phospholipid (0.25 %), sodium (0.34 %), and chloride (0.35 %) is significantly more in the blood than in milk (i.e., 0.07 %, 0.05 %, 0.035 %, 0.05 %, and 0.11 %, respectively, in milk). The alveolus is considered as the primary physical location of milk production. It is a small bud-shaped chamber lined internally with the secretory mammary cells and externally with a network of capillaries and muscle cells. The capillaries transport blood and precursors to the udder for milk production.

Milk Composition and Constituents

Chemically milk is a heterogeneous mixture which can be defined as a complex chemical substance in which fat is emulsified as globules (2,000–6,000 nm), major milk protein (casein, 50–300 nm), and some mineral matters in the colloidal state and lactose together with some minerals and soluble whey proteins (4–6 nm) in the form of true solution. The lactose, salt, and other minor soluble constituents have a diameter of 0.5 nm. The number of milk fat globule, casein micelles, whey protein, and lactose per ml of milk is 10^{10} , 10^{14} , 10^{77} , and 10^{19} , respectively. The multiple constituents in different states and phases exist in a delicate balance that provides stability of milk under normal condition. The pH of normal milk varies from 6.5 to 6.7.

Table 1 Gross composition of milks of selected species

Species	Average composition (%)				
	Water	Fat	Protein	Lactose	Ash
Buffalo	84.20	6.6	3.9	5.0	0.7
Cow	86.30	4.9	3.4	4.1	0.7
Sheep	83.70	6.0	4.8	4.9	0.8
Goat	86.50	4.5	3.5	4.7	0.8
Camel	87.61	5.38	2.98	3.20	0.70
Human	87.43	3.75	1.63	6.98	0.21

Milk, a biological fluid, is a highly variable and complex raw material for further processing. The gross composition of milk from different species of mammals is given in Table 1.

Milk Fat

Fat is one of the most important constituents of milk and is considered as economics of milk and milk products. Most price plans for milk are based largely on milk fat content. It serves as a rich source of energy (~9 kcal per gram) and carrier of fat-soluble vitamins (A, D, E, and K). It contains significant amount of essential fatty acids. The milk fats play an important role in desirable or undesirable flavor development in dairy products as well as impart soft, smooth, and rich tasting qualities and overcome watery, flat, hard, grainy characteristics which are normally encountered in their absence (Shivashraya 2014a).

Level of fat can vary from below 3.0 % to more than 6.0 %, a much greater range than that of any milk constituent. Triglycerides (96–98 % of milk fat) are the main constituent of milk fat. Triacylglycerols containing three different fatty acids have a chiral carbon at the *sn*-2 position of the glycerol skeleton. Over 400 different fatty acids have been identified in bovine lipids (Swaisgood 2008). These fatty acids can differ in chain length of carbon atoms (i.e., short-, medium-, and long-chain fatty acids) and numbers of double bonds (i.e., saturated or unsaturated). Milk fat has a great diversity of fatty acids with chain length varying from four carbons up to more than 20 carbons. Some of the fatty acids like conjugated linoleic acid (CLA) and short-chain fatty acids have some possible health-beneficial properties found in milk fat. Milk fat also contains low levels of mono- and diglycerides and minor constituents such as phospholipids, sphingolipids, and cholesterol. Milk fat contains a greater portion of saponifiable materials accounting almost 99–99.5 % that include glyceride (monoglycerides, diglycerides, and triglycerides), phospholipids, cholesterol ester, and FFAs. Unsaponifiable matter of milk fat falls within the range of 0.30–0.45 % that includes largely cholesterol which is quite apolar and easily associates with phospholipids. The part of the cholesterol is in the fat globule membrane and the remainder being dissolved in the fat. Some other unsaponifiable matters are carotenoids (β -carotene), fat-soluble vitamins, traces of squalene (hydrocarbon), and waxes. β -Carotene is responsible for the yellow color of the fat.

The concentration of various components of milk lipids in tabular form can be found in ► [Chap. 13, “Chemical Composition of Fat and Oil Products”](#) in this book.

Triglycerides make up the bulk (>98 %) of the lipids that largely determine the properties of milk fat. These properties vary with the fatty acid composition. The distribution of fatty acid residues over the position in the triglyceride molecule is far from random that affects the crystallization behavior of milk fat. Triglycerides are very apolar and not surface active and they act as a solvent for many other apolar substances like sterols, carotenoids, and tocopherol. A small amount of water (~0.15 % at room temperature) dissolves in liquid milk fat (Walstra et al. 2006). Di- and monoglycerides as well as free fatty acids occur in fresh milk fat and lipolysis increases their quantities. Diglycerides are apolar and properties of them are almost similar to that of triglycerides. Monoglycerides are present in small quantities and are somewhat polar. Monoglycerides are surface active and accumulate at an oil–water interface. Most lipolytic enzymes preferably attack the 1 and 3 position of the triglyceride molecule, yielding most monoglycerides having fatty acid residue at the 2 position. The short-chain fatty acids are water soluble and are responsible for soapy-rancid flavor after lipolysis.

The polar lipids, also called as compound lipids, contain charged groups. They are strongly amphipolar and are virtually insoluble in water as well as in oil. They are highly surface active and form the typical bilayers. In milk, they are mainly present in the fat globule membrane and in the poorly defined lipoprotein particles. The most polar lipids found in milk fat are phospholipids. Total phospholipid content of cow milk fat averages 33.71 mg/ml, whereas for cow milk the corresponding value is 21.04 mg/100 ml (Shivashraya 2014a).

Milk fat is present in the milk as milk fat globules (MFG) with diameters ranging from 0.1 to more than 22 μm (average size is 4–6 μm). The globules contain a nonpolar core of triglycerides and cholesterol esters. The core of milk fat is protected and stable in the aqueous environment of milk by the presence of a protective coating on the surface of the spherical globules known as the milk fat globule membrane (MFGM). The MFGM stabilizes the emulsion and protects the triglycerides from degradation by lipase. The MFGM consists of a double-layer phospholipid membrane into which different proteins are embedded. Butyrophilin and the enzyme xanthine oxidase are some of the major proteins, and many other minor proteins are also associated with the MFGM (Jensen 2002; Huppertz and Kelly 2006). Density of fat is lighter than the surrounding aqueous serum. This leads to relatively rapid separation of unprocessed milk into a phase enriched in milk fat globules (i.e., cream) and a phase largely depleted of such globules (i.e., skim milk). Upon centrifugal force on milk, the rate of separation of milk fat can be accelerated. On the other hand, reduction in the size of fat globules using homogenization hugely retards the separation of milk fat.

Milk Fat Globule Membrane

The milk globules (micro lipid droplets) are encircled by a special membrane composed of lipid bilayer and proteins. This membrane is known as milk fat/lipid

globule membrane or milk fat globule membrane (MFGM). The MFGM is a surface-active membrane surrounding each of the milk fat globules allowing them to remain dispersed in milk. The MFGM originates from several distinct trilayers with total thickness of 10–20 nm (Walstra et al. 1999). The MFG core is essentially composed of triacylglycerides (TG), while the MFGM envelope is a true polar lipid bilayer with proteins, enzymes, neutral lipids, and other trace components (Danthine et al. 2000; El-Loly 2011). An electron dense material on the inner membrane face is composed of proteins and polar lipids; the outer part of the MFGM corresponds to a biological membrane organized as a bilayer. The phospholipid bilayer serves as a backbone of the membrane.

The lipids of the MFGM are primarily polar lipids, although neutral lipids (triglycerides, diglycerides, monoglycerides, cholesterol and its esters) can also occur. Phospholipids of bovine MFGM constitute almost 30 % of the total MFGM lipids. The MFGM consists of a complex mixture of (glyco)proteins (20–60 %), glycerophospholipids (15–33 % of the MFGM), sphingolipids, glycolipids (i.e., cerebrosides and gangliosides), high-melting TGs, and sterols (Wooding and Kemp 1975; Keenan and Patton 1995). MFGM contains high-melting TGs (Wooding and Kemp 1975; Keenan and Mather 2002). These MFGM-associated TGs contain higher proportions of palmitate and stearate than do TGs of the core fat (McPherson and Kitchen 1983). MFGM lipids contain about 60 % triglycerides and 0.3–2.3 % sterols, 90 % of which is cholesterol (Keenan and Patton 1995; Walstra et al. 1999; Keenan and Mather 2002). The MFGM contains approximately 0.5–1 % of the total lipids in milk, of which the majority are sphingolipids and phospholipids (phosphoglycerides) which show polar properties (Christie 2003; Vanhoutte et al. 2004; Fong et al. 2007; Dewettinck et al. 2008). The glycerophospholipids of the MFGM are phosphatidylcholine (35–36 %), phosphatidylethanolamine (27–30 %), phosphatidylinositol (5–11 %), and phosphatidylserine (3–4 %). The major sphingolipid (highly bioactive molecule) of the MFGM is sphingomyelin (25 % of polar lipids) (Christie 2003; Lopez et al. 2008), which accounts for up to one third of the MFGM polar lipid fraction. There are also glycosphingolipids in the MFGM, such as glucosylceramide and lactosylceramide with amounts in the range 3–6 % (Deeth 1997; Danthine et al. 2000). Cerebrosides are glycolipids containing a hexose residue. MFGM contains mainly glucosylceramide and galactosylceramide. Gangliosides are another type of glycosphingolipids composed of a ceramide unit and an oligosaccharide chain linked to one or more sialic acids and several sugars (Keenan and Patton 1995; Jensen 2002). The long-chain fatty acid phospholipids will make the membrane thicker and more rigid. The fatty acid composition associated with the MFGM triglyceride was found to contain higher proportions of palmitic and stearic acids and fewer unsaturated fatty acids, such as C_{14:1}, C_{16:1}, C_{18:1}, and conjugated C_{18:2} (Fong et al. 2007; Lopez et al. 2008; Lopez 2011).

Depending on the method of isolation, proteins account for 25–70 % of the MFGM (Danthine et al. 2000; Walstra et al. 2006; Fong et al. 2007) but they only

represent 1–2 % of the total milk proteins (Ricchio 2004). Reinhardt and Lippolis (2006a) resolved up to 120 proteins in the MFGM. The main protein of the MFGM is the glycoprotein such as butyrophilin (BTN), heavily glycosylated mucin (MUC)-like glycoproteins MUC1 and MUC15, periodic acid–Schiff (PAS) 6/7, adidophilin (ADPH), and cluster of differentiation (CD36) (Keenan et al. 1983; Kim et al. 1992; Reinhardt and Lippolis 2006b), and the second representative protein of the MFGM is xanthine oxidase/oxidoreductase (Mather 2000; McManaman et al. 2002). Other proteins are present in the MFGM, each at 5 % or less (Kim et al. 1992; Spitsberg et al. 1995). Furthermore, it is assumed by several authors that parts of the proteose-peptone fraction, like proteose peptone 3 (PP3), originate from the MFGM (Girardet et al. 1995; Campagna et al. 2004).

The different enzymes or enzymatic activities like xanthine oxidoreductase, catalase, acid phosphatase, alkaline phosphatase, NADPH/NADH oxidase, cholinesterase, hexosaminidase, plasmin, aldolase, protein kinases, adenosine triphosphatase, etc., have been detected in the MFGM (Fox and McSweeney 1998). The miscellaneous components such as cholesterol and its esters, carotenoids, vitamin A, Fe, and Cu are present (Mulder and Walstra 1974). Mono- and diacylglycerols might be true MFGM components or products from the degradation of glycerolipids (Keenan and Dylewski 1995).

The health-beneficial components of the MFGM have anticarcinogenic properties, antimicrobial activity, inhibitor of *Helicobacter pylori*/prevention of stomach diseases, antiinflammatory effect, anticholesterolemic effect, effect on autoimmune disorders, anticoagulant and coagulant properties, and use in bone treatment (Kanno 1990; Singh 2006). Spitsberg (2005) proposed the use of MFGM as a potential nutraceutical.

Milk Proteins

Proteins are made up of amino acids, more precisely, L- α -aminocarboxylic acids. Proteins constitute an important class of compounds that are essential to all living processes. Milk proteins represent one of the greatest contributions of milk to human nutrition, and more than 200 types of proteins have been characterized in bovine milk. About 95 % of the nitrogen in milk is in the form of proteins. When total nitrogen content is multiplied with 6.38, a Kjeldahl factor, it gives the total protein content in milk and milk products. Nonprotein nitrogen components comprising about 5 % of the total nitrogen in fresh milk are equally important.

The proteins of milk are classed into two major groups, i.e., casein and whey proteins, which differ fundamentally in their properties, in particular their solubility when the pH of milk is adjusted to 4.6 (isoelectric point). Casein constitutes over 80 %, while whey proteins contribute 20 % of the total protein of milk. Casein is insoluble and either precipitates or forms a gel, depending on whether the rate of pH drop is rapid or slow. Whey proteins remain soluble in this pH. The properties of milk proteins are shown in Table 2.

The amino acid composition of the bovine milk protein is presented in Table 3.

Table 2 Properties of milk proteins

Property	Average concentration (%)	Molar mass	(Residues/molecule)			Isoelectric pH
			Amino acid	Phosphoserine	Cysteine	
α_{s1} -Casein	1.1	23,614	199	7–9	0	4.5
α_{s2} -Casein	0.3	25,230	207	10–13	2	5.0
β -Casein	0.9	23,983	209	5	0	4.8
κ -Casein	0.3	19,023	169	1	2	5.6
β -Lactoglobulin	0.32	18,283	162	0	5	5.2
α -Lactalbumin	0.12	14,176	123	0	8	4.3
Serum albumin	0.04	66,267	582	0	35	4.8

Table 3 Amino acid composition of the total protein, casein, and whey protein of bovine milk

Amino acid	g amino acid/100 g protein		
	Total protein	Casein	Whey protein
Alanine	3.7	3.1	5.5
Arginine	3.6	4.1	3.3
Aspartic acid	8.2	7.0	11.0
Cystine	0.8	0.3	3.0
Glutamic acid	22.8	23.4	15.5
Glycine	2.2	2.1	3.5
Histidine	2.8	3.0	2.4
Isoleucine	6.2	5.7	7.0
Leucine	10.4	10.5	11.8
Lysine	8.3	8.2	9.6
Methionine	2.9	3.0	2.4
Phenylalanine	5.3	5.1	4.2
Proline	10.2	12.0	4.4
Serine	5.8	5.5	5.5
Threonine	4.8	4.4	8.5
Tryptophan	1.5	1.5	2.1
Tyrosine	5.4	6.1	4.2
Valine	6.8	7.0	7.5

Adapted from Belitz et al. (2009)

Caseins

Caseins display a distinctive structure as well as physical, biological, and nutritional properties. Casein is not a globular protein. Casein associates extensively and is present in milk in large aggregates, casein micelles, which also contain the colloidal calcium phosphate (CCP). The casein colloidal particle contains calcium and phosphate as inorganic ions. The casein micelle consists of about 94 % proteins (as submicelles) and 6 % minerals. Casein is a mixture of α_{s1} -, α_{s2} -, β -,

and κ -casein. The weight ratio of α_{s1} -, α_{s2} -, β -, and κ -caseins is 3:0.8:3:1. Most of the κ -casein molecules are glycosylated, while α_s - and β -caseins are phosphoproteins (Table 2). Phosphoproteins have a number of groups esterified to serine residue and they precipitate with Ca^{+2} ions. The κ -casein protects α_s - and β -caseins from precipitation by Ca^{+2} ions. But κ -casein is easily attacked by the rennet enzyme, which splits off a portion of the κ -casein molecules; it thereby loses its protective ability. Hence, the casein precipitates in the presence of Ca ions and this is the basis of cheese making. Caseins are considered as naturally denatured proteins due to high content of proline; they tend to have very little secondary structure. Heating at above $\sim 120^\circ\text{C}$ causes the casein to slowly become insoluble due to chemical changes (Walstra et al. 2006).

α_{s1} -Casein

It contains 199 amino acid residues with a molecular weight of 23 kDa. It contains eight phosphoserine residues and does not have cysteine residue. Proline is uniformly distributed along the chain and hinders the formation of a regular structure. In the presence of Ca^{+2} ions, α_{s1} -casein forms an insoluble Ca salt (Belitz et al. 2009).

α_{s2} -Casein

It contains 207 amino acid residues and a pronounced dipolar structure with a concentration of anionic groups in the region of the N-terminus and cationic groups in the region of the C-terminus. It contains 11 phosphoserine and 2 cysteine residues and is even more easily precipitable with Ca^{+2} than α_{s1} -casein (Belitz et al. 2009).

β -Caseins

It is a peptide chain consisting of 209 residues and has a molecular weight of 24.0 kdal. It is the most hydrophobic casein. It has no cysteine and a high proportion of proline (35 residues), which has a profound effect of its structure. Five phosphoserine residues are localized in positions 1–40; these positions contain practically all of the ionizing sites of the molecule. Positions 136–209 contain mainly residues with apolar side chains. The molecule has a structure with a “polar head” and an “apolar tail,” thus resembling a “soap-like” molecule. The amphipathic nature of β -caseins forms micellar aggregates in solution. β -Casein contains about 9 % of α -helix structure and about 25 % of β -structure. An increase in temperature results in an increase in the β -structure. The self-association of β -casein is an endothermic process. The protein precipitates in the presence of Ca^{+2} ions at the levels found in milk. However, at temperatures at or below 1°C , the calcium salt is quite soluble. A hydrolytic product of β -caseins is known as γ -caseins. The γ -caseins corresponding to residues 29–209, 106–209, and 108–209 of β -caseins are present in the precipitate at pH 4.6. Other fragments of β -caseins (residues 1–28, 1–105, and 1–107) are found in whey and they constitute part of a fraction formerly known as proteose peptone (Belitz et al. 2009; Shivashraya 2014a).

Table 4 Composition and component distribution of casein micelles (%)

Casein micelles				Distribution of components				
Composition (%)				Component	Ratio number			
Casein	93.2	Citrate	0.4	α_{s1}	3	6	9	12
Ca	2.9	K	0.3	β	1	1	4	4
Phosphate (inorganic)	2.9	Mg	0.1	γ	–	1	1	1
Phosphate (organic)	2.3	Na	0.1	κ	1	3	3	3

Adapted from Belitz et al. (2009) with modification

κ -Caseins

It consists of a peptide chain with 169 residues and has a molecular weight of 18 kdal. The monomer contains one phosphoserine and two cysteine residues. A κ -casein occurs as a trimer or as a higher oligomer in which the formation of disulfide bonds is probably involved. κ -Casein is the only protein that is glycosylated. The protein contains varying amounts of carbohydrates (average values, 1 % galactose, 1.2 % galactosamine, 2.4 % *N*-acetylneuraminic acid) that are bound to the peptide chain through Thr-131, 133, 135, or 136. κ -Casein constitutes 10–12 % of the whole casein. It plays a crucial role in stabilizing the casein micelles in milk. It remains soluble in the presence of Ca^{+2} ions. Aggregation of α_{s1} - and β -caseins with κ -casein prevents their coagulation in the presence of Ca^{+2} ions (Belitz et al. 2009).

The molar ratio of the main components $\alpha_{s1}/\beta + \gamma/\kappa/\alpha_{s2}$ is on an average 8/8/3/2. All casein forms contain phosphoric acid, which always occurs in a tripeptide sequence pattern (phosphoserine). Only up to 10 % of the total casein fraction is present as monomers. They are usually designated as serum caseins. The main portion is aggregated to casein complexes and casein micelles. The diameter of the micelles in skim milk varies from 50 to 300 nm (average diameter of 140 nm), the micelle volume is $1.4 \times 10^6 \text{ nm}^3$, and the particle weight is 10^7 – 10^9 dal. This corresponds to 25,000 monomers per micelle. The ratio of monomers in micelles varies to a great extent (shown in Table 4). The micelles are not tightly packed and so are of variable density. They are strongly solvated (1.9 g water/g protein) and hence are porous (Belitz et al. 2009). The composition of casein micelles is presented in Table 4.

The monomers are kept together with (i) hydrophobic interactions, (ii) electrostatic interactions, mostly as calcium or calcium phosphate bridges between phosphoserine and glutamic acid residues, and (iii) hydrogen bonds. On a molecular level, different micelle models have been described in the literature. The models may be divided into three categories:

1. Coat-core models: Based upon the formation of coat around the core (nucleus).
2. Internal structure models: Based upon known properties of isolated casein components.
3. Subunit (submicelle) models: Considered an aggregate of submicelles, and colloidal calcium phosphate acts as cementing material.

The κ -casein molecules are arranged on the surface of the corresponding submicelles, and other fractions are inside the micelles. At various positions, their hydrophilic C-termini protrude like hairs from the surface, preventing aggregation. Indeed, aggregation of the submicelles proceeds until the entire surface of the forming micelle is covered with κ -casein, i.e., covered with “hair,” and, therefore, exhibits steric repulsion.

For further reading, readers are advised to refer Waugh (1958) and Schmidt (1980) for earlier models, for *submicelle model* refer Slattery and Evard (1973) and Walstra (1990, 1999), for *dual bonding model* consult Holt (1992), and for *interlocked lattice model* refer McMahon and Oommen (2008) papers.

Whey Proteins

Whey (or serum) protein accounts for ~20 % of the total protein and comprises the non-casein proteins that remain soluble when caseins have been precipitated at its isoelectric pH 4.6. Whey proteins have more heterogeneous group of compounds. Whey proteins are globular proteins and have more organized secondary and tertiary structures. Four major whey proteins include β -lactoglobulin (~50 %), α -lactalbumin (~25 %), immunoglobulins (~9 %), and bovine serum albumin (~6 %).

More than half of the whey protein is β -lactoglobulin. The monomeric β -lactoglobulin has a molecular weight of 18 kdal and consists of 162 amino acids. By self-associating, it forms dimmers in the pH range of 3–7. It has five cysteine residues (one of which being free) capable of forming disulfide bonds. In the native protein, however, this cysteine is buried within the structure. This SH group is exposed on partial denaturation and can participate either in protein dimerization via disulfide bridge formation or in reactions with other milk proteins, especially with κ -casein and α -lactalbumin, which proceed during the heating of milk.

α -Lactalbumin is the smallest of the major whey proteins that plays important role in the biosynthesis of lactose. It contains 123 amino acids and eight cysteine residues. Minor forms of α -lactalbumin have carbohydrate moiety. α -Lactalbumin is a rich source of tryptophan, which has been shown to improve sleep quality, cognitive performance under stress, and mood under stress through the formation of the neurotransmitter serotonin (Farkye and Shah 2015).

Bovine serum albumin is identical to blood serum protein. It is a single polypeptide of 582 amino acid residues. It has molecular weight of 66,433 Da. It contains 17-S-S-, 1-SH, and no phosphorus group. The structure is stabilized by internal network of disulfide bonds. The secondary structure has 76 % helix, 10 % turn, and 23 % extended chain with no β -sheet. It is a carrier of fatty acids in blood plasma.

The largest and most heterogeneous of the major whey proteins belongs to a group known as immunoglobulins which has molecular weight of >1,000 kDa. They have been classified as IgG, IgM, IgE, and IgD. They all exist as either monomers or polymers of a basic unit made up of two light and two heavy chains. The immunoglobulins are antibodies synthesized in response to stimulation by specific antigens (Belitz et al. 2009; Shivashraya 2014a).

Table 5 Fraction of proteose peptones

Proteose-peptone fraction	Molecular weight (Da)
PP3	20,000
PP5	13,000
PP8f	3,900
PP8s	9,900

Proteose-peptones fraction of milk has been defined as those proteins that remain in solution after milk is heated at 95 °C for 20 min followed by acidification to pH 4.7 (Farkye and Shah 2015). The proteose peptones (PPs) in milk are precipitated by 12 % TCA and represent ~1.1 % of the total milk protein and about 10 % of total whey proteins (Innocente et al. 1998). Depending on mobilities in free-boundary electrophoresis, there are four major PPs: designated component 3 (PP3), component 5 (PP5), component 8 fast (PP8f), and component 8 slow (PP8s) which are presented in Table 5.

PP3 (also known as lactophorin) is a small phosphoglycoprotein containing over 17 % carbohydrate. PP5 contains phosphorus; it represents β -CN (f1–105) resulting from plasmin proteolysis. PP8f represents β -CN (f1–28), whereas PP8s represents β -CN (f29–105). The PPs are heat resistant and very surface active partly because of their low molecular weights and also to the carbohydrate present in PP3. PP3 has emulsifying properties and foaming ability in milk (Girardet and Linden 1996).

Glycomacropeptide (GMP) is a hydrophilic peptide, κ -CN (f106–169), that provides stability to the casein micelles in milk. When chymosin acts on κ -casein during the manufacture of cheese, GMP is released into the whey. GMP makes up about 15–20 % of the whey proteins (Farkye and Shah 2015). GMP contains high levels of branched-chain amino acids (leucine, isoleucine, and valine) but does not contain phenylalanine, tyrosine, and tryptophan. The phenylalanine is not digested by phenylketonuria (PKU) patients, and hence GMP is one of the few peptide sources PKU patients can tolerate (van Calcar and Ney 2012). GMP is reported to enhance the growth of bifidobacteria in probiotic fermented milk (Janer et al. 2004).

Lactoferrin (LF), an iron-binding glycoprotein, is a single polypeptide chain containing ~689 amino acid residues, having a molecular weight between 76 and 80 kDa. It has five potential glycosylation sites. Bovine LF has antibacterial, antiviral, antioxidant, immune modulation, anticancer, and antiallergic properties. Lactoperoxidase [EC 1.11.1.7] is an enzyme present in colostrum, milk, and whey, with a MW of ~77.5 kDa. Bovine colostrum and milk contain about 11–45 mg/L and 13–30 mg/L of lactoperoxidase, respectively. In whey, lactoperoxidase constitutes ~0.5 % of total whey proteins. It functions as a natural antibacterial agent (Farkye and Shah 2015).

Lactose

The main sugar in milk is lactose, an *O*- β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucopyranose, which is 4.2–5 % of milk. Lactose is a disaccharide and comprises α -D-glucose (or β -D-glucose) and β -D-galactose which is shown in

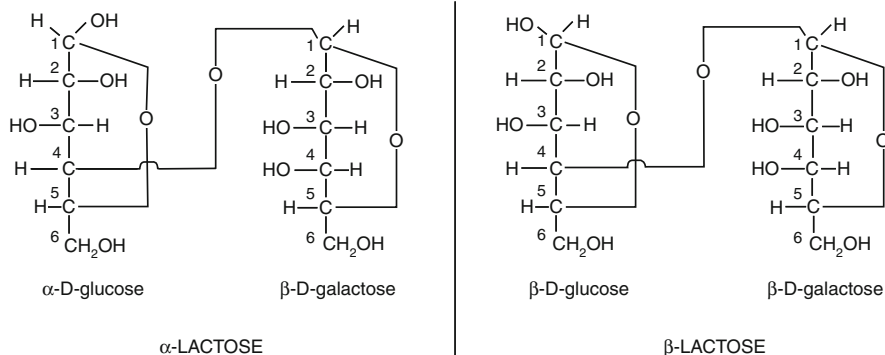


Fig. 1 Structure of lactose

Fig. 1. The aldehyde group of galactose is linked to glucose, through a β -1,4-glycosidic linkage.

The C_1 atom of glucose can easily change from the α - to the β -form and vice versa via aldehydes form; this process is called mutarotation and will eventually lead to equilibrium. α -Lactose and β -lactose differ in their specific rotation to polarized light, i.e., $+89.4^\circ$ or $+35.0^\circ$, respectively, at 20°C . An aqueous solution of lactose in equilibrium at 20°C contains 37.3 % α -lactose and 62.7 % β -lactose and thus has an optical rotation of $+55.7^\circ$ (Huppertz and Kelly 2009). Lactose occurs in both α - and β -forms, with an equilibrium ratio of $\beta/\alpha = 1.68$ at 20°C . Lactose is normally found in dairy products in either of the two forms.

1. Crystalline forms: (a) α -hydrate or α -lactose monohydrate and (b) anhydrous β -lactose
2. Amorphous “glass” mixture of alpha and beta lactose

Alpha hydrate or α -lactose monohydrate is obtained by concentrating an aqueous lactose solution to supersaturation and allowing crystallization to take place at a moderate rate below 93.5°C . It is the stable solid form of lactose, and its crystals are hard and less soluble and feel gritty when placed in the mouth, similar to sand particles. This is the origin of the term “sandy” to describe the defect in texture of ice cream, condensed milk, or processed cheese spread that contains perceptible alpha hydrate crystals. This sandiness defect depends on both the size and the number of crystals. Crystals of anhydrous β -lactose are soft, more soluble, and sweet. They are obtained by crystallization above 93.5°C from supersaturated solution. The anhydrous lactose glass (or amorphous noncrystalline glass) is obtained when a lactose solution is dried rapidly; its viscosity increases so quickly that crystallization cannot take place. This is very hygroscopic, rapidly takes up moisture from the air, and becomes sticky that leads to problem (cakiness) in stored milk powder.

Lactose is only 25 % as sweet as sucrose. Lactose makes a major contribution to the colligative properties of milk: osmotic pressure freezing point depression and boiling point elevation. Lactose is fermented by lactic acid bacteria to lactic acid that reduces the pH of milk. While uncontrolled or unwanted fermentation clearly results in spoilage of the milk, controlling this fermentation is the basis of production of cheese and yogurt. Lactose is undergoing Maillard reactions at high temperature, leading to color changes in milk heated to very high temperatures. Thermal degradation of lactose also leads to production of various organic acids that affect the heat stability of milk.

Minerals (Salts)

Milk contains inorganic and organic salts. The concept of salts thus is not equivalent to mineral substances. Ash (a gray-white residue obtained after incineration of milk at ~500 °C to 550 °C) contains minerals or inorganic constituents of milk. Ash is the indicator of mineral load in milk. Ash does not represent the real status of salts in milk. Salts are by no means equivalent to “ash” because ashing of milk causes loss of organic acids including citrate and acetate and because organic phosphorus and sulfur are transferred to inorganic salts during ashing. Mineral in ash (0.7 %) is less than in salt (0.9 %). Ash is alkaline, whereas salt is acidic in nature (Walstra et al. 2006).

Minerals in milk consist principally of the bicarbonates, chlorides, citrate, and bicarbonates of calcium, magnesium, potassium, and sodium. All of the minerals are distributed between a soluble phase and a colloidal phase, and while monovalent ions exist largely, or totally, in the soluble phase as much as 66 % of the calcium and 55 % of the phosphorous may be in the colloidal phase. The distribution of calcium, citrate, magnesium, and phosphate between soluble and colloidal phase (i.e., associated with the casein micelles) and their interaction with milk proteins have important consequences for the stability of milk and milk products. The colloidal salts are commonly referred to as colloidal calcium phosphate (CCP) or micellar calcium phosphate (MCP), since calcium and phosphate predominate, although some magnesium and citrate are also present. CCP plays an important role in the structure and stability of the casein micelle. Calcium ions also bind to some caseins and complex strongly with citrate. The soluble (non-micellar) calcium concentration in milk correlates closely with the soluble citrate concentration, and the majority of the citrate exists as a complex with calcium. Half of the inorganic phosphate exists as colloidal calcium phosphate and only a small quantity (~6.6 %) of the calcium is present as soluble Ca^{+2} ion (Varnam and Sutherland 2001). The distribution of milk minerals between the colloidal and soluble phase is presented in Table 6.

The concentrations of minerals and trace elements in milk are presented in Table 7.

The monovalent ions, together with lactose and other low-molecular-weight components, maintain the osmotic pressure at a value iso-osmotic with that of

Table 6 Distribution of minerals (% of total minerals) in milk

Minerals	Soluble phase	Colloidal phase
Total calcium	33	67
Ionized calcium	100	0
Chloride	100	0
Citrate	94	6
Magnesium	67	33
Total phosphorus	45	55
Inorganic phosphorus	54	46
Potassium	93	7
Sodium	94	6

Adapted from Varnam and Sutherland (2001)

Table 7 Mineral and trace elements of milk

Minerals	
Constituents	g/100 ml
Sodium	0.048
Potassium	0.143
Calcium	0.117
Magnesium	0.011
Chloride	0.110
Phosphate	0.230
Citrate	0.175
Sulfate	0.0100
Trace elements	
Constituents	µg/l
Zinc	4,000
Aluminum	500
Iron	400
Copper	120
Molybdenum	60
Manganese	30
Nickel	25
Silicon	1,500
Bromine	1,000
Boron	200
Fluorine	150
Iodine	60

blood. Milk is an important source of dietary calcium and its association with caseins may improve absorption in the gastrointestinal tract. Trace elements are elements of which not more than a trace is found in milk. Trace elements are natural components in milk. Some parts of the elements are likely to be associated with protein, whereas most of the other elements are dissolved. About 10 % of copper and nearly half of iron are associated with fat globule membrane.

Table 8 Selected enzymes in milk

Name	EC number	Location
5'-Nucleotidase	3.1.3.5	F
Acetylcholine esterase	3.1.1.7	F
Acid phosphatase	3.1.3.2	F
Adenosine triphosphatase	3.6.1.3	F
Alkaline phosphatase	3.1.3.1	F
Carboxylesterase	3.1.1.1	S
Catalase	1.11.1.6	L
Choline esterase	3.1.1.8	S
Glucose-6-phosphatase	3.1.3.9	F
Lactoperoxidase	1.11.1.7	S
Lactose synthase	2.4.1.22	S
Lipoprotein lipase	3.1.1.34	C
L-Lactate dehydrogenase	1.1.1.27	P
Lysozyme	3.2.1.17	S
NADH dehydrogenase	1.6.99.3	F
Pancreatic ribonuclease	3.1.27.5	S
Phosphodiesterase	3.1.4.1	F
Phosphoprotein phosphatase	3.1.3.16	P
Plasmin	3.4.21.7	C
Xanthine oxidase	1.1.3.22	F
α -Amylase	3.2.1.1	S
γ -Glutamyltransferase	2.3.2.2	F

C: casein micelle, F: fat-globule membrane, L: leucocytes, P: plasma, S: serum

Adapted from Belitz et al. (2009) with modification

Enzymes

Milk contains a great number (~60) of enzymes. The indigenous or native enzymes are synthesized by the secretory cells, others derived from blood. In addition, enzymes of microbial origin may also be involved. The latter may be present in microorganisms, secreted by the organisms (e.g., lipases and proteinases), or released after lysis (Walstra et al. 2006). The selected enzymes in milk are shown in Table 8.

Indigenous enzymes in milk have no specific function in milk but enter milk owing to peculiarities of the mechanisms involved in the secretion and excretion of milk constituents. They originated from blood, somatic cells, MFGM, and cell cytoplasm. Milk is not a homogeneous solution of enzymes. The indigenous enzymes in milk are found in or associated with fat globule membrane, casein micelles, milk serum, and somatic cells. Indigenous enzymes in milk are of either beneficial or undesirable. The enzymes like plasmin, catalase, and *N*-acetyl- β -D-glucosaminidase are considered as indices of animal health (e.g., mastitis), while alkaline phosphatase, lactoperoxidase, and γ -glutamyl transpeptidase are indicators for thermal history of milk. The extent of deterioration of milk products such as

proteolysis (by plasmin), hydrolytic rancidity (by lipoprotein lipase), oxidative rancidity (by xanthine oxidase), and modification of casein (by acid phosphatase) can be monitored by estimation of the enzymes. The lactoperoxidase and lysozyme have protective effects. During cheese ripening, various enzymes like xanthine oxidase, plasmin, acid phosphatase, and lipoprotein lipase are responsible for desirable changes (Fox 2003). Readers can get further detailed information from some of the excellent review papers such as (Fox and Kelly 2006a, b; Kelly and Fox 2006).

Lipases and Esterases

Lipases are enzymes that catalyze the hydrolysis (lipolysis) of triglycerides (triacylglycerols) of milk. Lipases act at the lipid–water interface of emulsions of long-chain, insoluble triglycerides. Due to lipolysis, free fatty acids, partial glycerides (mono- and diglycerides) and, in some cases, glycerol are formed. The free fatty acids are responsible for various off-flavors (e.g., rancidity) in milk and milk products. Lipoprotein lipase (LPL) accounts for most of the lipolytic activity in bovine milk. LPL is a glycoprotein with two N-linked oligosaccharides. It exists as a homodimer with a molecular mass of ~100 kDa. It is associated with casein micelles. LPL is inactivated during pasteurization. LPL exhibits positional specificity. It catalyzes the hydrolysis of fatty acids from the sn-1 and sn-3 positions of the triglyceride molecule. It can cause rancid off-flavors in milk products as well as responsible for depression of the steam frothing capacity of milk. The spontaneous and induced lipolysis occurred in milk. Spontaneous lipolysis is initiated by the simple act of cooling raw milk to <10 °C soon after it is taken from the cow, while induced lipolysis is initiated by physical damage to the MFGM, which allows the lipase access to the fat substrate (Deeth 2006).

Esterases are distinguished from lipases by their preference for soluble rather than emulsified ester substrates. The arylesterases (EC 3.1.1.7), cholinesterase (EC 3.1.1.8), and carboxylesterase (3.1.1.1) are some of the esterases found in milk. Arylesterase activity is high in colostrum and during mastitis, but it probably has no technological significance (Fox and Kelly 2006a).

Lactoperoxidase (EC 1.11.1.7)

Lactoperoxidase (LPO) is synthesized in the mammary gland. LPO is the most abundant enzyme in milk. LPO has 78,030 Da mass which includes sugars (8–10 % of the mass of the enzyme) and the heme group. It contains 612 amino acids. It is highly structured. It has 65 % β -structure, 23 % α -helix, and 12 % unordered structure. LPO binds a Ca^{+2} . LPO is constituting ~0.5 % of the total whey proteins (~0.1 % of total protein; 30 ppm). In the presence of low levels of hydrogen peroxide and thiocyanate, LPO exhibits very potent bactericidal activity. The combination of hydrogen peroxide, thiocyanate and LPO are collectively known as Lactoperoxidase-system (LP-system) (Fox and Kelly 2006a).

Xanthine Oxidoreductase (EC 1.13.22; 1.1.1.204)

Xanthine oxidoreductase (XOR) exists as two forms, XO (Xanthine oxidase) (EC 1.1.3.22) and xanthine dehydrogenase (XDH; 1.1.1.204), which can be interconverted by sulfhydryl reagents, and XDH can be converted irreversibly to XO by specific proteolysis. XOR is concentrated in the MFGM. It represents ~20 % of the protein of the MFGM (~0.2 % of total milk protein, ~120 ppm). XOR is a dimer of identical 146 kDa subunits, each containing ~1,330 amino acid residues; the subunits of the bovine milk enzyme contain 1,332 residues. Each XOR monomer contains one atom of Mo, one molecule of FAD⁺, and two Fe₂S₂ redox centers. XOR is considered as an indicator of milk heated in the temperature range 80–90 °C. XOR can excite stable triplet oxygen to singlet oxygen, which is a potent prooxidant. The H₂O₂ produced by the action of XOR can serve as a substrate for LPO in its action as a bactericidal agent. XOR reduces nitrate to nitrite, which is the bactericidal agent that can be useful in certain varieties of cheese to prevent late-gas blowing defect (Fox and Kelly 2006a).

Catalase (EC 1.11.1.6)

Catalases are heme-containing enzymes. Catalase catalyzes the decomposition of H₂O₂. The catalase activity in milk increases markedly during mastitis. The catalase is a useful indicator of mastitis. The molecular mass of 225 kDa is found using gel permeation technique. Catalase is relatively heat labile (Fox and Kelly 2006a).

Plasmin

Milk contains several indigenous proteinases, the principal of which is plasmin (EC 3.4.21.7). Milk contains the complete plasmin system which comprises of plasmin, plasminogen, plasminogen activators (PAs), and inhibitors of PAs and of plasmin. Bovine plasminogen is a single-chain glycoprotein containing 786 amino acid residues, with a calculated molecular mass of 88,092 Da. Plasmin is quite heat stable and partially survives ultrahigh-temperature (UHT) processing. Plasmin is highly specific for peptide bonds. The principal substrate for plasmin is β -casein in milk. Plasmin contributes to primary proteolysis in cheese, reduces the yield of cheese, and causes age gelation of UHT milk (Fox and Kelly 2006a).

Alkaline Phosphatase (EC 3.1.3.1) and Acid Phosphatase (EC 3.1.3.2)

Alkaline phosphatase is a membrane-bound glycoprotein. The enzyme is a homodimer of two identical subunits, each of molecular mass ~85 kDa; it contains four atoms of Zn which are essential for activity and is also activated by Mg⁺². It is optimally active at pH 10.5 and optimum temperature is ~37 °C. Alkaline phosphatase in milk is significant mainly because it is used as an index of HTST pasteurization.

Acid phosphatase is a glycoprotein with a molecular mass of ~42 kDa. Acid phosphatase is optimally active at pH 4.0 and is very heat-stable. The enzyme is not activated by Mg^{+2} but it is activated slightly by Mn^{+2} . Acid phosphatase is present in milk at a much lower level than alkaline phosphatase. Acid phosphatase is quite active on phosphoproteins (caseins) and hence it has been suggested that it is a phosphoprotein phosphatase. It has greater heat stability and lower pH optimum may make it technologically significant (Fox and Kelly 2006b).

Lysozyme (EC 3.1.2.17)

Lysozyme is also called *N*-acetylmuramidase or muramidase. Lysozyme is a hydrolyse-type enzyme that catalyzes the breakdown of peptidoglycan polymers of bacterial cell wall. The pH optimum is 6.35 and molecular weight is 18 kDa. The amino acid sequence of lysozymes is highly homologous with that of α -lactalbumin. Lysozymes are relatively stable to heat at acid pH values (3–4) but are relatively labile at pH > 7. Exogenous lysozyme may be added to milk for many cheese varieties, but at present, lysozyme is not used widely in commercial cheese making (Fox and Kelly 2006b).

γ -Glutamyltransferase (Transpeptidase) (EC 2.3.2.2)

γ -Glutamyltransferase (GGT) is found in the membrane material in skim milk or in the MFGM. The enzyme has been purified from the MFGM and has a molecular mass of ~80 kDa. It consists of two subunits which are glycoproteins. It is optimally active at pH 8.5–9. GGT is appropriate for monitoring heat treatments in the range of 70–80 °C for 16 s. GGT activity can also be used to distinguish breastfed from formula-fed infants (Fox and Kelly 2006b).

Vitamins

Milk is a source of the fat-soluble vitamins (A, D, E, and K). Vitamin A is a precursor of β -carotene which is responsible for the yellow color of cow milk. The water-soluble vitamins B₁, B₂, B₆, B₁₂, pantothenic acid, niacin, biotin, folic acid, and vitamin C are also present. During processing, the fat-soluble vitamins are retained by the cream, while the water-soluble vitamins remain in skim milk or whey. Milk contains all the vitamins in variable amounts which are shown in Table 9.

The detailed information on fat-soluble vitamins and water-soluble vitamins of milk can be found in Morrissey and Hill (2009) and Nohr and Biesalski (2009).

Vitamin A is present in cow's milk as retinol, retinyl esters, and carotenes. The content of vitamin A and β -carotene in cow's milk is 10–100 $\mu\text{g}/100\text{ g}$ (40 $\mu\text{g}/100\text{ g}$ as an average) and 3–50 μg (20 μg as an average), respectively. The levels of the

Table 9 Vitamin content of milk

Vitamin	mg/l
A (retinol)	0.4
D (calciferol)	0.001
E (tocopherol)	1.0
B ₁ (thiamine)	0.4
B ₂ (riboflavin)	1.7
B ₆ (pyridoxine)	0.6
B ₁₂ (cyanocobalamin)	0.005
Nicotinamide	1
Pantothenic acid	3.5
Biotin	0.03
Folic acid	0.05
C (ascorbic acid)	20

Adapted from Belitz et al. (2009)

fat-soluble vitamins and β -carotene in milk are highly dependent on the amount consumed in the feed. Vitamin D (mainly as vitamin D₃) is found in milk. Both ergo- and cholecalciferol-based compounds can be found in milk. The most important determinant of the vitamin D content of milk is sunlight exposure and seasonal variation. The concentration of vitamin E in animal products is usually low. The α -tocopherol levels vary between 0.2 and 0.7 mg/l in bovine milk. Colostrum contains about 1.9 mg/l of α -tocopherol. The concentration of vitamin E in milk appears to be dependent principally on the amount consumed by the cow. Milk is not a good dietary source of vitamin K, containing between 3.5 and 18 μ g/l as phyloquinone. The content of vitamin C in cow's milk varies from 1.65 to 2.75 mg/100 g (with an average of 2.11 mg/100 g). The concentration of vitamin C in cow's milk changes with season (Morrissey and Hill 2009).

Thiamine (vitamin B₁) is unstable and loses its biological activity in alkaline solutions (pH > 7) as well as in the presence of oxidants and radiation. The heat treatment, as well as storage conditions, can lead to losses of the thiamine content of milk and milk products. Riboflavin (vitamin B₂) is very heat stable but it is extremely photosensitive. Heat treatment has only negligible effects on riboflavin concentrations, whereas exposure of milk to sunlight results in a loss of 20–80 % of riboflavin. The photodegradation of riboflavin catalyzes photochemical oxidation and a loss of ascorbic acid. In cheese, most of the losses (66–88 %) of the original riboflavin of the milk appear to occur during whey drainage. Niacin (vitamin B₃) is stable to sunlight, various storage conditions, and heat treatments as used in dairy processing. Pantothenic acid (vitamin B₅) is highly hygroscopic and stable to heat and light. In milk, about 25 % of pantothenic acid is protein bound but this value rises to 40–60 % in cheese, depending on the type of cheese. Vitamin B₆ is sensitive to light and heat. In cow's milk, 14 % of vitamin B₆ is in the bound form and 86% in the free form. The loss of biotin (vitamin B₇) during processing or storage of food is generally small or negligible. Folates (vitamin B₉) are the most vulnerable vitamins as regards losses during processing and storage, as they are very susceptible to

oxidation, leakage, or enzyme activities. Cobalamin (vitamin B₁₂) is lost during high heat treatment. In cow's milk, the cobalamin content is very constant regarding feed, breed, season, or stage of lactation, except colostrum which has a very high level (Nohr and Biesalski 2009).

Factors Affecting the Composition of Milk

The composition and properties of fresh milk are not constant which is a challenging task for manufacturers of milk products. There are various factors which cause such variability. The main factors are (i) genetic factors (e.g., breed and individual), (ii) stage of lactation, (iii) health status of the animal, and (iv) environmental factors (e.g., climate, feed, method of milking). The list of the factors which are responsible for variation in composition of milk is given in Table 10.

1. *Species of animal*

Milk from all species contains the same kind of constituents but in varying amounts. The composition of milk of each species is designed in accordance with natural rate of growth of newborn mammals. The faster the rate of growth, the more concentrated are the milk components needed for this growth.

2. *Breed of animal*

There is a maximum variation in percentage fat content of milk due to breed variation. These differences are mainly due to the differences in milk production capacity of different breed of the animal.

3. *Individual variation within a breed*

Variation in composition of milk of the animal of the breed is due to the individuality of the animal.

4. *Breeding and crossbreeding*

Dairy cattle are usually selected and bred on the basis of their milk yielding capacity and fat-producing ability.

5. *Yield of milk*

A smaller yield gives milk with higher fat percentage, while larger yield gives milk with low fat percentage.

Table 10 Factors affecting the composition of milk

1. Species of animal	2. Breed of animal
3. Individual variation within a breed	4. Breeding and crossbreeding
5. Yield of milk	6. Age and number of lactations
7. Stage of lactation	8. Heat or estrum
9. Gestation	10. Infection of the udder
11. Intervals between milking	12. Variations during milking
13. Variability of milk from different quarters of udder	14. Excitement (frightening)
15. Administration of drugs and or hormones	16. Feeds and nutrition
17. Season and weather conditions	18. Exercise

6. *Age and number of lactations*

With increase in age and number of lactation of cow, there is a slight decrease in fat and SNF content of the milk.

7. *Stage of lactation*

In the initial and the last stage of lactation, there are profound changes in the composition of milk. Milk obtained immediately after calving (i.e., colostrum) has entirely different composition as compared to normal milk. During the last stage of lactation, there is a decrease in lactose content with concomitant increase in chloride content.

Colostrum (or post-parturient milk) is the milk (secretion) obtained from the lactating mammary gland immediately after calving. Colostrum of cow milk is richer in total solids, SNF, proteins (~20–21 %), and ash (~1.8 %) but poor in fat (~3.2–3.4 %) and lactose (~2.5 %). Colostrums contain very high amount of globulin which is most susceptible to heat and causes the colostrums to coagulate on heating. Colostrum is generally thick, viscous, and very slimy fluid with yellowish but sometimes slightly reddish in color. It has an abnormal odor and bitter taste. It is acidic in nature and acidity is ~0.3–0.6 % lactic acid.

8. *Heat or estrum*

There is usually much variation in the fat content of milk which may increase or decrease. These changes have been attributed to increased excitability and nervousness causing the cow to either hold up some of the milk or to secrete less milk.

9. *Gestation*

Gestation can affect the composition of milk in an indirect manner. An increase in milk solids, especially SNF, starts at about the fourth month of pregnancy and continues up to the end of the lactation.

10. *Infection of the udder*

Mastitis refers to the inflammation of udder. This disease is caused by different types of microorganisms. Microorganisms damage the tissue cells of the mammary gland and alter the composition and yield of secretory fluid (milk). The milk yield decrease ranges from 15 % to 33 % during mastitis. The principal effects are lowering of the concentration of fat, SNF, lactose, and casein and increasing the serum protein and chloride content. There is a decrease in casein content of the total milk protein with increase in whey proteins as well as nonprotein nitrogen. The high concentrations of phosphatase, xanthine oxidase, lipase, catalase, and cholinesterase have been observed. The pH of mastitic milk is slightly toward alkalinity.

11. *Intervals between milking*

A longer interval is associated with more milk, with a lower fat content.

12. *Variations during milking*

Total milking procedure can be divided into different parts like starting (i.e., fore-milking), middle, and end (i.e., stripping). Fat is the lightest constituent and hence accumulates in the upper part of the udder. The fore-milk has low fat, while stripping is rich in fat content.

13. *Variability of milk from different quarters of the udder*

The four quarters of the udder are separate units physiologically as well as anatomically. There are distinct differences among samples from different quarters of the udder. There are variations in gross composition as well as percentage of calcium and fat content.

14. *Excitement (Frightening)*

Cow holds up her milk that results in a lowered fat content during frightening.

15. *Administration of drugs and/or hormones*

Injection or feeding of hormones results in an increase of both milk yield and fat content. Moreover, certain drugs may affect temporary change in fat content.

16. *Feeds and nutrition*

Overfeeding does not cause consistent change in the composition of milk. Underfeeding, on the other hand, tends to reduce the yield of milk and its SNF content.

17. *Season and weather conditions*

With change in season, there will be a change in feed and weather that affects the percentages of both fat and SNF.

18. *Exercise*

Dairy cows are benefited by mild exercise. The feed consumption gets increased that leads to increase in fat percentage.

Fermented Milk Products

All sour milk products have undergone fermentation, which can involve not only lactic acid bacteria but also other microorganisms. Depending on the microorganisms involved, fermentation proceeds via the glycolysis pathway with the almost exclusive formation of lactic acid (homofermentation); via the pentosephosphate pathway with formation of lactic acid, acetic acid (ethanol), and possibly CO₂ (heterofermentation); or via both pathways. The formation of lactic acid is one of the important for lowering down the pH and affects the body and texture of the fermented dairy products. Moreover, the configuration of lactic acid formation depends on the microorganisms involved during fermentation. L-Lactic acid is metabolized easily by human, but high intake of D-lactic acid can result in enrichment in the blood and hyperacidity of the urine (Belitz et al. 2009). The total lactic acids in some dairy products are presented in Table 11.

Apart from the main products mentioned, various aroma substances are formed during fermentation. In addition, proteolytic and lipolytic processes occur to a certain extent. During proteolysis, peptides can be formed which have opiate activity and hypotensive, immune-stimulating, or antimicrobial effects (Belitz et al. 2009).

Table 11 Total lactic acid and L-lactic acid in selected dairy products

Fermented products	Total lactic acid (%)	L-Lactic acid (%)
Sour milk	0.97	88
Buttermilk	0.86	87
Sour cream	0.86	96
Yogurt	1.08	54
Curd	0.59	94
Cottage cheese	0.34	92
Emmental	0.27	76
Tilsit cheese	1.27	52

Adapted from Belitz et al. (2009)

Sour Milk

Sour milk is the product obtained by the fermentation of milk, which occurs either by spontaneous souring caused by various lactic acid-producing bacteria or an addition of mesophilic microorganisms into lactic acid, which coagulates casein at pH 4–5. The thick, sour-tasting curdled milk is manufactured from whole milk (at least 3.5 % milk fat), low-fat milk (1.5–1.8 % fat), or skim milk (at most 0.3 % fat), often by blending with skim milk powder to increase the total solid content and to improve the resultant protein gel structure. Sour milk contains 0.5–0.9 % of lactic acid (Belitz et al. 2009).

Dahi

Dahi is a product obtained by lactic fermentation of milk and one of the popular fermented dairy products of Indian subcontinent. Dahi is accepted as a dietary adjunct of high nutritive and therapeutic as well as medicinal values. In Ayurveda, an Indian system of medicine, dahi had been strongly used for curing ailments like dyspepsia, dysentery, and other gastrointestinal disorders. Dahi resembles plain yogurt in appearance and consistency and differs in having lesser acidity and use of different starter organisms in its manufacture. The composition of dahi depends upon the type of milk used, manufacturing conditions, and starter cultures used. With the exception of the conversion of part of lactose into lactic acid and small loss of water, there is no appreciable change in major constituents. The composition of dahi varies more or less with that of milk from which it is made. The water varies from 85 % to 88 %, fat, 5–8 %; protein, 3–3.4 %; lactose, 4.2–5.1 %; lactic acid, 0.5–1.1 %; ash, 0.7–0.73 %; calcium, 0.12–0.15 %; and phosphorus, 0.09–0.11 %, in dahi that can be prepared from whole milk (Shivashraya 2014a).

Lassi

Lassi (stirred dahi/yogurt) is a traditional beverage having its origin in India. Lassi is a ready-to-serve fermented milk beverage. It is a nutritious, refreshing, delicious,

and easily digestible beverage. Lassi is obtained as a by-product during the churning of dahi while making desi butter (i.e., *makhan*). Mild acidic flavor and sweetish taste of lassi make it a refreshing soft drink. The composition of lassi varies considerably since there is no standard method available for the preparation of this product. The composition of lassi has water 95–96 %, fat 0.6–0.8 %, protein 1.10–1.35 %, lactose 1.1–1.5 %, lactic acid 0.42–0.48 %, ash 0.38–0.43 %, calcium 0.60 %, and phosphorus 0.04 % (Shivashraya 2014a).

Shrikhand

It is semisolid, sweetish-sour fermented Indian milk product. Shrikhand is prepared by fermentation of milk with lactic acid bacteria and expulsion of whey from the curd, followed by mixing with sugar, flavoring, and spices. The proximate composition of this product is moisture 34–40 %, sugar (sucrose) 43–45 %, fat 4–6 %, and milk solids-not-fat 10–12 %, protein 3.6–3.9 %, ash 0.72 %, soluble nitrogen 0.21–0.29 %, free fatty acid 0.26–0.55 % oleic acid, titratable acidity 0.9–0.95 % lactic acid, and pH 4.4–4.6 (Shivashraya 2014a).

Yogurt

Yogurt cultures consist of thermophilic lactic acid bacteria that live together symbiotically (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). Incubation is conducted on addition of 1.5–3 % of the operating culture at 42–45 °C for about 3 h. The final product has a pH value of about 4–4.2 and contains 0.7–1.1 % of lactic acid. An essential part of the specific yogurt aroma comes from carbonyl compounds, predominantly acetaldehyde and diacetyl. In addition to 1-octen-3-one, 1-nonen-3-one has also been detected as an important odorant, which has an exceptionally low odor threshold (Belitz et al. 2009).

Kefir and Kumiss

Kefirs and kumiss are sparkling, carbonated, alcoholic beverages. Kefir contains lactic acid (0.5–1.0 %), noticeable amounts of alcohol (0.5–2.0 %), carbon dioxide, and some products of casein degradation resulting from proteolytic action of yeast. Normal kumiss contains 1.0–3.0 % of alcohol (Belitz et al. 2009).

Cheese

Cheese is the fermented milk-based food product. Preparation of cheese involves a complex process in which interdependent chemical, biochemical, and microbiological changes are involved. Cheese preparation is essentially a dehydration process

in which casein, fat, and colloidal minerals of milk are concentrated to 6–12-fold with removal of 90 % of water in milk and essentially all the whey proteins, lactose, and soluble milk salts. Generally cheese curd is obtained by coagulating the casein fraction of milk protein by acidification to about pH 4.6 or by limited proteolysis using enzymes (rennet).

There are many varieties (more than 1,000) of cheese and some varieties are consumed immediately after manufacture as fresh cheese, most undergo a subsequent period of aging or ripening, ranging from weeks to years depending on the variety (Kindstedt 2013). The main international varieties are Cheddar, Mozzarella, Gouda, Camembert, Grana types, Emmental, and Quark. Classification of cheese is extremely complicated. The International Dairy Federation reported the various cheese varieties under different heads like country of origin; raw milk (cow, buffalo, sheep, goat, etc.); type of cheese (hard, semihard, soft, fresh, acid coagulated, or whey cheese); internal characters (close or open texture; small, medium, or large eyes/holes; slit openings in curd; blue or white mold ripened; color of curds); external characters (rind hard, soft, smooth, or rough, smear or mold ripened, spices or herbal additions, type of coating); weight of cheese (shapes and sizes); fat-in-dry matter/fat-on-dry basis (minimum or maximum percentage); water (maximum percentage); and water-in-fat free substances/moisture-in-fat free substances. Most of the cheese varieties may be classified according to moisture content and according to the means by which any ripening is achieved (Varnam and Sutherland 2001) which is mentioned below.

1. Hard (26–50 % moisture)
 - (a) Internally ripened, no added ripening microorganism, e.g., Parmesan, Cheddar, Double Gloucester
 - (b) Internally ripened, added ripening bacteria, e.g., Emmental
 - (c) Internally ripened, secondary ripening by mold, e.g., Blue Cheshire
2. Semihard (42–52 % moisture)
 - (a) Internally ripened, no added ripening microorganism, e.g., Lancashire, Edam
 - (b) Internally ripened, ripening mold added, e.g., Stilton, Roquefort
3. Semisoft (45–55 % moisture)
 - (a) Surface ripened, ripening bacteria added, e.g., Limburger, Port du Salut
4. Soft (48–80 % moisture)
 - (a) Surface ripened, ripening mold added, e.g., Brie, Camembert
 - (b) Unripened, e.g., Cottage, Coulommier
5. Others, e.g., brined varieties, whey cheese

Chemical components of cheese are derived from milk components, other ingredients such as cultures and enzymes, and environmental contaminants. The diversity of chemical components is determined by ripening agents such as endogenous milk enzymes, milk coagulants, added proteases and lipases, enzyme systems of both starter and nonstarter bacteria, and other microflora (Hill 1995). The main objectives for cheese maker are to develop basic structure and optimum

composition (i.e., optimum levels of moisture, fat, pH, minerals (especially calcium), and salt) of cheese and obtain cheese composition required for optimum microbial and enzyme activity during curing. The acid production by bacterial cultures is essential to develop basic structure, composition, as well as cheese flavor and texture for most of the cheeses. Variations in the early stages of cheese making relate to different methods of controlling acid development and removal of water and lactose (whey) by curd syneresis. Special treatments and cultures such as surface ripening, eye forming bacteria, or alternative means of salting provide distinctive characteristics of many cheese varieties, but the basic composition and structure of cheese is mainly determined in the early manufacturing stages, especially up to the point of whey separation (Hill 1995). Most cheese varieties can be grouped into several categories according to the type of coagulation and procedures used for pH and moisture control. The compositions of some common varieties are given in Table 12.

Cheese composition depends on milk composition and on numerous process variables. The water, fat, and salt content of cheeses can be varied over a wide range. For several cheese varieties, legal standards may apply, such as maximum water content and a minimum and maximum fat content in the dry matter. The fat content is generally calculated on the dry matter because the water content of the cheese can change considerably during storage. Similar standards may apply to salt content. The composition of cheese tends to vary between batches and between cheese loaves within one batch, within a load, with aging time (Walstra et al. 2006).

During cheese making, partly lactose is washed out with whey. The rest is fermented into lactic acid, diacetyl, acetaldehyde, acetic acid, ethanol, and CO₂ during ripening. Cheese is a source of proteins. It provides all essential amino acids except methionine and cysteine. During proteolysis various bioactive peptides are formed. Bioactive peptides possess various biological activities that include blood pressure-lowering, anticarcinogenic, antiinflammatory, mineral binding, opioid, antimicrobial, cell-modulating, immunomodulating, anticariogenic, antithrombotic, and cholesterol-lowering activities. Another main component of cheese is fat. It varies between 20 % and 35 % of the dry mass. The fat does not change during ripening (except mold-ripened cheese). It has 60 % saturated, 23.5 % monounsaturated, and 4.6 % polyunsaturated fatty acids of the total milk fat. The palmitic acid is the most common saturated fatty acid that can contribute about 26 % of fat, while myristic acid and stearic acids contribute 9.8 % and 8.0 % of fat, respectively. The rest of the saturated fatty acid varies from 0.02 % to 3.1 % of fat. The oleic acid (16.5 % of fat) is the most common unsaturated fatty acid. Cheese is a good source of calcium. The calcium content in semihard and hard cheese varies from about 0.6 % to 1.1 %. Cheese is also a good source of phosphorus, zinc, and magnesium (Walther et al. 2008).

Several flavor compounds are found in cheeses. The flavor compounds in *Cheddar cheese* (e.g., isovaleric acid, 3-methylbutanal, methional, methanethiol, dimethyltrisulfide, propionic acid, diacetyl, butyric acid, acetic acid, 1-octen-3-one, butanone, ethyl butyrate, and ethyl hexanoate), *Gouda cheese* (3-methylbutanal, 3-methylbutanol, methanethiol, dimethylsulfide, 2-methylpropanol, dimethyltrisulfide, diacetyl, butyric acid, butanon, hexanal, pentanal, ethyl butyrate, limonene),

Table 12 Chemical composition (% by weight) of selected cheese varieties

Type	Cheese	Moisture	Protein	Total fat	Total carbohydrate	Fat in dry matter	Ash	Calcium	Phosphorus	Salt
Acid coagulated	Cottage (dry curd)	79.8	17.3	0.42	1.8	2.1	0.7	0.03	0.10	Nil
	Creamed cottage	79.0	12.5	4.5	2.7	21.4	1.4	0.06	0.13	1.0
	Quark	72.0	18.0	8.0	3.0	28.5	—	0.30	0.35	—
	Cream	53.7	7.5	34.9	2.7	75.4	1.2	0.08	0.10	0.73
	Neufchatel	62.2	10.0	23.4	2.9	62.0	1.5	0.07	0.13	0.75
Heat-acid coagulated	Chhana	53.0	17.0	25.0	2.0	53.2	—	—	—	—
	Queso blanco (acid)	55.0	19.7	20.4	3.0	44.8	—	—	—	3.0
	Ricotone – from 3 % fat milk	72.2	11.2	12.7	3.0	45.7	—	—	—	<5.0
	Ricotone – from whey and milk	82.5	11.3	0.5	1.5	2.9	—	—	—	<5.0
	Queso blanco-rirennet	52.0	23.0	20.0	—	42.0	—	—	—	2.5
Unripened-rirennet coagulated	Queso de freir	52.4	23.0	19.5	—	41.0	—	—	—	3.0
	Italian fresh cheese	49.0	28.0	16.0	—	31.4	—	—	—	Nil
Soft ripened high acid	Camembert	51.8	19.8	24.3	0.5	50.3	3.7	0.39	0.35	2.1
	Feta	55.2	14.2	21.3	—	47.5	5.2	0.49	0.34	—
	Blue	42.0	21.0	29.0	2.3	50.0	5.1	0.53	0.39	3.5
	Gorgonzola	36.0	26.0	32.0	—	50.0	5.0	—	—	—

Semihard washed	Colby	40.0	25.0	31.0	2.0	51.7	3.4	0.68	0.46	0.65
	Gouda	41.5	25.0	27.4	2.2	46.9	3.9	0.70	0.55	0.82
	Edam	41.4	25.0	27.8	1.4	47.6	4.2	0.73	0.54	0.96
	Fontina	42.8	24.2	25.5	—	44.6	3.3	—	—	1.2
	Havarti-Danish	43.5	24.7	26.5	—	46.9	2.8	—	—	2.2
	Munster	41.8	23.4	30.0	1.1	51.6	3.7	0.72	0.47	1.8
	Cheddar	36.7	24.9	33.1	1.3	52.4	3.9	0.72	0.51	1.8
	Manchego – Spain	37.9	28.1	26.9	—	45.2	3.6	—	—	1.5
	Provolone	40.9	25.6	26.6	2.1	45.1	4.7	0.76	0.50	2.2
	Mozzarella	54.1	19.4	21.6	2.2	47.1	2.6	0.52	0.37	1.0
Hard cheese high temp.	Parmesan	29.2	35.7	25.8	3.2	36.5	6.0	1.18	0.69	3.0
	Romano	30.9	31.8	26.9	3.6	39.0	6.7	1.06	0.76	3.0
	Swiss	37.2	28.4	27.4	3.4	43.7	3.5	0.96	0.60	1.2
	Kefalotyri – Greece	34.2	24.8	28.3	—	—	4.7	—	—	—

Adapted from Hill (1995)

Swiss type (methional, 3-methylbutanal, skatole, propionic acid, diacetyl, ethyl butyrate, ethyl hexanoate, ethyl-3-methylbutanoate, phenylethyl acetate), and *Camembert* (3-methylbutyrate, 3-methylbutanal, methional, methanethiol, benzaldehyde, phenylacetaldehyde, 2,3-butanedione, 1-octen-3-ol, butyric acid, 1-octen-3-one, 2-undecalactone, γ -decalactone, phenylethyl acetate) are the metabolism products produced from amino acids, sugar, fat, and other pathways during ripening of cheese (Smit et al. 2005).

Concentrated and Dried Milks

Concentrated milks can be made by partial removal of water. The main purpose is to diminish the volume and to enhance the shelf life of the products. The concentrated milk products give considerable saving in the cost of packaging, storage, and transportation. Water is removed by evaporation under vacuum, i.e., lower temperature, to prevent damage caused by heating. The basic principle underlying the production of concentrated milk is that milk of high quality is filtered or clarified, standardized, forewarmed (i.e., preheated), and condensed to the desired level. Preservation is achieved either by sterilization (i.e., evaporated milk) or addition of large quantity of sucrose (i.e., sweetened condensed milk). The proximate composition of concentrated milk products is shown Table 13. Moreover, the mineral compositions of concentrated milk products are also shown in Table 14.

Evaporated Milk

Evaporated milk is concentrated milk which can be homogenized and sterilized. The product can be kept without refrigeration. Milk can be concentrated up to 2.6 times, beyond that the heat stability of milk will be affected. The milk is first heated, e.g., to 120 °C for 3 min, to kill unwanted microorganism and reduce the danger of delayed thickening as well as improve the heat stability of milk. The evaporation is carried out in a continuously operated vacuum evaporator at 40–60 °C. The film evaporators are widely used. Several units (up to seven stages) are usually connected in series, each unit being heated by the vapor from the previous stage. The temperature and pressure decrease from stage to stage. Fat separation is prevented by homogenization at 40–60 °C (12.5–25 MPa). The resulting evaporated milk, with a solid content of 24–31 % or more, is homogenized, poured into lacquer (enamel)-coated cans, and is sterilized in an autoclave at 115–120 °C for 20 min. Continuous flow sterilization followed by aseptic packaging is also used. To prevent coagulation during processing and storage, Na-hydrogen carbonate, disodium phosphate, and trisodium citrate are incorporated into the condensed milk. These additives have a dual effect: pH correction and adjustment of free Ca^{+2} ion concentrations, both aimed at preventing casein aggregation and improving the heat stability. The additives are in the range of 0.02–0.08 % (Belitz et al. 2009).

Table 13 Proximate composition (%) of concentrated and dried milk products. Adapted from Deeth and Hartanto (2009). Modified and reproduced with permission from Wiley-Blackwell Publishing Ltd (A John Wiley & Sons Ltd), UK

Products	%				
	Water	Fat	Protein	Carbohydrate	Ash/ minerals
Concentrated milks					
Evaporated whole milk					
American standard	72.7–74.7	7.5–8.0	6.5–7.1	9–10	1.3–1.6
British standard	67–69	9–10	8–9	11–12.5	1.9–2.1
Evaporated skim milk	79.5	0.3	7.6	11	1.6
Sweetened condensed milk	27	9	8	55	1.8
Sweetened condensed skim milk	28	0.3	10	59	2.3
Milk powders					
Whole milk powder	2–4	25–28	25–27	37–38	6–7
Skim milk powder	3–5	0.7–1.3	35–37	49–52	7.5–8.0
Buttermilk powder	2.8–3.8	3–6	33–36	47–49	7–8
Cream powder	2.6–3.0	55–70	12–15	13–24	2.0–3.5
Milk and whey protein powders					
MPC 42	3.5	1.0	42	46.0	7.5
MPC 70	4.2	1.4	70	16.2	8.2
MPC 75	5.0	1.5	75	10.9	7.6
MPC 80	3.9	1.8	80	4.1	7.4
MPC 85	4.9	1.6	85	1.0	7.1
High milk protein powder	5.3	2.3	88	0.7	7
Caseinate (Ca, K, Na)	3–5	0.9–1.5	89–95	0.2	3.3–5
Casein (acid)	9.5	0.8	97	0.1	1.8
Casein (rennet)	9.5	0.8	90.5	0.1	8.5
Low-protein WPC	4.6	2–4	34–36	44–53	7–8
Medium-protein WPC	4.3	5	53	35	7
High-protein WPC	3–4	4–6	59–65	21–22	3.5–4
Very high-protein WPC	4–5	0.3–7.0	72–81	2–13	2.5–6.5
Whey protein isolate	2.5–6	0.1–0.7	89–93	0.1–0.8	1.4–3.8
Fractionated whey proteins					
α -Fraction	4.5	1.0	81.5	7	3.4
β -Fraction	4.5	0.4	87	0.5	3.0
Milk/whey protein hydrolysates	4	5	81.5	3	4.5
Whey powders					
Whey powder (acid)	≤ 3.5	0.8	9–12	65–69	11–12
Whey powder (sweet)	3–6	0.8–1.5	12–13	70–73	7.5–8.5
Whey powder (demineralized)	≤ 3	≤ 1.5	≥ 11	78–82	≤ 4

(continued)

Table 13 (continued)

Products	%				
	Water	Fat	Protein	Carbohydrate	Ash/ minerals
Whey powder (demineralized)	≤3	≤1.5	≥11	80–84	≤1.5
Whey powder (deproteinized)	3	0.2–1	2.5	80–85.5	8.5–10
Whey powder (lactose reduced)	2–3	1–4	18–25	40–60	11–27
<i>Miscellaneous products</i>					
Lactose (food grade)	0.5	0.1	0.1	99	0.1–0.3
Infant formula	2–3	26–39	10–18	40–60	8

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The CODEX Standard described the evaporated milks as milk products which can be obtained by the partial removal of water from milk by heat or by any other process which leads to a product of the same composition and characteristics. The fat and/or protein content of the milk may have been adjusted, only to comply with the compositional requirements of this Standard, by the addition and/or withdrawal of milk constituents in such a way as not to alter the whey protein to casein ratio of the milk being adjusted. The standards for composition of evaporated milks are shown in Table 15.

Sweetened Condensed Milk

Sweetened condensed milk is milk that is concentrated by evaporation, to which sucrose is added to form an almost saturated sugar solution, after which it is packed. The high sugar concentration is primarily responsible for keeping the quality of the product and for its fairly long shelf life, but once the container (can) has been opened, the product eventually become moldy. In the production of sweetened condensed milk, after a preheating step (short-time heating at 110–130 °C), sucrose is added to a concentration of 45–50 % of the weight of the end product. Homogenization and sterilization steps are omitted. To avoid graininess caused by lactose crystallization – the solubility limit of lactose is exceeded after sucrose addition – the condensed milk is cooled rapidly and then seeded with finely pulverized α -lactose hydrate. Seeding ensures that the lactose crystal size is 10 μm or less (Belitz et al. 2009).

Codex standards described the sweetened condensed milk are milk products which can be obtained by the partial removal of water from milk with the addition of sugar, or by any other process which leads to a product of the same composition and characteristics. The fat and/or protein content of the milk may have been adjusted, only to comply with the compositional requirements of this Standard,

Table 14 Mineral composition of concentrated and dried milk products. Adapted from Deeth and Hartanto (2009). Modified and reproduced with permission from Wiley-Blackwell Publishing Ltd (A John Wiley & Sons Ltd), UK

Products	Sodium (mg/100 g)	Potassium (mg/100 g)	Calcium (mg/100 g)	Phosphorus (mg/100 g)	Total ash (g/100 g)
<i>Concentrated milks</i>					
Evaporated whole milk	100–108	300–368	255–263	220–247	1.5–1.7
Evaporated skim milk	91–110	324–330	246–290	190	1.5–1.7
Sweetened condensed whole milk	105–130	357–402	268–300	240–250	1.8
Sweetened condensed skim milk	125–130	445–475	335–340	230–280	2.3
<i>Milk powders</i>					
Whole milk powder	310–400	1,157–1,300	875–910	800	5–6
Skim milk powder	428–530	1,603–1,790	1,183–1,260	970–1,103	7.9–8.5
<i>Casein and whey protein powders</i>					
Ca-caseinate	50–100	100	1,000–1,500	800	3.5–4.5
K-caseinate	60	1,650	300	800	3.3–4.0
Na-caseinate	1,200–1,300	20	100	800	3.3–4.0
Casein (acid)	100	–	80	900	2.2
Casein (rennet)	20	30	300	1,500	7.5
Low-protein WPC (~35 g/100 g)	460	1,190	480	500	5.7–7.8
High-protein WPC (~65 g/100 g)	280	650	350	330	3.5–3.9

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by the addition and/or withdrawal of milk constituents in such a way as not to alter the whey protein to casein ratio of the milk being adjusted. The standards for composition of sweetened condensed milks are shown in Table 15.

Dried Milks

Removal of water to such a low level that the product becomes solid (dust) like is called drying. Drying is usually applied to make a product that is easy to handle and, after reconstitution with water, is very similar in properties to the original material. The resulting powders are generally in the glassy state. Drying is achieved by vaporization of water, usually from concentrated milk. Drying is applied to products

Table 15 Standards for composition of evaporated and sweetened condensed milks

Constituents	Evaporated milk	Evaporated high-fat milk	Evaporated skim milk	Evaporated partly skim milk	Sweetened condensed milk	Sweetened condensed high-fat milk	Sweetened condensed skim milk	Sweetened condensed partly skim milk
Milk fat	Min. 7.5 % (m/m)	Min. 15 % (m/m)	Max. 1 % (m/m)	More than 1 % and less than 7.5 % (m/m)	Min. 8 % (m/m)	Min. 16 % (m/m)	Max. 1 % (m/m)	More than 1 % and less than 8 % (m/m)
Milk solids ^a	Min. 25 % (m/m)	–	Min. 20 % (m/m)	Min. 20 % (m/m)	Min. 28 % (m/m)	–	Min. 24 % (m/m)	Min. 24 % (m/m)
Milk protein in milk solids- not-fat ^a	Min. 34 % (m/m)	Min. 34 % (m/m)	Min. 34 % (m/m)	Min. 34 % (m/m)	Min. 34 % (m/m)	Min. 34 % (m/m)	Min. 34 % (m/m)	Min. 34 % (m/m)
Milk solids- not-fat ^a	–	Min. 11.5 % (m/m)	–	–	–	Min. 14 % (m/m)	–	Min. 20 % (m/m)
Sugar	–	–	–	–	–	–	–	–

CODEX Standard 281-1971 (for evaporated milks) and CODEX Standard 282-1971 (for sweetened condensed milks)

^aThe milk solids and milk solids-not-fat content include water of crystallization of the lactose

For all sweetened condensed milks, the amount of sugar is restricted by good manufacturing practice to a minimum value which safeguards the keeping quality of the product and a maximum value above which crystallization of sugar may occur

Table 16 Proximate composition (%) of selected dried products

Powders	%					
	Fat	Casein	Other proteins	Lactose	Ash	Water
Whole milk	26	19.5	5.3	38	6.3	2.5
Skim milk	1	27	6.6	51	8.5	3
Whey	1	0.6	8.5	72–74	8	3
Sweet cream buttermilk	5	26	8	46	8	3

like milk, skim milk, whey, infant formulas, cream, ice cream mix, and protein concentrates (Walstra et al. 2006). Some special dried products are rich in proteins that are known as milk protein concentrate (MPC) and whey protein concentrate (WPC) used as functional/nutraceutical foods.

The main drying process used is spray drying. However, drum drying (with and without vacuum) and fluid-bed drying (foaming with inert gas N₂ or CO₂) are used for special purposes. Using film evaporating systems, the milk is first pre-concentrated to 30–55 % solids. In drum drying, the liquid (30–40 % solids) is applied in a thin layer to a heated drying cylinder (100–130 °C) and, after a defined residence time (rotation, 2–3 s), removed with a scraping knife. The liquid film can be applied in various ways. In drum drying, relatively large particles are obtained. The thermal exposure (temperature, time) is considerably higher than in spray drying, which is consequently preferred. The solubility is poor due to the denaturation of whey proteins. The product is clearly brown owing to the Maillard reaction. In spray drying, the milk concentrate (30–55 % solids) is finely dispersed in the spray tower by centrifugal atomization or by nozzle atomization and dried with hot air (150–220 °C) concurrently or countercurrently. The water content drops to 6–7 % in 0.5–1 s. A further decrease to 3–4 % is achieved by after drying in a vibration fluid bed with hot air (130–140 °C). Particles with a diameter in the range of 5–100 µm consist of a continuous mass of amorphous lactose and other low-molecular components, which includes fat globules, casein micelles, whey proteins, and usually vacuoles. When the powder absorbs water, lactose crystallizes at aw >0.4, causing agglomeration. During drying, the temperature of the particles normally does not rise above 70 °C. Therefore, the whey proteins do not denature and remain soluble. Many enzymes are still active. Storage problems are caused by the Maillard reaction and by fat oxidation in the case of fat-containing powders. Foam-dried products can have excellent properties (aroma, solubility). Other dehydrated dairy products, in addition to whole milk or skim milk powders, are manufactured by similar processes. Products include dehydrated malted milk powder, spray- or roller-dried creams with at least 42 % fat content of their solids and a maximum 4 % moisture, and butter or cream powders with 70–80 % milk fat. Dehydrated buttermilk and lactic acid-soured milk are utilized as children's food (Belitz et al. 2009). The proximate composition of selected dried products is shown in Table 16.

Readers are advised to refer to the excellent book chapter written by Deeth and Hartanto (2009) for chemical components of concentrated and dried milk products. The proximate composition of dried milk products is shown in Table 13. Moreover, the mineral compositions of dried milk products are also shown in Table 14.

Table 17 Composition of some varieties of cream (%)

Constituents	Whipped cream	Coffee cream	Plastic cream
Water	56–58	78–81	18.0–18.5
Fat	35–38	10–12	75–80
Protein	1.8–2.2	3.0–3.2	0.6–0.8
Lactose	2.2–3.0	4.2–4.7	1.0–1.2
Ash	0.5–0.6	0.70–0.72	0.1–0.2

Fat-Rich Dairy Products

Fat-rich dairy products such as cream, *makhan* (freshly churn butter), butter, butter oil, and ghee have played an important role in dairy industry. Buffalo milk is better suited for the manufacture of fat-rich dairy products as compared to cow milk due to its higher fat, bigger size of fat globules, and higher proportion of solid fat leading to higher yield, lesser loss of fat in buttermilk or skim milk, and better texture. *Malai* (cream) is a traditional Indian delicacy and symbol of richness. A form of white-clotted cream, malai is formed as a thick layer/skin of fat and coagulated proteins on the surface of the milk. It should contain a minimum of 25 % milk fat. The malai is used to make butter and ghee. Cream is another term which is associated with a premium milk fat product. Cream is generally part of milk in which the milk fat is concentrated into a fraction of the original milk, either as a result of standing or by means of centrifugal force. Cream consists of fat, together with the decreased proportion of other solids and the water of milk. Cream is a typical oil-in-water emulsion. Cream having 10–18 % fat content referred to as half cream or coffee cream and 35–40 % fat content is usually considerably thicker (or whipped cream whipped into a thick froth). The low-fat cream contains fat in the range of 25–39 % (e.g., table cream, light cream, whipping cream). Medium-fat cream contains 40–59 % fat (e.g., heavy cream), and high-fat cream contains >60 % fat (e.g., plastic cream) (Shivashraya 2014b). The compositions of some varieties of cream are shown in Table 17.

The characteristic flavor and aroma of cream are derived primarily from constituents of the fat phase, although there is also a contribution from constituents of the aqueous phase and the milk fat globule membrane. Alkanoic acids (C_{10,12}), δ -lactones (C_{8,10,12}), indole, skatole, dimethyl disulfide, and hydrogen sulfide, at the levels commonly present in cream, are considered to contribute to the desired flavor, there being a marginal contribution from phenol and phenolic compounds such as *o*-methoxyphenol. Oxidation during whipping may improve flavor, and 4-*cis*-heptanol, if present, contributes to the full flavor (Varnam and Sutherland 2001). The detailed information on other fat-rich dairy products mainly butter, butter oil, and ghee can be found in ► [Chap. 13, “Chemical Composition of Fat and Oil Products”](#) in this book.

Ice Cream

Ice cream is a frozen dairy product made by freezing a mix with agitation, to incorporate air and ensure uniformity and consistency. Ice cream contains whole milk, skim milk products, cream or butter, sugar, vegetable oil, fruit and fruit ingredients, coffee, cocoa, aroma substances, and approved food colors. Ice cream is comprised of a mixture of air, water, milk fat or nondairy fats, milk solids-not-fat (MSNF), sweeteners, stabilizers, emulsifiers, and flavors. An ice cream mix is the unfrozen blend of the ingredients used to supply these constituents, except the air and flavoring materials. Mix formulations are defined as percentages of the constituents, e.g., percentage of fat, MSNF, sugars, stabilizers, and emulsifiers (the sum of which equals the total solids). These components can be combined in varying proportions within acceptable ranges. The composition of ice cream varies in different countries and in different localities and markets within each country. The best ice cream composition for a manufacturer to produce is often difficult to establish. Consideration must be given to legal requirements, quality of product desired, raw materials available, plant equipment and processes, trade demands, competition, and cost. The milk fat content of ice cream may vary from less than 1 % to 20 %, depending upon such factors as regulations, expected characteristics, price, and competition. Within the ice cream category, usually at >8–10 % fat, as the fat content of ice cream is increased, the MSNF must be decreased so as to avoid high viscosity and the potential for “sandiness” (i.e., the crystallization of milk sugar or lactose in the finished ice cream) (Goff and Hartel 2013). The approximate compositions of commercial ice cream are shown in Table 18.

The structural elements of ice cream are ice crystals (~50 μm), air bubbles (60–150 μm), fat globules (<2 μm), lactose crystals (20 μm in length), and aggregated fat globules (5–10 μm). The fat is mostly attached to the air bubbles. The air bubbles have a threefold function: they reduce the nutritional value, soften the product, and prevent a strong cold sensation during consumption (Belitz et al. 2009). The aqueous layer forming thin lamellae between air cells (mean thickness 9 μm) and even thinner layers between ice crystals also carries some fat clumps besides the casein micelles. This structure is believed to be achieved, as a result of partial destabilization of ice cream emulsion.

Traditional Indian Dairy Products

Traditional Indian dairy products represent the most prolific segment of Indian dairy industry. These products are also getting popular in various countries. Some of these products like heat-desiccated products (e.g., khoa, khoa-based sweets, *rabri*, *basundi*), heat- and acid-coagulated products (e.g., paneer, *chhana*), and fat-rich dairy products (e.g., desi butter, ghee) are widely manufactured. The detailed information on traditional dairy products can be found in Aneja et al. (2002) and Shivashraya (2014b).

Table 18 Approximate composition (% by wt.) of commercial ice cream by formulation category

Group	Milk fat	Milk solids-not-fat	Sweeteners ^a	Stabilizers ^b and emulsifiers	Total solids
Nonfat ice cream	<0.5	12–14	18–22	1.0	28–32
Low-fat ice cream	2–5	12–14	18–21	0.8	28–32
Light ice cream	5–7	11–12	18–20	0.5	30–35
Reduced-fat ice cream	7–9	10–12	18–19	0.4	32–36
Economy ice cream	10	10–11	15–17	0.4	35–36
Standard ice cream	10–12	9–10	14–17	0.2–0.4	36–38
Premium ice cream	12–14	8–10	13–16	0.2–0.4	38–40
Super premium ice cream	14–18	5–8	14–17	0–0.2	40–42

Adapted from Goff and Hartel (2013) with modification

^aIncludes sucrose, glucose, corn syrup solids, maltodextrins, polydextrose, and other bulking agents, some of which contribute little sweetness

^bIncludes ingredients such as locust bean gum, guar gum, carrageenan, cellulose gum, and cellulose gel, as stabilizers, and also mono- and diglycerides and polysorbate 80 as emulsifiers

Heat-Desiccated Products

Khoa is an important traditional dairy product obtained by partial dehydration of milk. It is conventionally prepared by continuous boiling of milk in an open kettle until desired desiccation (normally 72–75 % total solids) and texture is achieved. Generally, in khoa no sugar is added from outside. Khoa is a versatile intermediate base for wide varieties of sweets. The addition of sugar and other ingredients is required to prepare khoa-based sweets like burfi, peda, gulab jamun, kalakand, milk cake, and other products. Rabri and basundi are concentrated liquid forms of milk solids. Kalakand has more distinct cooked flavor, brown color, greasy or moist body, and grainy texture as compared to burfi. Rabri and basundi have added sugar to sweeten the product for direct consumption (Shivashraya 2014b). The chemical composition of market-prepared heat-desiccated milk products is shown in Table 19. This wide variability in compositions is due to method of preparation, locality, and preference for typical characteristics of products.

Heat-Acid Coagulated Products

Paneer and chhana are the two most important heat-acid coagulated Indian dairy products. They seem to be a kind of unripened cheeses. The manufacture of paneer and chhana consists of heating, coagulation of milk with an acid, draining of whey, consolidation of solid mass, chilling, and packaging. These products consist of entire milk casein, part of denatured whey proteins, almost all fat, and colloidal

Table 19 Chemical composition of heat-desiccated milk products

Constituents	%						
	Khoa	Peda	Burfi	Kalakand	Gulab jamun	Rabri	Basundi
Moisture	18–42	5–18	12–20	20–35	22–34	45–60	42–50
Fat	21–40	6–24	10–20	10–27	8–11	10–20	11–15
Protein	15–27	7–20	13–21	9.4–18	6–8	8–10.5	6.6–8.9
Lactose	16–34	15–19	12–18	10–22	8.2–11.8	10.2–14.2	9–12
Ash	3–5	1.2–3.6	1.5–3.1	1.7–3.4	0.8–1.1	1.2–2.0	1.2–1.6
Sucrose	–	30–55	17–42	16–43	37–52	10–14	20–23

milk solids in proportion to the moisture content retained (Shivashraya 2014b). Paneer is generally hard and prepared from buffalo milk, while chhana is relatively soft and prepared from cow milk. However, by standardizing the milk, these products can also be prepared from cow, buffalo, or mix milk. Paneer consists of 52–54 % moisture, 22–26 % fat, 16–18 % protein, 1.9–2.2 % lactose, and 1.3–1.7 % minerals. Chhana consists of 57–58 % moisture, 22–24 % fat, 15–16 % protein, 2.5–3.1 % lactose, and 1.5–2.1 % minerals. Rasogolla and sandesh are the most popular traditional Indian milk sweet prepared from chhana. The rasogolla consists of 44–46 % moisture, 4–6 % fat, 4–5 % protein, and 44–47 % sucrose, while sandesh consists of 15–33 % moisture, 15.5–21 % fat, 13–21 % protein, 1.4–1.9 % ash, and 33–47 % sucrose. This wide variability in compositions is due to method of preparation, locality, and preference for typical characteristics of products.

Conclusions and Future Directions

Milk is considered as nearly complete human food. Milk is a complex, nutritious product that contains more than 100 substances that are either in emulsion, suspension, or solution in water. Milk is a synthesis in the mammary glands. Milk, a biological fluid, is a highly variable and complex raw material for further processing. The milk fats play an important role in desirable or undesirable flavor development as well as body and texture in dairy products. The casein and whey proteins are the two main milk proteins. Casein gets precipitated out when pH drops to 4.6, while whey proteins remain soluble in this pH. Lactose is considered as milk sugar which provides sweetness to milk. It plays an important role in fermented dairy product, responsible for Maillard browning in high-heated milk and milk products as well as responsible for sandiness in sweetened condensed milk and ice cream. Ash is an indicator of mineral load in milk. The salt balance of milk affects the heat stability of milk during heat processing. Milk contains various enzymes that have significant role in dairy industry. The water- and fat-soluble vitamins are contributing nutritional significance to milk and its products. There are number of factors affecting the normal composition of milk. The various milk products like sour milk, yogurt, curd, dahi, lassi, shrikhand, kefir, kumiss, cheese, evaporated

milk, sweetened condensed milk, dried milk, cream, butter, ghee, ice cream, khoa, paneer, and chhana are manufactured. There are more variations found in the compositions of milk and milk products that depend on methods of preparation, varieties of products, consumer preference, legal standards, countries of origins, and type of milk used.

Cross-References

- ▶ [Chemical Composition of Fat and Oil Products](#)
- ▶ [General Properties of Major Food Components](#)
- ▶ [General Properties of Minor Food Components](#)

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Abstract

Organic food market is steady increasing worldwide. Consumers purchase organic food because they believed they are naturally produced, safe, healthy, and of higher quality. Organic agriculture methods are based on overall and specific principles that effectively can affect the chemical composition of the

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organic foods: the prohibition of the use of mineral fertilizers and synthetic pesticides. Organic livestock is an extensive production method based on pasture- and forage-based feeds.

In this chapter, the effects of the methods used in organic agriculture on various classes of chemical compounds in plant and animal origin food will be examined and illustrated through examples. Recent advances in analytical methods and their potential in discriminating between organic and nonorganic food will be also presented.

Introduction

Organic farming is growing at a steady pace worldwide. At the end of 2011, there were 37.2 million hectares of certified agricultural land, representing the 0.9 % of all agricultural land in the world, with an increase of 3 % compared to 2010. The regions with the largest areas of organic agricultural land are Oceania (12.2 million hectares) and Europe (10.6 million hectares). There were 1.8 million producers in 2011 (12 % more compared with 2010), the most of them in Asia (34 % of the world's organic producers) and Africa (30 %). In spite of the slowdown in the global economy, international sales of organic food and drinks have continued to rise reaching about 63 billion US dollars in 2011. The market has expanded by 170 % since 2002. Demand for organic products is mainly in North America and Europe (overall, more than 90 % of sales) (Willer et al. 2013). Increasingly, consumers are deciding to purchase organic products because they believe they are naturally produced, safe, and healthy and that they contribute to a form of production that is sustainable for the environment and society. For these reasons, consumers are willing to pay a premium price for organic products.

Are these consumer expectations supported by scientific evidence?

Qualitative and quantitative presence of chemical compounds and their interactions determine in part the quality of food and food products, their nutritional value, sensory characteristics, acceptability by consumers, and effects on human health.

Focusing on chemical composition of organic products, in the last 20 years, a number of comparison studies have been published on the differences between organic and nonorganic products. All comparative studies fall in one of the three following categories, each one with its *pros* and *cons* (Magkos et al. 2003):

- Retail market study. In these studies, the samples are represented by the products as they reach the consumer; the differences in the chemical composition of the products can be individuated; however, it is not possible to conclude whether the differences are due to the production method because the background of the products is unknown and impossible to be reconstructed.
- Farm comparison study. In this kind of studies, it is possible to record the conditions under which a product is obtained. Information about the production method used can be obtained directly from the farmers. By the selection of neighboring farms, the differences between environmental conditions can be

controlled but unlikely eliminated. Therefore, it is difficult to state that the selected farms accurately and realistically reproduce the two production systems.

- Research center study. These studies are considered the most accurate way to compare the effect on chemical composition of a food of the organic and nonorganic method. All the conditions and production procedures can be controlled and recorded. Therefore, the factors responsible for the differences measured can be identified. Apart from the possible limitation of the sample size that can be collected, results obtained cannot be generalized as reflective of the commercial production system.

Besides the approach adopted for the comparison, the studies in the literature differ one from the other because they were carried out in different locations, with different climate conditions and different soil characteristics, using different farming or rearing procedures, in different varieties or races, harvesting samples at different ripening stage, etc. That is why, even when the methodological approach of meta-analysis has been applied to data from the literature, inconclusive results have been often achieved so far regarding the differences of chemical composition between organic and nonorganic products (Benbrook et al. 2008; Dangour et al. 2009; Brandt et al. 2011; Smith-Spangler et al. 2012; Palupi et al. 2012). In truth, the different results obtained by the different meta-analyses depend also on the criteria set for the selection of the papers to collect and analyze. Based on the criteria set, a paper can be included into or excluded from the selected ones, and inevitably, this will affect the final results of the analysis.

However, a sound scientific background exists about the factors that can determine differences in the chemical composition between organic and nonorganic food.

In 2005, the International Federation of the Organic Agriculture Movements (IFOAM) established the principles that inspire the growth and development of the organic agriculture and are internationally recognized:

- Principle of health: organic agriculture should sustain and enhance the health of soil, plant, animal, human, and planet as one and indivisible.
- Principle of ecology: organic agriculture should be based on living ecological systems and cycles, work with them, emulate them, and help sustain them.
- Principle of fairness: organic agriculture should build on relationships that ensure fairness with regard to the common environment and life opportunities.
- Principle of care: organic agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment.

According to the definition of the International Federation of the Organic Agriculture Movements (IFOAM), *Organic Agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological*

processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic Agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved.

Principles and definition have been translated in national specific regulations in many countries and regions of the world (European Union, the United States, Japan, and Canada, to name but some). These regulations lay down process and production methods and include, among other things, the applicable administrative provisions, terminology, and labeling requirements. Organic agriculture relies on methods that are not commonly practiced in nonorganic agriculture:

- The use of mineral fertilizers is prohibited in organic agriculture. Due to the restrictions to the type and intensity of fertilization, soil fertility is enhanced by crop rotation, intercropping, covering crops, and mulching, green manure, manure, and compost.
- The use of synthetic pesticides is prohibited in organic agriculture. Pest control is achieved by using appropriate cropping techniques, biological control, and natural pesticides. Weed control is managed by appropriate rotation, seeding timing, mulching, etc.
- Organic livestock is an extensive production method based on pasture- and forage-based feeds and the observance of a high level of animal welfare. For example, this method has its roots in the principle of fairness that insists that animals should be provided with the conditions and opportunities of life that accord with their physiology, natural behavior, and well-being.

The adoption of these methods has an effect on food product chemical composition, thus potentially determining differences between organic and nonorganic food products.

The release of nitrogen from organic fertilizers is generally lower and/or slower than that of the mineral fertilizers. The difference in the availability of plant-available nitrogen has a range of indirect effects, due to the effect of nitrogen on plant metabolism and physiology (Brandt et al. 2011).

The prohibition of the use of synthetic pesticides could result in initially higher rates of attack by pests and pathogens in organic plants compared with corresponding nonorganic ones, triggering the formation of induced defense compounds, which then subsequently protect the plant against diseases or pests (Brandt et al. 2011).

For ruminants, extensive feeding systems based on pasture and forage significantly affect the fat composition of milk and muscle and the levels of dietary antioxidants compared to diets based on concentrate feeds (Benbrook et al. 2013).

In the following parts of this chapter, the effects of the methods used in organic agriculture on various classes of chemical compounds in plant and animal origin food will be examined and illustrated through examples. Recent advances in analytical methods and their potential in discriminating between organic and nonorganic food will be also presented.

Chemical Compounds

Water

Water plays many essential roles in plant growth and function. Chemically, water is a reactant in photosynthesis. The structural rigidity of the soft plant tissues is also conferred by water: the wilting of leaves or fruits is strictly related to water loss. Water is absorbed from the soil by the roots of a plant, and through the conduction by the xylem vessels, it is transported up to the leaves. Along with this movement, water transports nutrients: minerals from the soil up to the leaves and carbohydrates from the leaves, where they are synthesized through photosynthesis, throughout the plant.

It has been proposed that the different fertilization approaches used in organic and nonorganic farming can affect the moisture content of plant products. Plants grown with mineral fertilizer might absorb more water along with easily soluble mineral compounds. Therefore, nonorganic plant products should be characterized by a higher moisture content. As a consequence, a better performance of the organic plant products during postharvest storage could be associated with the potentially different moisture content. Indeed, inconsistent results can be found in the literature regarding the moisture content of the organic vegetables and fruits that do not allow to achieve a definitive conclusion.

Nitrogen Containing Compounds

Protein and Amino Acids

In the nonorganic farming system, nitrogen is supplied in a rapidly available form that facilitates its uptake by the plants, promotes the vegetative growth, and increases leaves production. In the organic farming system, only the use of organic fertilizers is permitted, and the release of nitrogen from them is slow and prolonged over the time. Generally, protein content increases with nitrogen uptake (Brandt and Mølgaard 2001), and this observation should imply a different protein content between nonorganically and organically grown crops.

Cereals, legumes, and pulses are the main sources of plant protein worldwide and part of the traditional diet in many countries. Most of the published studies on the effect of the organic farming on protein content of food is focused on cereals and derived products.

Comparison studies in the literature largely support the finding that nonorganically grown cereals show a higher protein content than their organically grown counterparts (Nitika Punia and Khetarpaul 2008). This finding is often associated with a lower yield obtained by the organic cultivation system (Mäder et al. 2007). However, there are few studies where no differences in protein content have been found (Mäder et al. 2007) or even where the protein content of nonorganic wheat was lower than that of the organically grown wheat (Kihlberg et al. 2004). As emphasized by Mäder et al. (2007), this latter result could be due to the use wheat varieties better adapted to low nitrogen input as commonly occurred in the organic farming.

Protein quality is largely determined by the amino acid pattern. Nutritional value of protein depends on the content of essential amino acids. Lysine, tryptophan, and threonine are the amino acids mainly determining the nutritional quality of cereal protein. Worthington (2001) reported that the protein quality in organically produced wheat was higher than in nonorganic one. No significant differences in the amino acid composition were instead found in other studies (Zörb et al. 2006; Mäder et al. 2007).

In a study of the effects of different strategies for soil fertilization on yield and composition of chickpea, a lower content of protein was found in chickpea grown with the application of a chemical fertilizer (Mohammadi et al. 2010).

If until now poor attention has been given to the effect of the organic growing methods on legumes' protein content, on the other hand legumes represent one of the most important crops in organic agriculture. In organic agriculture, the use of mineral fertilizers is prohibited and plants should be fed through the soil ecosystem. As reported in the EC Regulation N. 834/2007 in organic production and labeling of organic products, *The essential elements of the organic plant production management system are soil fertility management, choice of species and varieties, multiannual crop rotation, recycling organic materials and cultivation techniques*. The prohibition to use mineral fertilizers represents a relevant problem for plant nutrition in terms of nutrient availability, nitrogen in particular. Therefore, alternative strategies suitable for organic products, such as crop rotation, have to be used to overcome the nutrients' limitation. Crop rotation is the practice of growing a wide variety of crops in a sequential system throughout the field with the intention of avoiding a buildup of disease and pests associated with mono-cropping. Crop rotation promotes good soil health by alternating crops with different nutrient needs, therefore avoiding depletion of any one necessary element present in the soil. It can also benefit overall soil structure by alternating deep and shallow rooting plants and breaking up subsoil. Legumes have a major role in crop rotation. They derive most of their nitrogen requirements from the atmosphere and fix it on the root systems in the form of nodules. That is why legumes are defined as "nitrogen-fixing plants." Legumes fix nitrogen through a symbiotic relationship with fungi naturally occurring in the soil. This symbiotic association between a fungus and the roots of a vascular plant is known as mycorrhiza (from the Greek "μυκόης," fungus, and ρίζα, roots). The actual amount of fixed nitrogen depends on legume variety (e.g., faba bean fixes a higher amount of nitrogen than lentil), nodulation, soil moisture and temperature, and soil available nutrients such as nitrogen and phosphorus. While soil available N can boost legume growth and development in the early growth stages, excess nitrogen may delay the onset of nodulation, impeding nitrogen fixation.

Several evidences are available showing that rotation with legumes increases the N mineralizable from soil to support crop production (Carpenter-Boggs et al. 2000) and soil biodiversity (Brussaard et al. 2007) and reduce reliance on nonrenewable fossil fuels and petrochemicals (Doran 2002).

Protein content in animal origin food products seems not to be affected by the organic rearing methods. The concentration and composition of protein in milk are largely unresponsive to variation in nutrition and management. Stage of lactation is important in determining milk protein concentration, but has little influence on protein composition.

Nitrates

Nitrogen fertilization has been identified as one of the major factors influencing nitrate content in vegetables. Despite its abundance in the atmosphere, nitrogen is often the most limiting nutrient for plant growth, because most plants can only take up nitrogen in two solid forms: ammonium ion and nitrate ion. Ammonium is used less by the plants because it is extremely toxic in large concentrations. Nitrate is mobile in the soil and immediately available to the plant.

On nonorganic farms, the majority of the nitrogen available to plants is applied as fertilizer in a synthetic form that is rapidly and readily available. On organic farms, nitrogen is supplied in a complex matrix involving nitrogen stored in the soil, affixed by legumes from nitrogen in the air, and nitrogen from composted manure and soil amendments. These forms and sources of nitrogen are more slowly delivered and available to the plant. It has been reported that the higher the amount of nitrogen available to the crops, the higher its uptake and, as a consequence, the higher the nitrogen and nitrate contents of the crop (Brandt and Mølgaard 2001). Based on this and on literature surveys, it has been concluded that organic production of plant foods does result in lower nitrate levels (Magkos et al. 2006), although some variation among the findings was individuated, with cultivar type and season being identified as two variables that may also influence the nitrate content (Magkos et al. 2006). Recently, the contents of nitrate in six different baby-leaf salads of a single species (green lettuce, red lettuce, watercress, rocket, chard, and corn salad) produced in organic and nonorganic agriculture system were evaluated. The nitrate levels from organic production were sevenfold lower than those from nonorganic production (Aires et al. 2013). In their 4-year study on potato varieties, Hajšlová et al. (2005) found that in most cases, nitrate content was lower in organically than in nonorganically grown tubers, with the exception of one experimental year for one variety. However, no significant differences were found in nitrate content of chicory grown under organic or mineral fertilization.

Approximately, 80 % of dietary nitrates are derived from vegetable consumption (Santamaria 2006). Generally, nitrate-accumulating vegetables belong to the families of Brassicaceae (rocket, radish, mustard), Chenopodiaceae (beetroot, Swiss chard, spinach), and Amaranthaceae, but also Asteraceae (lettuce) and Apiaceae (celery, parsley) include species with high nitrate contents (Santamaria 2006).

At high gastric concentrations, nitrate may cause methemoglobinemia among young children and infants and formation of carcinogenic *N*-nitroso compounds (Santamaria 2006). Nitrate per se has not been shown to produce a carcinogenic effect in animals, but can be converted into nitrite by bacteria in human saliva and in the intestine, which in turn may react with certain amines and amides, normally present in the body, to produce nitrosamines (Santamaria 2006). Nitrosamines have been shown to be able both of initiating and promoting the cancer process. Whether or not, however, dietary nitrate significantly contributes to human cancer is debatable. There is no sound evidence of a significant association between nitrate intake and gastric cancer risk in humans (Magkos et al. 2006), while an increasing number of studies indicate that plant-derived nitrate may provide significant benefits for human health (Brandt et al. 2011).

Carbohydrates

Nitrogen is one of the most important nutrients for plant yield and quality. However, the vegetable yield does not increase continuously by increasing nitrogen fertilization. Moreover, in environments rich in readily available nitrogen, such as those in which mineral fertilizer is used, the metabolism of plants is mainly oriented towards the production of nitrogen-containing compounds, i.e., free amino acids, proteins, nitrates, and alkaloids (Wang et al. 2008). Instead, increased application of nitrogen reduces the content of dry matter, potassium, sucrose, vitamin C, and fiber in leafy vegetables (Stamp 2003). The response of carbohydrates to fertilizer N application is essentially inverse to that observed for N-containing compounds.

In the organic system, the slower and prolonged release of nitrogen does not trigger a spike in plant growth, allowing more photosynthetic sugars to be available for other metabolic functions, such as producing more vitamin C and polyphenols (Benbrook et al. 2008). It has been observed that sugar content rises when phosphorous levels are low relative to other elements (Brandt and Mølgaard 2001). As reported elsewhere in this chapter (see section “Minerals”), the content of phosphorus in organically grown fruit and vegetables has been found higher than in the nonorganic counterparts (Dangour et al. 2009; Hunter et al. 2011; Smith-Spangler et al. 2012). However, available data are too little to make any speculation.

When investigating nonorganically and organically grown wheat on their content of carbohydrates (total soluble sugars, reducing sugars, starch), no or small differences were found (Zörb et al. 2006; Mäder et al. 2007; NitikaPunia and Khetarpaul 2008).

The carbohydrate content did not change in red rice varieties grown under organic and nonorganic farming systems (Hedge et al. 2013), whereas in organically cultivated potatoes, the content of starch was higher than in nonorganically grown, but no significant differences were found for reducing sugars between the cultivation systems (Järvan and Edesi 2009).

Lipids

The number of papers focused on the effect of the growing method on chemical composition of edible oils is still limited and the results inconsistent to have clear conclusions.

Anastasopoulos et al. (2011) studied the fatty acid profile and sterols in monocultivar virgin olive oil from organic and nonorganic growing methods according to fruit ripening and harvest year. They found that palmitoleic and oleic acids varied according to cultivation method, whereas sterols were not affected. A higher content of oleic acid and lower content of linoleic acid were observed in organic virgin olive oil compared to nonorganic (Gutiérrez et al. 1999). In a study aimed to compare the fatty acid composition of 59 organic edible oils and 53 nonorganic oils (coconut, olive, canola, mustard seed, sesame) from the market, no consistent results were obtained (Samman et al. 2008). In any case, these studies did not give any suggestion

about which is/are the factor(s) characterizing the growing method that could be considered as responsible of the detected effects on lipid composition.

Regarding the composition of fat from animal origin in relation to the rearing system, the picture is completely different, both in terms of number of scientific evidences and their soundness.

Milk and dairy products have always played an important role in human nutrition and, more recently, have also been described as an important source of a variety of relevant biologically active molecules (Pintus et al. 2013). Recently, the potential human health benefits of specific fatty acid, including benefits for conjugated linoleic acid (CLA), docosahexaenoic acid, and eicosapentaenoic acid, have been identified (Pintus et al. 2013). CLA comprises a group of unsaturated fatty acid isomers with a variety of healthy biological effects, primarily associated to *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA. The beneficial effects are proven in animals and not yet in humans, but the effect of CLA on human health is still in focus. Docosahexaenoic acid and eicosapentaenoic acid are essential for human growth, brain development, vision, and immunity and also play a vital role in the prevention and treatment of human diseases (Chilliard et al. 2001).

The content of this compound in fat from animal origin food products depends on several factors (forage species, breed, physiological stage, type of diet, etc.). However, several studies suggest that the nature of the diet is fundamental in determining the fatty acid composition (and not only) of the animal fat and animal-derived foods like milk (Chilliard et al. 2000, 2001; Jenkins et al. 2008; Luciano et al. 2013; Pintus et al. 2013).

The ω -3 fatty acids cannot be synthesized in the mammary glands of dairy animals since they lack of the enzyme able to make a double bond at the ω -3 position. Therefore, the ω -3 fatty acid content in animal food is dependent on the intake of these fatty acids through the diet. In addition, fatty acid composition depends also by the animal's digestive physiology. Pigs and poultry have a relatively simple digestive system and absorb fatty acids in approximately the same proportions as found in their diet. In ruminant, lipid absorption is heavily influenced by rumen microbial activity. Linoleic acid and α -linolenic acid from the diet are modified in the rumen through lipolysis and biohydrogenation processes to produce a saturated fatty acid, the stearic acid (C 18:0), as ultimate product (Jenkins et al. 2008). Intermediate products of this process, such as vaccenic acid and conjugated linoleic acid (CLA), can escape the rumen and be incorporated in the ruminant's tissues and milk (Chilliard et al. 2000). Fractions of linoleic and α -linolenic acid, not modified in the rumen, are also deposited in tissues and milk (Chilliard et al. 2000).

Therefore, changing the diet of the dairy cow, for example, will modify the fatty acid composition in milk.

A ruminant's diet based on pasture and forage (preferably, fresh forage) provides higher levels of ω -3 fatty acids and CLA when compared with a total mixed ration (TMR) diet with roughage based on maize, thus promoting the deposition of these unsaturated fatty acids in animal's tissues and milk and derived products, such as meat and cheese (Luciano et al. 2013; Pintus et al. 2013). Maize silage contains a

high concentration of linoleic acid, which affects the milk composition with an increase in this ω -6 fatty acid. However, in recent years, the intake of ω -6 fatty acids has risen dramatically in industrialized countries. Consumers should lower the intake of ω -6 fatty acids and increase that of ω -3 fatty acid, so that the ratio between these two fatty acids approaches the recommended, optimal of 1 (Wijendran and Hayes 2004).

What is reported above implies that the extensive feeding system based on pasture and forage, as called for the current regulation, drives organic farming to produce organic dairy products with a higher content of ω -3 fatty acids and CLA than the intensive systems. Actually, an enhancement of nutritional quality of milk and dairy products through organic production methods has been recently recognized (Palupi et al. 2012; Benbrook et al. 2013). A higher content of ω -3 fatty acids and CLA, a ratio between ω -3 and ω -6 fatty acid content more respondent to that recommended by nutritionists, may have beneficial effects on human health in terms of protection against cardiovascular and degenerative diseases (Pintus et al. 2013). Moreover, the ratio between ω -3 and ω -6 fatty acid content in organic milk and dairy products resulted higher than in nonorganic products during the whole season, that is, both in winter and summer periods (Palupi et al. 2012).

Finally, the different fatty acid profile of the diet between organic and semi-intensive rearing systems of sea bass significantly affected the fatty acid profile of fish fillets (Trocino et al. 2012). A higher content of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in the organic diet caused a significantly higher content of MUFA and PUFA in the fillets from organically reared sea bass than in nonorganic counterpart.

Organic Acids

Organic acids have a central role in plant metabolism. They are the early products of the photosynthesis and precursors for the synthesis of many other compounds, since they are part of different biological routes, among which is the cycle of Krebs. Organic acids contribute to the acidity and pH of fruits, parameters that are related to the ripening stage of fruit. The concentration of organic acids decreases during ripening, which could be due to the use of these compounds as respiratory substrates or their conversion to sugars.

Malic, citric, fumaric, and oxalic acids are the main organic acids in fruit and vegetables. They contribute to the acidic taste of fruits. Malic acid is an indicator of the freshness of fruit; oxalic acid forms insoluble salts with calcium and other divalent cations, thus decreasing the availability of this metal, and therefore, it is considered as an antinutrient compound.

In recent years, increasing experimental evidence has associated organic acid metabolism with plant tolerance to environmental stress (López-Bucio et al. 2000).

Data available in the literature on the organic acid content in organic and nonorganic fruits and vegetables are not consistent and do not allow to individuate a clear effect of the farming system.

From a meta-analysis, organic fruits and vegetables resulted higher in titratable acidity (Dangour et al. 2009). Titratable acidity measures the total amount of protons available in a solution, providing an approximate measure for the concentration of acidity to which also organic acids contribute.

Minerals

Soil management following the organic method procedures allows to increase soil biodiversity (Mäder et al. 2002). Soil microorganisms support plants in introducing active substances adsorbed by soil minerals, making them more available for plant roots. Based on this observation, a higher content of mineral elements in organic plant materials should be expected due to a higher content of microorganisms in organically cultivated soil. Hunter et al. (2011) through a meta-analysis of data in the literature found a significantly higher content of boron, copper, magnesium, molybdenum, potassium, phosphorus, selenium, sodium, and zinc in organic fruit and vegetables than in nonorganic counterparts. However, other two meta-analyses reported a significantly higher content only for phosphorus in organically grown fruit and vegetables (Dangour et al. 2009; Smith-Spangler et al. 2012).

Vitamins

Vitamin C

L-Ascorbic acid (vitamin C) performs many fundamental metabolic functions in the human body. It is essential for the synthesis and maintenance of collagen, of muscle carnitine, and neurotransmitters. Vitamin C is also necessary for the transformation of cholesterol to bile acids, thus contributing in preventing an accumulation of cholesterol in the liver (hypercholesterolemia). It accelerates the process of wound healing and has been shown to stimulate the immune system. It is known to prevent the oxidation of LDL and protect against the development of atherosclerosis. It enhances the availability and absorption of iron from nonheme iron sources and inhibits the formation of nitrosamines.

In plants, L-ascorbic acid participates in a variety of processes, including photosynthesis, photoprotection, cell wall growth and cell expansion, resistance to environmental stresses, and synthesis of ethylene, gibberellins, anthocyanins, and hydroxyproline.

In plants, L-ascorbic acid is produced from glucose. More precisely, its production was proposed to occur via GDP-mannose and L-galactose. L-Galactose is a minor component of cell wall polysaccharides.

It has been observed that plant accumulates vitamin C in higher extent whenever subjected to oxidative stress, which can be caused by different factors such as full sunlight, drought, herbicides, and nitrogen availability (Brandt and Mølgaard 2001). Regarding nitrogen availability, many studies have shown that increased fertilization tends to reduce the content of vitamin C (Lee and Kader 2000).

Most of the results from the literature reveal that organic crops are characterized by a higher content of vitamin C. For example, Järvan and Edesi (2009) found that organically grown potatoes were higher in vitamin C than those grown with mineral fertilization. Higher content of vitamin C was observed in sweet bell peppers grown in three organic farms compared with those from three conventional farms located in the same vicinity to ensure similar climate and soil conditions (Hallman and Rembialkowska 2012). Yet, the results of recent meta-analyses are controversial about a higher content of vitamin C in organic fruit and vegetables. While Brandt and coworkers (Brandt and Mølgaard 2001) concluded that organic fruit and vegetables have a significantly higher content of vitamin C than the nonorganic ones, according to Hunter et al. (2011) and Smith-Spangler et al. (2012), instead, organic vegetables and fruits showed on average more vitamin C (and other vitamins as well) than nonorganic raw materials, but the difference was not statistically significant.

Vitamin E

Vitamin E is the name given to a group of lipid-soluble, plant-derived substances. Vitamin E exists in eight naturally occurring isomers: four tocopherols and four tocotrienols. All have a chemical structure composed of a chromanol ring, with a hydroxyl group and a hydrophobic side chain. Both the tocopherols and tocotrienols occur in alpha, beta, gamma, and delta forms, determined by the number and position of methyl groups on the chromanol ring. The alpha-tocopherol is the most biologically active form and vitamin E activity is traditionally expressed in terms of equivalents of this isomer.

The main source of vitamin E is fats and oils. It is also found in some vegetables, meat, poultry, fish, and, to a lesser degree, cereals and dairy foods. About half the tocopherol in wheat germ, sunflower, safflower, canola, olive, and cottonseed oils is alpha-tocopherol, but soybean and corn oils contain about 10 times as much gamma-tocopherol as alpha-tocopherol.

The major role of vitamin E is to protect polyunsaturated fatty acids from oxidation. It acts as an antioxidant in the lipid phase of cell membranes.

Heterogeneous and not significant results were observed for the content of vitamin E in organic and nonorganic plant products (Smith-Spangler et al. 2012). Although feeding cattle on pasture confers higher level of vitamin E (Descalzo et al. 2005), inconclusive results have been obtained about the different content of this vitamin in organic meat and milk and dairy products compared to the nonorganic products (Palupi et al. 2012; Smith-Spangler et al. 2012).

Carotenoids

Carotenoids are terpenoids that have been identified in photosynthetic and non-photosynthetic organisms. Carotenoids include more than 600 compounds/pigments responsible of the red, orange, and yellow color of fruits and vegetables and also fungi, flowers, and animals. Their color in green leafy vegetables is

masked by the green chlorophyll. Carotenoids are isoprenoid compounds. The characteristic feature of the carotenoid structure is the long system of alternating double and single bonds that forms the central part of the molecule (polyene chain). This skeleton can be modified (a) by cyclization at one end or both ends of the molecule, (b) by changes in hydrogenation level, and (c) by addition of oxygen-containing functional groups. The group of carotenoids can be divided into carotenes, which contain no oxygen, such as α -carotene, β -carotene, and lycopene and xanthophylls, which contain oxygen, such as lutein, zeaxanthin, violaxanthin, capsanthin, and neoxanthin. Carotenoids are accumulated in chloroplasts. These pigments are found as complexes formed by a noncovalent bonding with proteins. In green leaves, carotenoids are nonesterified. Their role in the plant is to act as accessory pigments for light harvesting and in the prevention of photooxidative damage, as well as acting as attractants for pollinators. β -Carotene gives orange and yellow color to many fruits and green leafy vegetables, lycopene red color to tomatoes, and lutein and zeaxanthin yellow color to corn. Only microorganisms and plants can synthesize carotenoids *ex novo*; carotenoids in animals come from these two sources.

Among all carotenoids, about 50 compounds (retinoids) show the activity of vitamin A. For provitamin activity of carotenoid precursors, the presence of at least one un-substituted β -ionone ring attached to the end of molecule is necessary. The most widespread and important provitamin A is β -carotene. Dietary provitamin A carotenoids are a major source of vitamin A needs for humans. Vitamin A is essential for the promotion of general growth, maintenance of visual function, regulation of differentiation of epithelial tissues, and embryonic development. Vitamin A can be obtained from food, either as preformed vitamin A in animal products, such as eggs and dairy products, or as provitamin A carotenoids, mainly β -carotene in plant products.

In addition, dietary carotenoids play an important role for human health, in prevention of chronic diseases such as cancer, heart disease, and age-related macular degeneration. Moreover, they support the immune system. It is possible that carotenoids can play this role mainly by means of an antioxidant mechanism (Cooper 2004).

When rapidly available nitrogen is provided to plants, as it occurs in the intensive farming system, the vegetative growth is promoted, more leaves are produced and the production of chloroplasts within plant cells is increased. Being carotenoids accumulated in chloroplasts of all green plants, their content could be higher in plants that received ample to excessive nutrients, especially nitrogen. This is the reason why it has been suggested that nonorganic plant foods could contain a higher carotenoid content than the organic ones (Brandt and Mølgaard 2001).

Grass-based diets, especially pasture, lead to higher content of β -carotene in milk than diets rich in concentrates or corn silage. As a consequence, a higher content of carotenoids could be expected in organic cattle milk and meat due to the fact that the feeding is mainly based on green forage pasture. As mentioned elsewhere in this chapter, the organic livestock farming is based on an extensive feeding system (pasture and forage) that allows to obtain dairy products with a

higher content of unsaturated fatty acids. An increased proportion of unsaturated milk may increase the oxidative susceptibility of milk. Therefore, to maintain a high quality, the concentration of antioxidants in milk should be elevated. In milk, the concentration of carotenoids, and α -tocopherols as well, as antioxidant is important for the oxidative stability. β -carotene can act as scavenger of lipid peroxy radicals and quench singlet oxygen. High concentrations of β -carotene in milk can be obtained by high proportion of pasture and grass clover silage, because these types of forage are rich in antioxidants and, particularly, in β -carotene.

Nevertheless, a recent meta-analysis conducted on comparison studies published between 2008 and 2011 on chemical composition of milk and dairy products from organic and nonorganic husbandry systems did not confirm this expectation (Palupi et al. 2012).

Phenolic Compounds

Phenolic compounds are one of the main groups of secondary metabolites in plants. About 8,000 phenolics have been individuated deriving from shikimate pathway or malonate/acetate pathway. Secondary metabolites derive from primary metabolites. In the case of phenolics, the primary metabolite is glucose. Phenolics do not contain nitrogen.

Phenolic compounds are involved in many interactions of plants with their biotic and abiotic environment. These substances accumulate in different plant tissues and cells during ontogenesis and under the influence of various environmental stimuli, respectively.

One of the main factors affecting the chemical composition of plants, including the secondary metabolites, is the fertilization management. Organic fertilization management differs from nonorganic one because it involves the use of several methods to develop and maintain soil fertility (intercropping, N-producing plants, crop rotation, cover crops, and composted animal manure or green manure) instead of using mineral and synthetic fertilizers. With the organic methods, the release of nutrients to the soil occurs slowly; synthetic fertilizers in contrast are more easily soluble and will thus be more available to the plant and result in more effective uptake of nutrients.

A model has been suggested according to which when nutritive resource availability is high, the plant will allocate the nutrients to growth, but if nutritive resources are constrained, the plant will divert more of them to the synthesis of secondary metabolites associated with differentiation. That means that if N is largely available, the plant diverts more input into N-containing metabolites, such as protein, vitamin A, and nitrate, while more carbon-containing compounds are produced when N is more restricted, such as vitamin C and phenolic compounds (Stamp 2003). According to the model, this explains why organic produce usually shows a higher content of secondary metabolites and, in particular, phenolic compounds.

Another factor determining the production of secondary metabolites is plant protection by use of pesticides. Secondary metabolites are synthesized by the plant through defense mechanisms activated in response to a stress, such as an attack by pathogens, pests, herbivores, etc. The use of synthetic pesticides protects the plants against these attacks. Instead, the prohibition of the use of synthetic pesticides in the organic system could represent an additional factor, besides organic fertilization, inducing a higher content of phenolic compounds in organic plant foods than in nonorganic ones. However, according to Brandt et al. (2011), the effect of the use of pesticide on the synthesis of secondary metabolites as defense compounds seems to be negligible compared to that of fertilization.

In recent years, the role of phenolic compounds as protective dietary constituents has become an increasingly important area of human nutrition research. There is increasing evidence that long-term intakes of fruit and vegetables have a potential role for the prevention or reduction in the risk of degenerative diseases such as cardiovascular diseases, diabetes, and cancer. Since oxidative stress imposed by reactive oxygen species (ROS) is known to be associated with these pathologies, the potential mechanism of the protective effects of phenolic compounds is thought to be due to their antioxidant activity as free radical scavengers.

In general, the studies of comparison indicate a significant higher content of phenolic compounds, either as total polyphenols or individual phenolic compounds content, in organically cultivated fruit and vegetables than nonorganic counterparts. Brandt et al. (2011), who conducted a meta-analysis of the published comparative studies of the content of secondary metabolites in organic versus nonorganic crops, found that organic ones contained 12 % higher levels of secondary metabolites than corresponding nonorganic fruits and vegetables.

Flavonoids represent a large group of phenolic compounds, widely distributed in plants. They may be divided into subclasses: anthocyanidins, flavonols, flavanols, flavanones, flavones, and isoflavones. A great interest exists in the potential health effects of flavonoids associated with fruit- and vegetable-rich diets. Many of the biological effects of flavonoids appear to be related to their ability to modulate cell-signaling pathways and their antioxidant activity and free radical scavenging capacity. Therefore, flavonoids are considered able to contribute to the prevention of cardiovascular and degenerative pathologies.

A 10-year comparison study showed a higher level of quercetin and kaempferol (two flavonols), in organic tomatoes with values that resulted to be 79 % and 97 % higher than those in nonorganic tomatoes, respectively (Mitchell et al. 2007). Interestingly, this study demonstrated that the levels of flavonoids increased over time in the organic tomatoes, whereas the nonorganic ones did not show any variation. This increase corresponded not only with increasing amounts of soil organic matter accumulating in organic plots but also with reduced manure application rates once soils in the organic systems had reached equilibrium levels of organic matter. These results demonstrate the importance of long-term comparison studies to have results that can allow to draw meaningful conclusions about the effect of the organic growing methods on chemical composition of organic food products and the differences with their nonorganic counterparts.

Contaminants and Toxins

Contaminants

The occurrence of some chemical contaminants in food is not affected by the production method (organic or nonorganic). This is the case with compounds that only occur in food due to negligence, accidents or sabotage, etc., or from environmental pollution such as dioxins, PCBs, and heavy metals. The absence or presence and the relative amount of these contaminants in food are strongly dependent on farm location. It has been suggested that they may accumulate more in organic foods, because animals in organic rearing method have direct contact with soil. However, available data do not support this hypothesis (Magkos et al. 2006).

Since the 1960s, agricultural production has seen tremendous increase, thanks to the massive use worldwide of mineral fertilizers, water irrigation, and synthetic agrochemical. Mineral fertilizers and irrigation have been largely responsible of the increase in production from the same surface of land; the use of synthetic agrochemicals (insecticides, fungicides, herbicides, etc.) has allowed to reduce the losses through crop protection from pests (Carvalho 2006).

Pesticides can contaminate food through on-farm pesticide use, postharvest pesticide use, pesticide use on imported food, and canceled pesticides that persist in the environment (Magkos et al. 2006). The use of pesticides represents a very serious health issue for farm workers and others exposed to the compounds in concentrated form. Several papers suggested a possible contribution of the exposure to pesticides as an explanation of the observed higher prevalence of asthma and respiratory disorders among farmers than other categories of workers (Hoppin et al. 2002, 2007). Exposure to pesticides has been suggested also as a possible cause of the onset of Parkinson's disease (Firestone et al. 2010).

Nevertheless, the number of toxicological tests carried out on food for the presence of pesticide residues is still controversial whether or not dietary exposure to such chemicals indeed consists of a potential threat to human health (Magkos et al. 2006).

Synthetic agrochemicals are prohibited in organic production. However, even when grown and processed properly, organic food products are not necessarily free from pesticides. Contamination can occur due to several reasons (cultivation on previously contaminated soil, groundwater contamination, cross-contamination with wind drift, etc.). Anyway, the difference in the risk of contamination with detectable pesticide residue has been recently quantified to be 30 % lower among organic than nonorganic produce (Smith-Spangler et al. 2012).

Toxins

Mycotoxins are toxic compounds produced by the secondary metabolism of molds in the *Aspergillus*, *Penicillium*, and *Fusarium* genera occurring in food commodities and foodstuffs. There are several subgroups of mycotoxins, of which the major one are aflatoxins. Among the other mycotoxins sometimes found in specific foods, fumonisins, ochratoxin A, deoxynivalenol, and patulin can be mentioned. All have

shown carcinogenic activity in animal tests, and therefore, they are an ongoing animal health hazard and a constant risk for contamination of the food supply.

Fungal attack and mycotoxin contamination in organically and conventionally grown produce is an extremely controversial issue on theoretical grounds. In the field and after harvest, plant foods may be contaminated with toxin-producing fungi, and some formation of toxins may take place before harvest. The use of effective synthetic fungicides can prevent toxin formation through the elimination of the toxin-producing microorganisms. In organic production, the use of synthetic pesticides is prohibited, and it seems logical to assert that organic crops may be more susceptible to fungi contamination and, consequently, to a higher presence of mycotoxins. On the other hand, the greater availability of nitrogen in nonorganic crops seems to result in plants with a lower ability to fight these pathogens and, in particular, in thinner plant cell walls that are more susceptible to mold attack. It should be emphasized, however, that inadequately controlled storage conditions pose a major risk for formation of mycotoxins (Elmholt 2003). From a review of the scientific literature, however, it cannot be concluded that either type of farming leads to increased risk of mycotoxin contamination (Magkos et al. 2006; Smith-Spangler et al. 2012).

A range of bioactive compounds that plants produce for self-defense may be characterized as “toxins.”

Many plant toxins are phytoalexins (natural pesticides) that may accumulate at different degrees as a response to stress-related factors. The concentration of such compounds in food plants may vary with a factor of 100. Due to the prohibition of the use of agrochemicals, organic plants are thought to be more prone to insect and pest attacks and, therefore, to contain higher levels of natural pesticides, produced by the plants for self-defense, than the nonorganic counterparts. Most organic food plants show moderately higher median values, which do not represent any risk to man (Brandt and Mølgaard 2006). Humans have developed mechanisms to metabolize or excrete most of these compounds. Brandt and coworkers (Brandt and Mølgaard 2006) argue that many plant toxins show biphasic effects (*hormesis*) in bioassays with cells, implying positive effects on cell proliferation at low doses and toxic effects at high. Often the processing of plant food reduces the toxin levels in the raw material to below the threshold that can have negative effects. From a biochemical viewpoint, the toxicity of these natural pesticides is similar to synthetic pesticides, which has led many authors to believe that natural toxins constitute a serious risk to human health (Magkos et al. 2006).

Comparative studies of organically and conventionally produced vegetables tend to show moderately higher values in organic plants (Brandt and Mølgaard 2006). The presumed biological mechanism behind this has to do with how quickly plants grow. In nature, plant nutrients are released from decomposition of organic matter. Consequently, plants grow relatively slowly. This allows them to build up their chemical defenses to a level that prevents most diseases and pests. For edible crops, this normal level would not pose a human health threat after normal processing. However, if a plant is allowed to grow unusually fast by providing it with an abundance of nutrients, the accumulation of defense compounds is reduced (Stamp 2003).

Examples of naturally occurring toxins that might be elevated in organic foods include alkaloids and glycoalkaloids and furanocoumarins.

The alkaloids are plant secondary metabolites that, together with phenolic compounds and other secondary metabolites, are considered to constitute a plant protection system against adverse effects of light and mechanical injuries, as well as against damage caused by pests and pathogens. They are heterocyclic, nitrogen-containing compounds. Alkaloids are naturally occurring toxins and characterized by a bitter taste.

When tasted with the skin, organically grown potatoes were rated more bitter than the nonorganic ones by a trained sensory panel, and this result was related to a higher content of glycoalkaloids (Wszelaki et al. 2005). Instead, no difference in taste was detected in the flesh of organic and nonorganically grown potatoes. No statistically significant differences could be established in the content of glycoalkaloids in tubers produced by organic and nonorganic production systems in a 4-year study on eight varieties (Hajšlová et al. 2005). However, in another study, organically grown potatoes showed more glycoalkaloids than those nonorganically grown, possibly due to a more insect stress (Wszelaki et al. 2005). In any case, the glycoalkaloid content was lower than 200 mg/kg, which has been defined as safe threshold level (Wszelaki et al. 2005; Brandt and Mølgaard 2006). Organically produced potatoes were also found to be more resistant towards pest attacks (Brandt and Mølgaard 2006).

Furanocoumarins are secondary metabolites occurring in some edible food plants such as celery, parsnip, parsley, carrot, etc. belonging to the Apiaceae family. Lower levels of these phytochemicals are also contained in citrus fruits and other crops representing the Rutaceae family (Søborg et al. 1996). Furanocoumarins are natural toxic compounds, yielding reactive intermediates under UV light irradiation. Formation of adducts with DNA can take place (Søborg et al. 1996). Some of them are known for their ability to cause contact dermatitis. In addition, mutagenic and carcinogenic effects have been demonstrated in experimental animals when exposed to high doses of furanocoumarins. The lowest observed adverse effect level was estimated in the range 0.14–0.38 mg/kg body weight. Since their presence in human diet represents food safety issue of concern, more knowledge is needed to reduce consumers' exposure (Schulzová et al. 2007).

Their chemical structure is a furan ring fused with coumarin that belongs to phenylpropanoids. The fusion can generate different isomers, specifically, psoralen and angelicin, the precursors of linear (e.g., bergapten, xanthotoxin, trioxsalen, isopimpinellin, bergamottin, etc.) and angular (e.g., pimpinellin, sphondin, isobergapten, etc.) furanocoumarins, respectively.

Typical levels of furanocoumarins found in celery range between 4 and 40 mg/kg, in parsnip 3–60 mg/kg, and in carrot <0.004 to 2 mg/kg (Schulzová et al. 2002), but there are various factors influencing the concentration in fresh and processed vegetables. Furanocoumarins are heat stable and are not destroyed by cooking. Their accumulation during storage has been observed (Ostertag et al. 2002). Like other secondary metabolites, furanocoumarins are considered to constitute a plant protection system against pests and/or physicochemical factors.

Consequently, under stress conditions, furanocoumarin levels may increase significantly. Concentrations as high as 45, 145, and 112 mg/kg were found in celery, parsnip, and parsley, respectively (Schulzová et al. 2007). Regarding the influence of different farming systems, some studies show a smaller increase in furanocoumarin levels in organically grown celery and/or parsnip when injury or pest infestation occurs (Schulzová et al. 2007).

For the role of phenolic compounds in plant defense, see section “[Phenolic Compounds](#).”

Contamination of Animal Feed

Animal feeds can be contaminated by pesticide residues, agricultural and industrial chemicals, and heavy metals, thus giving rise to safety hazards in food of animal origin. Organic livestock are fed on organically produced feedstuff. Therefore, the level of contamination with pesticide residues and other agricultural chemicals is thought to be lower compared with conventional farming methods. From a review of the literature, contamination of animal feed ingredients with potentially harmful substances (e.g., mycotoxins) has been documented in both types of feedstuff (Magkos et al. 2006).

Volatile Compounds

Volatile compounds determine the aroma or odor of food and food products. They are perceived by the odor receptor sites of the olfactory tissue of the nasal cavity. They reach the receptors when drawn in through the nose as one sniffs a food (orthonasal detection) and via the throat after being released by chewing as one eats a food (retronasal detection). Volatile compounds are molecules which have a high tendency to evaporate (high volatility, high vapor pressure). They can be naturally produced by plants (flowers, fruit, vegetables, herbs, etc.) or animals, be the results of transformation and reactions during food processing (thermal processing, fermentation, etc.) or be artificially produced through chemical reactions designed for their production.

More than 7,000 volatile compounds have been identified in food. Their contribution to an aroma perception depends on their concentration and odor threshold. The odor (or recognition) threshold is defined as the lowest concentration of a compound that is just enough for the recognition of its odor. The human being is exceptionally sensitive to some volatile compounds (the odor threshold of R-(+)-1-p-menthene-8-thiol is 0.00002 ppb in water at 20 °C) but insensitive to many other volatiles (ethanol has an odor threshold of 100,000 ppb in water at 20 °C). The individual ability to detect odors depends on the personal aptitude, determined by genetic, and is influenced by many personal and environmental factors.

Often food aroma is a very complex mix of hundreds of volatile compounds. Fortunately, of all the volatile compounds, only a limited number are important for aroma. These compounds are those that provide the characteristic aroma of a specific food and are, consequently, called key odorants (character impact aroma

compounds). Examples of key odorants are (R)-limonene that provides a citrus-like aroma and characterized the orange juice, benzaldehyde that provides the bitter aroma of almonds, 2-furfurylthiol roasted aroma in coffee, or 2-acetyl-1-pyrroline roasted aroma in white bread crust.

Formation of volatile compounds during food processing is out of the scope of this chapter. Being plant nutrition and defense and animal feeding the main issues characterizing the organic growing and raising systems with respect to the nonorganic ones, this paragraph will be focused on the potential effects of these practices on the volatile compound composition of plant and animal origin organic foods.

Volatile Compounds in Animal Origin Foods

The role of pasture and grazing in determining the composition of the lipid fraction (polyunsaturated fatty acids, conjugated linoleic acid, and vitamin E and carotenoid content of milk and dairy products) has been highlighted elsewhere in this chapter. According to the organic feeding rules, livestock should have permanent access to pasture or roughage. This could result in a higher content of PUFA, CLA, etc., in organic foods of animal origin than in nonorganic ones.

In addition, animal feeding methods influence also the composition in volatile compounds of milk, dairy products, and meat, thus potentially affecting their aroma and flavor characteristics. Based on this observation, organic food of animal origin could be characterized by a different volatile compound composition and, consequently, aroma and flavor characteristics when compared to the nonorganic counterparts.

Terpenes and aldehydes are responsible of the typical odor of different plant species and could be found in milk and dairy products from animals fed on pasture.

Mono- and sesquiterpenes are hydrocarbons occurring in plants, based upon isoprene unit, a five-carbon-atom hydrocarbon (2-methyl-1,3-butadiene). Their structure may be open chain, closed chain, saturated, or unsaturated and may contain O, N, or S. Monoterpenes contain two isoprene units and sesquiterpenes three. Carotenoids are tetraterpenes and contain eight isoprene units.

Volatile terpenes are commonly associated with the flavor of citrus products, spices, and herbs. The main volatile terpenes include D-limonene, *p*-cymene, menthol, β -caryophyllene, β -cyclocitral, nerol, etc.

These terpenes can be transferred to milk through animal diet. Because they are not modified by animal metabolism, terpenes have been suggested as marker of an animal diet.

The content of monoterpenes, such as *p*-cymene, and sesquiterpenes, such as β -caryophyllene, was found to be higher in the subcutaneous fat from lambs raised and finished on grass than in animals fed in part or in toto and, for different durations, with concentrates. It was possible to discriminate among lambs fed with different diets on the basis of terpene content in the meat fat, thus suggesting the use of these molecules as a potential biomarker of grass feeding in milk and meat products (Prache et al. 2005).

Qualitative and quantitative terpene composition of a pasture is determined by its botanical composition. A pasture rich in Fabaceae, such as lucerne

(*Medicago sativa* L.), or Apiaceae family plants has a higher terpene content than a pasture rich in Gramineae. Milk terpene composition was shown to be linked to pasture terpene composition (Bugaud et al. 2001). Based on this observation, terpene composition of milk and meat has been suggested as a marker to trace these food products back to the origin. Traceability means the ability to track any food, feed, food-producing animal, or substance that will be used for consumption, through all stages of production, processing, and distribution. Specifically, traceability back to the origin refers to an animal's identity, breed, and also geographical origin which are often important considerations for quality labels (e.g., Protected Denomination of Origin label). In this way, consumers can easily identify the origin and authenticity of the food products they purchase.

In addition to terpenes, there are a number of volatile compounds in milk belonging to many chemical families (saturated and unsaturated hydrocarbons, benzene derivatives, furans, ketones, aldehydes, esters, etc.). For these volatile compounds, no correlation has been found between their content in pasture and milk, thus suggesting that they result from the animal metabolism or produced by milk microorganisms (Bugaud et al. 2001). Aldehydes in milk, such as hexanal and *trans*-2 hexenal, are the end products of oxidative reaction of fatty acids (linoleic and linolenic acids, respectively) produced by the grass enzyme when fresh pasture is cut and/or during mastication (Bugaud et al. 2001).

Do these differences in volatile compound composition related to the animal diet result in perceivable differences of sensory characteristics of aroma and flavor of milk, dairy products, and meat?

To individuate, the effect of the animal diet on aroma and flavor characteristics of food products of animal origin is a very complex task. For example, flavor compounds in cheese are also formed due to the degradation of milk components like lactose, lipids, and proteins during cheese aging. The perception of a food aroma profile is not the result of the sum of its single volatile compounds but of the interactions between the different volatile molecules and their concentration ratios. Technological factors seem to have major effect. Differences in flavor characteristics of cheese from raw milk disappeared when pasteurized milk was used (Martin et al. 2005).

Regarding the perception of difference in flavor between organic and conventional raw milk that can be related to animal diet, a slightly stronger hay or grass flavor was detected by a trained sensory panel in organic than in nonorganic milk (Bloksma et al. 2008). However, differences in volatile compound composition seemed not to affect consumer acceptance of milk from nonorganic and pasture-based production systems (Croissant et al. 2007).

Volatile Compounds in Plant Foods

Volatile compounds in plants have been associated with defensive and attractive roles. They probably serve to attract and guide pollinators and to protect the plants from enemies because some of them can act as direct repellents or toxicants for herbivores or pathogens. All plants are able to emit volatile compounds, and the content and composition of these molecules show both genotypic variation and

phenotypic plasticity. In fruits, volatile emission and accumulation have probably evolved to facilitate seed dispersal by animals and insects. Volatile compounds determine fruit aroma, one of the essential factors contributing to fruit quality and, consequently, consumer preference. Some volatile compounds are specifically formed only by ripe fruit, whereas vegetative tissues often produce and release volatile compounds only after their cells are disrupted.

Fruit volatile compounds are mainly comprised of diverse classes of chemicals, including esters, alcohols, aldehydes, ketones, lactones, and terpenoids. However, some sulfur compounds also contribute to the flavor of some fruit. The biosynthetic pathways of important plant volatiles have been elucidated. They derive from an array of compounds including phytonutrients such as carbohydrates, fatty acids, and amino acids.

Identification of key odorants that carry the unique character of the fruit is essential, as it provides the principal sensory identity and characteristic flavor of the fruit.

The qualitative and quantitative composition in volatile compounds of fruit depends on the cultivar, ripeness, pre- and postharvest environmental conditions, fruit sample (either intact fruit, slices, or homogenized samples), and analytical methods utilized.

Among the preharvest factors, the different approach to plant protection and nutrition adopted in nonorganic and organic agriculture could affect in a different way the biosynthesis of plant volatile compounds. Saturated and unsaturated volatile C6 and C9 aldehydes and alcohols are important contributors to the characteristic flavors of fruits, vegetables, and green leaves. The short-chain aldehydes and alcohols are produced by plants in response to wounding and play an important role in plants' defense strategies and pest resistance. Nitrogen deficiency can limit aroma potential in some fruit (Peyrot des Gachons et al. 2005). It has been suggested that an insufficient nitrogen supply due to reduced release rates by organic fertilizers could stimulate plant stress responses, resulting in an enhanced accumulation of phenolic flavonoids in fruits thus potentially affecting both flavor and nutritional quality (Lester and Saftner 2011). In a recent study, a limited impact of the use of different organic and nonorganic fertilizers on the volatile profile of apple fruits was observed, although significant differences resulted in the levels of available nitrogen in the soil (Raffo et al. 2014). However, a significant correlation between the nitrogen level in the leaves and the content of C6-aldehydes (1-hexanal and (E)-2-hexenal) straight-chain esters (hexyl acetate) in the fruit was observed. This result implies that a role of nitrogen nutrition on the formation of some apple volatile compounds cannot be completely ruled out. In a study in which quality characteristics were determined on carrot grown under different farming practices (nonorganic and three organic cropping systems with increasing levels of nutrient recycling), a lower content of total sesquiterpenes and β -caryophyllene resulted in the nonorganic carrots, whereas the organic carrots grown using fertility-building crops (catch crops, green manure, living mulch) showed a higher content of these compounds (Paoletti et al. 2012).

Analytical Methods to Discriminate Between Organic and Nonorganic Food Products

Due to the lower yield, the more labor-intensive production, and the cost of certification, organic products usually retail at a higher price than their nonorganic counterparts. Notwithstanding, the market for organic food has grown constantly in the last 20 years. The increasing demand and the premium price associated with make the organic food products susceptible to fraud. Although the “added value” of these products is guaranteed by a system of certification, nevertheless a confirmatory assessment of the identity of organic food products in addition to administrative controls is needed. From an analytical point of view, however, the authentication of organic food products is a challenging problem. A different content of a single compound or a limited number of compounds between organic and nonorganic food products is not sufficient for the authentication of the organic food products. Analytical methods involving the analysis of a range of compounds (fingerprinting) which are considered potential discriminators seem to be the more promising approach. As these techniques result in a high number of variables, the application of multivariate statistical methods is essential for evaluation of the data.

Stable Isotopes

The distribution of isotopes is not the same throughout the earth and among different organisms, thus giving rise to distinct isotopic signatures in plants and animals which may be useful also in the differentiation between farming regimes.

Special attention has been given to the potential application of N isotope analysis in discriminating between organic and nonorganic plant products based on the assumption that the $^{15}\text{N}/^{14}\text{N}$ ratio ($\delta^{15}\text{N}$) of a plant reflects that of the soil in which the plant has grown and depends on the fertilization strategies adopted. While synthetic fertilizers exhibit $\delta^{15}\text{N}$ close to 0 ‰, organic fertilizers show much higher $\delta^{15}\text{N}$ values. It has therefore been suggested that plants grown under a nonorganic regime will exhibit lower $\delta^{15}\text{N}$ values than plants grown under an organic regime (Choi et al. 2003).

The potential of the nitrogen isotope ratio for the authentication of organic crops has been thoroughly investigated with controversial results (Capuano et al. 2013). In nature, $\delta^{15}\text{N}$ abundances in plants depend on many factors such as the type and degree of mycorrhization, the type of synthetic fertilizers used, the amounts and timing of fertilizer application, the use of organic manure in nonorganic growing systems, the produce considered, etc. Furthermore, the use of organic fertilizers to grow a crop does not imply that the crop can be classified automatically as organic, because other rules of the organic regulation could not have been respected.

A multi-method approach is often necessary to solve the authentication problem.

Plants have a different $^{13}\text{C}/^{12}\text{C}$ ratio ($\delta^{13}\text{C}$) according to their differences in photosynthesis. C3 plants, such as grass and hay, show the lowest $\delta^{13}\text{C}$ values, whereas C4 plants, such as maize, exhibit the highest; intermediate values are

shown by CAM (crassulacean acid metabolism) plants, such as succulents (O'Leary 1988). Since pasture is almost exclusively made up of C3 plants, $\delta^{13}\text{C}$ values are expected to be lower in meat and milk from pasture-fed and organically reared cows. In fact, there are studies in the literature showing that the use of $\delta^{13}\text{C}$ is able to discriminate between organic and nonorganic meat (Piasentier et al. 2003) and organic and nonorganic milk and dairy products (Molkentin 2013). However, a marked effect of season on $\delta^{13}\text{C}$ values was observed both for meat and milk. For milk an improvement of the discrimination potential was observed when $\delta^{13}\text{C}$ values were used in combination with the α -linolenic acid (C18:3 ω 3) content (Molkentin 2013).

Interestingly, the combined measurement of $\delta^{15}\text{N}$ values and α -linolenic acid content allowed the correct classification also of samples of organically and nonorganically farmed Atlantic salmon (Molkentin et al. 2007).

A multi-isotopic analysis based on a combination of several techniques, such as isotope ratio mass spectrometry (IRMS), multicollector-inductively coupled plasma-mass spectrometry (MC-ICP-MS), and compound-specific isotope analysis (CSIA), has been recently proposed for discriminating organic and nonorganic plant products (Laursen et al. 2013). The study was based on wheat, barley, faba bean, and potato produced in rigorously controlled long-term field trials. Hydrogen isotope analysis $\delta^2\text{H}$ allowed the discrimination between organic and nonorganic cereals, whereas oxygen isotope analysis of nitrate was successful for potato tubers.

Metabolomics

Recent developments in analytical techniques have provided new options for the authentication of organic products based mainly on profiling/fingerprinting approaches. However, since these approaches involve the analysis of a range of compounds which are considered potential discriminators, the application of multivariate statistical methods for the evaluation of the collected data is needed.

Metabolomics represents one of these approaches.

In the same way as the genome is the genetic material of an organism and the proteome is the entire set of protein expressed by a genome, the metabolome is the complete set of small molecule metabolites. Plants react to any change in their surroundings; everything the plant does can be followed by looking at the changes in the low molecular weight chemicals. Metabolites are the end products of cellular functions, and their levels can be viewed as the response of biological systems to environmental or genetic manipulation or may be a reflection of molecular changes induced during growth, postharvest, and processing. Important small metabolites include amino acids, organic acids, sugars, volatile metabolites, and most of the diverse secondary metabolites found in plants, such as alkaloids, phenolic compounds, and colored metabolites such as carotenoids and anthocyanins.

Metabolomics is the study of all the chemicals in a plant that have a low molecular weight aimed to gain the broadest overview possible of the biochemical composition of complex biological samples in just one or a small number of analyses. Metabolomics can be divided into:

- (a) Targeted analysis, that is, a qualitative and quantitative evaluation of one or a few metabolites related to a specific metabolic reaction
- (b) Nontargeted analysis when as many as possible metabolites are detected

In turn, nontargeted analysis can be divided into:

- (i) Metabolic profiling that involves the accurate determination (identification and quantification) of a selected number of predefined metabolite level, generally related to one or more specific metabolic pathway(s)
- (ii) Metabolic fingerprinting that is a high-throughput, rapid, global analysis of samples to provide a specific recognition and shape of a sample, without proper quantification and metabolite

The typical analytical equipment used for metabolomics includes nuclear magnetic resonance (NMR), Fourier transform infrared (FT-IR) spectroscopy, and mass spectrometry, often combined with chromatography (GC-MS, LC-MS).

Metabolomic approach has been recently adopted for the discrimination between organic and nonorganic food products with alternating fortunes. Three different maize varieties grown under organic and nonorganic conditions were not differentiated through the metabolite profile obtained by GC/MS, because the genetic differences and seasonal effects resulted stronger than the differences due to growing method (Rohlig and Engel 2010). Instead, using an untargeted approach based on MS fingerprint measured by flow injection electrospray ionization ion trap mass spectrometry (FI-ESI-IT-MS) and flow injection electrospray ionization time-of-flight mass spectrometry (FI-ESI-TOF-MS), Chen et al. (2010) were able to obtain a complete separation of the samples of organic and nonorganic grapefruit based on the growing method. Through the determination of the triacylglycerol profile of egg yolk, Tres et al. (2011) were able to discriminate between organic and nonorganic eggs. Novotná et al. (2012) employed ambient mass spectrometry consisting of a direct analysis in real-time (DART) ion source and a time-of-flight mass spectrometer (TOF-MS) to analyze tomato and pepper samples grown under organic and nonorganic farming method. The statistical models used by the Authors allowed a clear separation between the samples according to the farming method both for tomato and pepper and showed a satisfying prediction ability. However, the authors emphasized that the year of production had stronger influence on the metabolomic fingerprints compared with the farming method. A systematic impact of the farming system on the metabolome of white cabbage was observed by Mie et al. (2014), and this imprint was retained over two harvest years.

Conclusions and Future Directions

The overall and specific principles of organic farming and their application in practice have the potentiality to determine differences in chemical composition between organic and nonorganic foods. Based on this, a number of comparison

studies have been published during the last 20 years, from which some trends can be individuated. In comparison with nonorganic counterpart, (a) organic cereals show a lower protein content; (b) organic plant foods are characterized by a higher content of phenolic compounds; (c) organic plant foods show a higher vitamin C content; and (d) organic milk and dairy products show a higher content of ω -3 fatty acids, conjugated linoleic acid, and a ratio between ω -3 and ω -6 fatty acid content more respondent to that recommended by nutritionists.

The clear finding emerging from these studies is that several factors can affect the food chemical composition and that very often their effects are more relevant than those caused by the farming systems. Notwithstanding, an experiment can be accurately designed to reduce the influence of the most of these other factors; some of them are unpredictable and uncontrollable, such as the year-to-year weather variation. Therefore, whenever the effect of a farming system on food chemical composition has to be determined, a long-term study has to be adopted to reduce the influence of all the other factors. Only in this way the objective of an unambiguous assessment of the influence of organic farming practices on food chemical composition can be achieved.

Fingerprinting analytical approaches, such as metabolomics and multi-isotopic analysis, coupled with multivariate statistical analysis methodologies (chemometrics) seem promising in discriminating between organic and nonorganic foods. However, also in this case, seasonal effects resulted to impair the discrimination ability of these methods. To develop robust statistical models able to correctly classify unknown samples, it is necessary to design experimental studies which include all possible sources of variation for the target markers and to have at disposal comprehensive and well-structured databases.

Cross-References

- ▶ [Agricultural Chemical Pollutants](#)
- ▶ [General Properties of Major Food Components](#)
- ▶ [General Properties of Minor Food Components](#)
- ▶ [Plant-Associated Natural Food Toxins](#)

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Abstract

This chapter is focused on chemistry of carbohydrates present in sugar and confectionery products. The introductory part brings the basic knowledge about the classification, chemical structure, properties, and reactions of carbohydrates, including the chirality, stereoisomerism, mutarotation, caramelization, and Maillard reactions. Nutritional properties, occurrence and importance of simple sugars, and the basic characteristics and physiology of polyols and sugar substituents in confectionery are specified as well.

The reader will find information about the classification and preparation of different kinds of confectionery products, such as candies, fondants, caramels, nougat, gums, jellies, chewing gums, and many others. Chocolate and its chemical composition and preparation are described in an individual chapter with an emphasis on chocolate nutritional aspects and benefits. Sources and production of table sugar, its properties, quality, and important functions in food production are mentioned as well. The last part is dedicated to the structure, properties, and reactions of other substances and ingredients used in confectionery, such as pectin, gelatin, agar, lecithin, vanilla, peppermint, and more.

Introduction

Carbohydrates, also called saccharides, are widespread organic substances in nature. Together with proteins, fats, and their derivatives, carbohydrates are one of the three main groups of constituents in foods.

From nutritional point of view, carbohydrates are mainly the source of energy but also affect the food texture and color, give food its sweetness, and act as preservatives or flavor enhancers. Their composition, chemical changes, as well as function in sugar and confectionery products are the main topic of this chapter.

Sugars in Confectionery and Sugar Products

In nature, carbohydrates are produced by photosynthesis in cells of autotrophic organisms (green plants) by assimilation of carbon dioxide in the presence of water using energy from sunlight. Plants are therefore able to transfer the light energy into a chemical one and store it. Tissues of animal cells contain few percents of sugars. Plant tissues, on the other hand, are formed mostly from carbohydrates (85–90 % of carbohydrates in dry solid).

Carbohydrates play many roles in biological systems:

- They form energy reserves (such as starch in plants or glycogen in animals) because they can be oxidized to release energy. Glucose, the main source of energy in organisms, provides through an aerobic respiration 17 kJ/g, i.e., 4 kcal/g, whereas sugar alcohols provide only about 10 kJ/g or 2.4 kcal/g.
- They may serve as structural or protective components (e.g., cellulose).
- They can act as biologically active compounds or be part of active and functional components in nucleic acids, vitamins, hormones, glycoproteins, coenzymes, etc.

The name carbohydrates means “hydrated carbon” and can be expressed by the basic formula $C_x(H_2O)_y$. Hence, carbohydrates contain only atoms of carbon, hydrogen, and oxygen. In recent years, carbohydrates have been classified on the basis of their structures (Bodner 2014), and other compounds derived from carbohydrates or formed from sugars by oxidation, reduction, substitution, or other reactions belong to the group of carbohydrates as well.

According to their structures, carbohydrates can be classified in three groups: monosaccharides, oligosaccharides, and polysaccharides:

- Monosaccharides contain only one sugar unit. They are basic building blocks of more complex carbohydrates.
- Oligosaccharides consist of two to ten monosaccharides linked together with acetal linkage. If two monosaccharides are linked together, a molecule of water is released to form disaccharide. Similarly, trisaccharides, tetrasaccharides, etc. can be formed by linking three, four, etc. monosaccharides. Both mono- and disaccharides are called sugars or simple carbohydrates. They are typical with their sweetness, water solubility, and rate of fermentation by microorganisms. Sugars with more than three linked monosaccharides are called complex sugars.
- Polysaccharides are built from more than ten sugar units in a polymer-like fashion. The chain length can vary from eleven to several hundreds of sugar units and usually is not exactly determined. They can break down in the presence of acids or specific enzymes to smaller molecules, including their simple sugars.

A special category is conjugated sugars containing, apart from the sugar, other compound such as lipid, protein, or peptide.

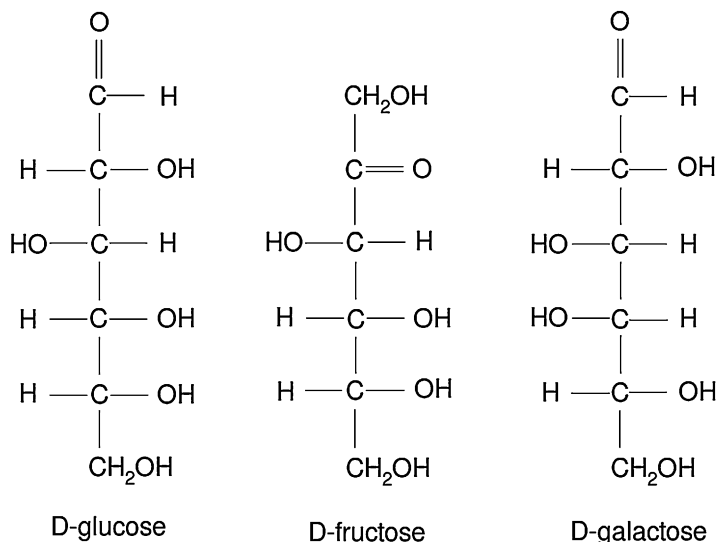


Fig. 1 Fischer formulas for basic monosaccharides

Monosaccharides

Monosaccharides are white, crystalline solids that contain a single aldehyde or ketone functional group. Therefore, they are subdivided into two classes; compounds containing aldehyde functional group ($\text{H}-\text{C}=\text{O}$) are called aldoses, and compounds with ketone group ($\text{C}=\text{O}$) are ketoses.

The structure of monosaccharides can be written using the so-called Fischer projection formulas, which are by convention written vertically, with the aldehyde or ketone on the top. Fischer projections for glucose, fructose, and galactose are in Fig. 1.

Due to the presence of asymmetric (chiral) carbon, carbohydrates exhibit optical activity as they rotate plane-polarized light. The hydrogen ($-\text{H}$) and hydroxyl ($-\text{OH}$) groups attached to the carbon at position 2 can occur on both sides of the skeleton structure. If the hydroxyl group is written on the left side of the skeleton structure, the structure is called L isomer because it rotates the plane of the light left (levorotatory). Similarly, if $-\text{OH}$ is on the right side, the D isomer is dextrorotatory. Fischer projections for the two isomers of glyceraldehyde are shown in Fig. 2. Although the sugars can exist in both forms, most of the monosaccharides found in nature are in the D configuration. The isomers are like mirror images. This type of isomerism is called enantiomorphism. The chemical and physical properties of enantiomers are identical apart from optical rotation.

Monosaccharides contain from three to nine carbon atoms and can be classified according to the number of C atoms in a molecule as trioses, tetroses, pentoses, hexoses, etc. The molecules of trioses and tetroses are mostly linear but if the carbon chain is long enough, the hydroxyl group at one end of a monosaccharide

Fig. 2 Fischer projections of D-glyceraldehyde and L-glyceraldehyde

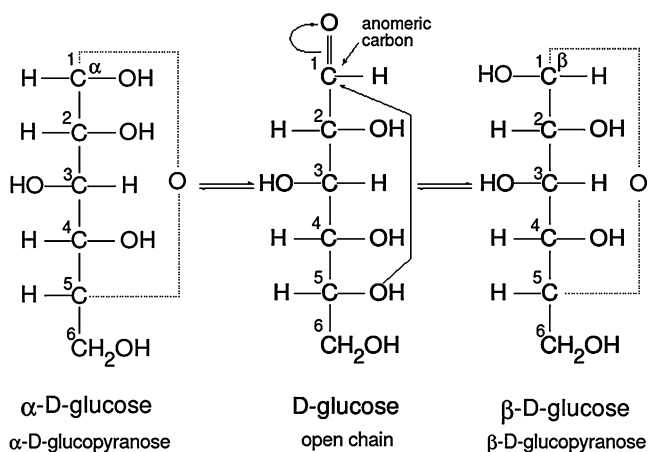
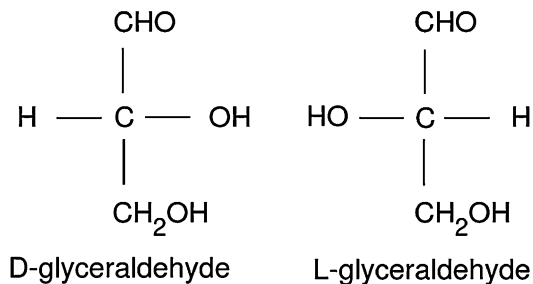
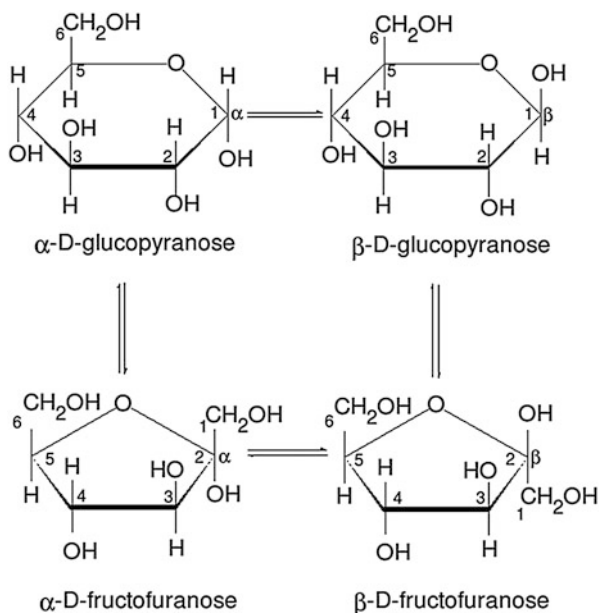


Fig. 3 Formation of cyclic hemiacetal in D-glucose

can attack the carbonyl group to form a cyclic compound called a cyclic hemiacetal (see Fig. 3), which is a preferred structure due to the low angle and eclipsing strain (Farmer and Reusch 2014).

The six-member ring is called a pyranose; if only five atoms form the ring, the product is named furanose. Both pyranose and furanose forms are often depicted in a flat projection known as a Haworth formula (see Fig. 4). By convention, the anomeric carbon atom is placed on the right. The anomeric atom is the carbon originating from the carbonyl group (C1 carbon in aldose, C2 carbon in ketose). According to the position of hydroxyl group on the anomeric carbon (hemiacetal or hemiketal carbon), there are two possible structures for the pyranose and furanose forms, which are called the α - and β -anomers. If the hydroxyl on the anomeric carbon is oriented above the ring plane, the anomer is called β -anomer, and vice versa.

While reducing sugars dissolve in water, the ring opens due to hydrolysis and then closes again producing different tautomeric forms. Therefore, D-glucose can occur as α -D-glucopyranose, β -D-glucopyranose, α -D-fructofuranose, or β -D-fructofuranose (see Fig. 4). This effect is called mutarotation. Since every of these forms have different physicochemical properties, such as optical rotation, solubility, reactivity,

Fig. 4 Mutarotation of glucose**Table 1** Occurrence of sugar tautomers in aqueous solution

Sugar	Temperature (°C)	α -Pyranose (%)	β -Pyranose (%)	α -Furanose (%)	β -Furanose (%)	Open chain (%)
Glucose ^a	20	36.4	63.6	–	–	0.02–0.06
Glucose ^a	31	37.5	61.5	0.5	0.5	0.002
Galactose ^a	20	32	63.9	1	3.1	–
Galactose ^a	31	30	64	2.5	3.5	0.02
Galactose ^b	40	27	73	<1	<1	–
Fructose ^a	27	–	75	4	21	–
Fructose ^a	31	2.5	65	6.5	25	0.8
Mannose ^b	40	67	33	<1	<1	–

^aWrolstad (2012)^bVelíšek and Hajšlová (2009)

or sweetness, and their distribution in a solution depends on temperature, the solution can exhibit various properties at different temperature. Table 1 shows a distribution of some D-sugar tautomers in aqueous solution. In a glucose solution at 20 °C, there is 63.6 % of the β -anomer and 36.4 % of the α -anomer.

The number of open-chain forms increases with higher temperature. Many sugar reactions will run at higher rates which are proportional to the number of sugar molecules in open-chain form. That is why pentoses are more reactive than hexoses and react more readily in Maillard reactions (see section “[Nonenzymatic Browning](#)”).

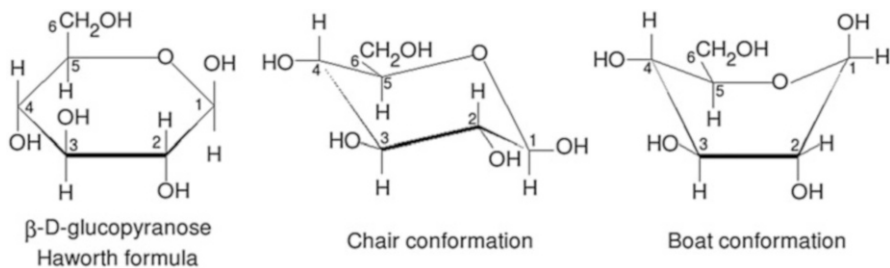


Fig. 5 Typical glucose conformations

Nevertheless, neither Haworth formulas show the true shape of the molecules, because the ring cannot be flat. The carbon-carbon bonds rotate and the ring can take several nonplanar shapes called conformations. In glucose, the two typical conformations called “chair,” which has the lowest energy, or the “boat” conformations are shown in Fig. 5.

Glucose, Fructose, and Galactose Structure and Properties

Glucose, fructose, and galactose represent the three most important simple sugars. They all contain six carbon atoms (belong among hexoses) and have the same molecular formula, $C_6H_{12}O_6$. However, these sugars have different characteristics and properties. This is caused by their different structural arrangements due to the formation of a wide range of spatial and geometric configuration. The ability to form such forms is called stereoisomerism.

Glucose

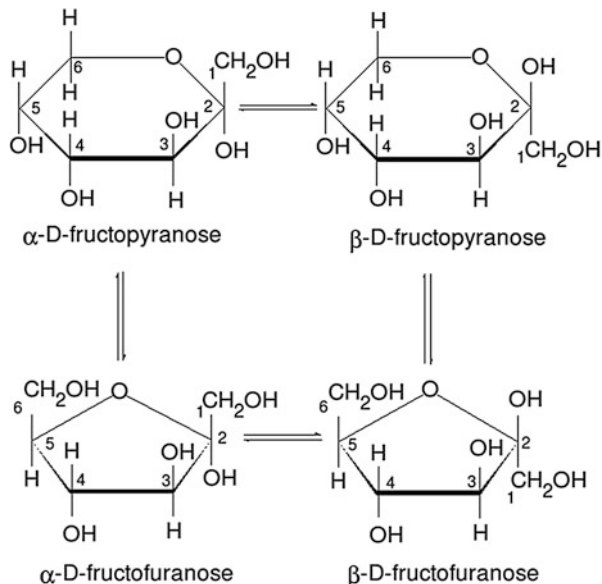
Glucose, also known as dextrose, grape, corn, or blood sugar, is white crystalline compound widely present in plant or animal tissues. Glucose is the source of energy for all kinds of cells. The energy from glucose can be provided either by aerobic respiration, anaerobic respiration, or fermentation. Its name originates from the Greek word “glykys” which means “sweet.” Glucose is soluble in water forming colorless and odorless solution. It is a reducing sugar, which also reacts in Maillard reactions. Glucose sweetness is 74–80 % of the sweetness of sucrose, and the glycemic index has been set as a standard with a value of 100. Glycemic index (GI) describes the speed of how the blood sugar level increases after consumption of certain type of food.

From a chemical point of view, glucose has an aldehyde group attached on C-1 carbon in an open-chain configuration. Due to the presence of chiral carbon atom, glucose can exist in a dextrorotatory (D) or levorotatory (L) form. In a solution molecule of glucose mostly occurs in the form of cyclic isomers, which are all chiral (see Fig. 4 and Table 1).

Fructose

Fructose (fruit sugar) is also a very common simple six-carbon ketose in our diet (see Fig. 1). It is a white, crystalline, water-soluble compound, which undergoes fermentation, Maillard reactions, and dehydration. Fructose retains moisture and

Fig. 6 Mutarotation of fructose



acts as a humectant. That is why fructose addition into food products can improve the texture and the shelf life. In a combination with starch, fructose reduces the temperature necessary for starch gelatinization, which increases the viscosity of the final product.

Fructose is 1.74 times sweeter than sucrose at low temperature and has the lowest glycemic index (GI = 23) of all natural sugars (Wolever 2006); therefore, it has been introduced as a sweetener. For comparison, sucrose has a GI of 65 and honey (usually about 50 % fructose content) has a GI of 55.

In a solution, it can occur in the form of four isomers (Table 1, Fig. 6). The ring structure is made of four molecules of carbon and one oxygen forming 5-ring form (α -D-fructofuranose or β -D-fructofuranose) or forming 6-ring form (α -D-fructopyranose and β -D-fructopyranose). The increased sweetness of fructose is caused by the molecule structure (Wrolstad 2012). The β -D-fructopyranose, which is predominant in a solution at lower temperature, is intensively sweet. However, with higher temperature (60 °C), the β -D-pyranose form changes into β -D-fructofuranose and open-chain forms, and fructose is becoming less sweet than sucrose.

Galactose

Galactose is another important simple sugar which is mainly found in milk and milk products. Its name originates from the Greek “galaktos” which means “milk.” Galactose exists in both open-chain (L and D forms) and four cyclic forms (α -D-galactofuranose, β -D-galactofuranose, α -D-galactopyranose, and β -D-galactopyranose). Galactose is a hexose almost identical to glucose. The only difference is the position of -OH group on C-4 atom (see Fig. 1); it is a C-4 epimer of glucose. This little

structural difference gives galactose different properties. Galactose, which has a relative sweetness value of 60 % of the sweetness of sucrose, is less sweet than glucose and fructose. Its glycemic index (GI = 25) is also very low.

Occurrence of Monosaccharides in Food and Confectionery Products

Glucose is a main building block of disaccharides sucrose and maltose, polysaccharide glycogen, starch, and cellulose. Glucose occurs mainly in honey (30 %), fruits, vegetables, dried food (20–32 %), starch syrups (31 %), and cereals. Glucose content in fruit is very variable (0.5–32 %) and depends on the fruit type, degree of ripeness, and conditions during storage after the harvesting. Glucose is also one of the main ingredients in a confectionery technology.

Fructose is present mainly in fruits and honey and is one of the two sugars that are found in sucrose. It seems that content of monomeric fructose in human diet is relatively low, but the fructose uptake has increased during the last 40 years due to increased consumption of sucrose and introducing of high-fructose corn syrups (HFCSs) into human diet in 1970s. The annual fructose consumption in Europe was about 0.2 kg per person in 1970, and 27 years later, it was 28 kg (Kazdová et al. 2013). HFCS, also called isoglucose (in Europe) or glucose-fructose syrups, is produced by enzymatic hydrolysis and isomerization of corn or wheat starch. Due to its low producing costs and higher sweetness in comparison with sucrose, fructose has been introduced into production of many foods, such as soft beverages, jams, yogurts, bakery products, biscuits, and confectionery products. All forms of fructose, including fruits and juices, are used in confectionery technology to enhance the flavor, color, or palatability. HFCSs are used to provide a sweet taste, improve the texture, control graining, keep the quality, and prevent the crystallization of sugar and retain moisture. The average fructose content in 1 l of carbonated soft drink is about 50 g.

Another important monosaccharide, galactose, is rarely found as a simple sugar. Galactose is usually combined with other simple sugars in order to form larger molecules. The main source of galactose in confectionery product is a disaccharide lactose which originates from milk-based ingredients. Beside lactose, galactose is a part of complex carbohydrates and glycolipids. It also occurs in a complex polymer called pectin.

Physiology and Nutrition of Monosaccharides

In healthy person, adsorption of monosaccharides occurs mostly in the small intestine and is limited to the monosaccharides glucose, fructose, and galactose. Glucose and galactose are transferred through the intestinal lumen via the active transport membrane process driven by ATP (adenosine triphosphate).

When glucose is delivered to the circulation, the glucose concentration in blood is rising, activating a secretion of insulin. Insulin removes excess glucose from the blood that is why its regulatory functions in both carbohydrate and lipid metabolism are essential.

Galactose needs to be converted to glucose mainly in the liver and therefore produces less pronounced blood glucose elevation.

Fructose has different metabolism than glucose, and recent studies suggest that increased uptake of sucrose and HFCS leads to prevalence of obesity, metabolic syndrome, diabetes, and cardiovascular diseases both in adults and children (Bray 2007). Fructose is not transferred by this active transport but is moved by glucose transport proteins (GLUT5), which is dependent on a concentration gradient over the intestine cells. The gradient is maintained by GLUT2 and fructokinase. Fructokinase is produced in liver cells only. Therefore, fructose is transferred from portal blood into the liver to produce triacylglycerols. Unlike glucose, fructose is not a direct source of energy for tissues, and its metabolism is not controlled by insulin; therefore, fructose utilization is maintained even during starvation or diabetes when glucose utilization is reduced. Also, fructose in livers inhibits lipid oxidation. High capacity of the liver to produce precursors of triglycerides is the main reason of hypertriglyceridemic effect, which results in an increased synthesis of lipids and serum lipoproteins. Increased production of triglycerides enhances their transport into peripheral tissues and their accumulation in the muscles, myocardium, and pancreas (Rizkalla 2010) where other toxic lipidic metabolites are produced affecting negatively the transport of insulin signal (Shulman 2000).

Oligosaccharides

Oligosaccharides are oligomers of at least two monosaccharides linked by *O*-glycosidic bonds formed through dehydration of two hydroxyl groups; one hydroxyl is located on a hemiacetal group (C-1 atom) on one sugar and the other can be from any of the carbon atoms from the other sugar. The fact that monosaccharides have multiple hydroxyl groups means that various glycosidic linkages are possible. If the hydroxyl group on C-1 carbon is in a position α , the resulting bond is called alpha; if the C-1 hydroxyl is in β position, the linkage is called beta.

Sucrose, Maltose, Lactose, and Raffinose Structure and Properties

Sucrose, maltose, and lactose belong among the most common disaccharides.

Maltose

Maltose, or malt sugar, is formed by two *D*-glucose molecules joined by a glycosidic linkage between the α -anomeric form of C-1 on one sugar and the hydroxyl oxygen atom on C-4 of the adjacent sugar (Fig. 7). Such a linkage is called an

Fig. 7 Maltose

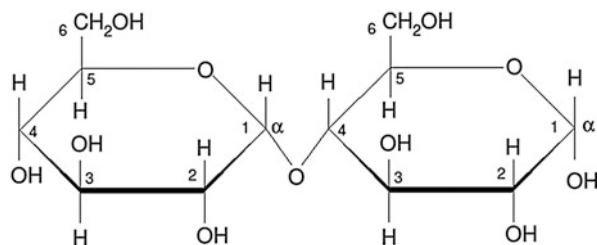
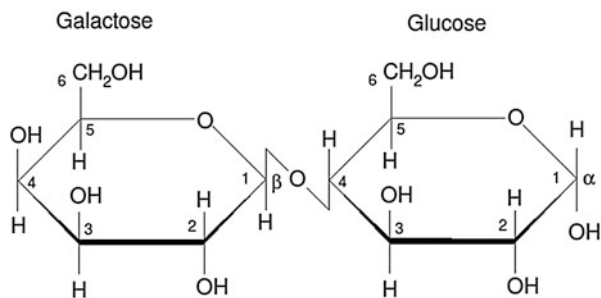
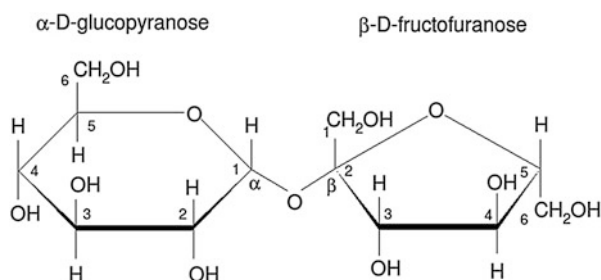


Fig. 8 Lactose**Fig. 9** Sucrose

α -1,4-glycosidic bond. Due to the free aldehyde group, maltose is a reducing sugar. It has relatively high GI (150) but low sweetness (30–60 % of sucrose sweetness).

Lactose

Lactose ($C_{12}H_{22}O_{11}$) is the reducing disaccharide in milk. It consists of galactose joined to glucose by a β -1,4-glycosidic bond (Fig. 8). Lactose is less soluble in water in comparison with other sugars. 60 % solution of lactose crystallizes easily at 25 °C (Čopíková 1999). Lactose sweetness is low (16 % of sucrose sweetness) and GI 45.

Sucrose

Disaccharide sucrose ($C_{12}H_{22}O_{11}$), known as table sugar, is the most abundant oligosaccharide in nature, synthesized by plants. In the sucrose molecule, two anomeric carbon atoms of a glucose unit and a fructose unit are joined together by α -1,2 glycosidic bond to form α -D-glucopyranosyl-(1 \rightarrow 2)- β -D-fructofuranoside (Fig. 9). Sucrose is a nonreducing sugar, since the glycosidic bond is formed between the reducing ends of both glucose and fructose. Its GI is 65 and the sweetness 100 %. Sucrose sweetness was established as a reference for sweet taste.

Sugar Production

Sucrose is industrially obtained from plants which are rich natural sources: sugarcane (*Saccharum officinarum*) and sugar beet (*Beta vulgaris*). About 23 % of commercial sugar is beet sugar and 77 % is cane sugar (Godshall 2013).

In a sugar beet processing, the sugar production is based on an extraction of sucrose from beet slices called cossettes by warm water. Obtained liquid, named diffusion or raw juice, is purified in so-called calco-carbonation process, where lime milk is added to precipitate present impurities, e.g., sulfate, phosphate, citrate, oxalate, proteins, saponins, and pectins. Also present glucose, fructose, and glutamine are transformed into carboxylic acids. Next step in this purification process is an introduction of carbon dioxide in two steps, which causes the precipitation of lime to calcium carbonate and its sedimentation. After the filtration, sugar solution of golden color called thin juice is obtained. Thereafter, the thin juice containing about 16 % of sucrose is concentrated on evaporators to the concentration of 60–67 %, which is golden brown thick juice. Thick juice is boiled in evaporation crystallizers under vacuum until it reaches the supersaturation stage. Then it is seeded with fine sugar crystals to start a crystallization process. The crystals are separated from the syrup in centrifuges and washed with water to remove residues of the syrup (mother liquor) from their surface. Pure product, white crystals clear as glass, called refined sugar, is obtained.

The syrup from centrifugation is boiled again to produce a second batch of sugar. This sugar contains more impurities; therefore, it is dissolved and crystallized again to obtain a pure product. The syrup obtained from the last crystallization, called molasses, is a valuable by-product containing mainly sucrose (60–65 % in dry solid) but also glucose, fructose, and the trisaccharide raffinose. It also contains other nonsugars, such as minerals (10 % in dry solid) calcium, potassium, oxalate, and chloride, and betaine (trimethylglycine).

In sugarcane processing, sucrose is extracted by warm water from the crushed cane in sugar mills. The juice is purified and clarified with heat (95 °C) and lime, and precipitate is removed on centrifuges or by sedimentation. Evaporation usually takes two steps; the first one is concentration of the juice in the evaporator and the second one is the crystallization in vacuum pans. Following evaporation, the syrup is clarified by adding lime, phosphoric acid, and a polymer flocculent, aerated, and filtered. From the clarifier, the syrup goes to the vacuum pans for crystallization. The crystallization and refining is similar to the sugar beet processing.

Sugar Quality

The quality of sugar is evaluated by analytical parameters, such as ash content, polarization (sucrose content), refractometric dry solid content, invert sugar content, moisture content, and color. The standard sugar quality according to the European Union is summarized in Table 2.

Granulated sugar can differ in crystal size, which is determined by sieving analysis (see Fig. 10).

Coarse sugar has the largest crystals and is highly resistant to color change or inversion at high temperature, which is important for making fondants, confections, and liquors. Regular, fine, or extra fine is the sugar best known to consumers and widely used in households.

Table 2 Crystallized sucrose quality in Europe (Council Directive 2001)

Parameter	Extra-white sugar	Sugar or white sugar	Semi-white sugar
Polarization (°Z)	99.7 min	99.7 min	99.5 min
Invert sugar content (%)	0.04 max	0.04 max	0.1 max
Moisture content (%)	0.06 max	0.06 max	0.1 max
Conductivity ash (%)	–	0.04 % max	–
Color of the solution	–	45 International Commission for Uniform Methods of Sugar Analysis units max	–

Sugar type	Sieve size range (mm)						
	2.00-1.00	1.00-0.80	0.80-0.63	0.63-0.40	0.40-0.32	0.32-0.16	0.16-0.00
Coarse sugar	70% min						
Regular sugar	5% max	70% min					
Powdered sugar			5% max	95% min			

Fig. 10 The crystal size distribution of different types of sugar

Ultrafine, superfine, or bar sugar is the finest of all the types of granulated sugar suitable for fine-textured cakes, fruit sweetening, or drinks because it dissolves easily. In England, the similar type of sugar is called caster (castor), named after the type of shaker.

Powdered, also icing or confectionery sugar, is a granulated sugar ground to smooth powder. It contains anticaking agents (e.g., cornstarch).

Brown sugar is a product where part of the mother liquor has not been washed out from the crystals and stayed on the crystal surface giving the crystal light brown color and pleasant flavor. Brown sugar contains more moisture than white (refined) one and tends to form clumps. Dark brown sugar has more color and stronger molasses flavor than light brown sugar. Dark brown sugar is usually used in full-flavored foods.

Muscovado or Barbados sugar is a British specialty brown sugar of very dark brown color and strong molasses flavor.

Demerara sugar, again popular in Britain, is light brown sugar with large golden crystals, which are slightly sticky (The Sugar Association 2014).

Liquid sugar can be either liquid sucrose prepared by sucrose dissolving in water with minimum content of solids 62 %, or it can be invert sugar prepared by enzymatic or acid hydrolysis of sucrose giving an equal mixture of glucose and fructose. The latter is called liquid invert sugar or invert sugar syrup. Liquid sucrose can have different colors from nearly colorless to light yellow or amber. Invert sugar is sweeter than crystal sugar and can be combined with dissolved sucrose.

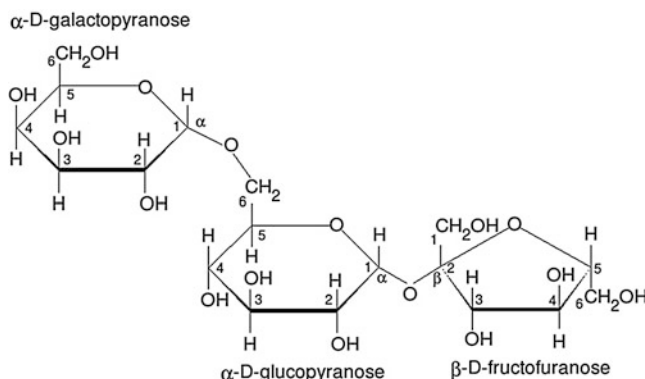


Fig. 11 Raffinose

Invert sugar with minimum amount of sucrose is called total invert sugar and is used in food products to retard crystallization of sugar and retain moisture.

Raffinose

Raffinose, also melitose or α -D-galactosylsucrose (Fig. 11), with molecular formula $C_{18}H_{32}O_{16}$, is a soluble trisaccharide composed of galactose, fructose, and glucose. Its sweetness is 20 %.

Occurrence of Main Disaccharides in Food and Confectionery Products

Sucrose is widely present in most eukaryotic cells. As a reserve-forming sugar, sucrose can be readily hydrolyzed by enzyme invertase. It occurs in large quantities in many plants, such as sugar beet (15–20 %), sugarcane (12–26 %), corn (12–17 %), fruits (up to 8 %), and vegetables (0.1–12 %). Palms are also a rich source of sucrose; for example, fruits of date palm can contain up to 81 % of sucrose, and the sap of some palm species (coconut, nipa, palmyra palm) or sugar maple tree (*Acer saccharum*) contains high amount of sucrose (Belitz et al. 2004).

Sugar is the main ingredient in confectionery products. Sugar concentration in sugar confectionery ranges from 85 % to 99 %. About 11 % of total sugar in the United States, and about 15 % in Europe, is used in confectionery (Godshall 2013).

Beside its sweetness and palatability, sucrose has many other important functions in food production, which are difficult to replace. Sucrose affects bulking, texture, and preservation; controls moisture; enhances, protects, and develops the flavor and color; and interacts with starch and other ingredients.

Sugar

- Caramelizes while heated, which provides food with pleasant aroma and color
- Interacts with macromolecules (protein or starch) during baking and cooking process
- Improves the tenderness and texture of the food products, due to water absorption, moisture retention, gluten development inhibition, and starch gelatinization delay

- Helps to integrate air during the creaming and whipping process
- Promotes the growth of yeasts
- Stabilizes beaten egg foams
- Slows down coagulation of egg proteins
- Controls the gelling of fruit jellies and preserves
- Inhibits microorganism growth and improves the shelf life of products
- Improves the tenderness and appearance of canned fruit
- Delays discoloration of frozen fresh fruit
- Provides various degree of crystallization which gives large variety of confectionery products
- Enhances smoothness and flavor in ice cream

Maltose originates from the hydrolysis of starch, and therefore it occurs in starch-containing products, such as in beverages, beer, cereal, pasta, potatoes, and many processed products which have been sweetened. Maltose is also a product of glucose caramelization. Maltose syrup, which is a dense, sweet, light brown syrup produced by enzymatic hydrolysis of starch, is widely used in confectionery. It gives the products brightness, color, and flavor. Maltose slows down the crystallization and improves the texture (reduces consequent hardening and provides smooth texture) and homogeneity. Due to its low viscosity, maltose syrup is used on the high-speed confectionery molding lines.

Lactose is mainly present in milk. Cow milk lactose content is 4–5 %; in human milk, it is slightly more (5.5–7 %). Therefore, lactose is present in all milk products including chocolate (Velíšek and Hajšlová 2009). Lactose in confectionery products is responsible for moisture and flavor and controls granularity. It also has a higher temperature of caramelization in comparison with other sugars; therefore, it provides an optimal color in caramels.

Raffinose is present mainly in legumes (5–15 % in the dry weight) and other vegetables. Sugar beet molasses contains about 1 % of raffinose too. Raffinose is a component of large group of oligosaccharides called raffinose family oligosaccharides (RFOs), which are known for their prebiotic effects.

Physiology and Nutrition of Main Oligosaccharides

Use of sugar (sucrose) is dictated mainly by consumer demands. While the consumption of sugar was few kilograms per year around 1850, the annual consumption in most western countries has risen to 40–50 kg per person (Table 3).

Sucrose is involved in tooth decay because it can be utilized by mouth bacteria after the hydrolysis into glucose and fructose, which leads to acid formation. However, sugar is not the only factor which can contribute to caries prevalence.

Table 3 Sucrose annual consumption in Europe (Kazdová et al. 2013)

Year	Consumption (kg per capita)
1800	8.1
1970	35.4
2002	45.3

Other sugars, including polysaccharides, can cause cavities too. Recent research suggests that dental caries is a multifactorial disease, depending more on how frequently the teeth are exposed to sugar, how quickly sugar is cleared from the mouth by saliva flow, or how good is the dental hygiene. This is confirmed by the 70–89 % reduction of caries prevalence in 12-year-old children over the period 1980–2006 (Organization for Economic Cooperation and Development [OECD] 2009), while the sugar consumption was stable. More about the effect of confectionery products on tooth health can be found in section “[Dental Caries](#)”.

Sugar consumption is often considered to be a significant contributor to rising obesity rate, cardiovascular diseases, and diabetes, as well as it is blamed to cause difficulties in the control of satiety and food intake.

Sucrose metabolism is related to the metabolism of glucose and fructose, and high doses of fructose in a diet were described to produce triglycerides, which can cumulate in an organism. However, it is rather difficult to find a clear relationship between a single dietary component and the diseases mentioned above. The prevalence of lifestyle diseases will be a more complex problem involving other elements, such as physical activity, stress, and overall diet. Nevertheless, according to the nutritional recommendations, the maximum intake of simple sugars should not exceed 90 g per day and added sugar less than 60 g, which corresponds to the annual sucrose consumption of 22 kg/person (Kadlec et al. 2014).

Lactose contains a β -anomer of galactose and most of the β -linked disaccharides are indigestible in humans. However, lactose is the only exception. At birth, lactose present in human milk is hydrolyzed by an enzyme lactase. The lactase activity can decline after weaning resulting in a low activity in an adult life. In humans whose digestive systems lack the ability to produce lactase, lactose is not digested and passes into the colon where it is fermented to lactic acid, carbon dioxide, and hydrogen. Such person can experience abdominal bloating and cramps, flatulence, diarrhea, nausea, and even vomiting. This is called lactose intolerance. It is estimated that 70–75 % of adults worldwide show some decrease in lactase activity (Pribila et al. 2000).

Raffinose has a prebiotic effect, which is caused by the raffinose indigestibility in the small intestine, due to the lack of the enzyme α -galactosidase. Raffinose passes to the large intestine where it is fermented by probiotic bacteria.

Changes in Confectionery Products

Chemical Reactions of Carbohydrates

The reactions of sugars are very essential in confectionery leading to many products which affect the sensory and nutritional quality of food.

Nonenzymatic Browning

Basically, four types of reactions occur during nonenzymatic browning (Davies and Labuza 1999):

- Caramelization
- Maillard reactions
- Lipid peroxidation
- Ascorbic acid degradation

Caramelization

At higher temperatures (more than 120 °C, but usually 150–190 °C), sugar-sugar reaction occurs forming brown amorphous substance called caramel (Velíšek and Hajšlová 2009). Caramel gives flavor and brown color to food products as well as helps to retain the moisture due to the crispy surface layer formation. Reaction products are similar to those of the Maillard reactions. Although, unlike the Maillard reactions, caramelization is a sugar pyrolysis followed by sugar-sugar polymerization, and no amine compounds are necessary to start caramelization and browning. However, in the presence of amines, the intermediates from the caramelization can react forming Maillard reaction-like products. Caramelization starts both in liquid and dry mixtures.

Maillard Reactions

In general, reducing sugars can react with an amine in a series of reactions to form a variety of products called Maillard reactions' products. These products give brown color, aroma, and flavor and, similarly like in caramelization, help to retain the moisture in confectionery products. The common reactive reducing sugars are xylose, ribose, glucose, fructose, lactose, and maltose. The amines may originate from free amino acids, N-terminal amine, N-terminal proteins or peptides, aspartame, and lecithin. Typical sources of amino groups in candies are milk and whey proteins, gelatin, eggs, nuts, cocoa solids, butter, fruit, and emulsifiers (e.g., lecithin).

The mechanism of reactions is very complicated; however, it is generally divided into three stages (Fig. 12).

The first stage involves a condensation of sugar and amino compound leading to N-substituted glycosylamine formation. The condensation occurs between the carbonyl group of the aldose and the three amino group of an amino acid. The product of condensation is transformed into a Schiff base. This substance is then rearranged into 1-amino-1-deoxy-2-ketose during a reaction called Amadori rearrangement. Therefore, glucose and glycine will convert into a 1-amino-1-deoxy-2-fructose. If ketoses (e.g., fructose) are involved in the reaction with amines, they form imines, which are not very stable and converted into Amadori products. The reactions of ketoses with amines are called Heyns reaction. No color substances are formed at this stage (Lee and Nagy 1983).

The second stage is followed by sugar dehydration and fragmentation and amino acid degradation especially at high temperatures, which leads to flavor component formation. The Amadori products formed in the first stage decompose yielding many different products, such as monofructoseamine, 3-deoxyosuloses, and 3,4-deoxyosulos-3-ene. In acidic conditions, 3,4-deoxyosulos-3-ene is formed. This product decomposes and provides color and flavor components. In neutral or slightly alkaline solution, the 2,3-enolization pathway is favored. Alternatively, the amino acids can oxidize in the Strecker degradation where carbon dioxide is formed.

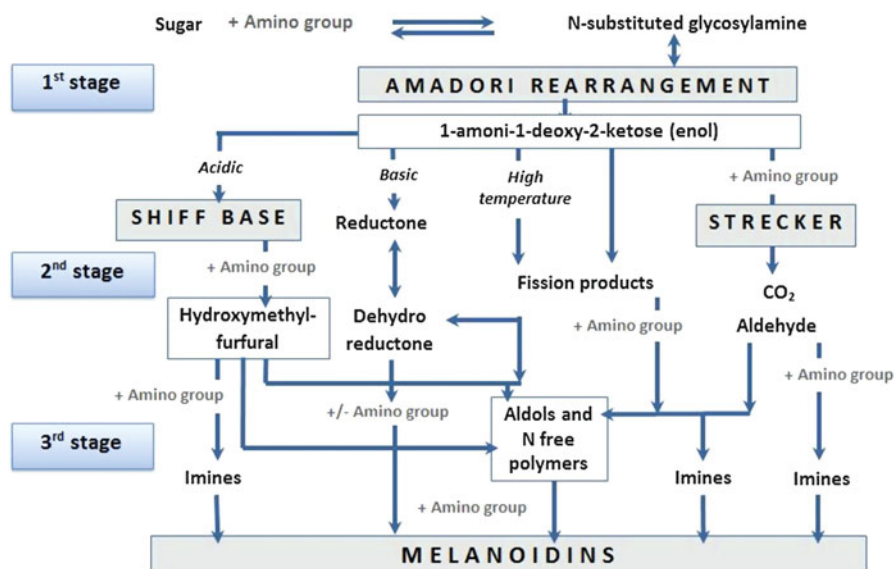


Fig. 12 Hodge scheme of Maillard reactions (Hodge 1953)

The last step is typical with a formation of heterocyclic nitrogen compounds and browning. Products of this stage, called melanoidins, are formed by polymerization reactions, and their molecular weights rise with time and temperature during browning. The main factors affecting the formation of color and flavor components are time, temperature, water activity, concentration of precursors, type of reactants, sugar to amine ratio, pH, concentration of the solution, and the consistency of the final solids (crystal, rubber, or glass state). In confectionery products it is difficult to interpret the Maillard reaction due to the large number of candies and recipes for their preparation.

Most of the components providing the sweetness in confectionery products (such as glucose, fructose, lactose, honey, molasses, maple syrup, and sweeteners) have Maillard reactivity. There are two exceptions: sucrose and sorbitol. However, if cane or beet sugar undergoes hydrolysis, products of hydrolysis (glucose and fructose) can react in Maillard reactions. Sucrose hydrolysis occurs at pH less than 3 at low temperatures; however, if the temperature increases to 70 °C, hydrolysis occurs even at pH 7. Therefore, the formation of color in candies produced from sucrose depends on the extent of inversion and the pH of the syrup.

Some products of Maillard reactions have a negative impact of the product quality. They can give the food unpleasant flavor or appearance. Especially in reaction with proteins, the Amadori products undergo further polymerization, reducing the solubility of the protein. Certain types of caramel have been reported to have mutagenic effect, and the carcinogenic effect of thermolyzed sucrose and casein in rats has been described too (Corpet et al. 1990).

Flavor components are formed by minor pathways in the Maillard reactions, and the compounds providing generally the typical food smell are pyrazines, pyrroles, pyridines, oxazoles, and oxalines. The produced volatile compounds are usually present in low concentrations; however, due to their low sensory threshold, the aroma can be really strong. A specific flavor formation can be difficult to control, since it may depend on the simultaneous generation of hundred or more individual compounds.

Lipid Peroxidation

Lipid peroxidation is caused by the oxidation reaction of fatty acids (especially unsaturated fatty acids). Fatty acids form aldehydes and ketones, which can subsequently react with amino acids to form brown products.

Ascorbic Acid Degradation

Ascorbic acid degradation is one of the nonenzymatic browning reactions which occur in food. It is a very reactive compound and degrades by two pathways, which both result in the formation of dicarbonyl intermediates leading to the formation of browning products (Wedzicha and Kaputo 1992).

Oxidation of Sugars

Aldehyde group of aldose sugars can be oxidized to form aldonic acids. Therefore, D-glucose will form D-gluconic acid. Free aldonic acids are not stable and in aqueous solution form γ -lactones (5-ring form) or less stable δ -lactones (6-ring form). Oxidation reaction of aldose sugars is used for determination of reducing sugars. So-called Fehling's test applies an alkaline Cu^{2+} solution to oxidize aldoses into aldonic acids. During this reaction, the cupric ions are reduced to cuprous hydroxide which forms red-colored cuprous oxide precipitate (Wrolstad 2012).

Another acid derived from carbohydrates are alduronic acids which are products of the oxidation of primary alcohol group. These acids are present in plant cells, and uronic acids (e.g., D-glucuronic, D-galacturonic, D-mannuronic acids) are basic structural unit of some polysaccharides, such as pectin.

Formation of Glycoside

Glycosides are formed during reactions of reducing sugars with hydroxyl group of other compounds, e.g., alcohols in acidic conditions. These reactions occur during corn syrup production, where high concentration glucose solution is heated in the presence of acid. Glycosides naturally occur in many plants where they can be parts of pigments, for example, vanillin is a glycoside present in vanilla beans and amygdalin can be found in bitter almonds.

Anhydro Sugars

Anhydro sugars, also called glycosans, are formed by intramolecular condensation of hemiacetal and hydroxyl groups during heating of aldose under acidic conditions. Therefore, nonreducing intramolecular glycosides are formed. 1,6-anhydro- β -D-glucopyranose (levoglucosan) is derived from glucose and occurs

in caramel. Other glycosans are building unit of polysaccharides: 3,6-anhydro- α -L-galactopyranose in agar and 3,6-anhydro- α -D-galactopyranose in carrageenan (Velíšek and Hajšlová 2009).

Another example of a polyglucosan is polydextrose produced synthetically by pyrolysis of glucose, sorbitol, and citric acid. It is widely used as a non-dietary fiber and a low-energy bulking agent replacing sugars, starch, and fat in candies, desserts, cakes, breakfast cereals, puddings, and many others (Belitz et al. 2004). Polydextrose's energy value is 4.18 kJ/g and the E number for food additives is E1200.

Acid-Catalyzed Reaction of Sugars

In acidic solution, the glycosidic linkage both in reducing and nonreducing sugars is hydrolyzed. The reaction is catalyzed by protonation of the exocyclic group and free sugars are formed (Wrolstad 2012). Aldoses provide deoxy sugars and furfurals. Deoxy sugars are derivatives of carbohydrates where one or more hydroxyl groups (however, not a hemiacetal group) are substituted by hydrogen atoms.

Furfural naturally occurs in different foods (fruit, nuts, bakery products) and is used as a flavorant; however, it is toxic with an acute oral toxicity in rats LD₅₀ (lethal dose) of 127 mg/kg (Jenner et al. 1964).

Or the hydroxyl groups other than the one at the anomeric carbon atom can react with acidic compound resulting in the formation of sugar esters.

Alkaline-Catalyzed Reactions of Sugars

Reaction of carbohydrates in a strongly alkaline solution also occurs in food technology, e.g., after addition of lime in sugar refining or manufacture of alkali-baked products. Reducing sugars form various isomers in these 1–2-enolization reactions. Nonreducing sugars (sucrose) have no anomeric hydroxyl group; therefore, the hydrolysis will not take place (Belitz et al. 2004).

One of the practical applications of this reaction is formation of lactulose (4-*O*- β -D-galactopyranosyl-D-glucose) from lactose in the presence of sodium aluminate as a catalyst. Lactulose (Panesar and Kumari 2011) is a nondigestible and prebiotic sugar used in medicine as a laxative or in a treatment of encephalopathy. For its properties (good solubility in water, flavor enhancing), it is also applied in many food products as a sweetener or a sugar substitute in confectionery, beverages, desserts, bakery products, and yogurts.

Physical Changes in Confectionery Products

Sugar Crystallization

Sugar crystallization in confectionery may be desirable for certain products (fondant and fudge), but in many cases, it is seen as a quality defect. Undesirable crystallization of sucrose can occur during storage of sweets without proper packaging, especially in areas of high humidity, making the sweet sticky and grainy. This can be prevented by good quality packaging materials. Another example is the crystallization in the production of noncrystalline candies (see section “[Noncrystalline Candies](#)”).

Polyols

Sugar alcohols (polyols) are sugar derivatives formed by reduction of carbonyl group of aldoses and pentoses. They are naturally occurring compounds in food, mainly in fruit and vegetables. For their nutritional properties (see section “[Physiology and Nutrition of Polyols](#)”), they are widely used to replace sucrose in many confectionery products. Nowadays, they are mostly produced by catalytic hydrogenation of simple sugars using Raney nickel as a catalyst, although they (e.g., mannitol, sorbitol) can be isolated from natural sources (apples, pears, peaches, etc.) as well. Polyols have been classified as food additives associated with their E numbers; therefore, in the European Union, they are all approved by the European Food Safety Authority.

Main Polyol Properties

The mainly used polyols are monosaccharide-derived sorbitol, mannitol, xylitol, and erythritol and disaccharide-derived maltitol, lactitol, and isomalt.

Sorbitol (D-glucitol; E420) is a product of our metabolism; however, it is not taken up in the small intestine. One gram provides 11 kJ and the relative sweetness is 50 %. In large doses, it can cause abdominal pain, diarrhea, and pathological changes in tissues.

One gram of **mannitol** (E421) provides 7 kJ and its relative sweetness is 50 %.

Xylitol (E967) is the sweetest polyol with a relative sweetness similar to glucose. It has very low glycemic index of 7 and displays no known toxicity or carcinogenicity. In metabolism, xylitol is partially fermented by colon bacteria and in higher doses can have laxative effect.

Erythritol (E968) is mostly absorbed in the small intestine into the bloodstream and consequently extracted by the kidneys in urine. That is why, unlike xylitol or maltitol, it does not have a laxative effect and is “generally recognized as safe” (GRAS).

Maltitol (E965), **lactitol** (E966), and **isomalt** (E953) are polyols of very similar structure derived from disaccharides, all having very low glycemic index of 9. They do not occur naturally and are mostly produced synthetically. They are all GRAS; however, if consumed in large quantities, they can cause gastric distress.

Physiology and Nutrition of Polyols

The main nutritional benefit of alditols is they are low in calories (see Table 4); therefore, they have been introduced into a human diet as sweeteners. However, their relative sweetness is lower than the sweetness of sucrose (see Table 4). They are suitable for diabetics, like fructose, but unlike fructose they show non-cariogenic properties, which means they cannot be fermented by most of the bacteria in the mouth to produce harmful acids. Xylitol and sorbitol in chewing

Table 4 Physical properties of polyols

Alditol	Relative sweetness ^a (%)	Energy (kJ/g)	Laxation threshold ^b (g/day)
D-Sorbitol	40–70	11	50
D-Mannitol	40–70	7	20
Maltitol	70–90	12	60–90
Xylitol	90–120	12	50–90
Lactitol	30–40	8	20–50
Isomalt	30–60	8	50–70
Erythritol	60–70	0	125

^aKadlec et al. (2014)

^bČopíková et al. (2006)

gums can reduce the plaque formation in teeth and lower the tooth decay. Xylitol also shows bactericidal properties and increases the production of saliva in mouth, which helps to remove food residues from teeth.

In metabolism, some sugar alcohols are partially absorbed from the small intestine into the blood, where they can be converted to obtain energy. This may require insulin. Others pass to the large intestine where they are metabolized by bacteria. Therefore, in high doses they have laxative effect (they cause diarrhea) or they can cause bloating and flatulence. The laxative threshold of some polyols is shown in Table 4.

Occurrence and Changes of Polyols in Confectionery Products

Sugar alcohols have been widely used as sugar replacers in food industry, and the confectionery production is no exception. They are added into candies, ice creams, chewing gums, chocolate, and many other products, when it is required to make low-energy or sugar-free products. They have a cooling effect on tissues. This is caused by dissolving the crystals in mouth, which requires energy. The energy is provided by reducing the temperature of the solution. Every polyol has different level of cooling sensation. Maltitol has the lowest cooling effect, xylitol performs the highest sensation, and erythritol, mannitol, and sorbitol have moderate properties. Due to its highest cooling effect, xylitol is often used to enhance mint flavors. On the other hand, low solubility of mannitol inhibits the cooling sensation of mint candies.

Another common attribute of polyols is they do not caramelize or brown when heated. However, some of them require to be processed at relatively low temperatures, to prevent them from forming aggregates.

Mannitol exhibits a very low hygroscopicity and it is used for coating hard candies, dried fruits, and chewing gums. It also provides the mouthfeel sensation (the physical sensation of food in the mouth) used in chewable candies.

Maltitol with its high sweetness and properties similar to sucrose is often used without being mixed with other sweeteners in production of sugarless hard candies, chewing gums, chocolates, and ice cream. Its main advantage is that, unlike sucrose, maltitol crystallization is less likely. Maltitol may also be used as a plasticizer in gelatin capsules or as a humectant.

Sugar Substituents

Sugar substituents are food additives characterized by sweet taste and low caloric values. Originally they have been introduced as sweeteners for diabetics, but nowadays they are mainly used as cheap replacements of table sugar. Some of them, produced synthetically, are called artificial sweeteners. They all have been approved for food and are generally considered as safe by authorities such as the Food and Drug Administration (FDA) in the United States or by the European Food Safety Authority (EFSA).

Artificial Sweeteners

Sucralose

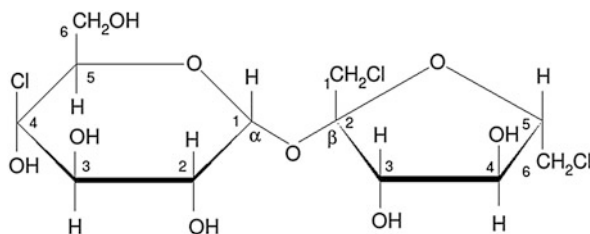
Sucralose (E955) is a synthetic derivative of sucrose, where three of the hydroxyl groups are substituted with chlorine (Fig. 13). Its relative sweetness is high, approximately 500–650 times higher than the sweetness of sucrose; therefore, it can be added into food in very low concentrations. Sucralose does not build up in the organism and is excreted mainly in feces and urine (Roberts et al. 2000). Sucralose is a highly heat-stable artificial sweetener; however, in a pure state and temperatures above 119 °C, sucralose decomposes into polychlorinated dibenzo-*p*-dioxins and other highly toxic substances (Bannach et al. 2009).

Aspartame

Aspartame (E951) is a methyl ester of the dipeptide of aspartic acid and phenylalanine. It was firstly synthesized in 1965 and became a topic of many controversies over its safety; however in 1981, it has been approved for use in food. As other peptides, aspartame can hydrolyze at higher temperature or pH into its components, such as phenylalanine, and therefore is not suitable for people suffering from phenylketonuria. Methanol, which can be generated in the digestive system, is another product of the aspartame decomposition under strongly acidic or alkaline conditions.

Aspartame is one of the most widespread sweeteners. Its relative sweetness (180–200) is high in comparison with sucrose, the caloric value is the same as glucose, and hence aspartame can be used in small amounts.

Fig. 13 Sucralose



As a donor of amino group, aspartame can react in Maillard reactions. Aspartame is not stable at high temperatures and undergoes decomposition and therefore is not suitable for use in bakery products. In confectionery, it is added to chewing gums.

Saccharin

Saccharin (2-benzothiazol-1,1,3-trione, E954) is the first known artificial sweetener synthesized in 1878. During the long period of its use, saccharin was several times investigated about its possible harmful effect on human health. In 1958, it was not approved by FDA for its possible carcinogenic effect, because some studies described it might cause bladder cancer in rodents. Saccharin was labeled with a warning about its potential harmfulness. However, the warning labels have been removed in 2000, because further research revealed that the urine composition in rodents was quite unique in comparison with humans (Whysner and Williams 1996). Therefore no elevated risk of bladder cancer development in humans was confirmed.

Saccharin is 300–500 times sweeter than sucrose, but for its slightly bitter aftertaste, it is often blended with other sweeteners. When heated, it is not stable and decomposes.

Acesulfame Potassium

Acesulfame potassium (acesulfame K or ace K; E950) was synthesized in 1967. Like saccharin, acesulfame molecule contains sulfonamide group and is slightly bitter in higher concentration. Therefore, it is often mixed with other sugar substitutes. Unlike saccharin, acesulfame is stable at higher temperature. Its sweetness is 200. Acesulfame does not cumulate in the organism and is eliminated in urine. It is considered as safe; however, there are still controversies over its safety. Some studies on mice suggest that acesulfame K may affect prenatal development, or it may affect the neurological functions. Its harmful effect to humans has not been confirmed.

Sodium Cyclamate

Cyclamate is sodium or calcium salt of cyclohexanesulfamic acid. Sodium cyclamate (E952) is 30–60 times sweeter than sucrose and stable when heated. It has been synthesized in 1937 and approved as a sweetener in over 55 countries since that time. There are concerns over its safety, for its potential carcinogenicity, and its production of potentially toxic cyclohexylamine by some intestinal bacteria. That is why it has been banned in the United States.

Natural Sweeteners

Steviol Glycosides

Steviol glycosides (E960) are naturally occurring sweeteners, which can be extracted from the leaves of stevia plant (*Stevia rebaudiana*). The steviol glycosides are mixtures that contain not less than 95 % stevioside and/or rebaudioside A.

The sweetness can vary in dependence on composition from 40 to 450 times sweeter than sucrose. The glycosides are heat and pH stable, non-fermentable, and not inducing a glycemic response when ingested. They can be used in confectionery with no added sugar. EFSA established an acceptable daily intake (ADI) for steviol glycosides of 4 mg per kg of bodyweight and day (Aguilar et al. 2010).

Classification, Preparation, and Composition of Confectionery Products

The range of sugar confectionery products is very large, because sugar, the basic ingredient in these products, shows various chemical and physical properties. The form of confectionery largely depends on the cooking stage (temperature) of the sugar-liquid mixture. Due to the careful control of temperature, sugar concentration in solution, type of heat, stirring, and addition of other substances, many different confectionery products can be made. Table 5 shows the boiling range for a selected range of confectionery products.

The production of candies usually starts by dissolving sugar in water at room temperature, then the solution is heated, and more sugar is added to create a saturated solution. This solution is stirred well and heated to boil, getting a supersaturated solution. Supersaturation is defined as the ratio of sugar in solution to sugar needed to saturate the solution at the same temperature.

If the supersaturated solution is heated even more, some water evaporates and solution gets more concentrated. The degree of sugar concentration of the supersaturated solution can determine the final consistency of the candy; however, this solution is unstable and prone to crystallize especially if small crystals or foreign particles are introduced or the solution is agitated. Table 6 shows the boiling point of sucrose solution in dependence on pressure and concentration.

Candies can be divided into several categories according to different parameters. One of them is the structure. If the sugar is present in the form of crystals, the candies are called crystalline candies; if the sugar is present in an uncrystallized (amorphous) form, the candies are called noncrystalline.

Table 5 Boiling temperatures for some confectionery products

Type of sweet ^a	Temperature range for boiling (°C)
Fondants ^a	116–121
Fudge ^a	116
Caramels and regular toffee ^a	118–132
Hard toffee (e.g., butterscotch) ^a	146–154
Hard-boiled sweets ^a	149–166
Nougat ^b	120–132

^aFellows and Hampton (1992)

^bNPCS Board of Food Technologists (2013)

Table 6 Boiling points of pure sucrose solutions as a function of concentration and pressure (Saska 2002); w_{Suc} = sucrose mass percents in a solution

Pressure (kPa)	19	30	46	69	101	145	205
Water boiling point (°C)	60	70	80	90	100	110	120
w_{Suc} (%)	Boiling points of sucrose solutions						
10	60.15	70.15	80.16	90.17	100.18	110.19	120.20
20	60.37	70.39	80.41	90.44	100.46	110.48	120.51
30	60.68	70.72	80.76	90.80	100.85	110.89	120.94
40	61.12	71.19	81.26	91.33	101.40	111.48	121.56
50	61.78	71.89	82.00	92.11	102.23	112.35	122.47
60	62.83	73.00	83.17	93.35	103.54	113.73	123.92
70	64.68	74.96	85.25	95.55	105.85	116.16	126.49
80	68.64	79.16	89.70	100.25	110.81	121.39	131.98
90	81.77	93.07	104.42	115.80	127.23	138.68	150.18

Crystalline Candies

There are two groups of crystalline candies: candies with perceivable crystals (e.g., rock candy) and candies with very small crystals which cannot be recognized by the tongue (fondant and fudge).

Rock Candies

This type of candy is prepared by immersing a string into a supersaturated sugar solution and heating. Then the solution is cooled down and the sugar from the solution will recrystallize on the string. The sugar crystal will slowly grow as the molecules of sugar stick on the crystal surface. This type of candy contains pure sucrose, because only sucrose will recrystallize.

Cream Candies

Cream candies are confectionery products containing only small (imperceptible) crystals. These small homogeneous crystals are formed by controlled growth of crystals from supersaturated solution. The solution is cooled down to certain temperature and beaten. This “creaming” process depends on the presence of other interfering agents, which interact with sucrose molecule, preventing sugar from creating conglomerates and bigger crystals. Proteins and fats from milk, butter, cream, chocolate, or cold gelatin are examples of agents which facilitate creaming. Invert sugar, which is formed naturally during candy production, can also act as an inhibitor of recrystallization. The sucrose breakdown depends on the intensity and length of cooking and can be accelerated in the presence of acids from other ingredients, such as fruit, honey, cream of tartar (potassium bitartrate), or chocolate. A certain amount of invert sugar in candies is necessary to give them moisture and prevent them from formation of large crystals.

Fondant and Creams

Fondant is a confectionery product, which is often used as an ingredient in other sweets. Its production starts by dissolving sugar in water. If all crystals are perfectly dissolved, corn syrup is added and the mixture is boiled. The typical cooking range is 116–121 °C and the typical composition is 60–80 % of sucrose and 20–40 % of syrup. After boiling, the batch is cooled and beaten in order to control the crystallization process and reduce the size of crystals. It can be done by a wooden spatula or in a special beating machine resulting in a formation of very small crystals. Fondant can be stored and remelted before further use. Different flavors such as peppermint, chocolate, fruit, vanilla, or flower (rose or violet) can be added to get even more varieties of cream confectionery products.

Fondants, diluted with a weak sugar solution or water, are called creams. These products have shorter shelf lives in comparison with other sugar confectionery products, due to their high water content. Both fondants and creams are commonly used as soft fillings in chocolates and other sweets. Fondants can be immersed into supersaturated sugar solution, which will form a hard layer on the top, or can be used as a coating for Brazil nuts.

Fudge

Fudge is a soft confectionery product made from similar ingredients as toffees and caramels; however, unlike toffees, the crystallization is initiated by the addition of fondant (15–25 %). Therefore, fudge production starts with caramel, made by boiling sugar, glucose syrup, invert sugar, milk, fat, and emulsifiers with water. Vanilla can be added as well as a flavoring agent. Due to the Maillard reactions between present sugars and milk proteins, brown color and characteristic flavor components are formed. When the caramel is cooled down to 80 °C, about 10 % of fondant is added and stirred well. The mixture is then poured on the table and let to cool and finish the crystallization.

Marshmallow

Marshmallows differ from other sweets because of their soft and aerated texture. Marshmallow manufacture starts with a preparation of base syrup by mixing glucose syrup and sugar. Aerating agents, such as gelatin or albumen, are added, and the whole mixture is beaten in a mixer or under pressure to produce foam. The marshmallow can have various textures; it can be extruded, spongy, covered by starch, or made into a short consistency by inclusion of icing sugar.

Noncrystalline Candies

Amorphous or noncrystalline candies are much easier to be produced. Interfering agents are added into the sucrose solution to prevent crystallization, or the solution must be heated at very high temperature. Noncrystalline candies can be also cooked by dry heat as well as moisture heat.

Hard Candies (Boiled Sweets)

Hard candies represent a large group of different sweets, such as suckers, lollipops, drops, candy canes, etc. Hard candies are supercooled liquids in a noncrystalline state. They can be considered as highly viscose liquid. The water content can range from 1.5 % to 5 % and depends on the cooking temperature, vacuum pressure, and ratio of sucrose to corn syrup.

Their production starts with granulated sugar, glucose syrup, and water, which are heated in a steam-heated vessel to dissolve the sugar. The mixture is then brought to a vacuum cooker and boiled until most of the water evaporates. The cooking temperature is higher than 140 °C at atmospheric pressure. Then the vacuum is applied to remove the excessive water and establish the desired humidity. The liquid is placed on a cooling table where it gets plastic, single-phase character. Flavoring and coloring agents and acids are incorporated by turning and folding. In the next producing step called tempering, the amorphous product is mixed in a kneading machine to ensure a homogeneous temperature. Then hard candies are shaped and cooled down in a cooling tunnel.

Caramels and Toffees

Caramel is not just a name for a brown matter which is formed during heating of sugar (section “[Nonenzymatic Browning](#)”) but also a confectionery product. Caramels, as well as toffees, and butterscotch are made from sugar solutions with the addition of ingredients such as milk solids (butter, cream, condensed milk, etc.), fats, and emulsifiers. Invert sugar is necessary to prevent the crystallization. It can be added in the form of molasses or corn syrup into the sucrose solution, or invert sugar is formed after addition of acids.

The structure of caramels is made by a highly concentrated sugar solution containing emulsified fats and dispersed milk proteins. Milk proteins (typically 1–4 %) and moisture (1–20 %) affect the texture and hardness; moisture in medium-hard caramels is 5 %, in soft caramels 7–11 %. Low moisture also prevents crystallization. The texture ranges from semifluid to hard and can be controlled by boiling temperature, which is higher than in the crystallized candies. The typical cooking range for medium caramel is 122 °C and soft caramels 118 °C (NPCS Board of Food Technologists 2013).

Hard caramels can have very low moisture content (about 1 %), which leads to the formation of noncrystalline rubbery or glassy state. Hard caramels in this state are called toffees. Toffees are usually boiled at high temperatures (121–130 °C) and contain less milk. As the product does not need to be clear, it is possible to use unrefined sugar, instead of white granular sugar. Due to presence of milk protein, Maillard reactions occur giving the typical color and flavor in caramels and toffees.

Brittles

Brittles are made by melting dry sugar or by boiling sugar solution to very high temperature (149 °C). Due to the low water content, no crystallization occurs and

sucrose takes the form of a noncrystalline glassy solid. Caramelized sugar is mixed with nuts (almonds, peanuts, sesame, or pecans), other leavening agents can be added, and the mixture is cooled down.

Nougat

Nougat is an aerated confectionery product containing sugar, syrup, fat, and a whipping agent (albumen, gelatin, milk proteins, soya protein, starch, or gum arabic), with 8–11 % moisture and rubber texture. The typical cooking range is 120–132 °C. There are three types of nougat: white one, which is made from sugar and egg albumen mixed with nuts or sometimes dried fruit. Brown nougat is produced from sugar and nuts (hazelnuts, almonds, walnuts, pistachios). The cream of tartar and glucose can also be added. The last one is the chocolate nougat, which is made from nuts, sugar, and chocolate.

Jellies and Gums

There is a wide range of jelly candies, such as jelly beans, gums, Turkish delights, or gumdrops. Jelly candies come with various textures, which depend both on water content and gelatinizing agent used. Pectin-based jelly manufacture requires precise control of the technological process (section “[Pectin](#)”). Pectin is dissolved in water, sugar and syrup are added, and the mixture is boiled followed by the addition of either citric acid to set pH to 3.3–3.6 or calcium salt for setting.

In gelatin jellies, gelatin (section “[Gelatin](#)”) is soaked first and then added to the syrup boiled to the required concentration. The amount of gelatin affects the consistency and usually is 4–10 %.

Agar jellies are made from agar, a polymer obtained from seaweed (section “[Agar](#)”). Only a small concentration of agar (0.2 %) can create a jelly texture; however, acids must be added to provide the firmness.

Starch is also a cheap alternative for jelly production. Usually, the modified starch (high-amylose starch) is used for gelatinization. High-pressure steam is applied to initiate gel formation in a slurry containing starch and syrup.

Gums are produced by similar technology like jellies, but after the gelatinization, they are stored at 50 °C in starch for several days and then are glazed by steam and lubricants.

Nut Pastes (Marzipan)

In general, marzipan is a nut paste made from blended almonds mixed with sugar. However, there have been many discussions about the marzipan definition regarding the ratio of almonds to sugar or addition of other substances.

High quality or “raw marzipan” usually contains 66 % of almonds, but the ratio of almonds to sugar can be 1:1 or even less in favor of sugar (NPCS Board of Food Technologists 2013). Some other substances, such as coconut, apricot, or peach kernels, can be added as well. For its high price, almonds are often being replaced by soy paste and almond essence. Almonds need to be sterilized to inhibit enzymes

and microorganisms. Sugar can be added in the form of granulated sugar or a syrup prepared by mixing of sugar with glucose syrup or invert sugar. The blend is mixed thoroughly and passed through a roll refiner to provide smooth paste-like texture. To evaporate excess water and ensure the stability of the product, the paste is boiled in kettles at 82–85 °C.

Licorice

Another popular confectionery product uses extract from the roots of the licorice plant (*Glycyrrhiza glabra*) as a flavoring agent. Licorice sweets are made of sugar and starch, which can be substituted with gelatin or gum arabic or combination of all of these. Another compounds added are ammonium chloride, molasses to provide the black color, and the beeswax to get the shining surface. Fruits, such as strawberries, cherries, raspberries, or cinnamon, and/or anise oil can also be used as flavoring agents instead of the licorice extract.

Chewing Gums

According to the name, chewing gum is a confectionery product which is not supposed to be swallowed. Traditionally, it has been produced as a mixture of finely pulverized sugar (58–60 %) and glucose syrup impregnated in a natural gum base (chicle) originating from *Manilkara* trees. Chewing gum production is the fastest growing confectionery segment globally, experiencing 4.8 % growth worldwide in 2011 (Godshall 2013). Therefore, natural gum supply was not sufficient, and new polymers (butadiene-based synthetic rubber) were introduced to substitute the natural gum and reduce the production costs as well. Nowadays, sugar-free chewing gums, where sugar is replaced by sugar alcohols and other sweeteners, gain the popularity. The production is based on mixing of sweetener (see section “[Artificial Sweeteners](#)”), sugar alcohols, and flavoring agents with shredded gum base. The mixture is very sticky and needs application of dusting powder, which is a combination of starch and fine sugar. Flavoring agents are peppermint and spearmint oils, strawberry (methylphenylglycidate), apple (malic acid), banana (isoamyl acetate), or pineapple (allyl hexanoate) flavors.

Frozen Desserts

Frozen desserts such as ice creams, ice lollipops, or sherbets (sorbets) are made by freezing a liquid mixture of cream, milk, fruit juice, or fruit puree with sugar. Sugar is a preferred sweetener for ice cream because it gives a clean, sweet taste with no “syrupy” attributes. Sugar also has an important role during the freezing process.

Sucrose binds the present water thereby reduces the freezing point. When the liquid mixture starts to freeze, the sugar remaining in the unfrozen solution becomes even more concentrated, lowering the freezing point. Therefore, the temperature is much lower than the freezing point of the mixture resulting into a formation of smaller water crystal, providing a smooth, creamy texture. If fructose or HFCS is substituted for sucrose on a one-to-one basis, the freezing point is suppressed almost twice, which can lead to development of ice crystals and an unpleasant icy texture. Liquid sucrose is often used in food industry instead of dry sugar for ease of pumping high-bulk quantities. The recommended concentration of sugar in ice cream is about 16 %. In low-fat frozen desserts, it is even higher, in order to counterbalance the reduced amount of fat and ensure a smooth flavor (The Sugar Association 2014).

Sugar also finds its application in frozen fruit production where it helps to protect the surface of the frozen fruit against air and inhibits enzymatic browning caused by oxidation. Sucrose reduces flavor changes by retarding the fermentation and improves the fruit texture after thawing.

Jellies, Preserves, and Jams

Sugar in jellies, preserves, and jams protects the aroma and color, and the fruit flavor is enhanced and concentrated. Transparent jellies are made with pressed fruit juices. Preserves are traditionally made from whole or large piece of fruit, whereas jams are made with crashed fruit. Sugar is necessary for the gelling process to provide the desired consistency. During the gel formation (gelatinization), pectin makes gel in the presence of acids (pH is usually between 3 and 3.5) and sucrose. Sugar absorbs water; therefore, it serves as a preservative. A concentrated sugar solution of at least 65 % inhibits the growth of microorganisms due to the increased osmotic pressure. Water is retained by sugar and microorganisms become dehydrated. HCFSSs can be also used for jam or preserve production; however, it contains as much as 29 % of water which needs to be evaporated in the final stage production causing some of the flavor components to be lost.

Chocolate

One of the most favorite confectionery products, chocolate, is made out of the seeds of the tree *Theobroma cacao* L. which grows in South and Central America, West Africa, and East Asia. It has been named by the Swedish botanist Carl von Linné and the meaning of *Theobroma* is “food of the gods.” Nowadays, the main cultivated variety of cocoa is called *Forastero* (Winkler 2014). Another variety is *Criollo*, which used to dominate the market, and the third variety is *Trinitario* (ICO 2013).

Cocoa Beans Processing

After harvesting, the beans are separated from cocoa pods, and the subsequent fermentation involving yeasts and bacteria takes place. This is an important step due to the flavor precursor development and creating a nonperishable commodity. After the fermentation, the beans are dried (moisture content drops from 60 % to 7 %) and hand sorted to remove damaged beans and debris. After cleaning, the beans are processed by different manners.

The beans are dry roasted at the temperature 110–140 °C for 10 up to 30 min. During the roasting, the degradation of volatile acids occurs, lowering the acidic and bitter taste of unroasted beans. However, the most important reactions to provide flavor and brown color are the Maillard reactions.

The beans are broken and the shells and nibs (cotyledon) are separated through sieves and by air currents, and germs are winnowed away. Nibs can undergo different heat treatment, such as alkalization (called Dutch processing) by sodium hydroxide and calcium or potassium carbonate. The alkalization process is used mainly in cocoa powder production and gives the roasted nibs a distinctive darker color. Nevertheless, this process also contributes to a weaker flavor and destroys valuable biologically active components. That is why it is not applied by all manufacturers (McFadden 2000).

The nibs are ground in mills into a paste called cocoa mass (cocoa liquor, cocoa paste, or chocolate paste). The usual process consists of two steps: coarse and fine grinding. Cocoa liquor is then separated into cocoa butter and cocoa cakes by hydraulic pressing. The cocoa cake is ground into the fine cocoa powder.

Cocoa butter is filtered to remove small particles and stored. Or it is processed by deodorization, which removes odors by heating to high temperature. Cocoa butter is a yellowish fat typically having a brittle fracture below 20 °C, softening between 30 °C and 32 °C, and having a sharp melting point at 35 °C. Cocoa butter occurs in six polymorphic forms, which are designated as I–VI. They have different stability and different physical characteristics such as gloss or hardness. Every form also has slightly different melting point ranging between 17.3 °C and 36.3 °C (Roth 2005).

Chocolate Preparation

There are many different ways to produce chocolate depending on the final product. The main scheme is shown in Fig. 14. The process starts with chocolate crumb making where cocoa liquor is mixed with other ingredients (milk, sugar, etc.). Water can be added and the mixture is heated to caramelize present sugar. The excess water is then evaporated under vacuum and dried mixture undergoes the refining process.

The refining is necessary to reduce the particle size. It is performed on three to five metal rollers. Another important step in making delicious chocolate is the conching. The aim is to develop desirable flavors and fine aroma and give the chocolate viscosity and smooth liquid consistency. During this process, the refined mass is mixed with other ingredients like cocoa butter, emulsifiers, flavors (vanilla, cinnamon), nut pastes, etc.

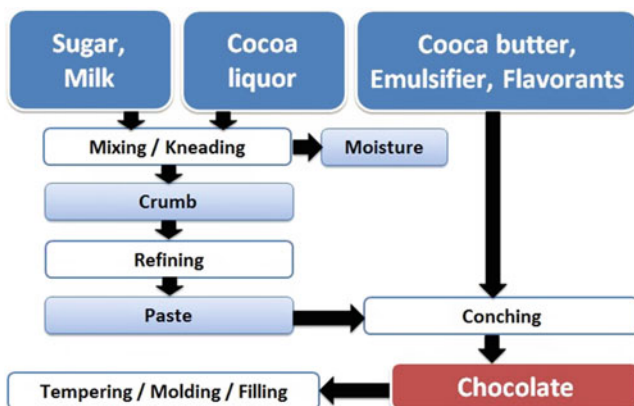


Fig. 14 Chocolate production scheme

Conching usually takes from several hours up to 2–3 days and is performed at double-jacketed vessels called conches at various temperatures (50–90 °C) depending on the type of the chocolate produced. Milk chocolate is heated up to 50 °C, while the dark one requires higher temperatures up to 70–90 °C. Conching can be a two-step process; in the first step, the water and undesirable products of fermentation are evaporated and all present solid particles are coated with fat. In the second step, emulsifiers and additional fat are added to obtain a homogeneous liquid paste.

In the last technological process called tempering, the temperature of the product is gradually reduced from 50 °C until the desired consistency is obtained. During the cooling process, the crystals can start to form. The tempering machine is a large tank with several cooling zones equipped with mixing discs.

Produced liquid chocolate is stored in heated tanks from where it is pumped and transported into the molding lines, which need to be kept warm too. The chocolate can be transferred into temperature-controlled rooms to prevent condensation. After demolding, chocolate is ready for packaging.

Filled chocolate is made by several steps involving molding, filling, and cooling, which can be repeated until the demanded product is made. Filling and inclusions in chocolate are very various involving dried fruits, spices, herbs, nuts, and seeds. Two main categories are recognized: the fat-based fillings (nougats, nut pastes, marzipan, and cream) and the water- and sugar-based fillings, such as caramel and marshmallow. Also chocolates with exotic flavor are becoming very popular. New combination of flavors appeared, such as Himalayan pink salt, apple smoked bacon, chili, burnt sugar caramel with stout beer, lavender with cardamom, or crushed black pepper.

Other Chocolate Products

Soluble cocoa drinks are produced in a process called agglomeration where the surface structure of the cocoa powder is changed by applying steam into a free-flowing powder.

Table 7 Nutritional composition of dark, milk, and white chocolate

	Dark chocolate ^a	Milk chocolate ^b	White chocolate ^b
Energy value (kJ/100 g)	2,180	2,238	2,255
Dry cocoa solids (%)	66.0	25.0	0.0
Fat-free dry cocoa solids (%)	28.0	2.5	0.2
Total proteins (g/100 g)	8.1	7.6	6.0
Sugars (g/100 g)	37.4	51.5	59
Dietary fiber (g/100 g)	10.5	3.4	0.2
Total fats (g/100 g)	37.7	29.7	32.0
Total vitamins (mg/100 g)	2.75	1.42	2.62

^aLettieri-Barbato et al. (2012)

^bUSDA [U.S. Department of Agriculture] (2013)

Chocolate truffles, or **pralines**, are usually small chocolate products where the inner center (filling, inclusion) is coated by chocolate. The center is usually made from another ingredient, such as caramel, cream, nut pastes, nougat, nuts, fruits, and berries. The technology is rather complicated, which makes this confectionery product more expensive. In the manufacture, chocolate is usually deposited by a nozzle on the filling placed either in a mold or on a belt.

Nutrition and Physiological Benefit of Chocolate

Chocolate contains all the essential components of food, i.e., proteins, carbohydrates, and fat, however, not in ideal proportions. Chocolate is a rich source of energy. On average, 100 g of milk chocolate will provide approximately 26 % of recommended daily energy intake, 63 % Guideline Daily Amounts (GDAs) of sugar, 43 % of GDA for fats, and only 16 % of GDA for proteins. Typical nutritional values of these components for different types of chocolate are given in Table 7.

Fat originating from cocoa butter and milk usually forms approximately 30 % of chocolate weight. It is the major energy-rich component of chocolate. Chocolate butter contains a number of glycerides of fatty acids, e.g., stearic, palmitic, oleic, and linoleic. Triglyceride composition varies according to the growing area, and typically the content of fatty acids is 1.0–1.6 % of trisaturated, 64.2–77.7 % of monounsaturated, 15.3–29.8 % of diunsaturated, and 4.4–8.0 % of polyunsaturated. Fatty acid composition of cocoa butter and its effect on cholesterol levels is summarized in Table 8 (Beckett 2008).

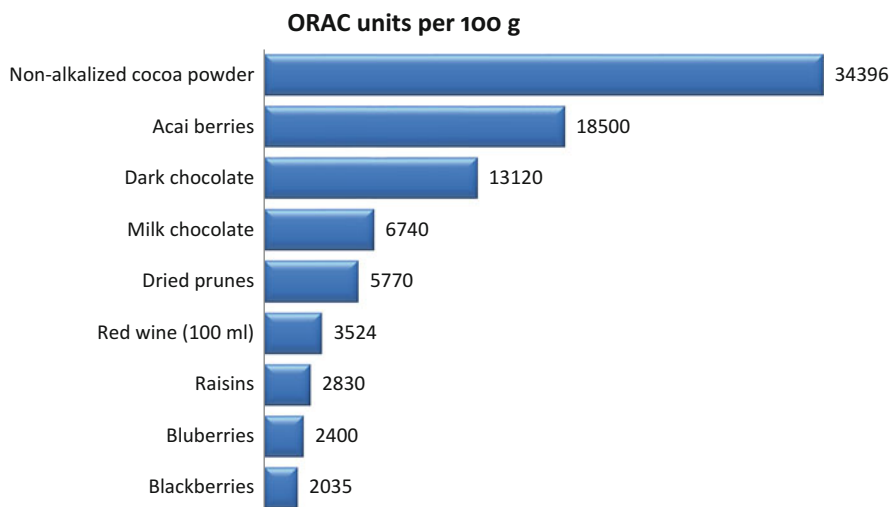
Lots of studies have been written on nutritional properties of chocolate and their positive effect on human health. Chocolate displays adaptogenic properties. The term adaptogen, used by herbalists, describes the ability of natural product to stabilize physiological processes, promote homeostasis, and increase the resistance to stress.

Antioxidants

In chocolate, the adaptogenic properties are related to plant compounds called antioxidants. Because plant foods contain many different classes of antioxidants,

Table 8 Fatty acid composition of cocoa butter (Beckett 2008)

Component	Content in chocolate	Effect on cholesterol levels
Stearic acid (%)	34	Little
Oleic acid (%)	35	Neutral or lowering
Palmitic acid (%)	26	Moderate
Polyunsaturated fats (%)	8	

**Fig. 15** Oxygen radical absorbance capacity in food (Pech 2010)

the cumulative capacity of food components to bind free radicals is expressed by their total antioxidant capacity (TAC) or oxygen radical absorbance capacity (ORAC). Antioxidants were found to be responsible for:

- Improvement of markers of cardiovascular functions
- Decrease in blood pressure
- Improvement of the protection against oxidative modification of low-density lipoproteins (LDL)
- Helping the body reduce the damage caused by free radicals, which can lead to diseases such as cancer
- Reduction of deterioration of cognitive functions in elderly

In chocolate and cocoa, the present polyphenols are more specifically called flavanols. The main flavanols in cocoa are epicatechin, catechin, and procyanidins. All of them are absorbed by the organism in proportion to the amounts eaten. Chocolate displays high levels of ORAC in comparison with other food products (Fig. 15). Due to its higher cocoa content, dark chocolate has more polyphenols

Table 9 Methylxanthine content in chocolates and beverages

	Serving	Caffeine ^a (mg)	Theobromine ^b (mg)
Milk chocolate	50 g	10	70
Dark chocolate	50 g	43	209
White chocolate	50 g	0	1.1
Strong ground coffee	Cup	95	–
Instant coffee	Cup	63	–
Black tea	Cup	47	2
Cola	Can (370 g)	33	–

^aUSDA (2013)^bBeckett (2008)

than milk chocolate. The technology affects the level of flavanols in chocolate. Cocoa fermentation in particular can destroy a lot of the flavanols, as well as the alkalization process.

Psychoactive Compounds

Chocolate contains alkaloids caffeine and theobromine (see Table 9). Theobromine and caffeine are related alkaloids, both belonging to the group of chemicals called methylxanthines. They are capable to have various physiological effects. Both stimulate the central nervous system, but theobromine effect is lesser. In high doses both alkaloids can cause sleeplessness and tremors. Both have diuretic effects and theobromine is used as a vasodilator (a blood vessel widener) and heart stimulant.

Another physiologically active compounds present in cocoa are phenylethylamine (an amphetamine-like compound), anandamide (a cannabinoid), and tryptophan, which all have been thought to induce cravings for chocolate, but it has not been confirmed. Phenylethylamine is a neurotransmitter and stimulant but is rapidly oxidized to phenylacetic acid in liver, which prevents significant concentrations from reaching the brain. Anandamide is an endogenous cannabinoid neurotransmitter affecting the nervous system affecting many biochemical processes. Its specific effect on human health and behavior (pain killing, eating, and sleeping patterns) is currently under study.

Mineral Substances

Chocolate is a rich source of mineral substances and vitamins. Table 10 summarizes content of physiologically important elements in dark chocolate and percentage of their GDAs.

Dental Caries

Sugars present in chocolate are cariogenic; however, the chocolate's negative effect on teeth is not that bad, because chocolate contains other components with anticaries activity. Milk proteins can play a positive role in limiting the tooth decay. Glycoproteins have been proved to reduce the formation of a dental plaque,

Table 10 Content of physiologically important elements in dark chocolate, 45–59 % cocoa solids (USDA 2013)

Minerals	mg/100 g	% Guideline Daily Amounts
Calcium	62	8
Iron	6	45
Magnesium	176	59
Phosphorus	260	32
Sodium	10	1
Potassium	567	24
Zinc	3	16

and casein is able to remineralize the tooth enamel. Tannins are the components which give the color and flavor to chocolate. They are part of a group called polyphenols and have antibacterial effect on plaque bacteria in mouth; therefore, they reduce the plaque formation.

Other Substances Used in Sugar and Confectionery Products

Beyond carbohydrates, there are many other ingredients used in confectionery products. Here is a brief overview of some of them.

Pectin

Pectin is a structural heteropolysaccharide rich in galacturonic acid linked by means of α -(1–4) glycosidic bond. It occurs in fruit (apples, apricots) and vegetables (carrot) or sugar beet and is widely used in food industry and confectionery. Pectin is a complex biopolymer containing several polysaccharides, such as homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). Furthermore, other substituted galacturonans have been described, such as xylogalacturonan and apiogalacturonan (Fraeye et al. 2010).

Carboxyl groups of galacturonic acid are esterified with methanol, and the ratio of esterified to nonesterified galacturonic acid affects the behavior of pectin in food applications. Therefore, pectins are categorized as high-ester (high-methoxy, HM) or low-ester (low-methoxy, LM) pectins (Srivastava and Malviya 2011).

Pectins are mainly used as gelling, thickening, and stabilizing components. The viscosity and stability of a pectin solution is related to many parameters, such as the molecular weight, degree of esterification, concentration, pH, and presence of counter ions in the solution. LM pectins form thermoreversible gels in the presence of calcium ions. HM pectin is stable at room temperature and pH 5–6 only, but in the presence of sugar, it forms thermally irreversible gels even at low pH (<3.5). The gel strength is not only affected by the sugar concentration but also depends on the type of the sugar added. Sucrose increases the gel strength more than glucose,

which has a larger effect than fructose. On the other hand, some polyols (such as sorbitol) may compete with pectin for binding of calcium ions. This may reduce the gel-promoting effect (Grosso and Rao 1998).

Gelatin

Gelatin (or gelatine) is a mixture of proteins and peptides prepared by the hydrolysis of collagen from animal skin and bones by alkali or acid solutions. Gelatin contains mainly glycine, proline, and 4-hydroxyproline residues. The main use in confectionery (marshmallows, gummy bears, fruit snacks, and jelly babies) and food industry is based on forming transparent elastic thermoreversible gels on cooling below about 35 °C, which simulates the mouthfeel of fat or cream.

Agar

Agar is a strong gelling hydrocolloid from marine red algae (Rhodophyta). Chemically, it is a mixture of agarose and agaropectin in variable proportions. The main structure is formed by repetitive units of D-galactose and 3–6,anhydro-L-galactose and a low content of sulfate esters. Agar's digestibility is less than 10 % of what is ingested (Armisen and Galatas 2009). That is why it is often used for dietetic formulas and foods for diabetics because the gelatinization does not depend on sugar addition. In confectionery, agar is used as a gelatin substitute in confectionery for vegetarians and in fruit preserves, ice cream, and jellies.

Gum Arabic

Gum arabic, or acacia gum (E414), is another natural polymer used in confectionery. It is obtained as hardened sap from trees *Acacia senegal* or *Acacia seyal* grown in the Sahel of Africa. Gum arabic is a mixture of high-molecular-weight polysaccharides and glycoproteins. The content of saccharides (arabinose, galactose, rhamnose, and 4-O-methyl glucuronic acid) varies according to the climate, variety, season, etc. Gum arabic is used primarily in the confectionery as a stabilizer or as a coating agent. It delays crystallization of sugar in gumdrops and acts as an emulsifier in caramel and toffee (Williams and Phillips 2009).

Nuts

Nuts (walnuts, peanuts, almonds, hazelnuts, macadamia, cashew, Brazil nuts, pistachio, pecan, etc.) are used as healthy fillings and inclusions in many confectionery products. From the nutritional point of view, nuts are rich sources of

vitamins (vitamin A, thiamine, riboflavin, and niacin), antioxidant (α -tocopherols), unsaturated fatty acids, phytosterols, and physiologically important elements (phosphorus, calcium, iron, sodium, potassium, magnesium, and even copper).

Lecithin

Lecithin (E322) is the most common surface active agent naturally occurring in soya, egg yolks, milk, rapeseed, and other plant or animal tissues. It is a fatty substance binding strongly to the sugar; therefore, it is widely used as emulsifier in chocolate production. An addition of between 0.1 % and 0.3 % soya lecithin reduces the viscosity of cocoa butter by more than 10 times and hence improves the rheological properties (Beckett 2008). In confectionery, it controls sugar crystallization, enhances shelf life, and improves the homogenization of ingredients.

Vanilla

Vanilla is a flavor obtained from pods of orchids of the genus *Vanilla planifolia* and belongs among one of the most expensive spices. It can be used either in the form of a whole pod, powder, or extract or as a vanilla sugar. Vanilla products contain hundreds of compounds; however, vanillin (4-hydroxy-3-methoxybenzaldehyde) gives primarily the characteristic flavor and smell of vanilla.

Peppermint and Spearmint

Peppermint and spearmint oils are ingredients which can also benefit to human health. Candies or chewing gums that primarily consist of peppermint oil, which is obtained from a plant *Mentha piperita*, as well as spearmint oil, which is present in *Mentha spicata*, have digestive benefits and can kill bacteria (Anonym 2014). Mint-flavored products showed a positive effect on a short-term memory, heart rate, and the amount of oxygen in the brain. Peppermint oil is rich in menthol but contains many other substances, such as menthone and methyl esters. Spearmint oil contains mostly terpenoid carvone, which provides the characteristic smell. Unlike peppermint oil, spearmint oil has minimal amounts of menthol and menthone.

Milk

Milk and its components improve the nutritional content of the chocolate and confectionery products, and they are important for the flavor and texture. Milk proteins are important donors of amino groups in Maillard reactions. More on milk composition and chemistry can be found in ► [Chap. 17, “Chemical Composition of Milk and Milk Products”](#).

Conclusion and Future Directions

Sugar products have a persistent history in human consumption and nutrition. Long before the Common Era, people craving for sweet taste have found many sources of sugar, not only fruit and berries but also honey and sugarcane and sugar beet later on. Sugar is not just a main nutrient providing energy and a vital ingredient in our diet but has many other important functions in food production.

Due to the prevalence of obesity, metabolic syndrome, diabetes, and dental caries in our population, there are concerns about high consumption of free sugars. Simple sugars have been accused to be the main reason for this fact, which brought the sugar use and nutrition into reexamination. The evidence that a single dietary component could cause all these diseases is still insufficient, and other factors need to be taken into consideration, such as reduced physical activity, excess energy intake irrespective of the nutrition source, low energy expenditure, or bad dental hygiene, in order to find out the reason for high rate of lifestyle diseases.

In the last decades, high but steady consumption of sugar can be noticed in urbanized and developed countries; however, the tooth decay in children in OECD countries has dropped during the last 25 years. The decreasing consumption of added sugars among more educated citizens, who may regard sugar as less healthy, is also one of the recent trends. Nevertheless, per capita consumption of sugar is growing in emerging economies, where the demand for sugar will continue.

Confectionery products benefitting from their indulgence and popularity will remain stable segment of the market, and manufacturers are already looking for innovations, such as new and healthier ingredients, reduced amount of fats and sugars, or introduction of organic, fair-trade products and crops from sustainable agriculture.

Cross-References

- ▶ [Chemical Composition of Eggs and Egg Products](#)
- ▶ [Chemical Composition of Fat and Oil Products](#)
- ▶ [Chemical Composition of Milk and Milk Products](#)

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Abstract

Vegetables and their products (salads, fermented and nonfermented pickles, prepared sauces, and pickled, conserved, frozen, marinated, and dried vegetables) are a good source of compounds that are involved in pharmacodynamic activity. Humans are inseparably linked to the existence of vegetables, as they are the source of several bioproducts essential for the survival of the animal kingdom. The importance of vegetables from the point of view of the food industry is determined by their complex chemical content that is important to the human body and includes organic substances (carbohydrates, proteins, lipids, and organic acids), phytoncides and antimicrobial substances, a high content of minerals (Ca, P, Fe, K, Mg, S, Cl, Zn, and Cu), and a high content of vitamins (A, B complex, C, E, F, K, P, and PP). The complex chemicals in vegetables have a favorable effect on the human body because they provide hydration due to their high water content, regulate metabolism in general, stimulate the muscular and skeletal systems, internal glands, and enzymatic activity, and have high nutritional and energy value. Vegetables are characterized by high nutritional density with low energy input and contain a variety of biological phytonutrients that make them an important part of the basic diet. It is recommended that vegetables be consumed fresh when their nutritional value is at its highest; some methods of processing and storage cause some water-soluble vitamins and nutrients to be lost. With energy from the sun, vegetables synthesize the basic compounds necessary for their survival (carbohydrates, lipids, and proteins), and a variety of organic phytochemicals can be extracted as raw materials that have important applications in dermatology, cosmetics, medicine, technology, and commerce.

Introduction

Vegetables and their products are low in calories but rich in vitamins and are essential for a balanced body. Of the 1,200 plant species that are considered “vegetable plants,” only 250 species are cultivated. Vegetables are sources of nutrients but are also 75–95 % water. The dry matter of vegetables consists of biomolecules (lipids, proteins, and carbohydrates), minerals, vitamins, and phytonutrients. Vegetables have a great variety of flavors, provide energy, provide hydration for the body which is 80–90 % water, are rich in liposoluble and water-soluble vitamins and fibers such as cellulose and pectin that facilitate intestinal transit, and are rich in minerals.

Vegetables are foods from plants with different uses and are a significant source of nutrients. The edible part of a plant – root, stem, bulb, fruit, flowers, seeds, leaves, or the whole plant – varies from one species to another. The intake of nutrients from vegetables depends on the consumed organ of the plant. Tubers are rich in starch but have few vitamins; roots and stems are high in fiber (cellulose and hemicellulose) but do not contain vitamin C; leafy vegetables are very rich in vitamin C, Mg, chlorophyll, and carotenoids; flowers, which are rarely consumed as such (e.g., cauliflower), are high in vitamin K and B-complex vitamins.

The carbohydrate content (cellulose in large amounts) of a plant varies by species. Lettuce, zucchini, tomato, eggplant, cucumber, greens, onion, okra, carrots, leeks, beets, celery, green peas, Brussels sprouts, potatoes, and dried beans contain sucrose, starch, and cellulose. In general, carbohydrates found in vegetables, such as starch, pectin, and cellulose, are mono- and disaccharides. Glucose is rarely consumed in its monosaccharide form but was determined to be a free compound in carrots. Fructose (the sweetest monosaccharide) makes up approximately 3 % of dehydrated vegetables by weight; in general, vegetables contain between 1 % and 7 % fructose.

Inulin-type oligosaccharides are natural polymers of fructose, a form of carbohydrate storage (onions and asparagus), and since they are not proximally absorbed, they produce 1 kcal/g. Other vegetables rich in fructo-oligosaccharides are tomatoes and garlic. Sucrose is found in large quantities in unripened vegetables but the quantity decreases as the fruit ripens, with sucrose replaced by glucose and fructose. Some vegetables do not contain sucrose.

Starches are insoluble compounds with a complex nature and formula $(C_6H_{10}O_5)_n$. Starch is a reserve substance in vegetables such as carrots, potatoes, and dried legumes (beans and peas). In vegetables, starch granules are encapsulated in rigid cellulose walls, making them inaccessible to digestive enzymes, which is why raw grains and potatoes are difficult to digest. Heat causes the granules to expand, the starch gels, and the cell wall softens and breaks, thus making digestion possible. Starch is found in large quantities in a plant before it matures, decreasing the extent of fruit maturation by its transformation to low sugar. Carrot starch can be eaten raw and usually is recommended for infants. Vegetable cellulose has a finer structure than that of grains and is recommended for those with some digestive diseases. In vegetables, cellulose combines with pectic substances to form pectin-cellulose complexes with the potential to stimulate motility and digestive secretions. Vegetable cellulose content ranges from 0.3 % (zucchini) to 3.6 % (parsnip). Hemicelluloses retain water in the intestine and fix cations; they are in carrot, beet, and zucchini. An indigestible polysaccharide, hemicellulose is associated with cellulose and lignin except in fiber-rich vegetables such as radish, cabbage, and eggplant. Pectins are characteristic to carrots and beets; they are used in medicines for the intestines (e.g., to treat enteritis), and they are used to make antiseptics and absorbent gels.

Lipids are in vegetables in low amounts (except oilseeds). Among the saturated fatty acids, palmitic, myristic, and stearic acids can be found in peas, beans, and spinach, and low amounts of saturated fatty acids are in almonds and peanuts. Of the monounsaturated fatty acids, oleic acid was identified in peas and palmitoleic acid in

spinach (23 %). The polyunsaturated fatty acid omega-3 is found in soybeans and beans. Moreover, soybean is a known source of lipids (47 mg/100 g; with a linoleic to linolenic acid ratio of 7.5:1) and glycerophospholipids such as lecithin.

The protein content of most vegetables ranges from 0.5 % to 1.5 %; the exceptions are green legumes (5–6 % in peas and beans) and dried legumes (20–34 % in lentils, dried beans, and soybeans which contain globulin-type protein: phaseolin in beans, legumelin in peas and lentils, and glycine in soybeans). From a nutrition point of view, soybean proteins are incomplete proteins because they contain all of the essential amino acids but not in equal proportion (methionine and lysine are in limited amounts). A protein is considered incomplete if it contains all essential amino acids but not in equal proportion, such as dried legumes which has methionine in limited amounts, or if it is missing one or more essential amino acids. Soybean is used to make flour and concentrate/protein isolates are used in the food industry for the production of soy milk, energy drinks, and other products (Butu et al. 2014a).

Vegetables contain minerals, mainly K, Ca, Mg, P, and Fe. They also contain traces of oligoelements (Cr, Cu, I, F, Zn, Mg, Mo, and Se), which are absorbed from the soil together with water; therefore, their proportions vary. Ca is found in vegetables such as cabbage, cauliflower, broccoli, parsley, onions, peas, and beans (with an absorption rate >50 %). Soybeans and beans contain relatively large amounts of Ca. Oxalic acid in spinach and tomatoes limits the bioavailability of minerals by forming insoluble calcium oxalate salts. Ca:P ratios are optimal in cabbage, lettuce, and onions which helps their uptake. The vegetables provide <30 % of P needed by the body. Phosphorus has been identified in dried legumes in the form of phytic acid, which forms insoluble complexes with a predatory effect on Ca, Fe, Zn, and Mg. Potassium is found in large quantities as carbonate and organic acid salts and is solubilized in water and gastric juice. Because of the high content of water and K, vegetables in general have a diuretic effect. Important sources of K are dried beans (1,500 mg/100 g), spinach (700 mg/100 g), and potatoes (500 mg/100 g).

Good sources of Mg are the darker green vegetables, such as lettuce, spinach, green onions, and nettles, and dried legumes in which Mg is an essential constituent of chlorophyll. After meat, vegetables are a good source of Fe, particularly dried beans and lentils. A high vitamin C content in vegetables makes the Fe more available for absorption, even for vegetables with a lower Fe content (carrots, broccoli, potatoes, tomatoes, cauliflower, cabbage, and sweet peppers). Vegetables with high concentrations of phytate, phosphate, cellulose, tannin, and oxalic acid have a decreased amount of Fe available for absorption. Fragmentation caused by boiling and excessive refining increases the loss of Fe. The most important sources of S are dried beans, broccoli, and cauliflower. Vegetables with high concentrations of Zn are usually rich in chlorophyll (spinach, dried beans, lentils, and peas). The Cu content in vegetables, which provide about 2 mg Cu/day, is lower than that in animal products, except for dried legumes. The presence of cellulose or thiocyanate (as in cabbage, cauliflower, turnips, and kale) reduces Cu absorption. Iodine is found in varying amounts in vegetables. Vegetables have a low Se content; it is found in soy, garlic, beans, and broccoli. These foods are a poor source of Na and are indicated for salt-restricted diets needed by hypertensive patients or patients

with renal insufficiency or those with edema). High-Na-content vegetables are winter radishes, celery, carrots, beets, spinach, and turnips.

The amount of vitamins in a vegetable depends on its type, ripening stage, the soil it grows in, and mode of conservation. Vitamin C from vegetables is more active than synthetic vitamin C, and when accompanied by vitamin P and other antioxidants, its uptake is enhanced. Vitamins and minerals have a significant presence in most green vegetables. Large amounts of ascorbic acid (vitamin C) are found in leafy greens and some vegetables (tomatoes and peppers), tubers (asparagus and potato), and bulbs (onions). The concentration of vitamin C in vegetables depends on the development and maturation of crops. Vitamin C is present in higher quantities in the shell than in the core, so when the outer layer is removed vitamin C is lost. The presence of vitamin C in plants depends on ascorbic oxidase activity (metalloenzymes containing Cu), which oxidizes vitamin C to dehydroascorbic acid with reduced vitamin activity or to compounds with no vitamin activity (dicetogulonic acid). The amount of vitamin C is inversely proportional to the amount of ascorbic oxidase (Xu et al. 2007). Vegetables with a high proportion of ascorbic oxidase (cucumbers, zucchini, and carrots) are almost devoid of vitamin C. Vitamin C in the form of dehydroascorbic acid is found in red pepper, parsley, dill, and horseradish.

Vitamin C is destroyed by oxidation, and sodium bicarbonate added to preserve and enhance the color of cooked vegetables causes loss of vitamin C. Refrigeration and freezing of foods can help preserve vitamin C, but cooking causes the loss of up to 45 % and 52 %, respectively, of vitamin C in those foods (Butnariu et al. 2013).

Deficiency in Fe and vitamin C may affect the status of folic acid in an organism. Vitamin B₁ is in legumes (dried beans at 0.6 mg%), leafy vegetables (spinach and lettuce), cabbage (0.5–1.5 mg%), and potatoes (0.56 mg%). B₂ is found in green leafy vegetables (broccoli, spinach, and parsley at 0.2–0.3 mg%) and pulses (lentils at 0.2–0.3 mg%). Niacin in the form of nicotinic acid is in dried legumes (2–3 mg% in lentils) and other vegetables such as potatoes, tomatoes, eggplant, spinach, cauliflower, green peas, and beans. The most important plant sources of vitamin B₅ are broccoli, potatoes, and tomatoes. Vitamin B₆ is present in pulses (soybeans, lentils, and beans) and vegetables such as potatoes, cauliflower, and spinach. The bioavailability of vitamin B₆ in vegetables is lower than that in animal sources because it is affected by long-term storage, prolonged heat treatment, and freezing techniques. Vitamin B₇ (H) is in vegetables such as cabbage, spinach, beans, peas, carrots, and tomatoes. Leafy green vegetables (spinach, lettuce, broccoli) are a good source of folic acid (vitamin B₉), which is also found in potatoes and pulses. Reduced B₉ from vegetables is oxidized and destroyed at a rate of 50–90 % during boiling. The bioavailability of B₉ varies depending on the presence or absence of B₉-conjugated metal chelates, inhibitors, and chelating agents (B₉-linked vegetables), the form of which determines an individual's nutritional status. B₉ uptake from lettuce is twice as small as that of yeasts or bananas. Cyanocobalamin (vitamin B₁₂) is found in vegetables in small amounts and is synthesized by bacteria. Vegetarians have lower circulating concentrations of vitamin B₁₂ after 5–6 years if they do not take a supplement.

Table 1 Stability of vitamins when exposed to certain conditions

Phytochemicals	Air	Light	Heat	Maximum loss from cooking (%)
Vitamin A	A	A	U	40
Vitamin C	A	A	A	100
Vitamin D	A	A	U	40
Vitamin K	U	A	U	5
Vitamin B ₇	U	U	A	60
Vitamin B ₆	U	A	A	40
Vitamin B ₂	U	A	A	75
Vitamin B ₁	A	A	A	55

U unalterable (no considerable destruction), *A* alterable (considerable destruction)

Vitamin D is found in vegetables, with the highest content in spinach and cabbage. Tocopherols/tocotrienols are synthesized only by plants, with the oils extracted from plants being the richest sources. Soybean oil contains 80–95 % α -tocopherol/ γ -tocopherol. Vitamin E, known for its role as an antioxidant, is in seeds, legumes, spinach, lettuce, peas, cabbage, and celery. Vitamin K is found in green leafy vegetables such as parsley, spinach, broccoli, lettuce, turnips, and cabbage (40–50 % of total intake). The bioavailability of vitamin K₁ depends on the vegetable (e.g., higher in broccoli than in spinach) and the composition of the diet (e.g., the addition of fats increases absorption).

The stability of vitamins exposed to certain conditions is presented in Table 1.

Carotenoids, i.e., carotenoid hydrocarbons and their oxygenated derivatives, in vegetables are in free form or combined with holoproteins and carbohydrates to form carotenoproteins and carotenoid glycosides, respectively. Hydrocarbon carotenoids are carotenoids with 40 carbon atoms and the most important ones are lycopenes, α -carotene, β -carotene, and γ -carotene. Heating α -carotene may convert it into β -carotene. β -Carotene (the main precursor of vitamin A) is widespread in plants and permanently accompanies chlorophyll. By oxidative enzymatic hydrolysis, β -carotene is converted into two molecules of vitamin A₁. The bioavailability of β -carotene from plants compared to that of purified β -carotene is in the range of 3–6 % for leafy vegetables, 19–34 % for carrots, and 22–24 % for broccoli. Consuming broccoli and peas can cause an increase in retinol and β -carotene in plasma compared to consumption of spinach (Patel et al. 2001). It was demonstrated that β -carotene from some vegetables is 2.6–6 times more effective at increasing the plasma concentration of β -carotene than that from green leafy vegetables. The difference is due to intracellular localization of carotenoids; they are found in chloroplasts in vegetables and in chromoplasts in other products. γ -Carotene takes the form of red crystals with blue reflections and is less common in the plant kingdom (it is found mostly in carrots). Phytonutrients such as anthocyanins and carotenoids (carrots, spinach, green onions, lettuce, and tomatoes) are efficient protectors of cells because they inhibit destabilizing processes (Hendrickson et al. 2013; Butnariu 2014).

Isothiocyanates (ITCs) are found in cruciferous vegetables (cabbage, Brussels sprouts, cauliflower, and broccoli). When isothiocyanates split they form substances that cause hypertrophy and hyperplasia of the thyroid gland. Goitrin (L-5-vinyl-2-thioxazolidone) is found in plants in water-soluble form and its precursor is the inactive compound progoitrin; goitrin is released from progoitrin with the help of thioglucosidase. Heat treatment destroys thioglucosidase, so goitrin cannot be formed. Goitrin interferes with the synthesis of the thyroid hormones thyroxine and triiodothyronine. Thiocyanates inhibit inorganic iodine (I) intake, thereby contributing to the hyperthyroid effect of vegetables.

Insoluble fibers such as lignin and chitin bind biliary acids and reduce the absorption of cholesterol and lipids. Soluble fibers such as pectin and guar gum from dried legumes and other vegetables cause a decrease in LDL (low-density lipoprotein) cholesterol. The effects vary with the type and amount of fiber. Vegetables with a low glycemic index cause a low insulinemic response with decreased hepatic synthesis of VLDL (very low-density lipoprotein) and LDL cholesterol simultaneously.

Some components of vegetables, tannins, saponins, and phytates interact with macronutrients and vitamins or minerals in the diet, causing the reduction of their absorption. Phytate, found in the external layer of dried legumes, is a ring of six carbon atoms and each atom owns a phosphate radical (myo-inositol phosphate). It has the ability to bind to metal ions, especially those of Ca, Cu, Fe, and Zn. Ca catalyzes amylase action, and catalyzes excessive phytate contributing to the formation of resources starch hydrolisis. Phytate can be hydrolyzed by the phytase enzyme, which is produced by yeast fermentation. Hexaphosphoric ester of inositol (phytic acid or phytate) reacts with Ca salts and Mg to form phytin.

The constituents of fiber that may enhance absorption of nutrients, such as inulin-soluble fiber and sugar obtained from beets, have also been determined. They increase Ca absorption without adversely affecting minerals (Butnariu and Samfira 2013a). Beans, peas, lentils, and chick peas, all with high-protein content (23 %), carbohydrates (57 %), including starch (47 %), vitamins, and minerals have such fiber. If legumes are boiled too long they become bloated and their purine content increases.

Some vegetables contain antinutritive substances. Vegetables contain substances that inhibit or inactivate phytochemicals (vitamins) and are called antivitamin substances and antimineral substances. The former include oxalic acid (biologically inactive) and ascorbic oxidase, an enzyme which, in presence of oxygen, oxidizes vitamin C first to dehydroascorbic acid (active) and finally to 2,5-diketogluconic acid. The latter include phytic and oxalic acids, present in green vegetables and pulses (spinach and beans) and which bind Ca, Mg, and/or Fe into insoluble complexes, making their absorption and utilization in the human body impossible. Other antinutritive substances are antithyroid substances (ITCs) from cabbage, cauliflower, and kale that prevent iodine absorption in the thyroid gland, and some nonproteinogenic substances (e.g., trypsin inhibitor from beans) that inhibit pancreatic trypsin (Plaza-Díaz et al. 2013).

Table 2 Nutritional value of asparagus per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	85 kJ (20 kcal)	Folate (vitamin B ₉)	52 µg (13 %)
Carbohydrates	3.88 g	Choline	16 mg (3 %)
Sugars	1.88 g	Vitamin C	5.6 mg (7 %)
Dietary fiber	2.1 g	Vitamin E	1.1 mg (7 %)
Fat	0.12 g	Vitamin K	41.6 µg (40 %)
Protein	2.2 g	Minerals	
Vitamins		Calcium (Ca)	24 mg (2 %)
A equiv.	38 µg (5 %)	Iron (Fe)	2.14 mg (16 %)
β-Carotene	449 µg (4 %)	Magnesium (Mg)	14 mg (4 %)
Lutein/zeaxanthin	710 µg	Manganese (Mn)	0.158 mg (8 %)
Thiamine (vitamin B ₁)	0.143 mg (12 %)	Phosphorus (P)	52 mg (7 %)
Riboflavin (vitamin B ₂)	0.141 mg (12 %)	Potassium (K)	202 mg (4 %)
Niacin (vitamin B ₃)	0.978 mg (7 %)	Sodium (Na)	2 mg (0 %)
Vitamin B ₆	0.091 mg (7 %)	Zinc (Zn)	0.54 mg (6 %)

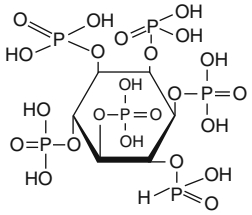
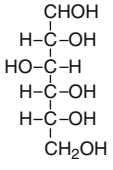
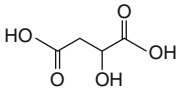
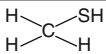
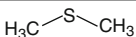
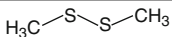
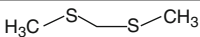
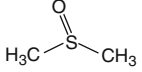
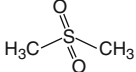

Vegetables have therapeutic value and are used as snacks (celery, peas, and chicory), cholagogue (artichokes), depuratives (radish, lettuce, asparagus, dandelion green, leek, celery, watercress, and chicory), diuretics (asparagus, dandelions, leeks, celery, and watercress), emollients (spinach, leeks, sorrel, lettuce, onions, carrots, and potatoes), expectorants (red cabbage), and vermifuges (garlic, onion, and cabbage). Vegetables can be used raw in salads or cooked in purees, puddings, and other foods. The water in which vegetables are boiled contains water-soluble vitamins (vitamin B complex, C), minerals, and other nutrients.

Asparagus

Asparagus officinalis contains folic acid, vitamin C, sulfur, and malic and citric acids. A quantity of 100 g of asparagus has 20 kcal of energy. In addition to proteins, lipids, carbohydrates, and cellulose, asparagus contains vitamins, phytohormones, enzymes, and minerals, and it has allelopathy potential for crop diseases and pests (Bostan et al. 2013). The chemical composition of asparagus is presented in Table 2.

Certain phytochemicals in asparagus are metabolized to yield NH₃ and diverse S-containing degradation products (various thiols and thioesters). Some volatile organic phytochemicals responsible for the odor of asparagus are methanethiol, dimethyl sulfide, dimethyl disulfide, bis(methylthio)methane, dimethyl sulfoxide, and dimethyl sulfone (Visavadiya and Narasimhacharya 2009). The first two are the most pungent, while the last two (sulfur-oxidized) have a sweet aroma (see Table 3).

Table 3 Some chemical components found in asparagus

			
Phytic acid	Fructose	Malic acid	
			
Methanethiol	Dimethyl sulfide	Dimethyl disulfide	Bis(methylthio) methane
			
Dimethyl sulfoxide	Dimethyl sulfone	Asparagusic acid	

Mixtures of these phytochemicals create an odor attributed to methanethiol. The phytochemicals known to originate in asparagus, such as asparagusic acid and its derivatives, are the only sulfur-containing phytochemicals and are unique to asparagus (Motoki et al. 2012). This vegetable is a source of vitamin E and fructo-oligocarbohydrates. Volatile oil contains methanethiol, dimethyl sulfide, dimethyl disulfide, bis(methylthio)methane, dimethyl sulfoxide, and dimethyl sulfone. Sulfate-active substances have a role in energy production, blood clotting, collagen synthesis (protein contents in bones, fibrous tissue, skin, hair, and nails), and enzyme synthesis (increase bioreactions). Sulfate-active substances contain P and vitamin B and have remineralizing and stimulating properties. The thiol-sulfide redox system is important in biological systems. Many peptides contain a free SH group and disulfide bonds can be achieved in the peptide chain. Asparagus is recommended for diabetics and diets for losing weight because it is low in calories: 14 cal per 100 g.

Beans

Bean (*Phaseolus vulgaris*) is an annual or multiannual plant cultivated for its edible pods or the seeds inside them. The chemical composition of beans is presented in Table 4.

Green beans are rich in carotene, vitamins, and minerals. Dried beans contain proteins (21 %), carbohydrates (56 %) as starch and cellulose, carotenes, vitamins, and albumin. Beans are a source of vitamins B and macro- and micronutrients. Dried beans contain K (177 mg%), Ca (195 mg%), P (420 mg%), Fe, and trace elements and has a protein content ten times higher than green beans. Because of

Table 4 Nutritional value of beans per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g(unit)	Phytochemicals	Value/100 g(unit)
Energy	71 kcal	Water	81.17 g
Protein	4.07 g	Vitamins	
Total lipid (fat)	0.29 g	Vitamin C, total ascorbic acid	7.3 mg
Carbohydrates, by difference	13.33 g	Thiamin	0.029 mg
Fiber, total dietary	3.6 g	Riboflavin	0.043 mg
Minerals		Niacin	0.532 mg
Calcium	28 mg	Vitamin B ₆	0.062 mg
Iron	1.61 mg	Folate, DFE	16 µg
Magnesium	34 mg	Vitamin B ₁₂	0.00 µg
Phosphorus	71 mg	Vitamin A, RAE	8 µg
Potassium	285 mg	Vitamin A, IU	150 IU
Sodium	252 mg	Vitamin D (D ₂ + D ₃)	0.0 µg
Zinc	0.64 mg	Vitamin D	0 IU
Lipids			
Fatty acids, total saturated	0.066 g	Fatty acids, total polyunsaturated	0.139 g
Fatty acids, total monounsaturated	0.016 g	Cholesterol	0 mg

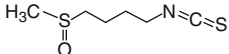
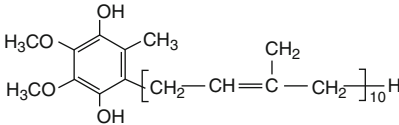
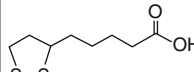
the silicic acid in the sheath, beans have many minerals to bring to an organism. One hundred grams of beans has 102 cal (Table 5).

White beans contain Ca, Fe, Mg, and other substances. Red beans are a good source of starch. A serving of 100 g red beans has 93 kcal (Mendes et al. 2012). The main components of green beans are vitamins, minerals, chlorophylls, carbohydrates, trace elements (Ni, Cu, and Co), amino acids (arginine, asparagine, tyrosine, tryptophan, betaine, and lysine), silicic acid, phosphoric acid, mineral salts, and about 50 % hemicellulose. A serving of 100 g dried red beans has 25 g starch and also contains phytin. The amino acid composition revealed that kidney bean had total essential amino acids of 48.8 %, including arginine at 65.5 mg/g protein, leucine at 62.6 mg/g protein, and lysine at 62.0 mg/g protein. Glutamic acid (134.1 mg/g protein) and aspartic acid (96.6 mg/g protein) are rich nonessential amino acids. Kidney bean is also rich in essential fatty acids (55.6 %) and contains soluble and insoluble cellulose and several antinutritional factors (ANFs), including saponin 1.24 %, tannic acid 0.77 %, phytin phosphorus 11.5 mg/g, phytic acid 40.8 mg/g, and oxalate 3.65 mg/g (Ashfield et al. 2012). The physicochemical features of kidney bean oil are that it has a significant saponification value (247 mg KOH/g) and a significant iodine value (89.4 mg I/g). Kidney bean has significant water and oil absorption capacities (165 % and 117 %) with a moderately low least gelation concentration (6.0 %) and significant emulsion capacity (34.5 %).

Table 5 Some chemical components found in beans

	lecithin
	Phaeosolin
	Betaine
	Phytin

Table 6 Some chemical components found in broccoli

		
Sulforaphane	Coenzyme Q10 (CoQ10, ubiquinone)	α -Lipoic acid

Broccoli

Broccoli (*Brassica oleracea* var. *botrytis italic*) is a source of vitamins and mineral components, β -carotene, and S compounds; it is one of richest sources of vitamins and minerals. A serving of 100 g broccoli has 30 cal. It is high in nutritional value due to its carbohydrates, vitamins (vitamin C content is equal to that of lemon), and provitamins. The Ca content of broccoli is equal to that of milk. Broccoli contains Q₁₀ coenzyme (a liposoluble substance with vitamin properties), which is present in every cell of the body, where it has a role in energy production and antioxidant activity. This vegetable contains α -lipoic acid as an antioxidant that increases the effects of other antioxidants like vitamins C and E (Guo et al. 2013) (Table 6).

Broccoli contains sulforaphane, hydrochinonglicosides, quercetin, and indoles, and its cellulose content is lower than that of cauliflower. Broccoli contains enzymes, chlorophyll and glucose enol, starch, minerals, and vitamins, has a strong effect on stress, and is used in the treatment of cancer. 3,3'-Diindolylmethane in broccoli is a strong modulator of the immune response system. Broccoli also contains glucoraphanin, which can be processed into the anticancer phytochemical sulforaphane. It is a source of indole-3-carbinol (I3C), a physicochemical that boosts DNA repair in cells. The chemical composition of broccoli is presented in Table 7.

Boiling broccoli reduces the levels of suspected anticarcinogenic phytochemicals such as sulforaphane, with losses of 20–30 % after 5 min, 40–50 % after 10 min, and 77 % after 30 min. It is rich in lutein and provides a moderate amount of β -carotene. Phytochemicals identified in broccoli are glucosinolates, dithiolthiones, indoles, glucoraphanin, S-methyl cysteine sulfoxide, and ITCs.

Cabbage

Cabbage (*Brassica oleracea* var. *capitata*) contains the carbohydrates pentosans, partially assimilated sugars, sucrose, glucose, and fructose. Red cabbage delivers 196.5 mg of polyphenols per 100 g (3.5oz), of which 28.3 mg are anthocyanins. Green cabbage yields 45 mg of polyphenols per 100 g, including 0.01 mg of anthocyanins (Table 8).

Table 7 Nutritional value of broccoli per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods/>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	35 kcal	Vitamins	
Water	89.25 g	Vitamin C, total	64.9 mg
Protein	2.38 g	Thiamin	0.063 mg
Total lipids (fat)	0.41 g	Riboflavin	0.123 mg
Carbohydrates, by difference	7.18 g	Niacin	0.553 mg
Fiber, total dietary	3.3 g	Vitamin B ₆	0.200 mg
Sugars, total	1.39 g	Folate, DFE	108 µg
Minerals		Vitamin B ₁₂	0.00 µg
Calcium	40 mg	Vitamin A, RAE	77 µg
Iron	0.67 mg	Vitamin A, IU	1548 IU
Magnesium	21 mg	Vitamin E (α-tocopherol)	1.45 mg
Phosphorus	67 mg	Vitamin D (D ₂ + D ₃)	0.0 µg
Potassium	293 mg	Vitamin D	0 IU
Sodium	41 mg	Vitamin K	141.1 µg
Zinc	0.45 mg		
Lipids			
Fatty acids, total saturated	0.079 g	Fatty acids, total polyunsaturated	0.170 g
Fatty acids, total monounsaturated	0.040 g	Cholesterol	0 mg

Table 8 Some chemical components found in cabbage

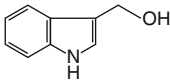
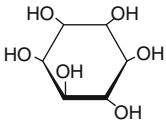
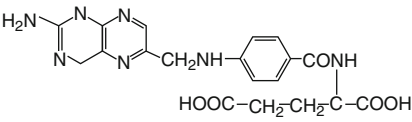
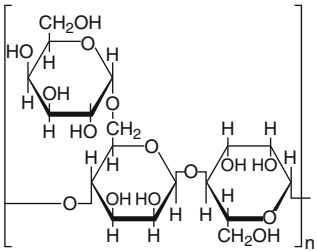
		
Indol-3-carbinol	Myo-inositol	Folic acid
		Guaran

Table 9 Nutritional value of cabbage per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	25 kcal	Vitamins	
Water	92.18 g	Vitamin C, total ascorbic acid	36.6 mg
Protein	1.28 g	Thiamin	0.061 mg
Total lipid (fat)	0.10 g	Riboflavin	0.040 mg
Carbohydrates, by difference	5.80 g	Niacin	0.234 mg
Fiber, total dietary	2.5 g	Vitamin B ₆	0.124 mg
Sugars, total	3.20 g	Folate, DFE	43 µg
Minerals		Vitamin B ₁₂	0.00 µg
Calcium	40 mg	Vitamin A, RAE	5 µg
Iron	0.47 mg	Vitamin A, IU	98 IU
Magnesium	12 mg	Vitamin E (α-tocopherol)	0.15 mg
Phosphorus	26 mg	Vitamin D (D ₂ + D ₃)	0.0 µg
Potassium	170 mg	Vitamin D	0 IU
Sodium	18 mg	Vitamin K	76.0 µg
Zinc	0.18 mg		
Lipids			
Fatty acids, total saturated	0.034 g	Fatty acids, total polyunsaturated	0.017 g
Fatty acids, total monounsaturated	0.017 g	Cholesterol	0 mg

Vitamin C equivalent – a measure of antioxidant capacity – of red cabbage is six to eight times greater than that of green cabbage. The chemical composition of cabbage is presented in Table 9.

Red cabbage is one of the most nutritious and best-tasting vegetables. Indole-3-carbinol (I3C) is not an isothiocyanate (ITC) but a benzopyrrole, and it is formed only when ITCs made by glucobrassicin are further broken down into non-sulfur-containing phytochemicals (Tarozzi et al. 2013). ITCs made from cabbage glucosinolates protect against cancer through a variety of different mechanisms. The cellulose and S content causes flatulence. Cabbage also contains substances that help the liver metabolize toxins, vegetal albumin, gum, gum extract, potassium nitrate, oxides of Fe, S, proteins, lipids, carbohydrates, and vitamins (Dong et al. 2013; Niu et al. 2012). The antioxidant I3C metabolizes excess estrogen (Duclos and Björkman 2008). A serving of 100 g cabbage has 25 cal (100 kJ) and 100 g Brussels sprouts has 25–34 kcal. Brussels sprouts are a source of folic acid, vitamin C, and antioxidants.

Carrot

Carrot (*Daucus carota*) contains the highest amount of β -carotene among all vegetables. Its consumption increases resistance to ultraviolet rays, giving the skin a smooth and healthy coloring. Carrot contains vitamins, levulose and dextrose, salts, minerals (Fe up to 7 % and K 235 mg%), carotene, asparagine, daucarine, and pectin (Mech-Nowak et al. 2012) (Table 10).

Carrot is a source of β -carotene as provitamin A and contains carbohydrates and minerals. A serving of 100 g carrot has 31 kcal. The chemical composition of carrot is presented in Table 11.

Polyacetylenes can be detected in carrots and are involved in cytotoxic activities. Falcarinol and falcarindiol are two such phytochemicals and they are antifungals. Falcarindiol is the main phytochemical responsible for the carrot's bitter taste (Singh et al. 2012). Other phytochemicals such as pyrrolidine (leaves), 6-hydroxymellein, 6-methoxymellein, eugenin, 2,4,5-trimethoxybenzaldehyde (gazarin), and (*Z*)-3-acetoxy-heptadeca-1,9-diene-4,6-dien-8-ol (falcarindiol 3-acetate) are also found in carrot.

Cauliflower

Cauliflower [*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *botrytis* L.] is high in nutritional value with its minerals, carotene, and vitamins. The chemical composition of cauliflower is presented in Table 12.

Cauliflower is easily digestible and has a high Ca content, which is good for building bone. Cauliflower has a sweet taste and is recommended to those who consume excessively acidic food. It has moderate energy value (100 g provides about 48.2 mg or 80 %) and provides needed vitamins and minerals, including a significant amount of vitamin C and many essential B-complex vitamins. It contains anticancer phytochemicals such as sulforaphane; vegetal sterols such as I3C, which is an antiestrogen agent; Mg, which is a cofactor for the antioxidant enzyme superoxide dismutase; and K, which is an intracellular electrolyte that helps counter the negative effects of Na such as hypertension (Table 13).

Cauliflower contains diindolylmethane (DIM), a lipid-soluble phytochemical contained in the *Brassica* group of vegetables in large amounts (Dhall et al. 2010). Cauliflower is low in calories but gives the feeling of satiation and thus is recommended as part of weight loss diets. It plays a role in the prevention of the development of genetically determined cancers and stimulates the natural antioxidant system with its glucosinolates and ITCs. It contains substances that can form phenols and hydrogen sulfide during heat treatment, and other substances that diminish the effectiveness of paracetamol analgesics. A serving of 100 g cauliflower yields 21 kcal of energy.

Table 10 Some chemical components found in carrot

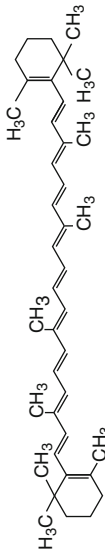
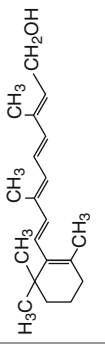
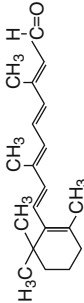
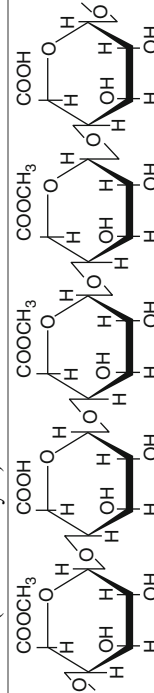
 <p>β-Carotene</p>	 <p>Vitamin A</p>
 <p>Retinal (retinaldehyde)</p>	 <p>Pectin</p>

Table 11 Nutritional value of carrot per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	41 kcal	Vitamins	
Water	88.29 g	Vitamin C, total ascorbic acid	5.9 mg
Protein	0.93 g	Thiamin	0.066 mg
Total lipid (fat)	0.24 g	Riboflavin	0.058 mg
Carbohydrates, by difference	9.58 g	Niacin	0.983 mg
Fiber, total dietary	2.8 g	Vitamin B ₆	19 µg
Sugars, total	4.74 g	Folate, DFE	19 µg
Minerals		Vitamin B ₁₂	0.00 µg
Calcium	33 mg	Vitamin A, RAE	835 µg
Iron	0.30 mg	Vitamin A, IU	16706 IU
Magnesium	12 mg	Vitamin E (α-tocopherol)	0.66 mg
Phosphorus	35 mg	Vitamin D (D ₂ + D ₃)	0.0 µg
Potassium	320 mg	Vitamin D	0 IU
Sodium	69 mg	Vitamin K	13.2 µg
Zinc	0.24 mg		
Lipids			
Fatty acids, total saturated	0.037 g	Fatty acids, total polyunsaturated	0.117 g
Fatty acids, total monounsaturated	0.014 g	Fatty acids, total <i>trans</i>	0.000 g
		Cholesterol	0 mg

Celery

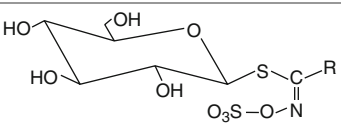
Celery (*Apium graveolens*) contains vitamins, minerals, and organic acids. Its volatile oil is rich in Ca and vitamins. Active phytochemicals identified in celery are falcariondiol, (9Z)-1,9-heptadecadiene-4,6-diyne-3,8,11-triol, oplopandiol, bergapten (5,8-dimethoxypsoralen) isofraxidin, eugenic acid, *trans*-ferulic acid, *trans*-cinnamic acid, p-hydroxyphenylethanol ferulate, caffeoylquinic acid, 5-*p-trans*-coumaroylquinic acid, sedanolide, lunularin, lunularic acid, 2-(3-methoxy-4-hydroxyphenol)-propane-1,3-diol, D-allitol, β-sitosterol, benzoic acid, and succinic acid (Jorge et al. 2013). The chemical composition of celery is presented in Table 14.

Celery contains 3-butylphthalide, a constituent of celery oil, and its leaves are high in minerals and vitamins and also contain essential oils, flavones, furanocoumarins (furocoumarins), and coumarins (obsthof, obsthofenol,

Table 12 Nutritional value of cauliflower per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	25 kcal	Vitamins	
Water	92.07 g	Vitamin C, total ascorbic acid	48.2 mg
Protein	1.92 g	Thiamin	0.050 mg
Total lipid (fat)	0.28 g	Riboflavin	0.060 mg
Carbohydrates, by difference	4.97 g	Niacin	0.507 mg
Fiber, total dietary	2.0 g	Vitamin B ₆	0.184 mg
Sugars, total	1.91 g	Folate, DFE	57 µg
Minerals		Vitamin B ₁₂	0.00 µg
Calcium	22 mg	Vitamin A, RAE	0 µg
Iron	0.42 mg	Vitamin A, IU	0 IU
Magnesium	15 mg	Vitamin E (α-tocopherol)	0.08 mg
Phosphorus	44 mg	Vitamin D (D ₂ + D ₃)	0.0 µg
Potassium	299 mg	Vitamin D	0 IU
Sodium	30 mg	Vitamin K	15.5 µg
Zinc	0.27 mg		
Lipids			
Fatty acids, total saturated	0.064 g	Fatty acids, total polyunsaturated	0.015 g
Fatty acids, total monounsaturated	0.017 g	Fatty acids, total <i>trans</i>	0.000 g
		Cholesterol	0 mg

Table 13 Some chemical components found in cauliflower

	$R-N=C=O$	$R-N=C=S$
Glucosinolates	Isocyanate	Isothiocyanate

umbelliferone) (Kolarovic et al. 2010). Furocoumarins and their chemical structures found in celery leaves are as follows (Table 15).

Volatile oil gives celery its characteristic taste and odor. The components of celery's volatile oil are p-cimol, α-santalol, limonene, β-pinene, β-caryophyllene, humulen, α- and β-selinen, and taxol, but the constituents that give celery its characteristic taste and smell are sedanolide and sedanonic anhydride.

Table 14 Nutritional value of celery per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

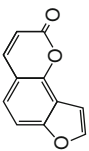
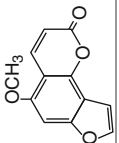
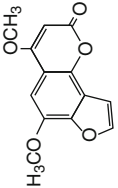
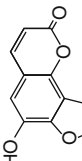
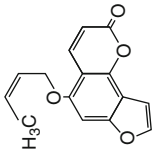
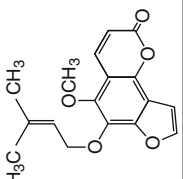
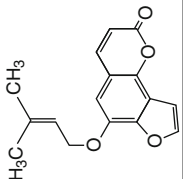
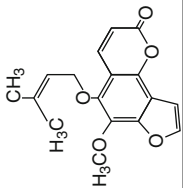
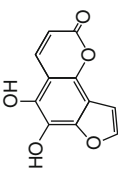
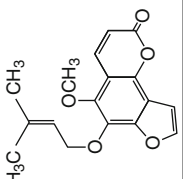
Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Water	95.43 g	Vitamins	
Energy	16 kcal	Vitamin C, total ascorbic acid	3.1 mg
Protein	0.69 g	Thiamin	0.021 mg
Total lipid (fat)	0.17 g	Riboflavin	0.057 mg
Carbohydrates, by difference	2.97 g	Niacin	0.32 mg
Fiber, total dietary	1.6 g	Vitamin B ₆	0.074 mg
Sugars, total	1.83 g	Folate, DFE	36 µg
Minerals		Vitamin B ₁₂	0 µg
Calcium	40 mg	Vitamin A, RAE	22 µg
Iron	0.2 mg	Vitamin A, IU	449 IU
Magnesium	11 mg	Vitamin E (α -tocopherol)	0.27 mg
Phosphorus	24 mg	Vitamin D (D ₂ + D ₃)	0 µg
Potassium	260 mg	Vitamin D	0 IU
Sodium	80 mg	Vitamin K	29.3 µg
Zinc	0.13 mg		
Lipids			
Fatty acids, total saturated	0.042 g	Fatty acids, total polyunsaturated	0.079 g
Fatty acids, total monounsaturated	0.032 g	Cholesterol	0 mg

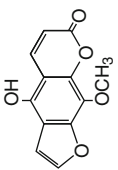
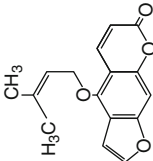
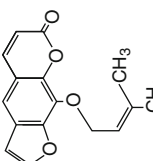
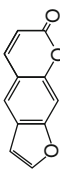
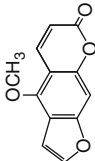
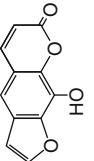
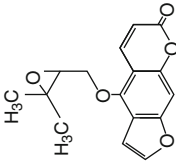
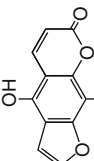
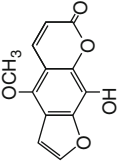
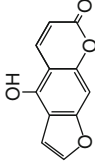
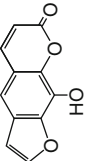
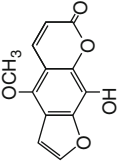
Cucumber

Cucumber (*Cucumis sativus*) is ~96 % water and contains minerals, vitamins, provitamin A, 11 % K, 2 % and 10 % NaCl. Cucumber also contains tartaric acid, which inhibits the synthesis of lipids from carbohydrates and provitamin A. The chemical composition of the cucumber is presented in Table 16.

Cucumber contains the following unsaponifiable matter: 24-ethylcholesta-7,22,25-trienol, 24-ethylcholesta-7,25-dienol, avenasterol, spinasterol, karounidiol, and isokarounidiol. The fatty acids in cucumber include myristic acid (0.12 %), palmitic acid (12.04 %), palmitoleic acid (0.09 %), heptadecanoic acid (0.06 %), stearic acid (5.64 %), oleic acid (6.95 %), linoleic acid (70.40 %), arachidic acid (0.19 %), and α -linolenic acid (0.51 %) (Bie et al. 2013). Specific phytonutrients provided by cucumbers include flavonoids (apigenin, luteolin, quercetin, and kaempferol), lignans (pinoresinol, lariciresinol, and secoisolariciresinol), and triterpenes (cucurbitacins A, B, C, and D) (Table 17).

Table 15 Some furancoumarins from celery

Angular furocoumarins	
	
Angelicin	Isobergaptien
	
Pimpinellin	Sphondin
	
Lanatin	Sphondinol
	
Heratomin	5-Isopentenyl oxyisobergaptien
	
5,6-Dihydroxyangelicin	6-Isopentenyl oxyisobergaptien

Linear furocoumarins				
	Psoralen		Isoimperatorin	
	5-Hydr oxyxanthoxin		Bergapten	
	Bergaptool		Xanthotoxin	
	Oxypeucedanin		Xanthoxol	
				8-Hydr oxybergapten

(continued)

Table 15 (continued)

				Ergamottin
				Bergamottin
				Oxypeuce daninhydrate
				Oxypeuce daninhydrate

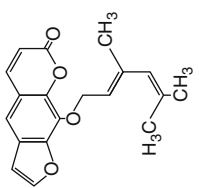
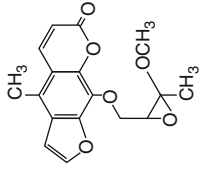
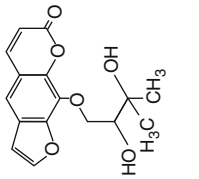
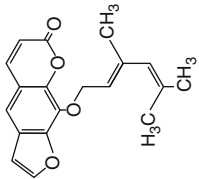
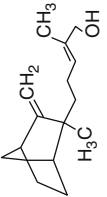
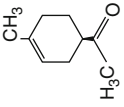
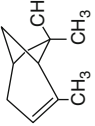
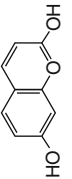
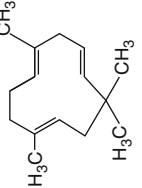
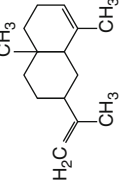
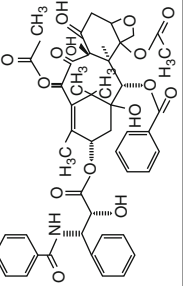
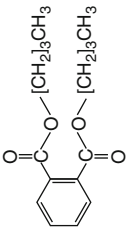
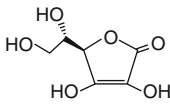
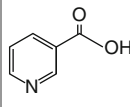
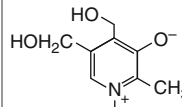
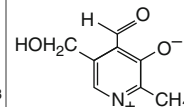
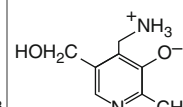
			
8-Geran oxypentalen	Byakangelicol	Heracelenol	Byakangelicin
			
Santalol	Limonen	β -Pinen	Isoimperatorin
			
Humulen	Selenin	Taxol	3-Dibutyl phthalate

Table 16 Nutritional value of cucumber per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

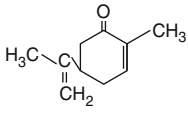
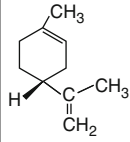
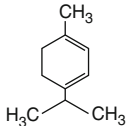
Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	15 kcal	Vitamins	
Water	95.23 g	Vitamin C, total ascorbic acid	2.8 mg
Protein	0.65 g	Thiamin	0.027 mg
Total lipid (fat)	0.11 g	Riboflavin	0.033 mg
Carbohydrates, by difference	3.63 g	Niacin	0.098 mg
Fiber, total dietary	0.5 g	Vitamin B ₆	0.040 mg
Sugars, total	1.67 g	Folate, DFE	7 µg
Minerals		Vitamin B ₁₂	0.00 µg
Calcium	16 mg	Vitamin A, RAE	5 µg
Iron	0.28 mg	Vitamin A, IU	105 IU
Magnesium	13 mg	Vitamin E (α-tocopherol)	0.03 mg
Phosphorus	24 mg	Vitamin D (D ₂ + D ₃)	0.0 µg
Potassium	147 mg	Vitamin D	0 IU
Sodium	2 mg	Vitamin K	16.4 µg
Zinc	0.20 mg		
Lipids			
Fatty acids, total saturated	0.037 g	Fatty acids, total polyunsaturated	0.032 g
Fatty acids, total monounsaturated	0.005 g	Cholesterol	0 mg

Table 17 Vitamins found in cucumber

				
Ascorbic acid	Vitamin PP (B ₃), niacin, niacinamide	Pyridoxine Vitamin B ₆	Pyridoxal	Pyridoxamine

Cucumber's plant lignans are converted into enterolignans (enterodiols and enterolactone) in the presence of digestive tract bacteria. Enterolignans bind to estrogen receptors and have both proestrogenic and antiestrogenic effects. A 100-g serving of cucumber has 15 kcal.

Table 18 Essential oil components found in dill

		
Carvone fungicides	d-Limonene insecticides	α -Terpinene

Dill

Dill (*Anethum graveolens*) contains monoterpenes, flavonoids, minerals, vitamins, fiber, α -phellandrene, eugenol, dillapiol, anethole, limonene, terpinene, myristicin, coumarins, triterpenes, phenolic acids, and umbelliferones. Dill oil is more than 50 % carvone and also contains limonene and terpinene. It is pale yellow, darkens over time, and has a fruity smell and a hot, acrid taste. Significant phytochemicals in dill are a mixture of a paraffin hydrocarbon and 40–60 % of d-carvone (23.1 %) with d-limonene (45 %). Other known phytochemicals in dill are oxypeucedanin, oxypeucedanin hydrate, falcariindiol, furanocoumarin, and 5-[4''-hydroxy-3''-methyl-2''-butenyloxy]-6,7-furocoumarin.

The seeds of dill contains about 3.5 % oil, with a specific gravity of volatile oil ranging between 0.895 and 0.915. Monoterpene hydrocarbons such as α -phellandrene, representing significant phytochemicals, are found in dill oil (Table 18).

Unsaponifiable matter and methyl esters in the aerial parts of the dill plant have been identified (Naseri et al. 2012). The chemical composition of dill is presented in Table 19.

β -Sitosterol (3.92 %) is the most significant sterol in dill, followed by campesterol (2.50 %), and stigmasterol (2.01 %). *N*-dotriacontane (58.40 %) is the most significant hydrocarbon in the unsaponifiable fraction, while linoleic acid (20.51 %) is the most significant fatty acid present, followed by nonadecanoic acid (9.95 %).

Eggplant

Eggplant (*Solanum melongena*) contains starch, minerals, and vitamins. A serving of 100 g eggplant has 20 kcal. The chemical composition of eggplant is presented in Table 20.

The significant phytochemicals in the leaves of eggplant are 4-ethylactechol; *trans*-caffeic, hydrocaffeic, protocatechuic, and chlorogenic acids; γ -hydroxyglutamic acid; lanost-8-en-3 β -ol; lanosterol; 24-methylene lanost-8-en-3 β -ol; cycloartanol; cycloartenol; 24-methylenecycloartanol; lupeol; β -amyirin; daturaolone; and daturadiol (Table 21).

Table 19 Nutritional value of dill per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	180 kJ (43 kcal)	Fat	1.1 g
Carbohydrates	7 g	Protein	3.5 g
Dietary fiber	2.1 g	Minerals	
Vitamins		Calcium	208 mg (21 %)
Vitamin B ₁₂	0 µg (0 %)	Iron	6.6 mg (51 %)
Vitamin C	85 mg (102 %)	Magnesium	55 mg (15 %)
Vitamin A equiv.	7,717 (154 %) IU	Manganese	1.3 mg (62 %)
Riboflavin	0.3 mg (25 %)	Phosphorus	66 mg (9 %)
Niacin	1.6 mg (11 %)	Potassium	738 mg (16 %)
Pantothenic acid	0.4 mg (8 %)	Sodium	61 mg (4 %)
Vitamin B ₆	0.2 mg (15 %)	Zinc	0.9 mg (9 %)
Folate	150 µg (38 %)	Copper	0.14 g (7 %)

Table 20 Nutritional value of eggplant per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	104 kJ (25 kcal)	Potassium	229 mg (5 %)
Carbohydrates	5.88 g	Zinc	0.16 mg (2 %)
Sugars	3.53 g	Vitamins	
Dietary fiber	3 g	Thiamine	0.039 mg (3 %)
Fat	0.18 g	Riboflavin	0.037 mg (3 %)
Protein	0.98 g	Niacin	0.649 mg (4 %)
Minerals		Pantothenic acid	0.281 mg (6 %)
Calcium	9 mg (1 %)	Vitamin B ₆	0.084 mg (6 %)
Iron	0.23 mg (2 %)	Folate	22 µg (6 %)
Magnesium	14 mg (4 %)	Vitamin C	2.2 mg (3 %)
Manganese	0.232 mg (11 %)	Vitamin E	0.3 mg (2 %)
Phosphorus	24 mg (3 %)	Vitamin K	3.5 µg (3 %)
Lipids			
Fatty acids, total saturated	0.034 g	Fatty acids, total polyunsaturated	0.076 g
Fatty acids, total monounsaturated	0.016 g	Cholesterol	0 mg

Eggplant also contains 4 α -methylsterols, vanillin, isoscopoletin, ethyl caffeate, *trans*-ferulic acid, and *p*-aminobenzaldehyde, along with four phenolic amides: *N-trans*-feruloyltyramine, *N-trans-p*-coumaroyltyramine, *N-trans*-feruloyloctopamine, and *N-trans-p*-coumaroyloctopamine. The eggplant fruit contains solasodin, antioxidants, and a number of nutrients. It is rich in fiber and low in fat and salt. Immature green fruit contains a toxin (solanine) that inhibits Ca (Ge et al. 2013). It also contains

Table 21 Toxin found in immature eggplant

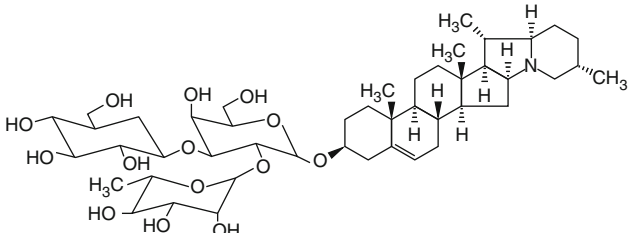
	α -Solanine
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Table 22 Nutritional value of garlic per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	623 kJ (149 kcal)	Protein	6.36 g
Carbohydrates	33.06 g	Fat	0.5 g
Sugars	1 g	Minerals	
Dietary fiber	2.1 g	Calcium	181 mg (18 %)
Vitamins		Iron	1.7 mg (13 %)
Thiamine	0.2 mg (17 %)	Magnesium	25 mg (7 %)
Riboflavin	0.11 mg (9 %)	Manganese	1.672 mg (80 %)
Niacin	0.7 mg (5 %)	Phosphorus	153 mg (22 %)
Pantothenic acid	0.596 mg (12 %)	Potassium	401 mg (9 %)
Vitamin B ₆	1.235 mg (95 %)	Sodium	17 mg (1 %)
Folate	3 μ g (1 %)	Zinc	1.16 mg (12 %)
Vitamin C	31.2 mg (38 %)	Selenium	14.2 μ g

dioscin, protodioscin, stigmasterol, stigmasterol- β -D-glucoside, β -sitosterol- β -D-glucoside, and methyl protodioscin, and is rich in polyphenols. The bark contains fibers and potassium.

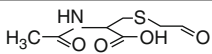
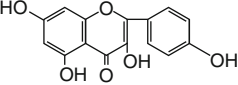
Garlic

Garlic (*Allium sativum*) is a “seleniferous” plant, i.e., it absorbs selenium from the soil even when Se concentration in the soil is not favorable. Garlic contains alliin and carbohydrates that give it a high nutritional value (Bagiu et al. 2012). The chemical composition of garlic (100 g/edible product) is presented in Table 22.

Garlic contains P and vitamin B₁ polysulfides, 1,2-DT (1,2-vinyldithiin), and thiacremonone, and its essential oils contain allyl polysulfides (i.e., a diversity of compounds) (Table 23).

Garlic bulbs contain anthocyanins, proteins, amino acids, glycosides of kaempferol and quercetin, saponin-like components, sterols (β -sitosterol, cholesterol, and campesterol), vitamins, polycarbohydrates, and prostaglandins A₂ and F₁; four steroidal saponins: protoisouruboside, eruboside B, isouruboside B, and sativoside C;

Table 23 Some chemical components found in garlic

$\text{H}_2\text{C}=\text{CH}-\text{H}_2\text{C}=\text{S}-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$		$\text{H}_2\text{C}=\text{CH}-\text{CH}_2-\text{S}(=\text{O})-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{COOH}$
Alliin	<i>N</i> -acetyl- <i>S</i> -allyl- <i>L</i> -cysteine	Alliin
$\text{H}_2\text{C}=\text{CH}-\text{CH}_2-\text{S}-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{CH}(\text{O})-\text{COOH}$	$\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$ S $\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$	
<i>S</i> -allyl- <i>L</i> -cysteine	Allitridum	Kaempferol

and two amino acids: adensine and tryptophan. An active component in garlic is scordinin A₁ isolated, which upon alkaline hydrolysis yields a peptide and allylthiofructosiduronic acid. Bulb yields have a mixture of polycarbohydrates containing pectic acid, a D-galactan, and a fructan component containing fructose. A 100-g portion of garlic leaves contains 83.2 mg kaempferol and salts of iodine.

Garlic (as well as onions and leeks) is rich in a variety S-containing compounds such as thiosulfates (allicin), sulfoxides (alliin), and dithiins (ajoene). These compounds are responsible for garlic's characteristic pungent odor. S-containing garlic constituents that help lower our risk of oxidative stress include alliin, allicin, allixin, allyl polysulfides, diallyl sulfide, diallyl disulfide, diallyl trisulfide, *N*-acetylcysteine, *N*-acetyl-*S*-allylcysteine, *S*-allylcysteine, *S*-allylmercaptocysteine, *S*-ethylcysteine, *S*-methylcysteine, *S*-propylcysteine, 1,2-vinyldithiin (1,2-DT), and thiacremonone.

Leeks

Leek (*Allium porrum*) has a more delicate flavor and is sweeter than onion. It contains proteins, carbohydrates, vitamins, minerals, mucilage, and cellulose. A serving of 30 g raw leek has 54 cal. The energy value is 22 cal per 100 g. The chemical composition of leek is presented in Table 24.

Leeks are a source of vitamin K, vitamin B₆, folate, vitamin C, vitamin E, vitamin A (in the form of carotenoids), Cu, Fe, Mg, Ca, dietary fiber, and omega-3 fatty acids as well as dietary fiber (Table 25). The leek's characteristic flavor is due to its essential oil content which is 0.02–0.08 % by weight. The folate is in the bioactive form of 5-methyltetrahydrofolate.

Four spirostanol saponins have been isolated from the bulb of *A. porrum* L. Leek contains (25R)-5 α -spirostan-3 β ,6 β -diol 3-*O*-{*O*- β -D-glucopyranosyl-(1 \rightarrow 2)-*O*-[β -D-xylopyranosyl-(1 \rightarrow 3)]-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside}, 6 β -diol 3-*O*-{*O*- β -D-glucopyranosyl-(1 \rightarrow 3)-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)-*O*-[β -D-xylopyranosyl-(1 \rightarrow 3)]-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside}, and (25R)-5 α -spirostan-3 β . It is rich in water at 87–90 % content and low in fat at ~0.4 % (Zhou et al. 2013). Leek also contains salts (oxalates) that can interfere with the absorption of Ca in the body.

Table 24 Nutritional value of leek per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	255 kJ (61 kcal)	Vitamins	
Carbohydrates	14.15 g	Vitamin A equiv.	83 µg (10 %)
Sugars	3.9 g	β-Carotene	1,000 µg (9 %)
Dietary fiber	1.8 g	Lutein/zeaxanthin	1,900 µg
Fat	0.3 g	Thiamine	0.06 mg (5 %)
Protein	1.5 g	Riboflavin	0.03 mg (3 %)
Water	83 g	Niacin	0.4 mg (3 %)
Minerals		Pantothenic acid	0.14 mg (3 %)
Calcium	59 mg (6 %)	Vitamin B ₆	0.233 mg (18 %)
Iron	2.1 mg (16 %)	Folate	64 µg (16 %)
Magnesium	28 mg (8 %)	Vitamin C	12 mg (14 %)
Manganese	0.481 mg (23 %)	Vitamin E	0.92 mg (6 %)
Phosphorus	35 mg (5 %)	Vitamin K	47 µg (45 %)
Potassium	180 mg (4 %)		

Lentils

Lentil (*Lens culinaris*) is rich in proteins, starch, cellulose, and minerals and has a low fat content. It also contains carotene and vitamins, uric acid, arsenic, kaempferol glycoside, and 3',4', 7-trihydroxyflavone (Flavonoids). A serving of 120 g lentils has 154 cal. The chemical composition of lentils is presented in Table 26.

A serving of 100 g dried lentils contains 6.8 % of meal nitrogen as nonprotein nitrogen. Lentil seed contains such phytochemicals as tricin, luteolin, a diglycosyldelphinidin, and two proanthocyanidins (Table 27).

The seeds also contain antinutritional phytochemicals (factors), including protease inhibitors, lectins, phytic acid, saponins, and tannins (moderate amounts) (Butu et al. 2014a). Lentil starch contains 36.1 % amylose. About half (47.1 %) of lentil proteins are soluble in 1.0 M sodium chloride, 3.8 % are soluble in water, 3.1 % are soluble in 70 % ethanol, and 14.9 % are soluble in diluted hydrochloric acid. Only 24.0 % of lentil proteins are not solubilized by these solvents. Lentils have all of the essential amino acids, especially glutamic acid, aspartic acid, arginine, leucine, and lysine. Minor amino acids in lentils are methionine, cysteine, and tryptophan. Lentils are a good substitute for meat (100 g cooked lentils contain the same amount of protein as 134 g beef). They have a low glycemic index and are rich in soluble and insoluble fiber and inositol, the C₆H₁₂O₆ part of B-complex vitamins.

Lentils contain *chiro*-inositol, a compound that lowers blood glucose. This vegetable also contains two antioxidants, quercetin and rutin. It is an excellent source of Mg and provides at least 20 % of the recommended daily dose. Lentils contain no cholesterol. The energy value is about 110–160 kcal/100 g, depending on the variety and how they are prepared.

Table 25 Dietary fiber components (nonstarch polysaccharides) found in leeks

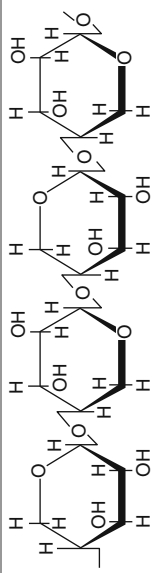
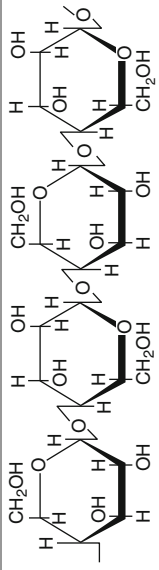
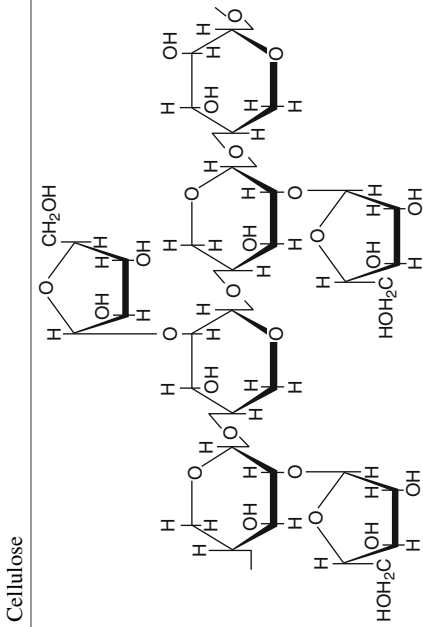
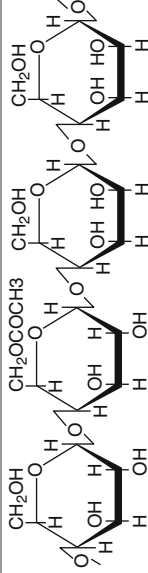
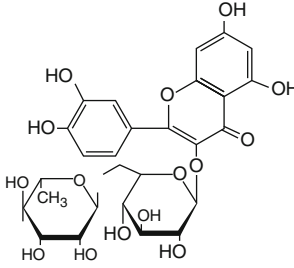
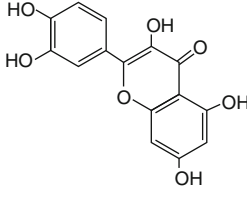
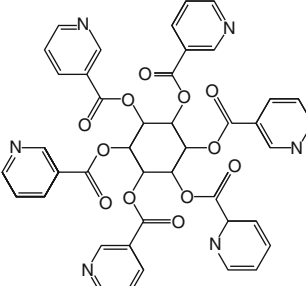
	
	<p>Xylan</p> <p>Arabinoxylan</p>
	<p>Glucomannan</p>

Table 26 Nutritional value of lentil per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	1,477 kJ (353 kcal)		
Carbohydrates	60 g	Fat	1 g
Sugars	2 g	Protein	26 g
Dietary fiber	31 g	Water	10.4 g
Vitamins		Minerals	
Thiamine	0.87 mg (76 %)	Calcium	56 mg (6 %)
Riboflavin	0.211 mg (18 %)	Iron	7.54 mg (58 %)
Niacin	2.605 mg (17 %)	Magnesium	122 mg (34 %)
Pantothenic acid	2.120 mg (42 %)	Phosphorus	451 mg (64 %)
Vitamin B ₆	0.54 mg (42 %)	Potassium	955 mg (20 %)
Folate	479 µg (120 %)	Sodium	6 mg (0 %)
Vitamin C	4.4 mg (5 %)	Zinc	4.78 mg (50 %)

Table 27 Phytochemicals found in lentils

		
Rutin	Quercetin	Inositol hexanicotinate

Lettuce

Lettuce (*Lactuca sativa*) contains starch, minerals, vitamins, and provitamin A. The chemical composition of lettuce is presented in Table 28.

The energy value of 100 g lettuce is 12 kcal. Lettuce contains flavonoids, anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and proanthocyanidin (Table 29).

Lettuce seeds contain japonicin A, isoquercitrin, flavonol glycoside (lactuca sativoside A), and caffeic acid. Lettuce contains triterpenoids, saponins, simple phenols, oxalate, tannin, phytate, ascorbic acid, chlorophyll (it is an antianemic), amino acids, and mucilage. The whole plant is rich in a milky bitter sap (lactucarium) that flows freely when the plant is wounded. Lactucarium, because it is a latex, is known as

Table 28 Nutritional value of lettuce per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods/>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	55 kJ (13 kcal)		
Carbohydrates	2.23 g	Water	95.63 g
Sugars	0.94	Fat	0.22 g
Dietary fiber	1.1 g	Protein	1.35 g
Vitamins		Minerals	
Vitamin A equiv.	166 µg (21 %)	Calcium	35 mg (4 %)
β-Carotene	1987 µg (18 %)	Iron	1.24 mg (10 %)
Lutein/zeaxanthin	1223 µg	Magnesium	13 mg (4 %)
Thiamine	0.057 mg (5 %)	Manganese	0.179 mg (9 %)
Riboflavin	0.062 mg (5 %)	Phosphorus	33 mg (5 %)
Pantothenic acid	0.15 mg (3 %)	Potassium	238 mg (5 %)
Vitamin B ₆	0.082 mg (6 %)	Sodium	5 mg (0 %)
Folate	73 µg (18 %)	Zinc	0.2 mg (2 %)
Vitamin C	3.7 mg (4 %)		
Vitamin E	0.18 mg (1 %)		
Vitamin K	102.3 µg (97 %)		

Table 29 Phytochemicals found in lettuce

Lactucin	Chlorophyll

“lettuce opium”; thus, lettuce has a mild narcotic effect due to two sesquiterpene lactones in lactucarium. Lettuce also contains lactucin (an ingredient of lactucarium) and chemicals that act as hormones (Ghorbani et al. 2013).

Lovage

Lovage (*Levisticum officinale*) contains etheric substances and volatile oils present in all plant parts and seeds, giving it the characteristic pungent and aromatic odor (Table 30).

Its chemical composition includes essential oil, organic acids, gumirezine, mineral salts, vitamins, coumarin (Dragan et al. 2008), and the tannins in lovage (containing several phenolic hydroxyl groups, and carboxyl groups). It is capable of precipitating

Table 30 Phytochemicals found in lovage

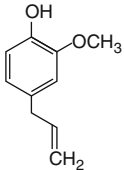
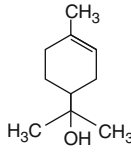
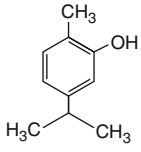
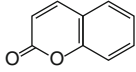
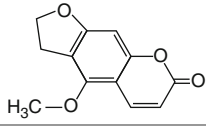
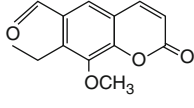
		
Eugenol	α -Terpineol	Carvacrol
		
Coumarin	Bergapten	Xanthotoxin

Table 31 Chemical composition of roots oil from *L. officinale*

Compounds	Value/100 g (unit)	Compounds	Value/100 g (unit)
<i>cis</i> -Ligustilide	42.0 %	α -Pinen	4.5–4.6 %
<i>trans</i> -Ligustilide	3.95 %	Camphene	1.0–1.1 %
3n-Butylidene phthalide E	1.75 %	β -Pinen	7.1–8.0 %
3n-Butylidene phthalide Z	0.73 %	Myrcene	0.9 %
3n-Butylidene-4,5-dihydrophthalide	4.9 %	α -Phellandrene	0.4–0.5 %
β -Phellandrene	0.28 %	α -Terpinene	0.1 %
α -Terpinyl acetate	0.08 %	Limonene	0.8–1.2 %
Palmitic acid	2.81 %	β -Phellandrene	8.7–10.7 %
Phytol	2.62 %	<i>cis</i> -Ocimene	0.2–0.4 %
Linoleic acid	3.52 %	γ -Terpinene/ <i>trans</i> -ocimene	0.2–0.3 %
Stigmasterol	11 %	Terpinolene	1.2–1.5 %
β -Sitosterol	1.28 %	Pentylcyclohexadiene	7.4–12.7 %
		Pentylbenzene	0.1–0.3 %

proteins that form insoluble, rot-proof, and waterproof precipitates with protein. The chemical composition of lovage is presented in Table 31.

The oil content of the dried cut roots of lovage is between 0.11 % and 1.80 % and that of the leaves, 0.09 %. Phytochemicals represent over 87 % of all the oil and include *trans-p*-mentha-2,8-dien-1-ol, iso-thujyl alcohol, *p*-mentha-1,5-dien-8-ol, bicycl[3.2.0]heptan-3-ol, 2-methylene-6,6-dimethyl, *trans*-carveol, perillaldehyde, sabinyl acetate, perillyl alcohol, methyl ester of methylpentadecate acid, and methyl hexadecadienate acid. Root oil contains high quantities of (E)-ligustilide and pentylcyclohexadiene, and leaf oil contains high amounts of α -terpinyl acetate and β -phellandrene. The content of (Z)-ligustilide in leaf oil was less than that in root oil. The plant's roots contain 0.6–1 % volatile oil. The oil obtained from lovage

Table 32 Nutritional value of onion per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	166 kJ (40 kcal)	Fat	0.1 g
Carbohydrates	9.34 g	Protein	1.1 g
Sugars	4.24 g	Water	89.11 g
Dietary fiber	1.7 g	Minerals	
Vitamins		Calcium	23 mg (2 %)
Thiamine	0.046 mg (4 %)	Iron	0.21 mg (2 %)
Riboflavin	0.027 mg (2 %)	Magnesium	10 mg (3 %)
Niacin	0.116 mg (1 %)	Manganese	0.129 mg (6 %)
Pantothenic acid	0.123 mg (2 %)	Phosphorus	29 mg (4 %)
Vitamin B ₆	0.12 mg (9 %)	Potassium	146 mg (3 %)
Folate	19 µg (5 %)	Zinc	0.17 mg (2 %)
Vitamin C	7.4 mg (9 %)	Fluoride	1.1 µg

lowers blood pressure and stimulates diuresis. Lovage essential oil has the following properties: $d_{15} = 1.00\text{--}1.05$, $\alpha_{20} = +6^\circ$, $n_D = 1.536\text{--}1.554$, and acidity index = 2–16. Consumption during pregnancy is not recommended since it stimulates uterine contractions.

Onion

Onion (*Allium cepa*) contains antibiotic substances. Several studies have shown the presence of albumin, sugar, some amounts of carbohydrates, vitamins, and flavonoids (Samfira et al. 2013). The chemical composition of onion (per 100 g of edible product) is presented in Table 32.

Known constituents of onion are sugar, vitamins, mineral salts, Na, K, phosphate and nitrate limestone, Fe, S, I, Si, phosphoric and acetic acids, allyl propyl disulfide, volatile oil, glucokinase, oxidase diastase (latter sterilized by heat), and antibacterial substances. It also contains carbohydrates, vitamins, and active substances that are antimicrobial agents (Putnoky et al. 2013). Onion contains alliinase, an enzyme; iso-alliin, a sulfur amino acid; and propanethial-S-oxide, which causes tearing (Table 33).

Onion also contains quercetin (the absorption of quercetin from onions is twice that of quercetin from tea and apples is absorbed at a greater rate than that from onions) and dihydroalliin (an antibiotic). Red onion contains sciliroside, a glycosidic insecticide, along with sciliroside aglycone. Onions contain phytochemicals known as disulfides, trisulfides, cepaenes, and vinyl dithiols. They are also a source of vitamin C, vitamin B₆, potassium, dietary fiber, folic acid, Ca, and Fe and are high in protein. Onions are low in sodium, contain no fat, are low in calories, and are cholesterol-free. Others include ryania, rotenoids, nicotinoids and a group containing strychnine and scilliroside.

Table 33 Phytochemicals found in onion

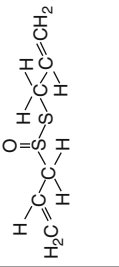
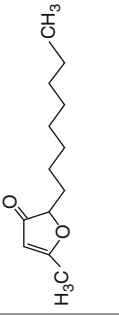
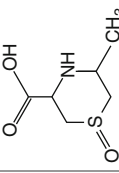
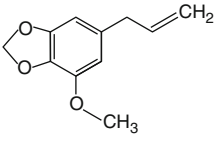
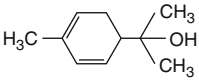
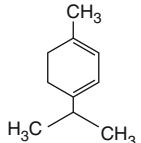
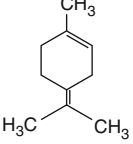
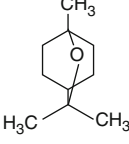
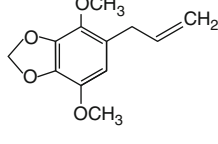
$\text{H}_3\text{C}-\text{CH}_2-\text{S}-\text{CH}_2-\underset{\text{NH}_2}{\overset{\text{H}}{\text{C}}}-\text{C}(=\text{O})-\text{OH}$				
Alliin	Alliinase	Cepanone	Cycloallin	

Table 34 Phytochemicals found in parsley

		
Myristicin	α -Felandren-8-ol	α -Terpinene
		
Terpinolene	p-Cineol	Apiol

Parsley

Parsley (*Petroselinum sativum*) is important for its roots, leaves, and seeds because they are rich in vitamin C and essential oil. Parsley is an antioxidant and contains vitamins A, C, complex B, and E, and minerals Fe and Mg. Other substances contained in its oil are myristicin allyl-tetramethoxybenzene, α -felandren, α -terpinene, caryophyllene, terpinolene, and p-cineole, and other components in small quantities such as apiol (Farshori et al. 2013) (Table 34).

Parsley contains three times more vitamins than an orange (100 g has ~150 mg vitamin C) and twice as much Fe as spinach. It contains flavonoids that neutralize free radicals and monoterpenes, coumarin, psoralen, 8-methoxypsoralen, 5-methoxypsoralen, oxypeucedanin, isopimpinellin, ficusin, bergapten, majudin, and heraclin. Parsley contains the estrogenic flavone glycosides acetylapiin and petroside (Butnariu 2012). The chemical composition of parsley is presented in Table 35.

Parsley contains polyacetylenes (Table 36):

and naturally occurring coumarins (Table 37):

A serving of 1 g dried parsley contains about 6.0 μ g of lycopene, 10.7 μ g of α -carotene, 82.9 μ g of lutein + zeaxanthin, and 80.7 μ g of β -carotene (Butnariu et al. 2014). Parsley is a source of flavonoids, luteolin, and apigenin.

Parsnips

Parsnip (*Pastinaca sativa*) is an aromatic herb that contains active phytochemicals, minerals, and vitamins. The dietary fiber in parsnip is part soluble and part insoluble (inulin, cellulose, hemicellulose, and lignin). The chemical composition of parsnip is presented in Table 38.

Parsnip contains furanocoumarins (psoralen, xanthotoxin, isopimpinellin, angelicin, and bergapten), which deter herbivores from eating its foliage. Parsnip also contains

Table 35 Nutritional value of parsley per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods/>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	151 kJ (36 kcal)	Carbohydrates	6.33 g
Fat	0.79 g	Sugars	0.85 g
Protein	2.97 g	Dietary fiber	3.3 g
Vitamins		Minerals	
Vitamin A equiv.	421 µg (53 %)	Calcium	138 mg (14 %)
β-Carotene	5,054 µg (47 %)	Iron	6.2 mg (48 %)
Lutein/zeaxanthin	5,561 µg	Magnesium	50 mg (14 %)
Thiamine	0.086 mg (7 %)	Manganese	0.16 mg (8 %)
Riboflavin	0.09 mg (8 %)	Phosphorus	58 mg (8 %)
Niacin	1.313 mg (9 %)	Potassium	554 mg (12 %)
Pantothenic acid	0.4 mg (8 %)	Sodium	56 mg (4 %)
Vitamin B ₆	0.09 mg (7 %)	Zinc	1.07 mg (11 %)
Folate	152 µg (38 %)		
Vitamin C	133 mg (160 %)		
Vitamin E	0.75 mg (5 %)		
Vitamin K	1,640 µg (1562 %)		

Table 36 Some polyacetylenes found in parsley

	
All- <i>cis</i> -polyacetylene (C ₂ H ₂) _n	All- <i>trans</i> -polyacetylene (C ₂ H ₂) _n

antioxidants such as falcarinol, falcarindiol, panaxydiol, and methyl-falcarindiol (Table 39).

Its volatile oil contains hexyl butyrate, octanol, octyl butyrate, octyl hexanoate, decyl butyrate, and phenylethyl butyrate (Carroll et al. 2000).


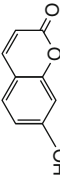
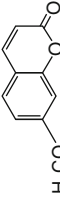
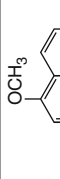
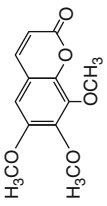
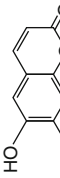
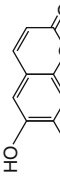
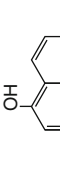
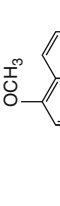
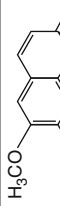
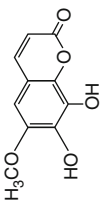
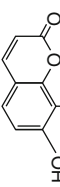
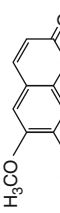
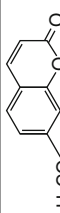
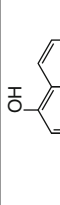
Peas

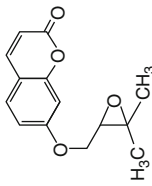
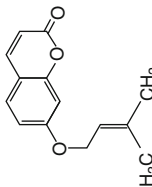
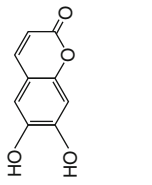
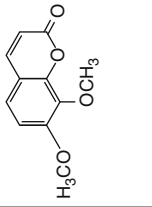
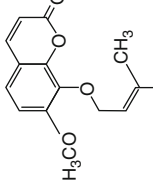
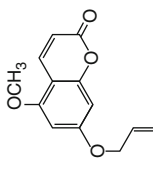

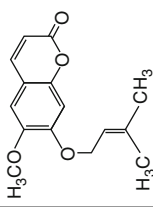
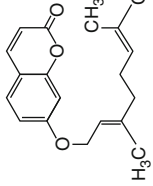
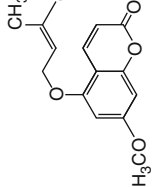
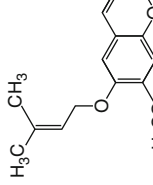
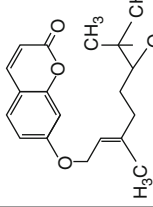
Pea (*Pisum sativum*) is rich in starch and contains proteins, vitamins, carotene, oil, cellulose, and minerals. A serving of 100 g fresh peas has 42 kcal. *P. sativum* consumption decreases cholesterol. The chemical composition of pea is presented in Table 40.

Yellow pea contains 22 % protein, 1 % fat, and 59 % carbohydrates and minerals and has high energy value (359 kcal/100 g).

Pea seeds contain starch, albuminoids, galactolipids, alkaloids, trigonelline and piplartine, essential oil and soluble carbohydrates, kaempferol-3-triglucoside, quercetin-3-triglucoside and p-coumaric acid esters, glycoside (pisatoside), pyrimidine derivatives and amino acids, pisatin and L-pipecolic acid, free homoserine, and methyl-4-chloindole-3-acetate. Germinating pea seedlings contain a high

Table 37 Naturally occurring coumarins found in parsley

					
Coumarin					
					
Umbelliferone					
					
7-Methoxy coumarin					
					
5-Methoxy-7-hydroxycoumarin					
					
Fraxetindim ethylether					
					
Isofraxitin					
					
Esculetin					
					
5,7-Dihydroxycoumarin					
					
Limettin					
					
Scopoletin					
					
Fraxetin					
					
Daphnetin					
					
Scoparone					
					
Daphnetin-7-methylether					
					
Hydroxy-7-methoxycoumarin					

	7-(2,3-Epoxy-3-methoxybutoxy) coumarin		7-Isoprenyl oxycoumarin		Isoscopoletin		Daphnetin dimethyl ether
	7-Methoxy-8-isoprenyloxy coumarin		5-Methoxy-7-isoprenyl oxycoumarin		6-Methoxy-7-isoprenyloxy coumarin		Epoxyauraptene
	Auraptene		5-Isoprenyl-7-methoxycoumarin		6-Isoprenyl-7-methoxycoumarin		Epoxyauraptene

(continued)

Table 37 (continued)

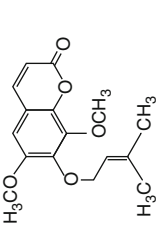
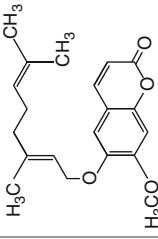
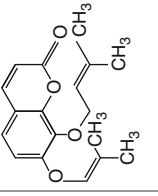
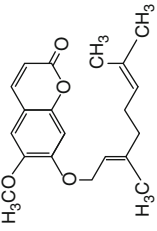
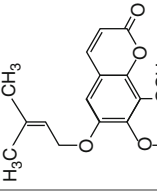
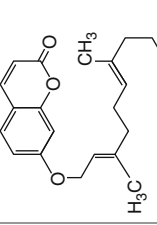
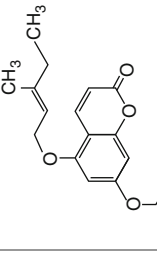
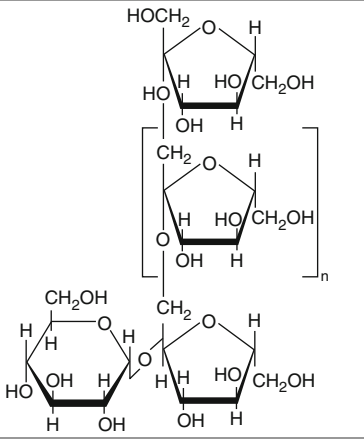
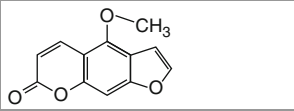
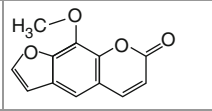
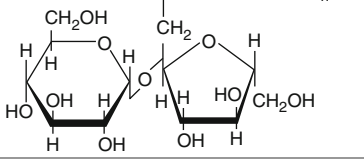
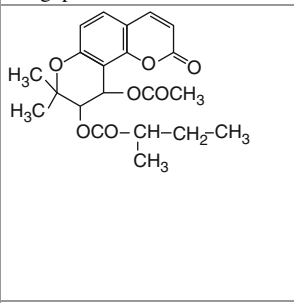
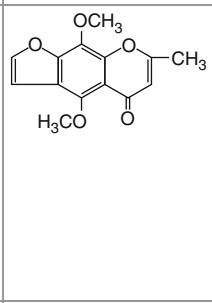
 <p>7-Isoprenyloxy-8-methoxycoumarin</p>	 <p>7-Methoxy-6-geranyloxycoumarin</p>	 <p>7,8-Diisoprenyloxycoumarin</p>
 <p>6-Methoxyauraptene</p>	 <p>6,7-Diisoprenyloxycoumarin</p>	 <p>Umbelliprenin</p>
		 <p>5,7-Diisoprenyloxycoumarin</p>

Table 38 Nutritional value of parsnips per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	314 kJ (75 kcal)	Fat	0.3 g
Carbohydrates	18 g	Protein	1.2 g
Sugars	4.8	Water	79.53 g
Dietary fiber	4.9 g	Vitamins	
Minerals		Thiamine	0.09 mg (8 %)
Calcium	36 mg (4 %)	Riboflavin	0.05 mg (4 %)
Iron	0.59 mg (5 %)	Niacin	0.7 mg (5 %)
Magnesium	29 mg (8 %)	Pantothenic acid	0.6 mg (12 %)
Manganese	0.56 mg (27 %)	Vitamin B ₆	0.09 mg (7 %)
Phosphorus	71 mg (10 %)	Folate	67 µg (17 %)
Potassium	375 mg (8 %)	Vitamin C	17 mg (20 %)
Sodium	10 mg (1 %)	Vitamin E	1.49 mg (10 %)
Zinc	0.59 mg (6 %)	Vitamin K	22.5 µg (21 %)

Table 39 Phytochemicals found in parsnips

		
	<p>Bergapten</p>	<p>Xanthotoxin</p>
		
	<p>Visnadin</p>	<p>Khelin</p>

concentration of D-alanine. A cerebroside has been isolated from pea seeds and upon hydrolysis yields hydroxytricosanoic acid, sphingosine base and glucose. Ferritin has been isolated from dried pea. *Cis*-, *trans*-, and *trans, trans*-xanthoxin were found in the roots of peas. Pea contains phytoestrogens or similar substances that are chemically isoflavones, phenylcoumarin, and naphthalene (Aughey et al. 2013). The maximum concentration of phytoestrogens is achieved during the flowering period.

Table 40 Nutritional value of pea per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods/>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	339 kJ (81 kcal)	Fat	0.4 g
Carbohydrates	14.45 g	Protein	5.42 g
Sugars	5.67 g	Vitamins	
Dietary fiber	5.1 g	Vitamin A equiv.	38 µg (5 %)
Minerals		β-Carotene	449 µg (4 %)
Calcium	25 mg (3 %)	Lutein/zeaxanthin	2,477 µg
Iron	1.47 mg (11 %)	Thiamine	0.266 mg (23 %)
Magnesium	33 mg (9 %)	Riboflavin	0.132 mg (11 %)
Manganese	0.41 mg (20 %)	Niacin	2.09 mg (14 %)
Phosphorus	108 mg (15 %)	Vitamin B ₆	0.169 mg (13 %)
Potassium	244 mg (5 %)	Folate	65 µg (16 %)
Sodium	5 mg (0 %)	Vitamin C	40 mg (48 %)
Zinc	1.24 mg (13 %)	Vitamin E	0.13 mg (1 %)
		Vitamin K	24.8 µg (24 %)

Peppers

Pepper (*Capsicum annuum*) fruits contain glucose (7.33 % fresh weight [fw]), fructose (1.99 % fw), sucrose (0.33 % fw), starch (1.78–4.40 % dry weight [dw]), hemicellulose (0.85–3.14 g% dw), pectic substances (7.8–9.8 g% dw), cellulose (14.83 g% dw in green fruit and 13.50 g% dw in dried fruit), fat (0.33 %), and carotenoids (127–284 mg/kg fw). Pepper contains coloring carotenoids with the following structures (Table 41).

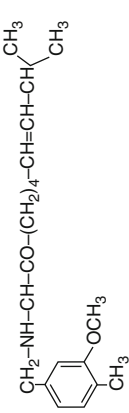
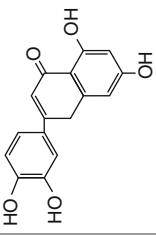
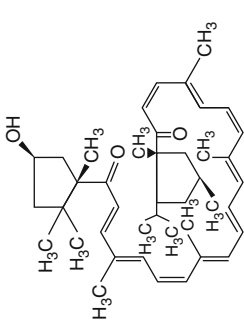
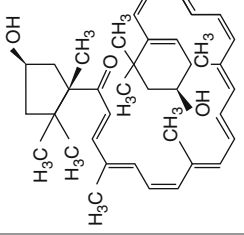
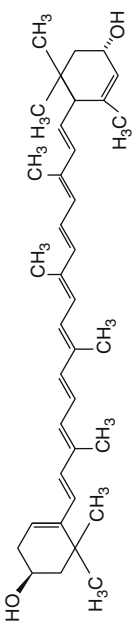
Pepper fruits contain small quantities of essential oil, vitamin C (139–160 % mg red fruits), vitamin B₁ (0.05 mg% g⁻¹ fw), vitamin B₂ (0.05 mg% g⁻¹ fw), vitamin PP (0.33 mg% g⁻¹ fw), vitamin E (0.65 mg% g⁻¹ fw green fruit and 1 mg% g⁻¹ fw red fruit), vitamin A (0.75–6.00 mg% g⁻¹ fw), vitamin P (5–300 g sp citrin, flavonoids that act synergistically with vitamin C), enzymes (peroxidase, lipoxidase, and cellulose), irritant capsaicin (hot variety), and macro- and trace elements. The energy value of pepper is 21 kcal/100 g.

Capsaicin [*N*-vanillyl-8-methyl-6-(E)-nonamide] is the main capsaicinoid in peppers, followed by dihydrocapsaicin, and is the cause of the sensation of heat of chilies. Others capsaicinoids in pepper are nordihydro-, homo-, homodihydro-, nor-, and nonnocapsaicin. Capsaicin is an important phytochemical condiment, and capsanthin and capsorubin are phytochemicals responsible for the red coloring present in chili peppers (Butnariu et al. 2012).

The chemical composition of pepper is presented in Table 42.

Capsaicin in red pepper is a powerful insecticide. It also gives chili peppers their hot taste and a small amount (0.01–0.25 %) causes local irritation. It can be extracted with organic solvent (Butnariu and Samfira 2013b). Pepper contains

Table 41 Color pigments found in peppers

 <p>Chemical structure of Capsaicin (8-methyl-N-vanillyl-6-nonenamide):</p> <chem>CC(C)C=CC(=C)C(=C)C(=C)C(=O)Nc1ccc(OC)c(O)c1</chem>	 <p>Chemical structure of Luteolin:</p> <chem>Oc1cc(O)c2c(c1)oc(O)c2OC</chem>
<p>Capsaicin (8-methyl-N-vanillyl-6-nonenamide)</p>  <p>Chemical structure of Capsorubin:</p> <chem>CC(C)C=CC(=C)C(=C)C(=C)C(=O)C1C(C)C(O)C1</chem>	<p>Luteolin</p>  <p>Chemical structure of Capsanthin:</p> <chem>CC(C)C=CC(=C)C(=C)C(=C)C(=O)C1C(C)C(O)C1</chem>
<p>Capsorubin</p>  <p>Chemical structure of Zeaxanthin:</p> <chem>CC(C)C=CC(=C)C(=C)C(=C)C(=O)C1C(C)C(O)C1</chem>	<p>Capsanthin</p> <p>Zeaxanthin</p>

(continued)

Table 41 (continued)

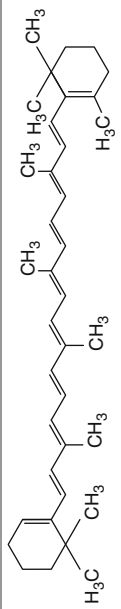
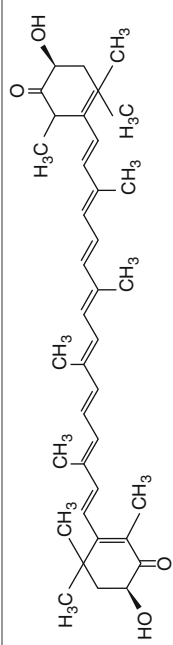
 <p>The structure shows the beta-carotene molecule, which consists of a long polyene chain with 11 conjugated double bonds and two non-conjugated double bonds. The chain is terminated at one end by a cyclohexene ring with two methyl groups (CH₃) and at the other end by a cyclohexene ring with one methyl group (CH₃).</p>	β-Carotene
 <p>The structure shows the Astaxanthin molecule, which features a polyene chain with 11 conjugated double bonds and two non-conjugated double bonds. The chain is terminated at both ends by cyclohexene rings. Each ring has a methyl group (CH₃) and a hydroxyl group (OH) attached to it.</p>	Astaxanthin

Table 42 Nutritional value of pepper per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods/>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	84 kJ (20 kcal)	Fat	0.17 g
Carbohydrates	4.64 g	Protein	0.86 g
Sugars	2.4 g	Vitamins	
Dietary fiber	1.7 g	Vitamin A equiv.	18 µg (2 %)
		β-Carotene	208 µg (2 %)
Minerals		Lutein/zeaxanthin	341 µg
Calcium	10 mg (1 %)	Thiamine	0.057 mg (5 %)
Iron	0.34 mg (3 %)	Riboflavin	0.028 mg (2 %)
Magnesium	10 mg (3 %)	Niacin	0.48 mg (3 %)
Manganese	0.122 mg (6 %)	Pantothenic acid	0.099 mg (2 %)
Phosphorus	20 mg (3 %)	Vitamin B ₆	0.224 mg (17 %)
Potassium	175 mg (4 %)	Folate	10 µg (3 %)
Sodium	3 mg (0 %)	Vitamin C	80.4 mg (97 %)
Zinc	0.13 mg (1 %)	Vitamin E	0.37 mg (2 %)
Fluoride	2 µg	Vitamin K	7.4 µg (7 %)

saponins, carbohydrates, and proteins. Green pepper contains more vitamin C, while yellow and red peppers contain more β-carotene. Pepper is a second source of vitamin C, immediately after parsley (200 g pepper gives the daily vitamin C requirement for an adult); it also contains vitamin A. The hot taste of chili peppers is measured in Scoville heat units (SHU).

Potato

Potato (*Solanum tuberosum*) contains provitamin A, vitamin K (antibleding and antianemia), S (combats excess oil and Fe), and tuberin (a nitric substance), and it is one of the main sources of vitamin C. By hydrolysis of tomatine, the α-glycone derivative tomatidine is obtained, and also two molecules of D-glucose and one molecule of D-galactose and D-xylose. The chemical composition of potato is presented in Table 43.

Four monosaccharide units form lycotetraose (tomatidine and lycotetraose, hydrolysis products of α-tomatine). Solasodine is the aglycone in solasonine and its carbohydrate component is identical to α-solanine (substane/solatrisoza). Potato is a source of complex carbohydrates (D-glucose, D-galactose, and L-rhamnose) and fiber and provides trace elements, minerals (K, Mg, Fe), vitamins, and especially vitamin C. Potato is about 80 % water, is a source of carbohydrates (23 g carbohydrates per 100 g), contains little protein (1.6 g/100 g), and has low fat. It is a source of vegetal protein and is low in fat and has no more than 80 cal per 100 g (Albiski et al. 2012). It contains minerals such as K (375 mg/100 g), Ca (411 mg/100 g), Mg and Fe. Potatoes are low fat in Na (only 7 mg/100 g) and a source of B

Table 43 Nutritional value of potato per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	321 kJ (77 kcal)	Fat	0.1 g
Carbohydrates	17.47 g	Protein	2 g
Starch	15.44 g	Water	75 g
Dietary fiber	2.2 g	Vitamins	
Minerals		Thiamine	0.08 mg (7 %)
Calcium	12 mg (1 %)	Riboflavin	0.03 mg (3 %)
Iron	0.78 mg (6 %)	Niacin	1.05 mg (7 %)
Magnesium	23 mg (6 %)	Pantothenic acid	0.296 mg (6 %)
Manganese	0.153 mg (7 %)	Vitamin B ₆	0.295 mg (23 %)
Phosphorus	57 mg (8 %)	Folate	16 µg (4 %)
Potassium	421 mg (9 %)	Vitamin C	19.7 mg (24 %)
Sodium	6 mg (0 %)	Vitamin E	0.01 mg (0 %)
Zinc	0.29 mg (3 %)	Vitamin K	1.9 µg (2 %)

vitamins and β -carotene. The vitamin C content of raw potatoes is high (30 mg/100 g), greater than that of carrots and green beans, but decreases significantly with time after harvest; 9 months after harvest this value decreases to 8 mg/100 g. Potato has a high microelement content, surpassing that of fruits like banana and apple. K in potato is involved in intracellular movement and the balance of muscles. The energy value of potato is 97 kcal/100 g.

The main glycoalkaloid in potato is α -solanine. The structures of glycoalkaloids in potato are as follows (Table 44).

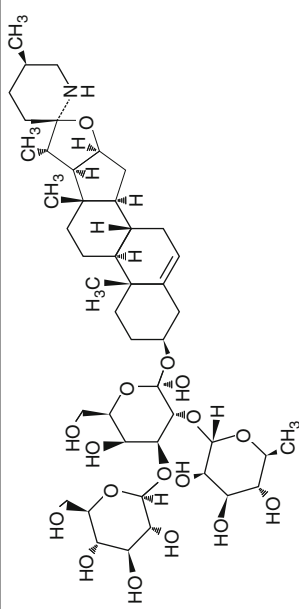
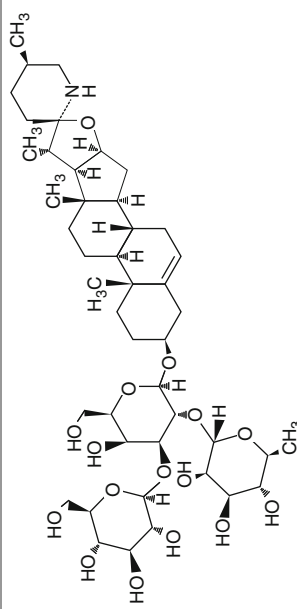
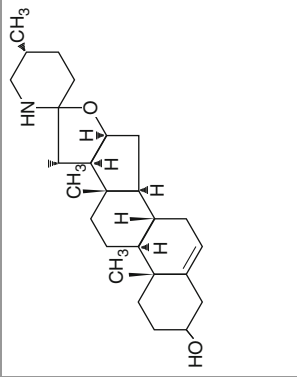
Pumpkin

Pumpkin (*Cucurbita pepo*) contains β -carotene, leucine, peporezina, tyrosine, carbohydrates, proteins and minerals, water, Ca, P, K, Mg, Zn, vitamins (folic acid, vitamin B₂, and B₁), unsaturated oils, and antioxidants. The fat content of pumpkin is 35–55 %. Pumpkin is a good source of K, starch, Ca, and P (Shapiro et al. 2013). The chemical composition of pumpkin is presented in Table 45.

Pumpkin seeds are rich in oil with medicinal properties. They contain anticancer substances such as fiber, pectin, sitosterol, and vitamin E in the following forms: α -tocopherol, γ -tocopherol, Δ -tocopherol, α -tocomonoenol, and γ -tocomonoenol. Pumpkin seed contains moderate concentrations of minerals, including P, Mg, and K. Pumpkin contains the antioxidant carotenoid lycopene as well as small amounts of provitamin A, vitamin C, K, and cellulose (including the pectin-soluble fraction) (Table 46).

The energy value of 100 g pumpkin is 11 kcal. The amino acid profiles indicate that methionine and tryptophan are the most limited, while arginine, glutamic acid, and aspartic acid are the most plentiful. Pumpkin contains the saturated fatty acids palmitic acid and stearic acid and the unsaturated fatty acids oleic acid and linoleic acid.

Table 44 Glycoalkaloid in potatoes

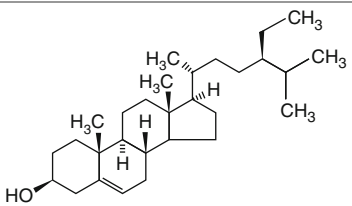
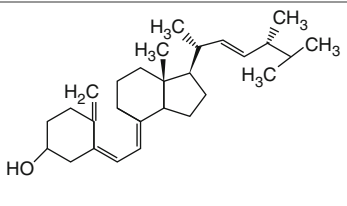
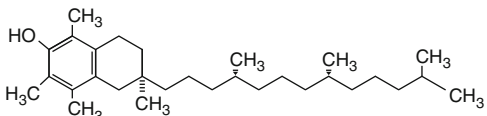
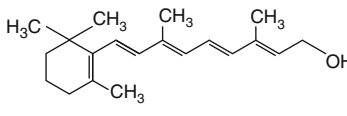
 <p>The structure shows a tropane alkaloid core (8-methyl-8-azabicyclo[3.2.1]octane) linked via an ester bond to a complex sugar moiety. The sugar moiety consists of a galactose unit linked to a glucose unit, which is further linked to a rhamnose unit. The tropane core has a methyl group at C-8 and a hydroxyl group at C-1.</p>	Solasonine
 <p>The structure is similar to Solasonine, but the tropane core is 8-methyl-8-azabicyclo[3.2.1]octane with a methyl group at C-8 and a hydroxyl group at C-1. The sugar moiety is identical to Solasonine.</p>	

(continued)

Table 45 Nutritional value of pumpkin per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods/>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	69 kJ (16 kcal)	Water	95 g
Carbohydrates	3.4 g	Vitamins	
Sugars	2.2 g	Vitamin A equiv.	10 µg (1 %)
Dietary fiber	1.1 g	β-Carotene	120 µg (1 %)
Fat	0.2 g	Lutein/zeaxanthin	2,125 µg
Protein	1.2 g	Thiamine	0.048 mg (4 %)
Minerals		Riboflavin	0.142 mg (12 %)
Iron	0.35 mg (3 %)	Niacin	0.487 mg (3 %)
Magnesium	17 mg (5 %)	Pantothenic acid	0.155 mg (3 %)
Manganese	0.175 mg (8 %)	Vitamin B ₆	0.218 mg (17 %)
Phosphorus	38 mg (5 %)	Folate	29 µg (7 %)
Potassium	262 mg (6 %)	Vitamin C	17 mg (20 %)
Zinc	0.29 mg (3 %)	Vitamin K	3 µg (3 %)

Table 46 Phytochemicals found in pumpkin

	
β-Sitosterol	Vitamin D ₂
	
Vitamin E	Vitamin A

Radishes

Radishes [*Raphanus sativus* (red radish) and *Raphanus sativus niger* (black radish)] contain mainly vitamins that are antiscorbutic and antisterility (tocopherol), vitamin B, minerals, and rafanol, a sulfur glycoside with bactericidal potential. Radishes contain Ca and vitamin D, which is needed by the body for Ca absorption. Nitrogen phytochemicals present in radish roots include pyrrolidine, phenethylamine, *N*-methylphenethylamine, 1,2'-pyrrolidin-3-yl-3-acid-carboxylic-1,2,3,4-tetrahydro-β-carboline, and sinapine.

Table 47 Phenylpropanoid glycoside from radishes

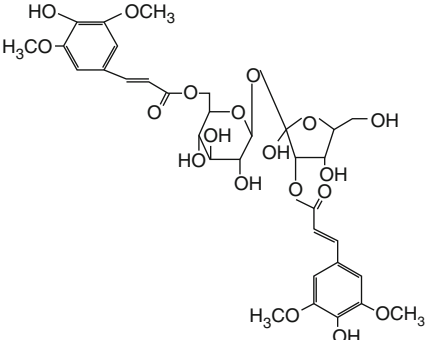
	Rasatiol
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Table 48 Nutritional value of radish per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods/>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	66 kJ (16 kcal)	Fat	0.1 g
Carbohydrates	3.4 g	Protein	0.68 g
Sugars	1.86 g	Calcium	25 mg (3 %)
Dietary fiber	1.6 g	Iron	0.34 mg (3 %)
Thiamine	0.012 mg (1 %)	Magnesium	10 mg (3 %)
Riboflavin	0.039 mg (3 %)	Manganese	0.069 mg (3 %)
Niacin	0.254 mg (2 %)	Phosphorus	20 mg (3 %)
Pantothenic acid	0.165 mg (3 %)	Potassium	233 mg (5 %)
Vitamin B ₆	0.071 mg (5 %)	Zinc	0.28 mg (3 %)
Folate	25 µg (6 %)	Fluoride	6 µg
Vitamin C	14.8 mg (18 %)		

Cytokinin (6-benzylamino-9-glucosylpurine) is a significant metabolite of 6-benzylaminopurine (6-BAP) in root radish. A minor biometabolite of 6-BAP in radish has been identified as 6-benzylamino-3-β-D-glucopyranosylpurine. In addition, the diamines diaminotoluene, 4,4'-methylenedianiline, and 1,6-hexanediamine were isolated during germination of young radish seeds (Roh et al. 2013). vitamin B₁ is produced at a greater rate during germination (Table 47).

The chemical composition of radish is presented in Table 48.

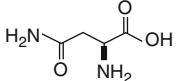
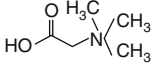
Red Beets

Red beet (*Beta vulgaris*) contains the following main components: 8–10 % carbohydrates, amino acids (betaine and asparagine), vitamins, provitamin A, micronutrients (minerals, with a small amount of rubidium and cesium), anthocyanin pigments (red color), and fiber (Hernández-Martínez et al. 2013). The chemical composition of red beets is presented in Table 49.

Table 49 Nutritional value of red beet per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	180 kJ (43 kcal)	Potassium	325 mg (7 %)
Carbohydrates	9.56 g	Sodium	78 mg (5 %)
Sugars	6.76 g	Zinc	0.35 mg (4 %)
Dietary fiber	2.8 g	Vitamins	
Fat	0.17 g	Vitamin A equiv.	2 µg (0 %)
Protein	1.61 g	β-Carotene	20 µg (0 %)
Water	87.58 g	Thiamine	0.031 mg (3 %)
Minerals		Riboflavin	0.04 mg (3 %)
Calcium	16 mg (2 %)	Niacin	0.334 mg (2 %)
Iron	0.8 mg (6 %)	Pantothenic acid	0.155 mg (3 %)
Magnesium	23 mg (6 %)	Vitamin B ₆	0.067 mg (5 %)
Manganese	0.329 mg (16 %)	Folate	109 µg (27 %)
Phosphorus	40 mg (6 %)	Vitamin C	4.9 mg (6 %)

Table 50 Phytochemicals found in red beets

	Asparagine		Betaine
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The protein in the red beet leaf comprises the following amino acids: threonine, valine, cystine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine, aspartic acid, serine, glutamic acid, proline, glycine, alanine, and tyrosine. The red beet leaf contains phenolic compounds, mainly betacyanins (betanin and isobetanine), and ferulic acid ester [β -D-fructofuranosyl- α -D-(6-O-(E)-feruloyl)glucopyranoside)] (Table 50).

The leaves of red beets contain fatty acids, especially polyunsaturated fatty acids of the omega-3 series such as α -linolenic acid. They are also particularly rich in vitamins and are a source of natural Ca and Fe. The red beet is a rich source of a group of red and yellow pigments known as betalains, including red violet betacyanins and yellow betaxanthins. Betanin is a major constituent (75–95 %) of the red pigment and vulgaxanthin I is the principal constituent of the yellow pigment. Betalains also include isobetanine, isobetanine, prebetanine, isoprebetanine, and vulgaxanthin II. Beet juice contains tetracyclitol (betitol). A 100-g portion of beet is 37 kcal.

Soybean

Soybean (*Glycine max*) is an excellent source of albumin, minerals, vitamins, including B vitamins, and Fe and is rich in fiber. A 100-g portion of soybean has 381 kcal. Soybean proteins are easily assimilated (77–97 %) and rich in essential

amino acids. The amino acid content of soybean is comparable to that of meat. Representative phytochemicals identified in soy include isoflavones, genistein, saponins, β -sitosterol, and daidzein (Table 51).

The chemical composition of soybean is presented in Table 52.

The principal soluble carbohydrates of mature soybeans are dicarbohydrate sucrose (2.5–8.2 %); triraffinose (0.1–1.1 %), composed of one sucrose molecule connected to one molecule of galactose; and tetracarbohydrate stachyose (1.4–4.1 %), composed of one sucrose connected to two molecules of galactose. Soybean oil or the lipid part of the seed contains four phytosterols: stigmasterol, sitosterol, campesterol, and brassicasterol, which account for about 2.5 % of the lipid fraction. Soybeans contain isoflavonoids (phytoestrogens, natural plant estrogens), which were identified in chickpeas, seeds, red wine, oats, legumes, and other vegetables (Kim et al. 2013a). The richest sources of isoflavonoids are soy milk, soy yogurt, and tofu. Soy also contains isoflavones like genistein and daidzein, and glycitein, an *O*-methylated isoflavone that accounts for 5–10 % of the total isoflavones in soy food products. Glycitein is a phytoestrogen with weak estrogenic activity, comparable to that of other soy isoflavones. The protein content of soybean (35–48 %) makes it first among plant and animal products. The fat content of soybeans is 17–37 %, and they are rich in unsaturated fatty acids, phosphatides (3.9 %), carbohydrates (20–32 %), B-complex vitamins, choline, minerals, and lecithin. Soybeans are used to prepare cereals, flour, protein isolate, vegetable oil, milk, and milk products. It contains 40 g albumin per 100 g. Soybean is a source of soluble dietary fiber and Ca, P, and K.

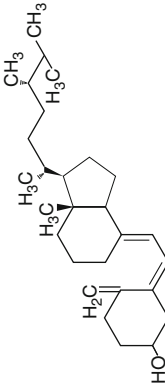
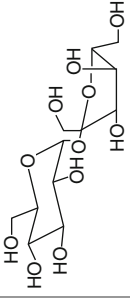
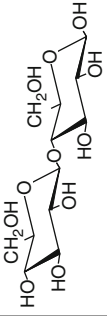
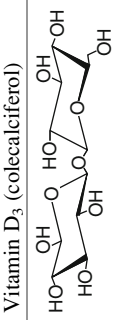
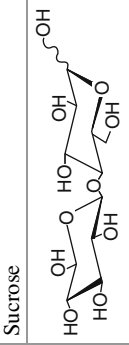
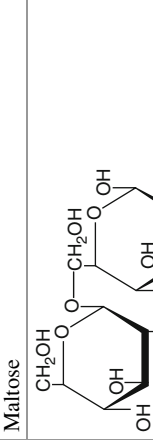
Soybeans can provide the human body with 18 amino acids, 9 of which are essential; soy protein contains all the essential amino acids needed by the human body. The seeds contain an estrogen analog molecule that attaches to the estrogen receptor to stop harmful substances (nonylphenol); this affects the genetic fingerprint.

Some negative effects of soy are that soy extracts contain large amounts of aluminum, which is toxic. Soy phytoestrogens disturb endocrine function. Soybean trypsin inhibitors interfere with protein digestion and can cause pancreatic disorders. At high temperatures, Soy protein isolate is a dry powder food ingredient that has been separated or isolated from the other components of the soybean and vegetal proteins (soya proteins with unstable structure) are skewed (Kumar et al. 2013). High amounts of phytic acid in soybeans trigger lower uptake of Ca, Mg, Cu, Fe, and Zn. The processing of soy protein results in the formation of carcinogenic nitrosamines and toxic lysinoalanine, and soy free of glutamic acid is formed, which makes it a neurotoxin. Soybean contains lecithin, a substance essential for brain function.

Spinach

Spinach (*Spinacia oleracea*) is rich in carotene, starch, minerals, vitamins, and oxalates, the latter of which prevent Ca absorption and so spinach is contraindicated for those with urolithiasis. The chemical composition of spinach is presented in Table 53.

Table 51 Phytochemicals found in soybean

 <p>Vitamin D₃ (coleciferol)</p>	 <p>Sucrose</p>	 <p>Maltose</p>
 <p>Trehalose</p>	 <p>Cellobiose</p>	 <p>Gentiobiose</p>

(continued)

Table 51 (continued)

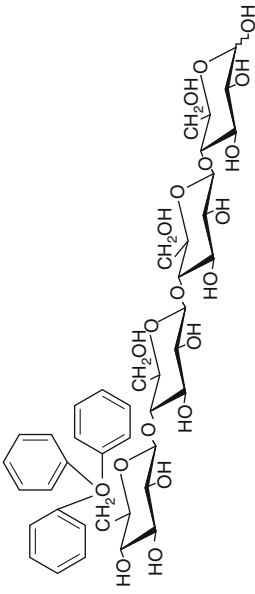
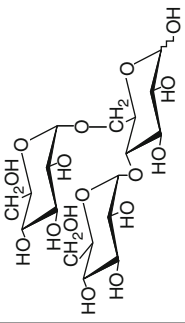
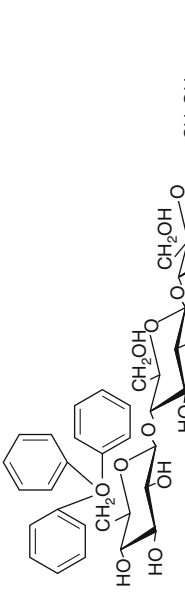
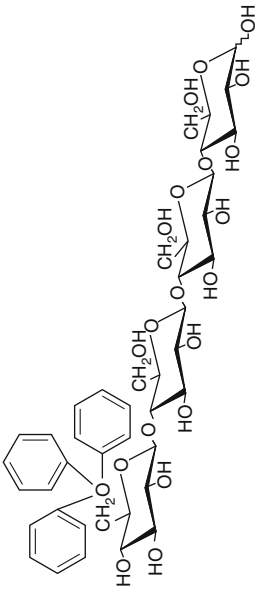
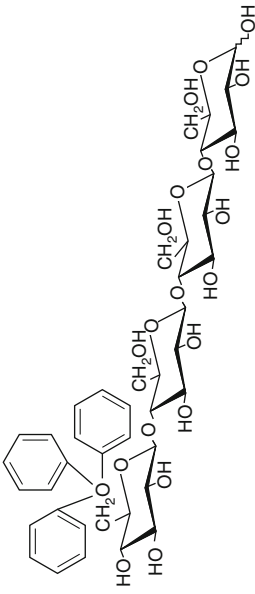
Panose	
Cellobiose	
Isopanose	
6-O-Tritylmaltotetraose	
6-O-Tritylmaltotetraose	

Table 52 Nutritional value of soybean per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods/>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	1,866 kJ (446 kcal)	Fat	19.94 g
Carbohydrates	30.16 g	Saturated	2.884 g
Sugars	7.33 g	Monounsaturated	4.404 g
Dietary fiber	9.3 g	Polyunsaturated	11.255 g
Protein (amino acid)	36.49 g	Water	8.54 g
Tryptophan	0.591 g	Vitamin A equiv.	1 µg (0 %)
Threonine	1.766 g	Thiamine	0.874 mg (76 %)
Isoleucine	1.971 g	Riboflavin	0.87 mg (73 %)
Leucine	3.309 g	Niacin	1.623 mg (11 %)
Lysine	2.706 g	Pantothenic acid	0.793 mg (16 %)
Methionine	0.547 g	Vitamin B ₆	0.377 mg (29 %)
Cystine	0.655 g	Folate	375 µg (94 %)
Phenylalanine	2.122 g	Choline	115.9 mg (24 %)
Tyrosine	1.539 g	Vitamin C	6.0 mg (7 %)
Valine	2.029 g	Vitamin E	0.85 mg (6 %)
Arginine	3.153 g	Vitamin K	47 µg (45 %)
Histidine	1.097 g	Calcium	277 mg (28 %)
Alanine	1.915 g	Iron	15.7 mg (121 %)
Aspartic acid	5.112 g	Magnesium	280 mg (79 %)
Glutamic acid	7.874 g	Manganese	2.517 mg (120 %)
Glycine	1.880 g	Phosphorus	704 mg (101 %)
Proline	2.379 g	Potassium	1,797 mg (38 %)
Serine	2.357 g	Sodium	2 mg (0 %)
		Zinc	4.89 mg (51 %)

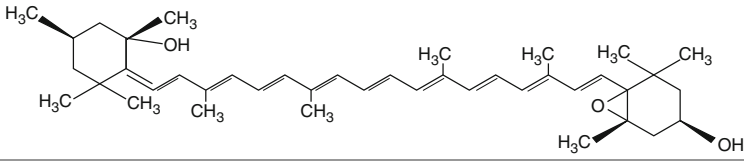
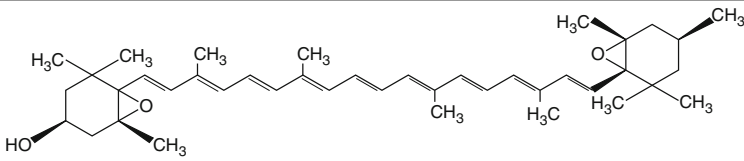
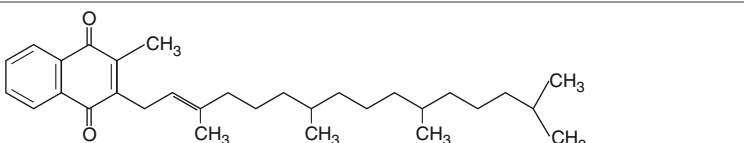
Polyglutamyl folate (vitamin B₉ or folic acid) is a constituent of cells and spinach is a source of folic acid. Spinach has a higher Fe content (2–5 mg/100 g) than any other vegetable, and a high content of β-carotene, a precursor pigment that once ingested is converted within the liver and small intestine into vitamin A. β-Carotene is an antioxidant that plays a role in preventing cancer of the lungs, oral cavity, stomach, and prostate. Spinach is rich in fiber and easy to digest, has a low protein content (2 %), low carbohydrate content (7 %), and low lipid content (0.5 %), and has a very low energy value of 17 kcal/100 g.

Spinach contains vitamin K, which affects bone strength. Because content of vitamins C and E, the substances folic acid, Zn, Mg, K and folic acid-soluble B vitamins have a important role for human consumption. Neoxanthin and violaxanthin are two anti-inflammatory epoxyxanthophylls found in spinach (Table 54). The opioid peptides called rubiscolins have also been found in spinach. The leaves contain phytonutrients such as mucilage, fat, sugar, oxalic acid (6–8 % in young leaves, 23–27 % in cotyledons), I, lecithin, chlorophyll, and carotene, and

Table 53 Nutritional value of spinach per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods/>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	97 kJ (23 kcal)	Vitamin A equiv.	469 µg (59 %)
Carbohydrates	3.6 g	Vitamin A	9,377 IU
Sugars	0.4 g	β-Carotene	5,626 µg (52 %)
Dietary fiber	2.2 g	Lutein/zeaxanthin	12,198 µg
Fat	0.4 g	Thiamine	0.078 mg (7 %)
Protein	2.9 g	Riboflavin	0.189 mg (16 %)
Water	91.4 g	Niacin	0.724 mg (5 %)
Calcium	99 mg (10 %)	Vitamin B ₆	0.195 mg (15 %)
Iron	2.71 mg (21 %)	Folate	194 µg (49 %)
Magnesium	79 mg (22 %)	Vitamin C	28 mg (34 %)
Manganese	0.897 mg (43 %)	Vitamin E	2 mg (13 %)
Phosphorus	49 mg (7 %)	Vitamin K	483 µg (460 %)
Potassium	558 mg (12 %)		
Sodium	79 mg (5 %)		
Zinc	0.53 mg (6 %)		

Table 54 Phytochemicals found in spinach

	Neoxanthin
	Violaxanthin
	Vitamin K ₁

flavonoids. They also contain stigmasterol, stigmastanol, α-spinasterol, patuletin, spinacetin, saponins, fatty acids, and hexadecenic acid. Spinacosides C and D have been isolated from the fresh aerial parts and spinasaponins A and B have been isolated from the roots of spinach, which also contain spilanthal (Park et al. 2013). Germinating seeds from spinach contain enzymes.

Tomatoes

Tomatoes (*Lycopersicon esculentum*) are red due to their lycopene content (Butnariu and Giuchici 2011). Lycopene in soups and sauces is assimilated more easily than that from raw tomatoes. The chemical composition of the tomato is presented in Table 55.

For an adult, the daily recommended 100 g of fresh tomatoes provides water (94 %), soluble sugars (glucose, fructose, and sucrose), organic acids, and zingiberene (Butu et al. 2014b). The fruit's color is the result of a combination of carotenoid pigments (red from lycopene and orange from carotene). In sunny and warm weather, more lycopene is synthesized so the fruit becomes redder.

Fruit firmness and consistency are functions of protein content, amino acids, pectic substances, cellulose, and hemicellulose (Khayat Nouri and Namvaran Abbas Abad 2013). The therapeutic quality and alimentary importance of tomatoes is due to its rich content of vitamins, carbohydrates, organic acids, minerals, and energy-giving compounds. The content of tomatoes depends on variety, cultivating area, variable climate conditions, and cultivation technology. Red tomato is the richest source of lycopene and yellow tomato is rich in carotene (Butnariu and Samfira 2012). Lycopene and carotene annihilate free radicals (Butnariu and Grozea 2012) and prevent formation of carcinogenic metabolites. Tomato juice and tomato paste block oxidation in body that predispose it to illness. Tomatidin, an aglycone steroid with fungicidal and insecticidal characteristics, is extracted from the tomato stem.

The energy value of tomato is relatively low (20 kcal/100 g fresh fruit).

Representative phytochemicals identified in tomato are phytoene, phytofluene, β -carotene, flavonoids, carotenoids, lycopene, quercetin, polyphenols, and kaempferol (Table 56) (Kim et al. 2013b). Leaves, stems, and green unripened fruit of the tomato plant contain small amounts of tomatine and solanine, toxic alkaloids found in potato leaves and other plants in the nightshade family.

Table 55 Nutritional value of tomato per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	74 kJ (18 kcal)	Potassium	237 mg (5 %)
Carbohydrates	3.9 g	Lycopene	2,573 μ g
Sugars	2.6 g	Vitamin A equiv.	42 μ g (5 %)
Dietary fiber	1.2 g	β -Carotene	449 μ g (4 %)
Fat	0.2 g	Lutein/zeaxanthin	123 μ g
Protein	0.9 g	Thiamine	0.037 mg (3 %)
Water	94.5 g	Niacin	0.594 mg (4 %)
Magnesium	11 mg (3 %)	Vitamin B ₆	0.08 mg (6 %)
Manganese	0.114 mg (5 %)	Vitamin C	14 mg (17 %)
Phosphorus	24 mg (3 %)	Vitamin E	0.54 mg (4 %)
		Vitamin K	7.9 μ g (8 %)

Table 56 Phytochemicals found in tomatoes

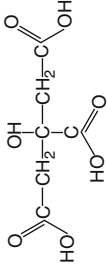
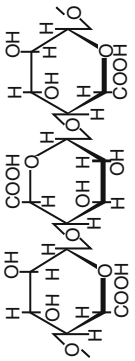
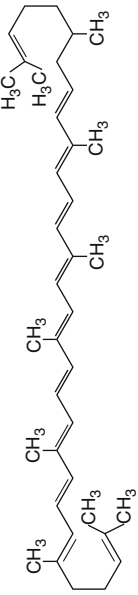
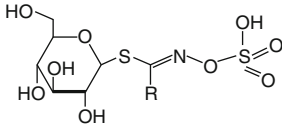
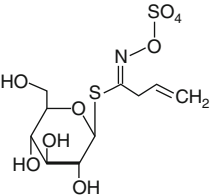
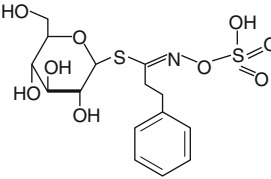
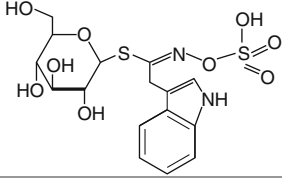
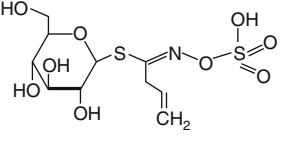
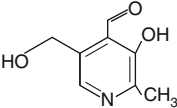
CO_2H CH_2OH CH_2 CO_2H		
Malic acid	Citric acid	
		Lycopene

Table 57 Phytochemicals found in turnip

		
General structure glucosinolates	Sinigrin	Gluconasturtiin
		
Glucobrassicin	Sinalbin	Piridoxal

Turnip

Turnip (*Brassica oleracea* var. *gongyloides*) has a sweet and delicate flavor, between that of radish and cabbage, with a high content of vitamin C and K and fiber. Vitamin C contributes to healthy bones, promotes Fe absorption, accelerates wound healing, and has antioxidant properties. Turnip is also a good source of P and contains minerals. The green leaves of the turnip top are a good source of vitamin A, folate, vitamin C, vitamin K, and calcium. A 100-g portion of raw turnip has 25 cal.

The chemical composition of the turnip is presented in Table 58.

Turnip is a vital vegetable and good for the diet. Its roots and leaves are consumed for their high content of carbohydrates, minerals (Table 57) (Bhattacharya and Malleshi 2012), vitamins, and cellulose. It contains glucosinolates that inhibit iodine uptake by the thyroid; turnip consumption is not recommended for people with thyroid problems (Butnariu and Caunii 2013). Like rutabaga, turnip contains bitter cyanoglucosides that release small amounts of cyanide. Sensitivity to the bitterness of these cyanoglucosides is controlled by a paired gene.

Consuming vegetables of cruciferous family (i.e., kale, cabbage, Brussels sprouts, cauliflower, broccoli, and radishes) helps prevent certain types of cancer such as lung cancer. Consumption of these foods is recommended for a low-calorie diet or to maintain a slim silhouette.

Conclusion and Future Directions

Vegetables, together with other components of the plant world, remain the only source of some secondary metabolites that are used by the pharmaceutical and food industries. This is because they cannot be synthesized chemically since they are

Table 58 Nutritional value of turnip per 100 g (3.5 oz) (Adapted after <http://ndb.nal.usda.gov/ndb/foods>)

Phytochemicals	Value/100 g (unit)	Phytochemicals	Value/100 g (unit)
Energy	84 kJ (20 kcal)	Vitamin A equiv.	381 µg (48 %)
Carbohydrates	4.4 g	β-Carotene	4,575 µg (42 %)
Sugars	0.5 g	Lutein/zeaxanthin	8,440 µg
Dietary fiber	3.5 g	Thiamine	0.045 mg (4 %)
Fat	0.2 g	Riboflavin	0.072 mg (6 %)
Protein	1.1 g	Niacin	0.411 mg (3 %)
Calcium	137 mg (14 %)	Pantothenic acid	0.274 mg (5 %)
Iron	0.8 mg (6 %)	Vitamin B ₆	0.18 mg (14 %)
Magnesium	22 mg (6 %)	Folate	118 µg (30 %)
Manganese	0.337 mg (16 %)	Vitamin C	27.4 mg (33 %)
Phosphorus	29 mg (4 %)	Vitamin E	1.88 mg (13 %)
Potassium	203 mg (4 %)	Vitamin K	368 µg (350 %)
Sodium	29 mg (2 %)		

stereocomplexes with chiral centers, which may be essential for biological activity. Primary and secondary plant metabolites with pharmacodynamic properties or that are used in the food industry have some common characteristics: most are nonprotein chemical compounds that can be extracted from plant material by steam distillation with organic solvents or water, and most are low-molecular-weight compounds, except biopolymers, condensed tannins, and some polycarbohydrates such as gums, pectin, and starch.

Carotenes are widespread in vegetal material (carrots, spinach, green onions, lettuce, and tomatoes). Leafy vegetables contain significant amounts of vitamin B₉, a vitamin involved in cell growth processes, the formation of red blood cells, and the prevention of anemia. Vitamin K, which is in the green parts of vegetables, is in sufficient quantities in spinach, kale, lettuce, and green onions; this vitamin is needed for blood clotting and to prevent bleeding. The vitamin inositol has a role in protecting the liver, heart, and digestive system. The carotenoids are red, orange, and yellow pigments that are mainly in vegetables (carrot, pumpkin, tomatoes, broccoli, and spinach) and fruits (papaya, orange, mango), but are also in animal products (fat, egg yolk) as provitamin A. Yellow and orange carotenoids are known as carotenes and the red carotenoid is lycopene. Carotenoids act as antioxidants and some are precursors of vitamin A (i.e., they biosynthesize vitamin A); therefore, they are called provitamins.

Because they are antioxidants, some carotenoids have anticancer properties and others protect against cardiovascular disease and eye diseases such as macular degeneration. α-Carotene helps to strengthen the immune system and reduces the risk of cancer. β-Cryptoxanthin is a precursor of vitamin A. Canthaxanthin helps the immune system and reduces the risk of skin cancer. Lutein (from broccoli) reduces the risk of lung cancer. Lycopene (from tomato) reduces the risk of bowel cancer.

Of the more than 600 carotenoids found so far, β-carotene is the most well-known since it is a precursor of vitamin A. β-Carotene is an isomer of carotene and

is the pigment that gives carrots their orange color. Metabolic bioreactions convert β -carotene into vitamin A as needed; β -carotene is processed by liver. When a large amount of alcohol is consumed, β -carotene becomes toxic, especially to the liver. β -Carotene consumption reduces the risk of cancer and cardiovascular incidents and improves the immune system, vision, and even bone growth. Vegetables with the highest content of β -carotene are carrots, sweet potatoes, pumpkins, broccoli, and spinach.

Vegetables are the most important source of phytochemicals used in pharmaceutical products, food, cosmetics, and agrochemicals; they have invaluable commercial value. Vegetables are irreplaceable sources of industrial oils (volatile and fixed), flavors, fragrances, resins, gum hydrocolloid, saponins and other surfactants, dyes, pesticides, drug substances, and many other special compounds.

The main properties of vegetables are summarized below:

- Alkaline action of organic acids that are metabolized in the body to alkali metal salts
- Astringent, constipating action, contain tannins
- Act as diuretics due to water content; this helps those with renal diseases, in which there is increased retention of water, and those with retention of nitrogenous substances in the blood (urea and uric acid)
- Diuretic effect of vegetables could have a role in the development of heart failure but the vitamins and carbohydrates they contain strengthen the heart muscle, thereby increasing the strength of the heart's contractions
- Hypocaloric characteristics and possess a small amount of protein, fat, and carbohydrates, thus indicated for diets to lose weight
- Low in salt, contain small amounts of Na; salt-restricted regimens may be used
- Act as laxatives, especially vegetables rich in ballast
- With a lower energy value, vegetables can replace concentrated and high-calorie foods, thus substantially reducing the energy level of the diet without reducing the volume of a meal
- Vegetables break down uric acid, thus preventing the formation of urate-based kidney stones
- Vegetables are indicated for those with anemia, they stimulate gastric secretion, promote Fe absorption, and stimulate formation of red blood cells
- Vegetables lower blood cholesterol and bile, thus preventing concentration and precipitation of in the gallbladder
- Vegetables, which are poor in carbohydrates, can be consumed by diabetics
- Carrots contain a large amount of carotene and thus are good for those with diseases due to lack or insufficiency of vitamin A
- Because vegetables lack lipids, they mitigate dyslipidemia and leave some of the cholesterol that reaches gut, preventing its reabsorption
- Black radishes are good for treating chronic cholecystitis and angiocholitis accompanied by constipation because they aid intestinal peristalsis and affect the smooth muscle of the bile channels, facilitating secretion of bile
- Tomatoes stimulate digestive secretions and are especially important in anemia and convalescence

- The sulfur derivative in white cabbage has antimicrobial properties, and salts with their antithyroid effect (prevent fixation the iodine) have a healing effect on gastroduodenal ulcers

Vegetable consumption stimulates metabolism by fighting free radicals, reducing water retention, improving tissue oxygenation and blood circulation, restoring intestinal bacterial flora, and revitalizing cells.

Vegetables do not provide a favorable environment for growing microorganisms (bacteria, viruses, or parasites). However, via vegetables, an organism can come into contact with the parasitic cysts of *Giardia*, tapeworm proglottids, and geohelminth eggs. Vegetables may be contaminated with bacteria from the soil, from fertilizer, from infested irrigation water, or by vectors.

Vegetables are basic nutrition that keeps cells young and vital. They have an important role in the diet because they are sources of vitamins, proteins, lipids, carbohydrates, enzymes, and minerals that protect the body from various diseases. Healthy eating requires a balanced and varied diet that includes large quantities of vegetables, fruits, fiber-rich cereals, protein, and moderate amounts of fat, salt, and sugar. Eating vegetables helps to reduce neuronal degeneration in the elderly.

Cross-References

- ▶ [General Properties of Major Food Components](#)
- ▶ [General Properties of Minor Food Components](#)
- ▶ [Plant-Associated Natural Food Toxins](#)

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Part IV

Chemical and Toxicological Aspects of Food Contamination

April R. Van Scoy, James N. Seiber, and Ronald S. Tjeerdema

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Abstract

There are a wide variety of chemicals, including fertilizers, soil amendments, and pesticides, used for agricultural purposes. Important in fending off pests and increasing crop yields, they also can leave residues that may be considered harmful for human consumption. This chapter aims to discuss the use of a few commonly used agricultural pesticides – spinosad, methyl bromide, mepiquat, and chlormequat.

Spinosad is a biologically derived insecticide used on numerous agricultural crops. It is a macrocyclic lactone containing two active components, spinosyn A and spinosyn D; these two components are responsible for spinosad's insecticidal properties. Currently, the US EPA has classified spinosad as a reduced-risk insecticide and has become an alternative to using highly persistent insecticides like organophosphates and carbamates.

Methyl bromide is a broad-spectrum fumigant used to control pests on both pre- and postharvest commodities. Due to its stratospheric ozone-layer-depleting capabilities, its production and import have been limited by the Montreal Protocol agreements of 2005. It has been classified as a restricted-use pesticide by the US EPA, and replacement fumigants, such as chloropicrin or 1,3-dichloropropene, are being considered.

Mepiquat and chlormequat are plant growth regulators often applied to many crops. The US EPA has classified plant growth regulators as pesticides due to their ability to alter the reproduction, growth, and flowering rates of plants. Plant growth regulators are designed to reduce longitudinal shoot growth and decrease the rate of cell division without impacting plant development or productivity. Use of these pesticides has been found to reduce crop maintenance costs and increase crop yields.

This chapter will further discuss the measured residues remaining from spinosad, methyl bromide, and mepiquat and chlormequat use on fruit, poultry, or grains. These residues, compared to regulatory limits established by individual countries, will provide insight into whether their consumption is safe. Furthermore, their toxicity to target and nontarget organisms is described.

Introduction

Chemicals used for agricultural purposes include a large variety of natural and synthetic compounds. Although there are many chemical classes, four examples will be discussed here – each having the potential to contaminate agricultural commodities impacting import and export practices, contaminating surface and groundwater, or adversely impacting consumers. Biopesticides represent a new concept in which the use of naturally produced compounds is employed in order to deter pest damage. According to the United States Environmental Protection Agency (US EPA), biopesticides are natural compounds or mixtures which manage pests without a specific toxic mode of action. However many chemicals, based on

natural compounds, are considered to fall into this category even though they have identified toxic modes of action. Generally they have low toxicity to nontarget organisms, are target-specific, and lack undue environmental persistence. These pesticides range from small compounds like methoprene to larger ones like spinosad, to be discussed here.

Fumigants, although restricted, have been under scrutiny regarding their use and subsequent environmental impacts, such as stratospheric ozone depletion. Poisonings can result from fumigant use since large quantities per acre are generally applied; the US EPA has categorized them as highly acutely toxic. Although fumigants volatilize following initial application, they have been found as residues sorbed to food and packaging materials. Agents such as hydrogen phosphide and methyl bromide have high vapor pressures and can penetrate soils and other materials rather quickly. A common fumigant, methyl bromide, used for nematode control in soil and to protect stored grain from a variety of pests and diseases, will be discussed.

There are many types of plant growth regulators, each having their own function such as stimulating cell division and/or ripening or inhibiting growth. The US EPA classifies plant growth regulators as pesticides due to their ability to alter the expected growth, flowering, or reproduction of plants. Chemicals that are categorized as such do not include fertilizers or other plant nutrients. These pesticides are often applied to control crop growth rate and size from germination to harvest. Commonly, enhancement of plant, flower, or fruit formation in addition to increased crop yields result. Since they impact normal plant function, it should be expected that they be translocated and metabolized within the plant or be transferred into the food. This will be considered when discussing two common growth regulators, mepiquat and chlormequat.

The overall aim is to provide information regarding the biosynthesis, mode of action, relative toxicities, and residual detections on foodstuffs of the pesticides and thus potential agricultural pollutants such as spinosad, methyl bromide, chlormequat, and mepiquat.

Biopesticides: Spinosad

Spinosad was first registered in the USA in 1997 by Dow AgroSciences, LLC. As of 2001, the insecticide has been registered in 37 countries with use on over 150 commodities (Cleveland et al. 2002). It is a biologically derived insecticide used on numerous agricultural crops, ornamentals, pet kennels, and aquatic sites. It has multiple formulations which can be applied as a spot treatment, via air or ground and chemigation (US EPA 2011). It is efficacious and has been used to control a variety of insects including Lepidoptera, Diptera, and Hymenoptera. Spinosad, a macrocyclic lactone, contains two active components, spinosyn A and spinosyn D (Fig. 1), which differ from each other by a single methyl group. Spinosyns, in general, have varied levels of methylation and are often less active than either spinosyn A or spinosyn D; insecticidal activity is driven by two deoxy sugar

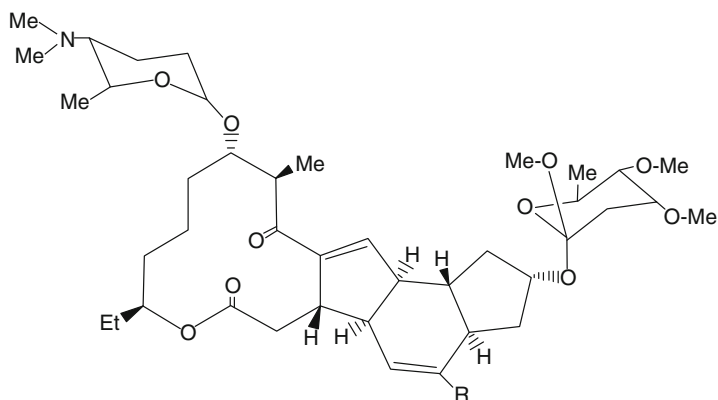


Fig. 1 Chemical structure of spinosad, where R = H (spinosyn A) or CH₃ (spinosyn D)

moieties. Spinosad is considered a reduced-risk insecticide by the US EPA and has become an alternative to using older pesticides of environmental concern, such as organophosphates and carbamates. Studies have shown spinosad to rapidly degrade via photolysis (half-life < 1 day); thus, it is a better alternative to using highly persistent insecticides (Cleveland et al. 2002). This section will provide details on the detections of this pesticide in goods, in addition to its overall toxicity.

Synthesis

Spinosad, like other biopesticides are naturally synthesized. This insecticide is a fermentation by-product of the soil actinomycete *Saccharopolyspora spinosa*. It is reported that spinosyn is synthesized via a polyketide pathway in which the nonglycosylated intermediate aglycone (AGL; macrolide ring system), tri-*O*-methyl rhamnose sugar, and forosamine sugar play an important role (Madduri et al. 2001a, b; Zhi-hua et al. 2006). Specifically, the polyketide pathway creates a lactone nucleus at which the tri-*O*-methyl rhamnose residue binds; this is followed by the binding of forosamine (Waldron et al. 2000). Waldron et al. (2000) have concluded that the genes involved in this synthesis do not include genes involved in tri-*O*-methyl rhamnose synthesis. Instead, tri-*O*-methyl rhamnose is important in both primary metabolism and secondary metabolic spinosyn synthesis and is derived from *S*-adenosylmethionine (Waldron et al. 2001).

Mode of Action

Insecticidal properties of spinosad are activated following ingestion or direct contact with a treated surface. The nicotinic acetylcholine receptor (nAChR) is the primary target site and is highly impacted (US EPA 2011). A secondary target

site is the γ -aminobutyric acid (GABA)-gated chloride channel. Exposure to this neurotoxin results in excitation of the nervous system, paralysis, and ultimately death (Kristensen and Jespersen 2004). Insect resistance has been reported in many species, for example, in the housefly, and is assumed to be linked to chromosome I and three nAChR subunit genes ($\alpha 5$, $\alpha 6$, and $\beta 3$); however, to actually pinpoint the mutation resulting in such resistance, further susceptibility and resistance studies are necessary (Scott 2008).

Toxicity

To determine the physiological responses brought on by exposure to spinosyn A, one of the two active components of spinosad, and spinosad itself, American cockroaches (*Periplaneta americana*) were injected with known concentrations; mechanisms of responses were recorded. Salgado (1998) observed both spinosyn A and spinosad to elicit identical responses; however, only observations from studies using spinosyn A are reported. Cockroaches experienced postural changes resulting from muscle contractions eventually causing paralysis. Salgado (1998) suggests paralysis is not the primary effect from spinosyn exposure, but rather an effect of the nervous system being overexcited for a prolonged period of time.

The toxic effect of spinosad, spinosad bait, and imidacloprid was investigated on the western cherry fruit fly, *Rhagoletis indifferens*. This insecticide was more effective toward adult and larvae fruit flies compared to imidacloprid; when ingested, greater mortality occurred than when topically applied. In addition, larval infestation was suppressed (Yee and Alston 2006). Maize flour granules, containing the pesticide (2–2000 mg/L), were fed to different insects in the laboratory; mortality affected the following insects from least sensitive to highly sensitive: *Aleochara bilineata* < *Chrysoperla carnea* < *Doru taeniatum* (Cisneros et al. 2002). Since *D. taeniatum* is a common maize predator, field studies were also conducted; a 94–96 % mortality 3 days post-application was reported. Further insecticide exposure was conducted by Getchell and Subramanyam (2008). The lesser grain borer (*Rhyzopertha dominica*) and the rice weevil (*Sitophilus oryzae*) were exposed to wheat, corn, and sorghum treated with liquid and dry spinosad formulations. It was found that *R. dominica* was more susceptible than *S. oryzae*, respectively. Susceptible and resistant houseflies were exposed via feeding bioassays. Kristensen and Jespersen (2004) recorded spinosad bioassay concentrations that were lethal to 50 % of the population (LC_{50}) after 72 h of 0.51 $\mu\text{g/g}$ for susceptible houseflies, whereas field populations had a 72 h LC_{50} of 1.1–3.8 $\mu\text{g/g}$ which is up to 7.5-fold higher than for susceptible insects.

Often, due to overuse of pesticides, species build a resistance in which the pesticide is no longer effective – the same is possible for biopesticides. In order to determine the potential for pesticide resistance, studies looking into the mechanisms responsible for such resistance have been conducted for select species. Multiple generations of tobacco budworm larvae (*Heliothis virescens*) were raised to be approximately 316-fold resistant to spinosad compared to the parental

budworms, when fed-treated diet. In addition, electrophysiological recordings were taken from neurons extracted from budworm thoracic ganglia; currents induced by spinosyn A were measured. Induced currents were observed in both susceptible and resistant neurons; however, resistant neurons produced smaller amplitude currents, indicating decreased sensitivity to spinosyn A (Roe et al. 2010). Moreover, Roe et al. (2010) did not observe spinosyn metabolism up to 96 h after exposure in either the susceptible or resistant tobacco budworms; thus, they conclude that the resistance mechanism cannot be explained by metabolism within the insect.

Other species, such as the oriental fruit fly (*Bactrocera dorsalis*), were studied for resistance to this biopesticide. Similar to the Roe et al. (2010) study, multiple generations of the fruit fly were reared; the eighth generation had a 408-times greater LD₅₀ (median lethal dose for the population) than the parental colony (Hsu and Feng 2006). In addition, they measured cross-resistance to other insecticides. They found the resistant line of fruit flies did not exhibit cross-resistance to organophosphates (malathion, fenitrothion, fenthion, naled, trichlorfon, and formothion), to methomyl (a carbamate), or to pyrethroids (cyfluthrin, cypermethrin, and fenvalerate). Although studies have been able to note resistance in laboratory-bred insects, the same has been identified in field populations. Houseflies (*Musca domestica* L.), in Denmark, were studied. Markussen and Kristensen (2011) identified involvement of cytochrome P450 genes in the acquired resistance to spinosad in Danish houseflies. Furthermore, they note a difference in resistance based on sex; females displayed a higher resistance than males. They also observed a lack of cross-resistance to the neonicotinoids, imidacloprid, and thiamethoxam.

The US EPA has set a reference dose as an estimate of a daily oral exposure (human) to be without deleterious effects during a lifetime; the reference dose which the general population could be safely exposed to is 0.027 mg/kg/day. Knowing this limit, subchronic tests were conducted with rats. Rats were fed feed containing 0–0.4 % spinosad for 13 weeks. Toxicity was observed and effects included vacuolation of cells within tissues along with degenerative and inflammatory responses (Yano et al. 2002). Overall, the no-observable-adverse-effect-level (NOAEL), for this study, was 24 mg/kg/day, which is much greater than the reference dose. The overall toxicity of spinosad does not reflect the toxicity of other biopesticides; however, it does prove that it is as potent to target pests as synthetic pesticides. Further toxicity data indicates this pesticide to be highly toxic to mosquitoes and oysters and less toxic to bluegill and rainbow trout (Table 1).

Table 1 Toxicity median lethal concentration (LC₅₀) values and their test time frames (in hours) for spinosad on select organisms^a

Species	Test	Value (mg/L)
Rainbow trout	96 h LC ₅₀	30
Bluegill	96 h LC ₅₀	5.9
Yellow fever mosquito	24 h LC ₅₀	0.00039
Oyster	96 h EC ₅₀	0.3
Water flea	48 h EC ₅₀	14

^aData from the Pesticide Action Network (PAN 2014)

Current Detection Techniques

Analytical methods for the detection of spinosad include liquid chromatography-mass spectrometry (LC-MS) or LC with ultraviolet (UV) detection. Spinosad, and its metabolites have been detected in environmental and food matrices via LC/UV at a limit of quantification (LOQ) of 0.01–0.04 $\mu\text{g/g}$ (West et al. 2000). Fortification studies have been conducted to develop a method suitable to measure residues and metabolites in chicken, beef, milk, eggs, and cream. West and Turner (1998), using LC/UV, have determined an LOQ for chicken fat to be 0.02 $\mu\text{g/g}$ and for other matrices (beef and chicken tissue, milk, cream, and eggs) to be 0.01 $\mu\text{g/g}$. With their analytical method, they are capable of identifying four analytes: spinosyn A and B, spinosyn B and *N*-demethyl spinosyn D. Although the use of an ultraviolet detector has been successful, further method development has led to a more sensitive detection with mass spectrometry. Method validations using spiked apple puree, lemon juice, canned peas, and tomato puree resulted in an LC-MS LOQ for spinosyn A and D of 1 $\mu\text{g/kg}$; this was more sensitive than for other macrocyclic lactones (Sannino 2007). Additional analysis with LC-MS utilizing atmospheric pressure chemical ionization has measured spinosad along with the three metabolites (spinosyns B, K, and *N*-demethyl spinosyn D) in hay, wheat straw, and corn stover; overall, LOQ was 0.01 $\mu\text{g/g}$ and the limit of detection (LOD) was 0.003 $\mu\text{g/g}$ (Schwedler et al. 2000). Residue methods utilizing magnetic particle-based immunoassay test kits have been developed specifically for spinosad in water, sediment, and foodstuffs. Following sample homogenization, extraction, and purification, total residue concentrations can be determined. This technique is capable of determining residues in water with an LOQ of 0.0001 $\mu\text{g/mL}$, in sediment (LOQ = 0.05 $\mu\text{g/g}$), and in commodities (LOQ = 0.01 $\mu\text{g/g}$). The use of spinosad immunoassays has been found to nicely correlate with results obtained through LC techniques (Young et al. 2000).

Levels of Residues

Maximum residue levels (MRLs) are based on the sum of both A and D homologues and have been set for many products throughout the world. For instance, the European Union (EU), USA, and Canada approved an MRL for grain at 1–1.5 mg/L, whereas Codex has established an international trade standard of 1 mg/L (Hertlein et al. 2011). Various commodities, such as honey, have set MRLs. Honey samples collected in Poland measured spinosad at 20.6 ± 0.1 ng/g which was above the set MRL (10 ng/g; Bargsanska et al. 2013).

The dietary risk posed by contaminated foodstuffs must be considered. A study in China was conducted by Gao et al. (2007) in which residues on cotton (0.2–0.39 kg/ha) and Chinese cabbage (0.1–0.15 kg/ha) were applied with spinosad and then measured. The overall residue concentrations were far below the supervised trials median residue levels (STMRLs) of 0.01 (cotton) and 0.103 mg/kg (cabbage), suggesting spinosad to be an unlikely health threat at normal consumption rates. Fruits and vegetables which are often applied with various pesticides must be

monitored for relatively high residue concentrations. Often it is unknown if these residues will remain at constant concentrations or will eventually dissipate over time. To investigate, Sharma et al. (2007) used high-performance LC (HPLC) to determine the persistence of spinosad in soil, cabbage, and cauliflower. Spinosad, applied at two different rates (17.5–35.0 g/ha), persisted up to 7 days in soil; however, the persistence in cabbage and cauliflower varied. At 17.5 g/ha, spinosad remained in the vegetables up to 7 days, whereas at 35.0 g/ha, it persisted up to 10 days in both.

Not only should produce be monitored for pesticide residues, but livestock can potentially be contaminated with inadvertent exposure to pesticides and to spinosad residues resulting from livestock consuming contaminated animal feed (Rutherford et al. 2000). Dairy cows (1, 3, 10 $\mu\text{g/g}$ doses, 28 days) and chicken (0.1, 0.3, 1, 5 $\mu\text{g/g}$ doses, 42 days) were dosed daily with gelatin capsules containing spinosad; milk and eggs were collected daily. Average residues in milk ranged from 0.071 to 0.797 $\mu\text{g/mL}$ within 14 days, whereas residues in eggs plateaued within 13 days to 0.227 $\mu\text{g/g}$, respectively. They also observed residues to concentrate more in samples high in fat content. Besides exposure to contaminated feed, animals may also come in contact with spinosad-containing products through the direct application to wounds. In Australia, a formulated product containing spinosad is often used to treat blowfly strikes (common to sheep), screwworm fly myiasis, and other general wounds found on livestock. In order to consume wounded livestock in Australia, they must be healed (Rothwell et al. 2005). Since products that contain insecticides are used as a treatment, Rothwell et al. (2005) investigated the residues of spinosad within sheep tissue. To do so, sheep were implanted with 200 first instar *Lucilia cuprina* larvae and treated with a spinosad-containing aerosol product 3 days later. Sheep were sacrificed, and the kidney, liver, muscle, perirenal fat, and subcutaneous fat were removed for analysis. Overall, they detected the highest concentrations within the fat 3 days posttreatment; however, they observed a decrease within 14 days; residues within the liver and kidneys were measured below the Australian MRL and US tolerances. These studies show that spinosad, even as a biopesticide, does accumulate within treated goods; however, further studies must be completed to show that using biopesticides poses less human risk than traditional pesticides.

Fumigants: Methyl Bromide

Methyl bromide (Fig. 2) was first registered in the USA in 1961. Due to its potential to deplete the stratospheric ozone layer, its production and import was limited by the Montreal Protocol agreements in 2005; however, there are several exemptions allowing the continued use of this product (US EPA 2009). Methyl bromide is a broad-spectrum fumigant, formulated as a pressurized gas, used to control

Fig. 2 Chemical structure of methyl bromide



nematode, spiders, fungi, insects, and rodents. It is most prevalently used as a soil fumigant during preharvest where it is injected into soil, at various depths, of either flat or raised bed fields. Often placement of tarps, over fields, has been accompanied with its use; methyl bromide may be injected into the soil first prior to tarp placement or can be injected under the tarps. Use of tarps is necessary to slow the movement of methyl bromide from the soil into the atmosphere; however, there is always the possibility of emissions once the tarps are removed. Many perishable goods are fumigated postharvest as well and include fumigating within an enclosed chamber at which methyl bromide is injected. Some commodities are fumigated multiple times during shipment and storage periods and may even receive methyl bromide treatment as part of a country's import process. Although methyl bromide is classified as a restricted-use pesticide, the use of replacement fumigants, such as chloropicrin or 1,3-dichloropropene, may result in higher material and labor costs and possibly reduced crop yields. This section will provide details on the detections of this fumigant in fruits and other crops, in addition to its overall toxicity.

Synthesis

Natural sources, such as oceans, salt marshes, and plants, release methyl bromide into the environment though the major sources in agriculture and food production are typically from intentional use of the synthetic material. Both abiotic and biotic methyl bromide production has been identified; however, atmospheric inputs are going to vary geographically due to temperature variations, plant species, and soil bromide content. In general, methyl halides, such as methyl bromide, are produced when the halide is enzymatically methylated by *S*-adenosyl-L-methionine (SAM); this process also utilizes methyl transferases. These enzymes have been identified in algae, fungi, and higher plants (Manley 2002). Studies which have measured the biosynthesis of methyl bromide in plants are presented here.

To demonstrate the natural production of methyl bromide from plants, genera from the Brassicaceae family, such as cabbage and broccoli, were grown in soil with varying bromide (Br^-) concentrations and then measured for methyl bromide emissions. Gan et al. (1998) reported an increase in methyl bromide emissions by Brassica plants that were proportional to an increase in soil Br^- levels. However, they also observed that plants consume the produced methyl bromide, showing plants act as both methyl bromide sources and sinks. To determine the emissions of methyl bromide from abiotic plant material, Horst et al. (2014) incubated (180°C ; 90–100 h) potassium bromide (KBr)-fortified pectin and a natural halophyte (*Salicornia fruticosa*). They found methyl bromide production to decrease as incubation time increased, where 85 % of the initial bromide (Br^-) was converted to methyl bromide by pectin and 30 % by *S. fruticosa*, respectively. Two wetlands in New Hampshire, USA, dominated by *Sphagnum* spp. were monitored for methyl bromide emissions. Varner et al. (1999) report direct emission measurements from the wetlands and suggest production may be common in saturated anoxic soils. Not only are plants and soils natural producers of methyl bromide, they also act as

methyl bromide repositories. Semiarid temperate grasslands have been found to oxidize methyl bromide; however, they are quite weak at doing so; water content within the soils increased oxidation compared to temperature (The et al. 2008). Besides plants, there is evidence that soils are an important sink for atmospheric methyl bromide as well. Shorter et al. (1995) showed methyl bromide uptake rates by temperate agricultural, sandy, boreal, tropical forest, and temperate grassland soils to be rapid and irreversible. Thus, soil can be considered an important sink in addition to oceans.

Laboratory studies have found that marine phytoplankton species such as *Chaetoceros calcitrans*, *Porphyridium* sp., *Synechococcus* sp., *Phaeodactylum tricornutum*, *Prorocentrum* sp., *Emiliana huxleyi*, and *Phaeocystis* sp. are capable of producing methyl halides (Scarratt and Moore 1998). During their stationary phase, the phase at which net growth and cell division halt, certain phytoplankton species accumulated more methyl bromide, even after cell division had stopped. However, some reduction was observed when the cultures became senescent resulting in decreased methyl bromide production (Scarratt and Moore 1998). Further observations revealed that the production mechanism is within the intact cells and production ceased when cell lysis occurred. The exact production mechanism is not well understood, with plant species demonstrating various mechanisms that may be contributing to methyl bromide synthesis. For example, Rhew et al. (2003) and Horst et al. (2014) noted a few mechanistic possibilities: abiotic conversion of halides by using pectin as a methyl donor undergoing a nucleophilic substitution reaction (S_N2) or an enzymatic production of methyl halides as a biotic production. Why methyl halides are produced by certain species and what their function is are unknown and can only be speculated. Scientists have proposed ways that species use methyl halide production in their favor. For instance, halide production may be a way to regulate its levels within plant tissues, thus removing any excess. Unfortunately, their true function is unclear. Manley (2002) states that synthesis of methyl halides, in general, may just be by-products of plant metabolism. With this said, further studies are required to determine (1) why plants produce these compounds and (2) the roles methyl halides play within the plant.

Mode of Action

It has been observed that this fumigant exhibits various responses among insects and that the exact mode of action is still unknown (Price 1985). It has been postulated that a major factor leading to methyl bromide toxicity is the rate at which an insect consumes oxygen at either atmospheric or reduced pressure. This is in addition to the fumigant's route of entry (Bond and Monro 1967). Bulathsinghala and Shaw (2014) note that methylation of glutathione occurs following methyl bromide exposure, leading to glutathione depletion. However, since neurological tissues lack methyl bromide accumulation, it is assumed glutathione depletion is only part of methyl bromide's overall toxicity. Methyl bromide has been observed to elicit genotoxic effects in exposed farm workers illustrating methyl bromide's

methylation abilities. Methylation of single-stranded nucleic acid bases may lead to alkylation mutations, thus aiding in carcinogenesis. Bulathsinghala and Shaw (2014) also note that methyl bromide's carcinogenicity may be explained by DNA alkylation through either a direct response to methyl bromide or an indirect response from the reduction of glutathione (GSH)-mediated detoxification. This suggests methylation to be a mechanism of in vivo mutagenicity and carcinogenicity. Furthermore, for animal species, cytotoxic actions are possibly due to alkylation of proteins, whereas in fish degenerative effects to gill epithelial and oral mucosa are linked to alkylation of cell membranes (US EPA 2009). Overall, the mechanism (s) of toxicity has not been established for all species. However, studies have illustrated that methyl bromide exposure results in the excretion of bromide (Br^-) and carbon dioxide (CO_2), suggesting multiple metabolic pathways are involved during the detoxification process (Garnier et al. 1996; Honma et al. 1985).

Toxicity

Human exposures are common for this pesticide. Agricultural workers can experience adverse effects from inhalation. Hustinx et al. (1993) investigated the occupational exposure of nine greenhouse workers following exposure to methyl bromide levels as high as 200 mg/L. Symptoms, such as headache, nausea, and an unsteady gait, were all observed and subsided within 3 weeks; in addition, two patients experienced convulsions. Serum bromide (Br^-) levels were not found to be related to neurological symptoms.

Since methyl bromide is used to control crop pests, studies have been conducted on both insects and rodents. The toxicity of methyl bromide to the red flour beetle (*Tribolium castaneum*) was found to be enhanced in the presence of carbon monoxide. Calderon (1992) recorded an increase in methyl bromide potency as carbon monoxide levels increased from 20 % to 40 % (1.7-fold increase in toxicity). This increase in toxicity may be influenced by the effect carbon monoxide has on the enzymatic systems of insects. Responses were thought to be attributed to inhibition of the insect's enzymatic system. Termites exposed to methyl bromide (0.4–3.25 mg/L doses, 20 h, 27 °C) also exhibit toxic effects. Examples of lethal accumulated doses for 50 % of the termite population (LAD_{50}) are reported in Table 2 (Scheffrahn and Su 1992). This study concluded that the current label application rate for termites is much higher than that needed to kill many termite

Table 2 Toxicity of methyl bromide for three termite species as reported in Scheffrahn and Su (1992). Values are reported as the product of exposure time (in hours) and concentration (in milligram per liter)

Termite family	Lethal accumulated doses (LAD_{50}) (mg·h/L)
Rhinotermitidae	11.4–35.8
Kalotermitidae	22.7–45.9
Termopsidae	26

Table 3 Toxicity LC₅₀ values and their test time frames (in hours) for methyl bromide on select organisms^a

Species	Aquatic or terrestrial	Test	Value
Rainbow trout	Aquatic	96 h LC ₅₀	3.9 mg/L
<i>Daphnia magna</i>	Aquatic	48 h LC ₅₀	2.6 mg/L
Algae (<i>Scenedesmus quadricauda</i>)	Aquatic	24 h LC ₅₀	2.2 mg/L
Bobwhite quail	Terrestrial	LD ₅₀ (single dose)	73 mg/kg
Rat	Terrestrial	LD ₅₀ (single dose)	86 mg/kg

^aData from US EPA (2009)

species; however, this could be remedied by monitoring concentrations applied and/or properly sealing structures to confirm proper doses are being delivered.

A reproduction study with parental female rats and pups was conducted by Kaneda et al. (1993). Methyl bromide-fumigated diet containing 80, 200 or 500 mg/L total bromine (Br₂) content was fed to the rats for two consecutive generations (18 weeks per generation). At the highest dose level, reduced food consumption by the parental females and lower body weights for the pups were observed. Kaneda et al. (1993) concluded that a dose of 200 mg/L was the no-observed-adverse-effect-level (NOAEL), whereas a dose of 500 mg/L was the minimum toxic level, respectively. A later study by Kaneda et al. (1998) looked at the effects of dosing on teratological effects to both rat and rabbit fetuses. Maternal toxicity occurred at high doses (10–30 mg/kg/day); decreases in body weight and food consumption resulted in addition to erosive lesions in the stomach and other organs. However, adverse effects were not observed in fetuses concluding that methyl bromide was not teratogenic or fetotoxic at doses responsible for maternal toxicity. Exposed Wistar rats, by inhalation to a range of doses (0, 3, 30, and 90 ppm; 29 months at 6 h/day, 5 days/week), were observed for carcinogenicity and methyl bromide toxicity. At the highest dose (90 mg/L), males and females exhibited a decrease in body weight, degeneration to the nasal olfactory epithelial, lesions in the heart, and hyperkeratosis in the esophagus (Reuzel et al. 1991). Reuzel et al. (1991) concluded that methyl bromide did not induce carcinogenic activity in rats. Further studies with male Fischer 344 rats identified histological responses from acute inhalation exposure. Rats were exposed to methyl bromide at concentrations up to 325 mg/L for 5 days, and selected tissues were dissected (nasal cavity, brain, adrenal glands, liver, kidney, and epididymides). Hurtt et al. (1987) reported dose-dependent responses. In particular, vacuolar degeneration within the adrenal glands, cerebellar granule cell degeneration, and nasal olfactory sensory cell degeneration resulted at concentrations greater than 90 mg/L. Both the kidneys and epididymides lacked changes. Hurtt et al. (1987) state that tissue responses observed by methyl bromide exposure are similar to those observed in methyl chloride exposures, thus possibly indicating similar modes of action.

Additional toxicity values, as reported in US EPA (2009), are provided in Table 3. Exposure to methyl bromide by aquatic and terrestrial organisms may occur through contamination of surface water from agricultural runoff, ingestion of contaminated drinking water or plant material, or possibly dermal absorption (US EPA 2009). Studies have shown methyl bromide exposure via inhalation

ultimately results in metabolism into carbon dioxide and bromide (Br^-); however, it is not thought that methyl bromide toxicity is related to bromide toxicity (Bulathsinghala and Shaw 2014). Based on toxicity values listed in Table 3, methyl bromide is moderately toxic to each test species.

Current Detection Techniques

Due to its gaseous nature, methyl bromide can be detected via gas chromatography-mass spectroscopy using an electron capture detector (GC-ECD). King et al. (1981) extracted and analyzed methyl bromide from grapefruits using a headspace assay. Collection of headspace gas was directly injected into a GC containing a nickel-63 ECD, with a detection limit of 0.002 mg/kg. Airborne methyl bromide can be coupled by adsorption on charcoal tubes followed by either thermal or solvent desorption (Woodrow et al. 1988). Use of headspace analysis, comparing charcoal sampling tubes to solvent phase, has been reported by Gan et al. (1995). Samples from both were analyzed on a GC-ECD, and resultant sensitivity was higher for the charcoal tubes (0.4 ng/tube) compared to the solvent phase (32 ng/tube). They note that the headspace method is a more rapid, time efficient method for methyl bromide analysis. Additionally, analysis using pre-concentration/capillary GC-MS has been successfully used to measure gaseous methyl bromide within air samples (Li et al. 1999).

Additionally, measurements of the bromide content of dried goods and cereals have been determined by using x-ray fluorescence spectroscopy equipped with a scintillation counter; the counts per second of bromine measured can determine the total bromine concentration (Getzendaner et al. 1968). Alternative instrumental techniques have been compared for the measurement of inorganic bromide in fumigated foodstuffs. Di Narda et al. (2003) compared cathodic stripping voltammetry (CSV), ion chromatography (IC), and spectrophotometry (SP) to the previously used inductively coupled plasma mass spectrometry (ICP-MS) technique. To do so, inorganic bromide was extracted from mushrooms (*Boletus edulis*) via a modified microwave digestion procedure. It was found that both cathodic stripping voltammetry and spectrophotometry may lead to overestimating bromide concentrations due to a coprecipitation of chloride and bromide. In addition, spectrophotometry tends to have a high detection limit and is negatively impacted by ammonium ions. However, out of the three techniques, ion chromatography was found to be a reliable alternative to using inductively coupled mass spectrometry; IC can determine bromide as low as 0.2 mg/kg. Overall, various methods have been successful in determining the off-gassing of methyl bromide from agricultural commodities and packaging and inorganic bromide concentrations remaining.

Levels of Residues in Foods

Methyl bromide residues have been detected in foodstuffs fumigated (either pre- or postharvest) with the pesticide. Often, fumigated crops are stored under varying

temperatures, and doses increase in order to eradicate insects. However, it is possible that a change in temperature will increase sorption by the crops/commodities. This has been investigated by Dumas (1973) who found that fumigated fruit did not retain higher levels of inorganic bromide (Br^- ; produced by methylation within the foodstuff) when fumigant concentrations increased and temperature decreased.

Often, produce to be exported undergoes fumigation and ventilation processes as part of a quarantine treatment. Fruit to be shipped to Japan, from Australia, was fumigated (2 h at 17 °C) and ventilated (2 h at 17 °C), and methyl bromide residues measured. Jessup et al. (1994) measured a decrease in residues (<0.002 mg/L) in 12 or more days while being stored. Measured bromide (Br^-) residues were below the maximum residue limit of 20 mg/L in all samples. Jessup and Slogett (1993) also reported a decrease in methyl bromide residues in apples and also showed that apple cartons contained residues, which then decreased within 7 days. Randomly selected vegetables from local markets in Spain were analyzed for bromide using a phenol red spectrophotometric method. Concentrations varied from 3.65 to 14.42 mg/kg for capsicum pepper (*Capsicum annum*), 4.5–9.3 mg/kg in potatoes (*Solanum tuberosum*), and 3.63–19.02 mg/kg in fungi (*Agaricus bisporus*, *Agaricus brunnescens* and *Pleurotus ostreatus*); these residues were measured below the maximum residue concentration residues established in Spain (20 mg/kg; Baso-Cejas et al. 2007).

When commodities are fumigated, portion of the parent methyl bromide will react forming inorganic bromide; however, often residues of both are measurable and at times persistent. Residues and overall persistence were investigated in rice, dried fruit, nuts, and seeds. Norman (2000) utilized an automated headspace gas chromatographic method to measure sorbed residues. It was found that commodities with a higher fat content (pumpkin seeds, walnuts, Brazil nuts, and groundnuts) contained high initial methyl bromide residues; due to high fat content and larger surface area, walnuts sorbed the highest amount. In nuts and seeds, methyl bromide was found to persist for approximately 10 weeks, whereas in dried fruit, persistence was found to be approximately 4 weeks (Norman 2000).

Investigation into the residues present in packaging materials and products was done by Scheffrahn et al. (1992). Packaged food, beverage, and medicinals, exposed to methyl bromide postharvest (8,640 mg/L target concentration, 20 h, 22 °C), were sampled and placed into 120 mL headspace serum bottles; sample matrix and headspace were equilibrated for 24 h. Following analysis, Scheffrahn et al. (1992) found residues to be commodity and packaging material specific. For example, higher residues were measured in fatty foods and their packaging (i.e., margarine and corresponding high-density polyethylene (HDPE) tub). It is thought that the residue accumulation was due to two factors, one where methyl bromide entered porous packaging via diffusion through air channels in closures and a second where methyl bromide permeated the polymer packaging, such as that of polyurethane bags.

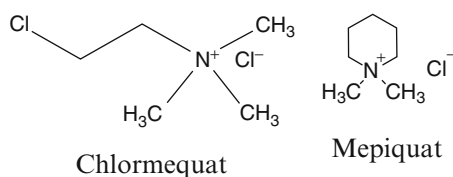
Further fumigation studies yielded various methyl bromide concentrations within grapefruit samples. Grapefruit, fumigated for 2 h (64 mg/L methyl bromide,

aerated for 15 min, stored at 24 ± 1 °C), had declining residues (time zero = 33.5 mg/kg) up to 48 h (time 48 h = 0.52 mg/kg) following fumigation (King et al 1981). Exposure to varying doses resulted in a similar decline over 48 h. The highest methyl bromide concentrations (3.94–18.8 mg/kg) were measured 2 hours after fumigation at doses of 16, 32, and 64 g/m³, respectively (King et al. 1981). Declining residues may be attributed to desorption from foodstuffs and packaging along with the possibility of hydrolysis occurring within food products. These studies show that methyl bromide does accumulate within products fumigated but not to the extent that harmful Br⁻ residues are produced in these products. However, residue concentrations have been determined to be below maximum residue levels at which consumption is acceptable.

Plant Growth Regulators: Mepiquat and Chlormequat

Mepiquat chloride (N, N-dimethylpiperidinium chloride, referred to as mepiquat) and chlormequat chloride (2-chloroethyl trimethylammonium chloride, referred to as chlormequat), both shown in Fig. 3, are agricultural plant growth regulators used in the USA and other countries. Mepiquat was introduced by BASF chemical company and registered in 1980, as Pix[®], for use on cotton. It is formulated as concentrates, granules, and pellets/tablets (US EPA 1997). In 1962 the US Department of Agriculture first registered chlormequat for use in greenhouses with further registration in 2006 when it was expanded to include bedding plants and nurseries (US EPA 2007). The US EPA has classified plant growth regulators as pesticides due to their ability to alter the reproduction, growth, and flowering rates of plants. Currently, use of these products in the USA is restricted with no application allowed on foodstuffs or residential areas. However, other countries have established maximum residue levels (MRLs) for individual food crops. Plant growth regulators are designed to reduce longitudinal shoot growth and decrease the rate of cell division without impacting development or productivity. More importantly, practical uses have been identified. For example, in grain cultivation, plant growth regulators prevent lodging during winter; vegetative growth is controlled in cotton, thus adjusting plant species to annual cultivation cycles, and fruit trees may become more compact, ultimately reducing maintenance and increasing fruit production (Rademacher 2000). This section will provide details on the detections of these chemicals in meat, poultry, fruit, and grains, in addition to their overall toxicities.

Fig. 3 Chemical structures of the discussed plant growth hormones



Synthesis

The biosynthesis of chlormequat and mepiquat is not currently known. However, mepiquat is known to be synthesized in plants. For example, mepiquat has been found to be formed in coffee and barley under high roasting temperatures. Wermann et al. (2013) examined the formation in cereal products under various temperature scenarios. At temperatures of 150–155 °C, it was not detected; however, at temperatures >200 °C, the cereal products did generate measurable quantities of mepiquat. This formation has been attributed to Maillard and methyl rearrangement reactions under high temperatures found in coffee-roasting processes (Wermann et al. 2013). Chlormequat, on the other hand, has not been observed to form in foodstuffs. Its method of synthesis is through the condensation of triethylamine and ethylene dichloride (Chlormequat Chloride 2014).

Mode of Action

Gibberellins are plant hormones that promote longitudinal growth as well as induction of enzymes in germinating seeds and plant development. Chlormequat and mepiquat are known to inhibit gibberellic acid biosynthesis in many plants. In particular, these compounds block the cyclases copalyl-diphosphate synthase and *ent*-kaurene synthase which are important in early gibberellic acid synthesis (Rademacher 2000). Not only has inhibition of gibberellic acid biosynthesis been identified, but both compounds also impact sterol and terpenoid synthesis in various plant species (Rademacher 2000). To investigate the impact on cotton growth and photosynthesis, Reddy et al. (1996) exposed cotton plants to various concentrations of mepiquat. Observed decreases in plant height and stem elongation were attributed to inhibition in gibberellic acid biosynthesis. In addition, plants had reduced ribulose biphosphate (RuBP) carboxylases activity relative to decreased photosynthetic rates (Reddy et al. 1996). Hedin et al. (1988) also looked at the effect both chemicals had on growing cotton. Overall yields decreased, but plants exposed to gibberellic acid increased yield. Furthermore, reduction of flavonoids, the secondary metabolites of a plant which are important in plant growth, development, and defense, was observed in plants treated with chlormequat.

Toxicity

The potential toxicity of commonly detected pesticides in foods was recently examined by Jones et al. (2013). Urine, collected from male Fischer 344 rats dosed with either chlormequat (0.05–100 mg/kg body weight) or mepiquat (0.6–150 mg/kg body weight), was analyzed by proton nuclear magnetic resonance

spectroscopy ($^1\text{H-NMR}$). The study showed that metabolic responses to the compounds resulted within 8 h after dosing, and recovery was observed to occur between 8 and 24 h. Lack of toxicity may be the result of mammals lacking target gibberellin receptors specific to bipyrilidylum herbicides.

Although these pesticides are designed to target plants, adverse effects may result with nontarget species. Insecticides in combination with plant growth regulators (chlormequat) have been tested to determine their impact on predatory species common to greenhouse crops. Natural enemies of the western flower thrips, *Neoseiulus cucumeris* (a mite predator) and *Grisu insidiosus* (a minute pirate bug), were exposed either through residuals or direct exposure to chlormequat. Oetting and Latimer (1995) found chlormequat to be compatible with both species and suggest it to be an acceptable chemical to use for thrip management. Since these pesticides impact all aspects of the applied plants, it is possible that an interaction between the treated plant and feeding insect may result. To investigate, Zummo et al. (1984) applied mepiquat to cotton (*Gossypium hirsutum* L.) to determine the effects on bollworms and corresponding plant. Bollworm larva (*Heliothis zea*) was placed onto treated cotton plants 14 days after the initial mepiquat application; significant decreases in larval growth and survival were observed. The cotton plants, however, responded to the mepiquat treatment by increasing tannin and terpenoid production; thus, bollworm resistance was induced. Further impacts of plant growth regulators were investigated. Biological control agents (use of other insects) are considered an important tool in controlling field pests. In particular, use of parasitoids which target the eggs of herbivorous pests has been released into cotton fields to control such pests as the cotton leafworm. However, it is unknown if these parasites are susceptible to pesticides or plant growth regulators. Bastos et al. (2006) conducted experiments to determine the impact pesticides and plant growth regulators have on the rearing of the parasitoid wasp *Trichogramma pretiosum* Riley when hosted by the Angoumois grain moth (*Sitotroga cerealella* Olivier) and the Mediterranean flour moth (*Ephestia kuehniella* Zeller). The parasitoid wasp was introduced into the host eggs and direct pesticide application was received. They found mepiquat did not affect the emergence of the wasps from the *S. cerealella* eggs or impact the percentage of emergent wasps from the *E. kuehniella* eggs. This study showed that the use of a biological agent, such as a parasitoid wasp, is not highly impacted by the exposure of plant growth regulators.

Experiments looking at the effect of 14 compounds on cell volume growth and cell division of green algae species, *Chlorella fusca* and *Chlorella sorokiniana*, were conducted by Thies and Grimme (1996). They found that both mepiquat and chlormequat exposure did not have an effect on either cell growth or cell division; thus, algicidal toxicity does not occur with these growth regulators. Toxicity values for both growth regulators are presented in Tables 4 and 5. Chlormequat and mepiquat are both of low toxicity to aquatic fish, aquatic invertebrates, and mammals; however, chlormequat appeared to be more potent to plants than other organisms.

Table 4 Toxicity values (lethal concentration, effective concentration, and lethal dose for 50 % of the population) for chlormequat for various organisms^a

Species	Plant or animal	Test	Value
Rainbow trout	Animal	96 h LC ₅₀	>100 mg/L
<i>Daphnia magna</i>	Animal	48 h EC ₅₀	31.7 mg/L
Duckweed	Plant	7 days EC ₅₀	5.3 mg/L
Microalgae (<i>Pseudokirchneriella subcapitata</i>)	Plant	72 h EC ₅₀	>100 mg/L
Japanese quail	Animal	LD ₅₀	441 mg/kg
Rabbit	Animal	LD ₅₀	115 mg/kg

^aData from IUPAC 2014**Table 5** Toxicity values (lethal concentration, effective concentration, and lethal dose for 50 % of the population) for mepiquat for various organisms^a

Species	Test	Value
Rainbow trout	96 h LC ₅₀	>100 mg/L
<i>Daphnia magna</i>	48 h EC ₅₀	68.5 mg/L
Northern bobwhite	LD ₅₀	>2,000 mg/kg
Rat	LD ₅₀	1,490 mg/kg

^aData from IUPAC 2014

Current Detection Techniques

Traditional analytical methods for both of these compounds have included the use of reversed phase liquid chromatography and ion pairing with heptafluorobutyric acid (HFBA), or liquid chromatography-mass spectrometry (LC-MS). However, using mass spectrometry coupled with HFBA has led to signal suppression and increased background noise. To overcome these analytical issues, additional method development for hydrophilic compounds has resulted in the use of hydrophilic interaction chromatography (HILIC) coupled with LC-MS. This new method has been applied for identification of chlormequat and mepiquat residues in many food products such as beer, bread, fruits, and vegetables (van Nuijs et al. 2011). With the use of high ionic strength mobile phases, baseline separation and increased peak shapes result. Furthermore, this method produces higher resolution of peaks, retention, and increased sensitivity of quaternary amine compounds. The method employed by Esparza et al. (2009) has achieved low parts per billion (ppb) level limits of detection and a separation of both chlormequat and mepiquat in only 4 min. Guo (2005) has also utilized the HILIC method and has successfully separated the two growth regulators without any ion-pairing reagents. It is also noted that the separation conditions including acetonitrile content, column temperature, and the type of buffer salt are all important in successfully separating these compounds.

Besides liquid chromatography, both compounds can be measured in water using capillary electrophoresis coupled to a mass spectrometer. Since these compounds are ionic species, using CE for their analysis is ideal since it does not require

the use of an ion-pairing reagent. The use of the mass spectrophotometer has allowed for analytical specificity and simultaneous analysis and determination of both growth regulators. Nunez et al. (2002) found this method, using an electrospray ionization source, to be reproducible, and ionic suppression of both chlormequat and mepiquat was avoided. Also in water samples, these growth regulators have been detected by micellar electrokinetic chromatography (MEKC). Through this method, micelles are formed, via addition of a surfactant, which interact with the compounds, thus enhancing the separation of similarly structured compounds (Eash and Bushway 2000). Although the use of capillary electrophoresis has been suitable for water samples, it may also become helpful for extracts from foods, further method development is required.

Another alternative for measurements of chlormequat in food utilizes ion chromatography. This method, although limited by its detection method, requires minimal sample extraction prior to analysis; Peeters et al. (2001) was capable of analyzing chlormequat residues in pears with this method. Cation-exchange chromatography includes an ion chromatograph coupled with a commercially available cation-exchange column and a suppressed conductivity detection system. This method was capable of measuring chlormequat at a limit of detection of 0.5 mg/kg; this is an alternative to traditional liquid chromatography methods. Colorimetric techniques have been used to determine chlormequat in wheat grain and plants. It is removed via adsorption chromatography and measured colorimetrically as a dipicyrlamine-chlormequat chloride complex; increasing the solution polarity and/or decreasing the alumina column length results in high recoveries (Mooney and Pasarela 1967).

Levels of Residues

Often growth regulators can improve crop yields. Countries have established maximum residue limits (MRL) in order to regulate application dosages of these compounds. Residues of chlormequat applied to two Chinese wheat crops postharvest at the recommended dosage of 2,000 mg/kg and 3,000 mg/kg were measured. Wheat powder extracts from the highest dose contained 0.04–0.07 mg/kg chlormequat in samples from Beijing and 0.31–3.51 mg/kg in samples from Changchun, respectively; only samples from Changchun had measurable concentrations from the lowest application dose (0.06–0.5 mg/kg). The results of this study indicate that chlormequat is safe to use at the recommended dosage on wheat since residues did not exceed China's MRL of 5 mg/kg (Guo et al. 2010).

Growth regulators applied to crop or plant products can also accumulate in meat and poultry products. To determine residue levels, Li et al. (2012a) collected live-stock and poultry meats from markets throughout Beijing, China, in 2011 and used organic raised meats as reference blanks. Chlormequat was detected in each sample at varying concentrations: goat meat (14.4–486 µg/kg), beef (0.6–27.8 µg/kg), pork (0.4–20.6 µg/kg), and chicken (0.5–636 µg/kg). Overall, measurements for both goat and chicken tissues were approximately 15.9 and 2.4 times greater

than the Codex maximum residue levels, thus demonstrating the accumulation of chlormequat in animal tissues after consumption of contaminated crop/plant product.

To further monitor for contamination of these compounds in food products, various commodities were purchased from a supermarket in Barcelona and evaluated for residues of chlormequat and mepiquat via LC-MS. Of the 28 food products sampled, chlormequat was only present in five products (bread, beer, apple juice, baby food, and pears) and detected at low ppb levels. In comparison, mepiquat was only detected in bread (0.9–2.9 ng/g) and coffee (41–166 ng/g). Residual levels for both compounds were below the set MRL; however, one coffee sample was greater than the 100 ng/g MRL (Esparza et al. 2009). Additionally, chlormequat residues have been measured in tomatoes that were randomly collected from tomato treatment plants. Tomato pulp, powder, and thick tomato sauce were homogenized and extracted with methanol prior to LC-MS analysis. Chlormequat was at measurable concentrations within the powder and sauce at maximum concentrations of 0.468 ± 0.014 and 0.285 ± 0.002 mg/kg, respectively (Careri et al. 2002). The maximum concentrations were measured in three powder samples and one sauce sample; thus, they were found to exceed the maximum residue limit set forth by the European Union Commission of 0.05 mg/kg for tomato products.

Finally, both chemicals have been identified in cereals, wheat, rye, and barley products during studies performed in Denmark. Chlormequat was detected in 83 % of cereal samples at an average concentration of 0.32 mg/kg, whereas mepiquat was only found in rye samples at concentrations one order of magnitude lower than those of chlormequat. Maximum residue levels (MRLs) have also been established by the European Communities (EC) and the Danish authorities for cereals. The MRL for chlormequat varies depending on the cereal's content. For cereal containing wheat, rye, and barley, the MRL is 2 mg/kg, for oat cereal it is 5 mg/kg, and an MRL of 0.05 mg/kg is for other cereals, whereas a mepiquat MRL 1 mg/kg has been set for barley and rye (Granby and Vahl 2001). Field studies investigating the dissipation and final residues of mepiquat, applied to wheat and potato plants, were conducted by Zhang et al. (2013). Using a fast hydrophilic interaction liquid chromatographic method (HILIC-LC-MS), with a limit of quantification of 0.007 mg/L, they were able to determine mepiquat in both wheat and potato samples. They found dissipation of mepiquat from the samples to be fast (on the order of a few days), with measurable residues from 0.052 to 1.9 mg/kg for wheat (4.8 g/ha and 7.2 g/ha doses) and below 1.17 mg/kg for potatoes (120 g/ha and 180 g/ha doses), respectively. The USA, Japan, and the European Union (EU) have established a maximum residue level of 2 mg/kg for wheat; however, the EU has established a level of 0.05 mg/kg specifically for potatoes (Zhang et al. 2013).

Not only can the food products contain these compounds, but it is possible for the seeds to contain residues. To determine residues on cotton seeds, field experiments in which mepiquat was applied at various concentrations to planted cotton during 2010 and 2011 was performed by Li et al. (2012b). Sample residues were measured at 14, 21, and 28 days following the final mepiquat application. In 2010 the seeds

contained various residual concentrations with the highest measured only 14 days post-spray (0.48–1.91 mg/kg) and the lowest 28 days post-spray (0.11–0.41 mg/kg), respectively. In comparison, in 2011 the residues were undetectable by the 28th day. These studies show that these plant growth regulators, although used to increase crop yields, can also accumulate within products and seeds. Although overall residue concentrations measured have been relatively low, some samples have been determined to be above the maximum residue levels set by certain countries. These pose an issue when importing fruits, grains, and coffee from other countries. The toxic effects of many pesticides have been assessed by the World Health Organization (WHO) in conjunction with the Food and Agriculture Organization (FAO). These organizations establish the acceptable daily intake (ADI) levels and maximum residue levels (MRLs) for concerning pesticides based on the pesticide's agricultural use, nature of residues, and toxicological studies (Lu 1995).

Conclusion and Future Directions

In this chapter, we have seen how commonly used agricultural pesticides have the potential to elicit adverse effects on both target and nontarget species and their potential to be present in the food we buy and consume. Many methods have been developed and validated in order to identify potential threats toward consumers. Such methods are capable of detecting many pesticides at a time, thus increasing efficiency and aiding in residual identification. This is just the beginning, but as the sensitivity of instrumental analysis increases, the more prepared we will be to determine how to limit residues on pre- and postharvest commodities, whether through a change in agricultural management practices or a shift in pesticide selection and use. Although many societies aim to reduce pesticide applications and use as a whole, studies have shown that currently, measurable residues of many pesticides are already below maximum residue limits and acceptable daily intakes (ADI) set forth by various countries and the World Health Organization (WHO). Moreover, for individual commodities, residues typically decrease over time following pesticide application. In essence, the risk presented by residues is far less than that perceived by consumers.

It would be ideal to reduce pesticide use throughout the USA and other countries not only to limit residues on food, but also to decrease the overall environmental impact. To do so, many factors may be considered, such as phasing out high use of synthetics such as methyl bromide; this is in effect due to the Montreal Protocol. In addition to phaseout, methyl bromide is being replaced by other fumigants with less environmental impact. Furthermore, the use of improved technology to deliver pesticides to the target crops, limiting environmental transport such as drift or runoff, and the use of integrated pest management tools such as intercropping, crop rotation, or biopesticides, together will work in tandem to increase pest control while reducing chemical application. Together, these tools will work in tandem to increase pest control while reducing chemical application. Although these are only a few suggestions, as the demand for pesticides change or as agricultural

technology advances, so will the future use, exposure, and potential impacts. However, it is most important to remember that the use of agricultural chemicals has aided in increasing crop production and lowering food costs; therefore, they are an important tool for consumers and local economies. Overall, there will always be potential agricultural chemical pollutants whether they are synthetic based or naturally produced, but the current trend in the USA and other developed countries is to use more biopesticides rather than synthetics.

Cross-References

- ▶ [Applications of Nanotechnology in Developing Biosensors for Food Safety](#)
- ▶ [Chemical Composition of Organic Food Products](#)
- ▶ [Contamination from Industrial Toxicants](#)
- ▶ [Model Fungal Systems for Investigating Food Plant Mycotoxins](#)

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Abstract

Industrial toxicants are classified as chemicals that are natural and mobilized through anthropogenic processes or anthropogenic-produced chemicals that are released into the environment, contaminating waterways and aquifers, soil, air, and organisms – including food. These contaminants can reach food directly from point source contamination or indirectly through long-range atmospheric and biogeochemical processes. This chapter will focus on a few of the industrial pollutants commonly found in the world's food supply and the mechanisms associated with their transport throughout the various environmental compartments. In addition, this chapter intends to touch upon toxicological effects in humans and the detection of these contaminants in food and the environment, which include: heavy metals, radionuclides, polycyclic aromatic hydrocarbons (PAHs), chlorinated solvents, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxin (PCDDs), polychlorinated dibenzofurans (PCDFs), and brominated flame retardants.

Introduction

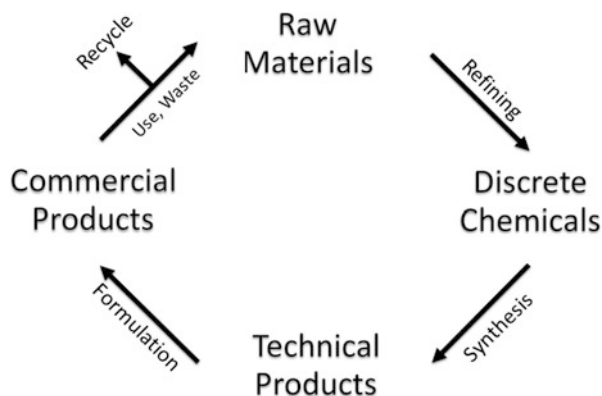
When addressing potentially harmful chemicals in the environment that can affect the health of humans and other organisms, the sources of these chemicals should be considered. These harmful chemicals are typically referred to as contaminants, pollutants, or most aptly, environmental toxicants. The sources also provide clues to the transport and subsequent fate of these chemicals, including exposure pathways. There are generally two categories of environmental toxicants: naturally occurring and anthropogenic.

Naturally occurring chemicals are those such as in poison ivy, poisonous mushrooms, and natural elements, such as mercury and arsenic. Certain measures can be employed to avoid or limit exposures to these natural toxins. General avoidance, or identifying and cordoning off the source (if possible), represents prudent, immediate measures that can be taken. Remediation may be needed in some cases as well. Anthropogenic chemicals, conversely, are those that society or its industries create, use, and dispose. Some undergo transformation in the environment; generally transformation is a detoxification process, but occasionally the transformation products are more harmful than what was initially released to the environment. A noteworthy example of this is the methylation of mercury by microorganisms to methyl mercury, which is significantly more toxic to humans than the parent compound. Unfortunately, it is difficult, and often not possible, to avoid incidental exposures to man-made chemicals without some form of regulatory action (Crosby 1998).

Sources of Anthropogenic Chemicals

Petrochemicals and subsequent petrochemical-derived products represent a major part of the Chemical Process Industry (CPI) and are sources of anthropogenic chemicals and contaminants. From about the 1940s to 1990s, it was not difficult

Fig. 1 The Chemical Process Industry derived from *Chemical in the Human Food Chain* (Winter et al. 1990)



to find emission sources in the air, water, soil, and occasionally foods. Industry is now much more cognizant and proactive in curtailing emissions, in large part due to the enactment of legislation (e.g., US Clean Water Act, US Clean Air Act) that placed the burden of preventing emissions on the manufacturers, transporters, and other industrial segments.

The Chemical Process Industry is a large sector of US and worldwide industry. It accounts for approximately 10 % of manufacturing activity in the United States. It is also a major employer of chemists, chemical engineers, chemical safety personnel, and toxicologists. These large companies can have manufacturing and formulation sites in numerous places, including highly populated areas such as California, Michigan, New Jersey, and the Gulf Coast states, among others. The Chemical Process Industry takes in raw materials (petroleum, coal, wood, air, salt, etc.) and refines these often-crude mixtures into discrete refined chemicals that are the building blocks for a variety of technical and commercial products (Winter et al. 1990). They include simple chemicals such as ethylene, propylene, toluene, styrene, formaldehyde, methanol, mineral acids, ammonia, and chlorine. Starting materials are used in chemical synthesis to produce solvents, monomers, fibers, paints, adhesives, detergents, pharmaceuticals, electronics, and explosives, as well as more finished consumer products such as tires, upholstery, flooring, paper, etc. (Winter et al. 1990). After use these products often are disposed of by recycling (a desirable option in many cases), incineration, landfilling, or, unfortunately, dumping in the ocean, waterways, or abandoned sites (Winter et al. 1990).

Accompanying a vast majority of these steps (Fig. 1) can be intentional or accidental release of the chemicals themselves or their by-products into the environment. Some infamous cases of release include the emissions of methyl isocyanate from a pesticide manufacturing plant in Bhopal, India, that killed an estimated 4,000 people and methyl isothiocyanate from a train derailment accident near Mount Shasta in California contaminating large parts of the Sacramento River. Similarly, the long-term release of methyl mercury from a manufacturing plant in Minamata, Japan, affected both humans and wildlife, notably from heavily contaminated seafood, and the contamination of soil and waters near toxic waste sites

like the Love Canal in upstate New York. The industry is now addressing some of the earlier mistakes by “going green” and manufacturing more bio-based products and recycling its wastes.

Chemistry of the Chemical Process Industry

The manufacturing routes for producing some high-volume petrochemicals are relatively simple. For example, ethane produced from petroleum refinement is dehydrogenated to produce ethylene, which can then be polymerized to polyethylene, or chlorinated to produce dichloroethane, vinyl chloride, and polyvinyl chloride (PVC) (Fig. 2).

Like any large industry, there are many chemicals made or used by the Chemical Process Industry that can potentially pollute the environment. In the United States, regulations were crafted that dealt with whole classes of chemicals. An example is the Priority Pollutant List, an outgrowth of the Clean Water Act, stimulated by the finding of simple chlorinated solvents and monomers in the Mississippi River water intake for the City of New Orleans in the early 1970s (Dowty et al. 1975). The Priority Pollutant List targeted 129 specific chemicals (or chemical mixtures, like polychlorinated biphenyls), which were among the chief water pollutants found in various analytical surveys. For chemicals on the list, industry was required to inventory and report emission and use best available technology (BAT) to minimize emissions. As an example, one chemical company went “off the river,” or in other words reduced disposal of effluent into rivers, at its large manufacturing complex in Midland, Michigan, to implement federal emission reduction requirements. Noncomplying companies were subject to fines and other penalties.

The Priority Pollutant List classification was based on how water was analyzed to determine levels of the regulated pollutants (Fig. 3). The purgeable organics include benzene, toluene, ethylbenzene, and xylene (BTEX), volatile organic compounds (VOCs), and volatile organic chlorinated compounds (VOCls). The base neutrals include polynuclear aromatic hydrocarbons (PAHs), semivolatile organic compounds (SVOCs), semivolatile organic chlorinated compounds (SVOCls), phthalate esters, pesticides, and polychlorinated biphenyls (PCBs). The acid extractables include phenols, cresols, etc., and the final aqueous layer includes metals, cyanides, and a few other mainly ionic chemicals. Asbestos was included in a “miscellaneous” category.

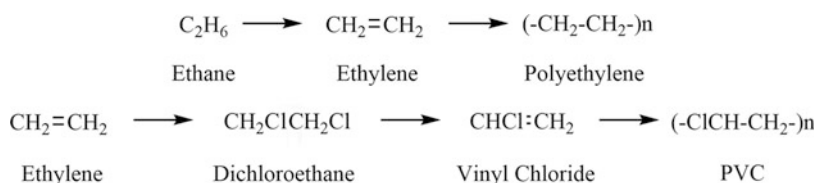


Fig. 2 Example of a Chemical Process Industry process: PVC production from ethane

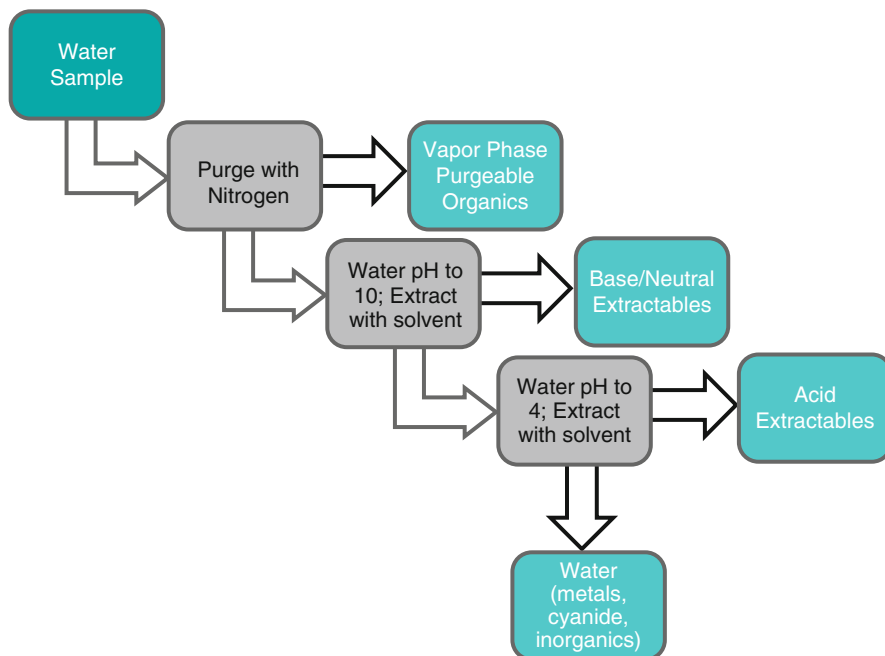


Fig. 3 Visualization of the different extraction steps of water samples that were used to help generate the Priority Pollutants List

The base–neutral compounds include polycyclic aromatic hydrocarbons from crude oil. The 2010 Deepwater Horizon crude oil platform accident in the Gulf of Mexico provides an example of petroleum products released into the environment. Of all of the oil-related contaminants resulting from the disaster, the polycyclic aromatic hydrocarbons were considered to pose the greatest long-term risk for consumers of fish and shellfish (Armbrust et al. 2013). Polynuclear aromatic hydrocarbons are also formed from combustion sources, cooking over fires, wild-fires, smoking, and from asphalt road sealants.

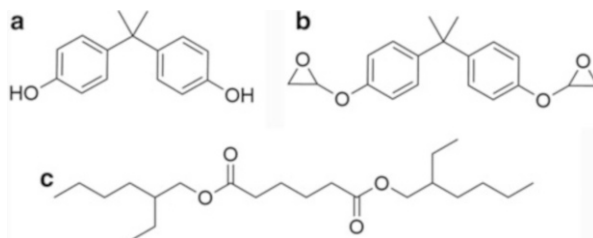
Polychlorinated biphenyls are products made by chlorination of crude-oil-derived biphenyl. They consist of congener mixtures and positional isomers of chlorinated biphenyls, many of which were produced and marketed under the trade name of Arochlors. These were once widely used in electrical transformers because of their stability, low dielectric constant, and effective thermal and electrical insulation and also as plasticizers and pesticide extenders. Polychlorinated biphenyl contamination in the environment is widespread; noteworthy is its bioaccumulation in seafood, because of the large usage and properties that favor stability, dispersion, and deposition from atmospheric transport and fate processes.

Persistent organic pollutants (POPs) address a special classification for compounds such as polychlorinated biphenyls and stable organochlorine. Properties that place a chemical on the persistent organic pollutants list include persistence,

Table 1 A group of polychlorinated, banned persistent organic pollutants coined the “Dirty Dozen”

Aldrin	Toxaphene
Chlordane	Hexachlorobenzene
DDT	Mirex
Dieldrin	Polychlorinated biphenyls
Endrin	Polychlorinated dibenzo- <i>p</i> -dioxins
Heptachlor	Polychlorinated dibenzofurans

Fig. 4 Depiction of common plasticizers used industry: (a) Bisphenol A (*BPA*) (b) Bisphenol A Diglycidyl Ether (*BADGE*) (c) Di(2-ethylhexyl)adipate (*DEHA*)



bioaccumulation, chronic toxicity, and the ability to undergo long-range transport (LRT), where they can inevitably reach organism and biomagnify up the food chain causing adverse effects (e.g., DDT causing egg thinning). A group of persistent organic pollutants have been singled out as the most notorious and are referred to as the “Dirty Dozen” (Table 1). Manufacture and use of the Dirty Dozen chemicals were banned by most of the developed nations. Note that all of the Dirty Dozen chemicals in Table 1 are polychlorinated – a feature that favors stability and persistence in the environment. Many of the compounds on this list are also frequently found on the National Priority List (NPL) of contaminants found at federal Superfund sites in the United States, like the Love Canal. Sites classified by the National Priority List are undergoing remediation and cleanup by the owners and users of these waste disposal sites or by the federal government if no responsible party can be located.

Many of the products of the Chemical Process Industry, particularly those associated with polymer and plastics manufacture, present exposures of serious concern. For example, phthalate esters are present throughout plastic consumer items including children’s toys and food containers, and include such chemicals as bisphenol A (BPA), bisphenol A diglycidyl ether (BADGE), and di(2-ethylhexyl) adipate (DEHA) (Fig. 4). Phthalates are added to plastics to manipulate the properties of plastics (e.g., malleability, strength). Concerns over plasticizers are primarily due to their endocrine-disrupting potential, which can result in tumors, birth defects, and developmental disorders (Fasano et al. 2012). BPA is a noteworthy example of a non-phthalate plasticizer that can be transported to foods and beverages through the hydrolysis of the ester moiety under heat, acidic, or basic conditions, which mimics the transport of phthalates into foodstuff (Cirillo et al. 2013; Fasano et al. 2012). Although synthetic plasticizers are found in foods, their primary pathway is from food packaging migration, thus exemplifying the fine line that distinguishes between industrial toxicants and food packaging. While these

two categories greatly overlap, this chapter will focus on an overview of toxicants in food from transport and fate processes, rather than direct contamination from processing and packaging practices.

Anthropogenic Chemicals

Anthropogenic chemicals include fertilizers, pesticides, and products or by-products of food processing, livestock and poultry operations, and many other sources, all of which now undergo routine monitoring and are subject to emission controls. At the heart of the nation's efforts to clean up the environment is a sophisticated system for analyzing, monitoring, and identifying potential problematic chemicals and sources of contamination and for assessing the health impacts of these chemicals to humans, wildlife, and plants. Herein we will present a brief overview of anthropogenic chemical toxins in foodstuffs, specifically resulting from industrial processes. They include persistent organic pollutants, heavy metals, and radionuclides.

Heavy Metals

The history of the toxicological and environmental concerns of heavy metals is well publicized throughout the media, with mercury contamination in fish being one of the more notorious examples. Heavy metals are toxic, even at low concentrations, and include classes such as metals, metalloids, and semimetals, therefore incorporating a long list of contaminants (DalCorso et al. 2013; Rascio and Navari-Izzo 2011). Zinc, copper, chromium, lead, cadmium, mercury, and arsenic are among some of the contaminants that fall under the heavy metal category, with mercury, lead, and cadmium being the more infamous and prevalent (Al Bakheet et al. 2013). Heavy metals are naturally found in the environment, where they can be liberated from the earth due to weathering processes and volcanic activity. Anthropogenic processes include engine exhaust, mining, smelting, and coking and are major contributors to heavy metal contamination (DalCorso et al. 2013; Vargas-García et al. 2012).

Fate and Transport into Food and the Environment

Anthropogenic sources of heavy metals enhance the load and transport of heavy metals that are naturally liberated into the environment. Atmospheric emissions of heavy metals are subject to long-range transport and deposition onto soils and waterways, as seen in the surrounding area of a copper mine in eastern Macedonia (Balabanova et al. 2011). Heavy metal transport is limited in soil, due to sorption, precipitation, cation exchange capacity, and other physiochemical properties of the soil, although heavy metals associated with the aqueous phase can be transported within the soil matrix (US EPA 1992). Metals in the soil solution are also subjected to plant uptake, leaching into groundwater, and volatilization, particularly for

arsenic, mercury, and selenium (US EPA 1992). For instance, lead is a noted air contaminant and has the ability to contaminate plants through deposition and leaf uptake, while cadmium is more likely to enter plants from the soil via root uptake (Hapke 1996). The presence of heavy metals in irrigation water has been noted as an important transport route of heavy metals to soils and crops. For instance, there have been recent concerns with the uptake of arsenic from soils and irrigation water into the rice grain (Khan et al. 2013; Rahman 2011). Heavy metal content of waters can be of particular concern for arsenic and mercury, for these metals have the ability to bioaccumulate, unlike nickel and zinc (Hapke 1996).

The disposal and removal of heavy metals from food and the environment provide immense challenges due to their inability to further degrade biologically or chemically. Biological reclamation has been gaining ground as an effective, nondisruptive, and clean method of removing a variety of heavy metals from soil (Vargas-García et al. 2012). In contrast, chemical removal processes have proven to be less effective, especially on low concentrations, and have high pollution potential (Vargas-García et al. 2012). Chemical removal processes include adding nontoxic materials (e.g., zeolite, peat, compost, clay minerals, phosphate, flying ash, or lime amendments) to reduce heavy metal toxicity in soil, but these processes may lead to secondary pollution and are expensive (Shi et al. 2009). Phyto- and micro-remediation are the major biological reclamation techniques currently available for heavy metals. Micro-remediation is the use of microorganism to biotransform and/or bioaccumulate heavy metals. *Vargas-Garcia* suggests that biosorption is the most common micro-remediation method because it does not have any energetic requirements. Here microbes are utilized to sorb heavy metals to the cell surface via physiochemical mechanisms (Vargas-García et al. 2012). Phytoremediation, specifically phytoextraction, is the act of using plants to extract heavy metals and other contaminants from the soil, which can then be harvested and disposed, although in some cases the metals may be recovered from the plants (DalCorso et al. 2013; Sylvania et al. 2005). Microorganisms can be used as synergists with phytoremediation, by increasing nutrient and metal bioavailability within the soil (Sylvania et al. 2005). It should be noted that although specific plants can be used for phytoremediation, the uptake of heavy metals to plants is not specific to these plants species. Therefore, plant uptake of heavy metals can occur in crops and other nontarget plants, thus providing another route of transport of heavy metals to food.

Food Contamination

Many metals and metalloids undergo bioaccumulation in the environment, including mercury in fish, cadmium in spinach, and selenium in algae and species that feed on algae. In dairy, bioaccumulation of zinc, copper, and chromium exists from contaminated feedstock (Li et al. 2005). Organic cucumbers have been observed to contain lead, cadmium, and chromium, with different levels at different times of the growing season (Mansour et al. 2009). To obtain a better idea on how to restrict accumulation, heavy metals should be monitored throughout the growing season since concentrations may fluctuate. The heavy metal content in fish, especially mercury, is a grave concern to many, particularly children and pregnant women, since they may be

susceptible to unsafe developmental and neurological effects caused by mercury. Additionally, mercury is especially high in gill of fish, which is where the mercury tends to accumulate, followed by the intestines, head, and muscles, respectively (Bennasir and Sridhar 2013). Arsenic, which is a naturally occurring contaminant, can also be apparent in fish (e.g., salmon) (Bennasir and Sridhar 2013).

Recently, the concentration of heavy metals in tea has gained significant interest, as tea has become a popular dietary source for essential elements such as potassium, zinc, and manganese (Szymczycha-Madeja et al. 2012). Plant uptake and translocation to the tea leaves comes from soil contaminated by air and water transport, such as *Camellia sinensis*, which has been found to contain elevated amounts of lead, aluminum, and fluorine (Szymczycha-Madeja et al. 2012). Due to the increased popularity of teas among consumers, levels of heavy metal as well as other forms of contamination could outweigh the beneficial nutrients and thus should be further studied.

Analytical Detection

Heavy metal detection in food such as crops, fish, seafood, and tea is important in order to determine safe levels. Detection methods include flame atomic absorption spectroscopy (FAAS), graphite furnace atomic absorption spectroscopy (GFAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) (Psoma et al. 2014; Szymczycha-Madeja et al. 2012).

Toxicology

Heavy metal contamination in food is of serious concern and results in a wide range of adverse health effects. It should be kept in mind that each heavy metal has its own set of toxicological end points and mechanisms. For instance, lead can replace zinc on heme enzymes and also is stored in bones, where it has a 20–30 year half-life (Yousef et al. 2013). Copper, on the other hand, can form the poorly absorbed copper molybdate or copper thiomolybdate, yielding copper that is unavailable for incorporation into copper-containing proteins (Yousef et al. 2013). Cadmium is noted as carcinogen (International Agency for Research on Cancer Class 1 carcinogen) by inhibiting genes responsible for DNA repair (Al Bakheet et al. 2013; IARC 2014). It has been noted to cause upper-gastrointestinal cancer at high concentrations (Al Bakheet et al. 2013). Highly concentrated nickel can cause headache, fatigue, and cancer (Al Bakheet et al. 2013). Other metals, such as mercury, can cause kidney damage, while chromium can decrease cellular mitochondrial activity and viability and, in addition, can induce the release of cytokines (Al Bakheet et al. 2013; Zhang et al. 2010). Exposure to lead, cadmium, and other heavy metals has been linked to learning disorders, cognitive impairments, and other neurotoxic effects, which is a particular concern for children (Yousef et al. 2013). Heavy metal contamination is heavily influenced by anthropogenic processes, which liberate these metals, allowing them to undergo long-range transport and bioaccumulation, among other transport processes. Their adverse health effects hold significant concern and thus require constant assessment in drinking water, air, and food, as well as continued regulatory oversight.

Radionuclides

Radionuclides, also known as radioactive nuclides or radioisotopes, are both natural and anthropogenic contaminants in the environment. Naturally occurring radionuclides include ^{40}K (potassium), ^{238}U (uranium), ^{210}Po (polonium), ^{210}Pb (lead), $^{220,222}\text{Rn}$ (radon), and $^{228,226}\text{Ra}$ (radium) (Beresford et al. 2012; Gwynn et al. 2013; Lehto et al. 2013; Ross et al. 2013), while anthropogenic radionuclides include $^{134,137}\text{Cs}$ (cesium), ^{90}Sr (strontium), $^{239,240}\text{Pu}$ (plutonium), ^{241}Am (americium), and ^{131}I (iodine). Both natural and anthropogenic radionuclides are toxic to organisms. However, anthropogenic radionuclides are typically more noteworthy due to various epidemiological studies and tracking of worldwide transport of radionuclides through the air, water, soil, and food webs from events such as the Chernobyl, Windscale, and Fukushima Daiichi disasters. In addition to the noted recent disasters, anthropogenic radionuclide contamination can be traced back to major historical events that include the use of atomic weapons during WWII, Chernobyl or Fukushima disasters, and nuclear weapons testing conducted during the 1950s and 1960s (Lehto et al. 2013).

Fate and Transport into Food and the Environment

The transport of natural and anthropogenic radionuclides can occur through all the environmental compartments, with atmospheric transport being one of the more significant routes. In addition to long-range transport, deposition is also a problem and is exemplified by the worldwide detection of radionuclides after nuclear disasters, including the more recent Chernobyl and Fukushima incidents (Crosby 1998). The unique signatures and location of anthropogenic radionuclides activity can be used to determine the radionuclide source. For instance, in Great Britain, ^{134}Cs was attributed to Fukushima, while ^{137}Cs was credited towards the Chernobyl meltdown and Windscale events (Beresford et al. 2012). Grass samples in Great Britain were found to contain ^{134}Cs and ^{137}Cs , in addition to ^{131}I , attributed to Fukushima, in grass and sheep's milk (Beresford et al. 2012). Contamination of crops is related to the amount of contamination of the soil, along with the soil physiochemical properties; however, root and vapor uptake, in addition to deposition, are the major routes of transport for radionuclide contamination in plants (Jandrić et al. 2013).

Microalgae and aquatic plants have been suggested as bioremediation methods for radionuclides. Small-scale experiments with microalgae show potential for cesium removal, although large-scale systems need to be further evaluated (Fukuda et al. 2014). Fish, crustaceans, and mushrooms contain radionuclides and are also used for biomonitoring purposes using ^{132}Cs and Radium, or in other words, the concentrations in mushrooms and seafood can be used as an indicator of radionuclide contamination in that particular environment (de Castro et al. 2012). Due to concerns of high levels of food radionuclide contamination after the Fukushima incident, fish, animal milk (cow and goat), grass, wild berries, and mushrooms have been monitored to assess contamination levels (Beresford et al. 2012; Gwynn et al. 2013; Ioannidou et al. 2012).

Food Contamination

Radionuclide contamination in the food chain is evident, but usually at low levels. However, concerns of higher levels become more apparent in the food chain top predators due to their ability to bioaccumulate. For instance, concentrations of radiocesium were studied in the web spider *Nephila clavata* L. Koch – a predator and food source in the forest ecosystem – after the Fukushima Daiichi Nuclear Power Plant disaster (Ayabe et al. 2014). Ayabe and coworkers found that spiders closer to the disaster had higher radiocesium concentrations than spiders farther away from the site. The authors went on to suggest that the higher radiocesium concentration was indicative that the radiocesium had traveled through the food chain to the higher trophic levels.

The radioisotopes ^3H , ^{89}Sr , ^{90}Sr , ^{131}I , ^{134}Cs , and ^{137}Cs are the most important radionuclides found in foodstuff (Nollet 2004). Nuclear power plants are noted as the primary source of anthropogenic radionuclide, food contamination, particularly for ^{137}Cs , although natural radionuclides such as ^{40}K can be found in foods eaten daily (Suzuki 2013). For example, after Soviet testing of nuclear weapons in the late 1950s and early 1960s, concentrations of ^{90}Sr and ^{137}Cs were observed in milk in New York, thus demonstrating the ability of long-range contaminant transport to food products and the environment (Crosby 1998). Additionally, as previously mentioned, animal milk, mushrooms, and wild berries have also been noted to contain radionuclide contamination.

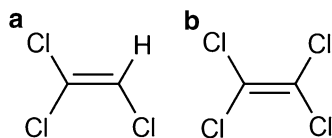
Analytical Detection

Given that radionuclide contamination in food is generally found at low concentrations, precise and sensitive radionuclide detection may be particularly important in some instances, such as evaluating levels of short- and long-range transport of radionuclides to food and the environment after nuclear disasters. Detection of radionuclides is determined using varying separation methods coupled with radiochemical detectors, including gamma spectrometry, alpha spectrometry, liquid scintillation counting, and gas proportional counting. In foodstuff, radio-HPLC and liquid scintillation counters are primarily used due to the low concentrations present (Nollet 2004). Radionuclides that are gamma-emitting are measured primarily through gamma-ray spectrometry of ^{131}I , ^{134}Cs , and ^{137}Cs (Nollet 2004). Beta-emitting radionuclides, ^{89}Sr and ^{90}Sr , are detected using gas proportional counting methods and other beta-detecting methods (Nollet 2004). Alpha spectrometry is used for alpha-emitting radionuclides such as ^{234}U and ^{235}U , while liquid scintillation is used for ^3H detection (Nollet 2004).

Toxicology

Although radionuclide contamination is an important concern, it is imperative to note that humans are exposed to radionuclides on a daily basis, with 81 % of all exposure being from natural and only 19 % from artificial sources (de Castro et al. 2012). Internally deposited beta- and alpha-emitting radionuclides are listed as Class 1 carcinogens by the International Agency for Research on Cancer (IARC 2014). Although the toxicological endpoints are similar for each

Fig. 5 (a) Trichloroethylene (TCE) (b) Perchloroethylene (PCE or Perc)



radionuclide, there are differences in mechanism due to routes of exposure, absorption mechanism, and other factors. For instance, due to radium's close chemical resemblance to calcium, it deposits to the bone, causing blood diseases and tumors (Crosby 1998). Uranium is also deposited into the bone, in addition to the lungs, and can cause skin lesions and kidney disease (Crosby 1998). Little plutonium is absorbed through the skin or GI tract; rather absorption primarily occurs through the lungs, causing lung and bone cancers (Crosby 1998). Radionuclides are ubiquitous natural and anthropogenic contaminants found throughout the environment, including food. Ultimately, controlling anthropogenic sources of radionuclides is the primary way to reduce its contamination, since long-range transport and nuclear fallout distributes radionuclides worldwide – leading to contamination in soils and waterways and eventually transporting to food.

Trichloroethylene and Perchloroethylene

Chlorinated solvents are notorious environmental contaminants in a variety of environmental compartments. For instance, trichloroethylene (TCE) and perchloroethylene or tetrachloroethylene (PCE or perc) (Fig. 5) are ground- and surface water contaminants regularly found in drinking water. The compounds 1,1,1-trichloroethane and Freon-113 (1,1,2-trichloro-1,2,2-trifluoroethane) are other chlorinated solvents that have contributed to ozone depletion in the upper atmosphere and were consequently banned in the Montreal Protocol, but will not be thoroughly discussed in this chapter due to their low relevance to food contamination (Morrison 1999). Chlorinated solvents are important industrial commodities not only as degreasers and cleansers but are also used intermediate compounds in industrial synthesis and in electronics and adhesives (Morrison 1999; US EPA 2011, 2012; US ATSDR 1997a, b). Exposure to chlorinated solvents mostly occurs from occupational exposure, inhalation, and contaminated drinking water but can also be present in foodstuff.

Fate and Transport in Food and the Environment

Chlorinated solvents tend to be volatile and are slightly to moderately soluble in water. In surface water the majority of the solvent evaporates to the atmosphere (Morrison 1999; US ATSDR 1997a, b; US ASTDR 2013). Chlorinated solvents can readily leach through the soil matrix and consequently into groundwater; however, the organic carbon content of the soil may decrease mobility and transport of the chlorinated solvents. For instance, trichloroethylene and perchloroethylene have the tendency to sorb to organic carbon; thus mobility decreases with an increase in organic matter (US ATSDR 1997a, b; US ASTDR 2013).

Degradation of chlorinated solvents in the atmosphere occurs via photooxidation with hydroxyl radicals ($\cdot\text{OH}$), the likely degradation pathway due to chlorinated solvent volatility (US ATSDR 1997a, b; US ASTDR 2013). Hydrolysis and microbial degradation are limited to certain conditions, but perchloroethylene has been reported to biodegrade to trichloroethylene and then further to dichloroethylene (US ATSDR 1997a, b; US ASTDR 2013). Biodegradation of haloethenes (trichloroethylene and perchloroethylene) may be biostimulated in contaminated soil. Aerobically, trichloroethylene can also be oxidized to the epoxide form in a process known as co-metabolism. The epoxide form can then either spontaneously bind to molecules or hydrolyze to a trichloroethylene diol and dichloroacetic acid (Sylvia et al. 2005). Anaerobically, trichloroethylene and perchloroethylene can be used as terminal electron acceptors, called dehalorespiration, but this process is limited by the availability of organic substrates or availability of more favorable electron acceptors (Sylvia et al. 2005). Microorganisms can reduce perchloroethylene to trichloroethylene to dichloroethylene, then vinyl chloride, and finally ethylene (Sylvia et al. 2005). Dehalorespiration must be stimulated in situ to bioremediate contaminated soils by drilling wells and injecting 1 % methane in air into the soil, thereby stimulating methanotrophic growth and increasing anaerobic dechlorination (Sylvia et al. 2005). This tactic has been successfully used to reduce haloethene soil and groundwater contamination in Aiken, South Carolina (Sylvia et al. 2005).

Plant uptake of trichloroethylene has been noted to occur primarily through foliage rather than roots. In contrast, the plant uptake for perchloroethylene has not been well researched, but plant uptake is a possible explanation for their presence in crops (US ATSDR 1997a, b; US ASTDR 2013). Water contamination is a concern with chlorinated solvents, with trichloroethylene posing a low to moderate bioaccumulation potential in aquatic organisms (US ATSDR 1997a, b; US ASTDR 2013). Perchloroethylene, however, is not considered to undergo biomagnification in the terrestrial food chain since it is successfully metabolized by animals to trichloroacetic acid (US ATSDR 1997a). Groundwater and surface water contamination with chlorinated solvents have been a recurring problem, resulting in exposure to humans through drinking water, foods in contact with water, and through bathing and showering.

Food Contamination

As noted, the highest concentration of trichloroethylene contamination is found in foods with high fat content, in the 1–100 $\mu\text{g}/\text{kg}$ range (US EPA 2011). A 5-year study (1996–2000) in the United States found that 30 off-the-shelf table-ready foods had trichloroethylene contamination, with an average between 2 and 10 ppb (US ASTDR 2013). Potato chips contained the highest concentration of trichloroethylene at 140 ppb, followed by beef frankfurters, 105 ppb; raw avocados, 75 ppb; and chocolate cake with icing, 57 ppb (US ASTDR 2013). Citing preliminary data, the US EPA estimates a human daily exposure of about 5 μg , with dairy products accounting for approximately 1.68 μg of the daily estimate (US EPA 2011).

Perchloroethylene, most notably used in dry cleaning, has been found at higher levels in stores and kitchens near dry cleaners (Grob et al. 1990). In margarine,

higher concentrations of perchloroethylene have been detected along the outside of the stick and decreasing towards the middle, showing that contamination is likely occurring post-processing (US ATSDR 1997a). Although perchloroethylene contamination is not considered a major exposure pathway, in a 1985 report, the US EPA estimated between 0 and 4 μg of average daily intake of perchloroethylene occurred from dairy products, while poultry, meat, and fish represent 0–1 $\mu\text{g}/\text{day}$, fats and oils exposure contributed to 0–0.95 $\mu\text{g}/\text{day}$, and beverage 0–0.06 $\mu\text{g}/\text{day}$ (US EPA 2012; US ATSDR 1997a).

Analytical Detection

Chlorinated solvent contamination has been observed in a variety of foodstuff and sources comprising transportation through air, irrigation water, food processing, and packaging (Grob et al. 1990; US ATSDR 1997a, b; US ASTDR 2013). These solvents have been found in fruits, vegetables, dairy, meat, beverages, and bread, but due to their lipophilicity, foods with higher fat content tend to have higher concentrations. Tracking the pathway of contaminants to food is often difficult, but as demonstrated by Grob and coworkers (1990), volatile compounds, such as perchloroethylene and trichloroethylene, can be absorbed by food from the air. Detection in food is primarily conducted with GC coupled typically with mass spectrometry, electron capture detector, or Hall's electrolytic conductivity detector (HECD) (US ATSDR 1997a, b; US ASTDR 2013). Headspace analysis provides a sensitive and effective means for analyzing trichloroethylene and perchloroethylene, but in trichloroethylene samples, increased lipid content may interfere with accuracy (US ATSDR 1997a, b; US ASTDR 2013).

Toxicology

It should be emphasized that trichloroethylene and perchloroethylene exposure is not primarily from food; rather air and drinking water are the largest exposure sources, outside of industrial sources and worker exposure to vapors and liquid phase solvents. Since levels are low in foods, more likely, any exposure from food would contribute to chronic exposure end points. The US EPA *Guidelines for Carcinogenic Risk Assessment* classifies trichloroethylene as a carcinogen by all routes of exposures while listing tetrachloroethylene as a "likely to be carcinogenic by all routes of exposure." The International Agency for Research on Cancer classifies trichloroethylene as a Group 1 carcinogen and perchloroethylene as a Group 2A or probably carcinogen (IARC 2014; US EPA 2011, 2012). Trichloroethylene has been linked to kidney cancer, non-Hodgkin's lymphoma, and liver cancer; in addition to non-carcinogenic endpoints, such as liver and kidney damage, impaired immune system, and, possibly, developmental toxicity (US EPA 2011). Chronic effects of trichloroethylene are not completely known, but it is linked to developmental toxicity and liver and kidney damage, in addition to liver and kidney cancers. The nervous system is one of the main targets for PCE and trichloroethylene, posing chronic, subchronic, and acute end points. These solvents act on sodium channels, neuronal calcium channels, nicotinic acetylcholine receptor, the GABA receptors, and glycine receptors (Bale et al. 2011). Both trichloroethylene

and perchloroethylene have been shown to block sodium and calcium channels, inhibit excitatory receptors, and potentiate inhibitory receptors (Bale et al. 2011).

Although the use of trichloroethylene and perchloroethylene has significantly declined, the chemical legacy of chlorinated chemicals in the environment means exposure will likely exist. Though exposure from food likely is not the major route of exposure, chlorinated solvents can still transport to food through contaminated air and may also occur from contaminated water or food processes.

Persistent Organic Pollutants

As defined by the World Health Organization (WHO), persistent organic pollutants or POPs are a class of organic compounds that are anthropogenically produced or influenced. These compounds are toxic and resist degradation. Many but not all of these compounds fall under the US Environmental Protection Agency's (EPA) list of persistent, bioaccumulative, and toxic (PBT) chemicals. Halogenated organic compounds comprise the majority of persistent organic pollutants, which include organochlorine pesticides, dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), dioxins, and brominated flame retardant, to name a few. This section serves to provide a brief overview of some common but critical persistent organic pollutants found in foods, humans, and the environment.

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons, or polynuclear aromatic hydrocarbons (PAHs), are among the most well-known industrial and cooking-related food contaminants. Polycyclic aromatic hydrocarbons were first recognized in the early 1900s as suspected carcinogens in soot and coal tar, responsible for scrotal cancer in chimney sweeps (Fu et al. 2012). The abundance of polycyclic aromatic hydrocarbons in the environment and their subsequent carcinogenicity were identified early in their history, and as a result they are among the most studied carcinogens. These generally planar and hydrophobic compounds are generated during incomplete combustion. While industrial processes are not the sole cause, they are a major source of polycyclic aromatic hydrocarbons in the environment, along with emissions from transportation exhaust. Additionally, volcanic activity, forest fires, the burning of plant material, crude oil and other fossil fuels, tobacco smoke, asphalt roadways, rooftop coverings, and food preparation processes all contribute to polycyclic aromatic hydrocarbon exposure and environmental burden. Essentially, under the right conditions, all compounds containing carbon and hydrogen are potential polycyclic aromatic hydrocarbon precursors (Purcaro et al. 2013). At higher temperatures (500–700 °C), organic compounds can fragment into radicals and then reconfigure into polycyclic aromatic hydrocarbons (Purcaro et al. 2013), but can also form under lower temperatures (100–150 °C) over geologic-scale time frames, with petroleum as an example (Purcaro et al. 2013).

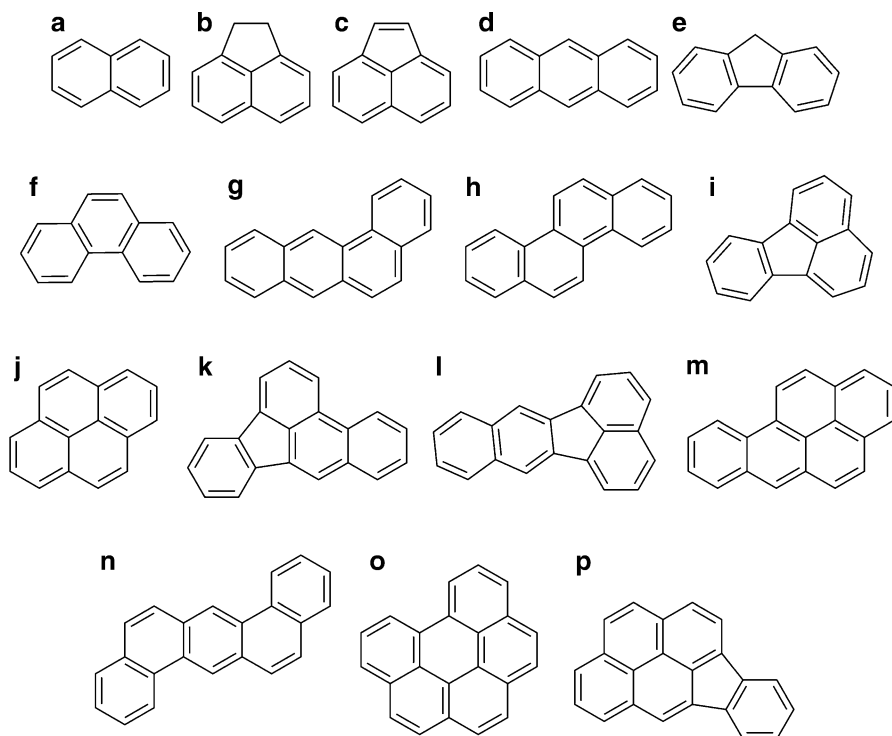


Fig. 6 The EPA's original 16 priority PAHs: (a) naphthalene, (b) acenaphthene, (c) acenaphthylene, (d) anthracene, (e) fluorene, (f) phenanthrene, (g) benz[*a*]anthracene, (h) chrysene, (i) fluoranthene, (j) pyrene, (k) benzo[*b*]fluoranthene, (l) benzo[*k*]fluoranthene, (m) benzo[*a*]pyrene, (n) dibenz[*a,h*]anthracene, (o) benzo[*ghi*]perylene, (p) indeno[1,2,3-*cd*]pyrene

The name polycyclic aromatic hydrocarbons is an overarching term that primarily describes two or more fused aromatic rings and is often delineated into light (less than four aromatic rings), intermediate (four aromatic rings), and heavy (more than four aromatic rings) subclasses (Fig. 6) but includes other related species such as nitro-PAHs, nitroxy-PAHs, and other oxygenated PAHs (Fig. 7) (Desalme et al. 2013; Fu et al. 2012; Kielhorn et al. 2003). Nitro- and nitroxy-PAHs are less abundant and primarily are a part of particulate matter or in the vapor phase (Fu et al. 2012; Kielhorn et al. 2003), and their formation is similar to typical polycyclic aromatic hydrocarbons. They are also formed through atmospheric reactions of polycyclic aromatic hydrocarbons with nitrogen dioxide (Fu et al. 2012; Kielhorn et al. 2003). Other aromatic rings may be present alongside of PAH mixtures, such as heterocyclic aromatic compounds, including carbazole and acridine (Plaza-Bolaños et al. 2010). Benzo(α)pyrene (Fig. 6), also known as B(α)P, B(a)P, or BaP, is a carcinogen and classic example of a polycyclic aromatic hydrocarbon that has been used as an indicator of overall contamination in foodstuff (Purcaro et al. 2013). Polycyclic aromatic hydrocarbons are not

Fig. 7 Examples of nitro- and nitroxy-PAHs, respectively: (a) 2-nitropyrene and (b) 3-nitrobenzanthrone

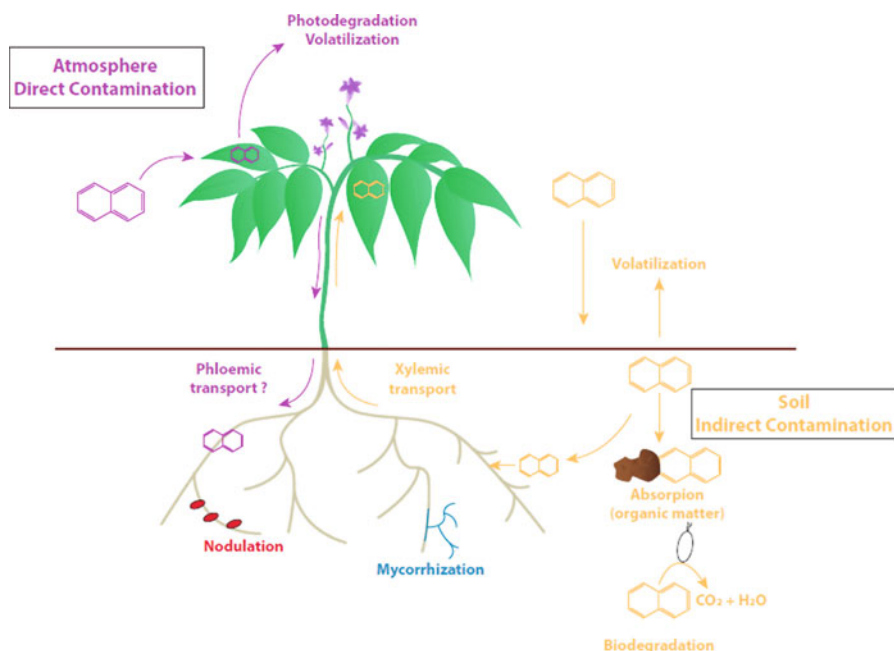
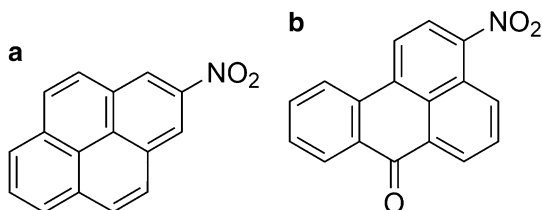


Fig. 8 This figure illustrates the fate and transportation of PAHs, including volatilization, deposition, plant uptake and translocation, soil transport, and degradation (Reprinted with permission from Desalme et al. (2013). Copyright 2013 American Chemical Society)

carcinogenic on their own but are activated via xenobiotic metabolism to their carcinogenic forms. Not all polycyclics are carcinogenic, some may act as synergists, and many others are not classified; albeit, the US EPA has defined priority polycyclic aromatic hydrocarbons with the original 16 seen in Fig. 5 (Hampikyan and Colak 2010). This topic will be covered extensively later in this section.

Fate and Transport into Food and the Environment

Polycyclic aromatic hydrocarbons are transported from industrial sources into food through all environmental compartments (Fig. 8), although emissions are primarily through air. Polycyclic aromatic hydrocarbons are semivolatile, hydrophobic compounds that may stick to particulates or remain in the vapor phase and travel great

distances. The size of the polycyclic aromatic hydrocarbons characterizes what phase will partition in the air – where light PAHs (two–three rings) will remain in the gaseous phase, intermediate PAHs (four rings) will occupy both gaseous and particulates, and heavy PAHs (greater than four rings) will primarily deposit onto particulates (Desalme et al. 2013). In the atmosphere, polycyclic aromatic hydrocarbons are subjected to photodegradation, radical, hydroxyl, nitrate and ozone reactions, dissipation, and deposition – depending on weather and climate conditions. Particulate polycyclic aromatic hydrocarbons deposit more rapidly compared to gaseous, where concentration in the air decreases with distance from the source, while gaseous polycyclic aromatic hydrocarbons are subject to long-range atmospheric transport and, therefore, are the dominant class of atmospheric polycyclic aromatic hydrocarbon exposure (Desalme et al. 2013).

Particulate and gaseous polycyclic aromatic hydrocarbons undergo wet and dry deposition, although wet deposition is more limited due to their hydrophobicity, onto surface waters and land. In addition to deposition, polycyclic aromatic hydrocarbons may also enter into waters through industrial discharges and wastewater treatment plants, where they tend to partition into the nonpolar, lipophilic fractions (Hampikyan and Colak 2010). Deposition onto land plays an essential role in food contamination and the polycyclic aromatic hydrocarbon burden present in the environment. The nonpolar, organic fraction of soil drives partitioning of polycyclic aromatic hydrocarbons into the soil and sediment organic matter, due to their lipophilicity (Hampikyan and Colak 2010).

Plants are susceptible to acquiring polycyclic aromatic hydrocarbons from contaminated soil and air. Polycyclic aromatic hydrocarbons can move from the roots up to the shoots, although the light and intermediate PAHs (ones with K_{ow} less than five) are the only ones capable of translocation in the plant (Desalme et al. 2013). Vascular plants may take up polycyclic aromatic hydrocarbons from the roots and transport them throughout the plant, although even the most water-soluble polycyclic aromatic hydrocarbons are tightly bound to soil particles and expected to have low desorption ability and bioavailability (Desalme et al. 2013). This contamination route is particularly important in soils with high PAH contamination (>10 – 100 mg PAH per kg of dry soil) (Desalme et al. 2013). Uptake of polycyclics by roots increases with increasing $\text{Log } K_{ow}$ (octanol–water partition coefficient), but is limited with increasing molecular weight, nonetheless, explaining why PAHs are positively influenced by increasing lipid root content (Desalme et al. 2013). Mycorrhizal fungi may also influence PAH concentrations; particularly, arbuscular and vesicle mycorrhizal fungi may enhance uptake and positively influence root-to-shoot translocation as seen in maize (Desalme et al. 2013). Microbial degradation by soil fungi and bacteria is the largest degradation pathway, occurring both aerobically and anaerobically (Haritash and Kaushik 2009).

Contamination from airborne polycyclic aromatic hydrocarbons may be one of the more significant routes for plants, occurring through three routes: diffusion, particle deposition, and gaseous deposition. Surface area, orientation, and hairiness of leaves influence the interception and uptake of PAH deposition, particularly the retention of particle-phased PAHs that are retained in higher amounts on leaves

with more hair (Desalme et al. 2013; Hampikyan and Colak 2010). The waxy hydrophobic cuticle on the surface of plants retains deposited PAHs and increases absorption either within the cuticle or into the plant tissue (Desalme et al. 2013). Absorption into plant tissue can also occur through the stomata; nevertheless, both methods are time dependent and related to molecular weight, where heavy PAHs remain absorbed to the cuticle and lighter PAHs are more easily absorbed due to their lower K_{ow} and subsequent increased water solubility (Desalme et al. 2013). Particulate matter containing higher molecular weight PAHs can wash off the leaf surfaces, since they are more likely to be present on the cuticle surface due to slow diffusion rates from particulate matter (Desalme et al. 2013). Similar to contamination from the soil, polycyclics absorbed into the plant may translocate systemically depending on the physiochemical properties of the PAHs (e.g., water solubility) and the plant species (Desalme et al. 2013).

Polycyclic aromatic hydrocarbon deposition and diffusion play important roles in contamination of produce and thus can be a significant source of human PAH exposure from food. This is primarily due to the ability of PAHs to sorb to the waxy surfaces of fruits and vegetables, particularly leafy vegetables (Hampikyan and Colak 2010; Plaza-Bolaños et al. 2010). Geographic location and proximity to pollution sources positively influences the concentration of PAHs found in foods. Vegetable oils and fats, such as olive oil, butter, margarine, coconut oil, etc., tend to contain higher levels of PAH contamination due to their lipophilicity (Hampikyan and Colak 2010; Purcaro et al. 2013). Among seafood, PAHs are typically low in fish, precooking, compared to invertebrates, likely due to their ability to metabolize and excrete PAHs unlike filter feeders, such as bivalves and mollusks (Purcaro et al. 2013). Additionally, milk contains an average PAH level of 0.99 $\mu\text{g}/\text{kg}$ according to one commercial milk study, which found that environmental conditions such as smoke exposure, grass PAH content, and feedstock affected the abundance of PAHs found in milk (Hampikyan and Colak 2010).

Food Contamination

The contamination of PAHs in food can generally be traced to either food preparation or environmental contamination. Organic compounds in food are converted to polycyclic aromatic hydrocarbons during roasting, smoking, grilling, frying, baking, and other cooking processes (Hampikyan and Colak 2010). The formation of PAHs in foods is dependent upon temperature, fat content, proximity from the heating source, duration of cooking, and method of cooking (Hampikyan and Colak 2010). The smoke from pyrolysis of the wood contributes to PAH content of foods, in addition to PAHs formed on the food itself, where there is a linear relationship between PAH generated in smoked between 400 and 1,000 °C (Hampikyan and Colak 2010). During grilling, PAHs are generated from the pyrolysis of fat from direct contact between the flame and meat; additionally, any fat that drips into the coals or flames can generate PAHs that may be transported back to the meat (Hampikyan and Colak 2010). Concentrations of nitro-PAHs in foods are typically less than 5 $\mu\text{g}/\text{kg}$, except grilled or smoked foods and spices (Kielhorn et al. 2003). Drying of foods, such as fruits, has been noted to result in significant PAH levels

(Purcaro et al. 2013). Contrarily, foods that are fried or roasted produce less PAH in comparison to charred foods (Hampikyan and Colak 2010). Additionally, polycyclic aromatic hydrocarbon content found in foodstuff may also be from contact with contaminated packaging or food-grade mineral oil and technological processes (Purcaro et al. 2013).

Although contamination and cooking processes can significantly influence PAH content, natural or background levels of PAHs do exist. For instance, after the Deepwater Horizon oil spill, several surveys were conducted in the Gulf of Mexico to determine the concentration of pollutants in seafood, including PAHs. Studies concluded that the oil spill had little impact on PAH concentration in fish, oyster, crabs, and shrimp, with concentrations <10 ppb for >90 % of the samples taken, which was within background PAH range and well below the US Food and Drug Administration's (FDA) Levels of Concern (LOC) (Armburst et al. 2013).

Analytical Detection

Polycyclic aromatic hydrocarbons are found as mixtures in foods and not as individual compounds. Therefore detection of polycyclics are often noted as total PAH concentration. As previously mentioned B(a)P has been used as a marker for overall PAH content, but it may represent only 1–20 % total PAH content and thus is not necessarily an effective marker of total PAH content (Plaza-Bolaños et al. 2010). Extraction is typically separated between fatty and nonfatty matrices, where lower concentrations are expected in nonfatty matrices due to their lipophilicity. Liquid–liquid extraction, Soxhlet, pressurized liquid extraction, and ultrasound extraction methods are used in nonfatty matrices, followed by solid-phase extraction (SPE) cleanup prior to chromatographic analysis (Plaza-Bolaños et al. 2010). PAH extraction from nonpolar matrices is typically more difficult and time-consuming due to the high lipophilicity of PAHs and thus can require saponification, liquid–liquid extraction, and solid-phase extraction or other cleanup steps (Plaza-Bolaños et al. 2010; Purcaro et al. 2013). Because of the semivolatile nature of some PAHs, solid-phase microextraction (SPME) and other volatile headspace collection methods can be used in some instances (Plaza-Bolaños et al. 2010). Analyses are usually conducted using high-performance liquid chromatography (HPLC) or capillary gas chromatography (GC) coupled with mass spectrometry (MS), fluorescence (FLD), or ultraviolet (UV) detectors, or using GC-MS, in which LC-FLD and GC-MS are the primary detector choices in food (Plaza-Bolaños et al. 2010; Purcaro et al. 2013). Liquid chromatography–fluorescence (LC-FLD) is the primary choice for many PAHs, including the 16 priority PAHs listed by the EPA. However, this method can be limiting for a small number of PAHs that have low fluorescence absorbance, and as such GC-MS has advantage over, in addition to having high chromatographic resolution (Plaza-Bolaños et al. 2010; Purcaro et al. 2013).

Toxicity

As discussed previously, the carcinogenicity of polycyclic aromatic hydrocarbons has been well studied (Table 2) and the polycyclics require metabolic activation in

Table 2 The International Agency for Research on Cancer's carcinogenicity classifications for the original 16 priority PAHs as defined by the US EPA (IARC 2014)

	Classification	Year
Naphthalene	2B	2002
Acenaphthene	3	2010
Acenaphthylene	(Not listed)	
Anthracene	3	2010
Phenanthrene	3	2010
Fluorene	3	2010
Chrysene	2B	2010
Benz[<i>a</i>]anthracene	3	2010
Fluoranthene	3	2010
Pyrene	3	2010
Benzo[<i>b</i>]fluoranthene	2B	2010
Benzo[<i>k</i>]fluoranthene	2B	2010
Benzo[<i>a</i>]pyrene	1	2010
Dibenz[<i>a,h</i>]anthracene	2A	2010
Benzo[<i>ghi</i>]perylene	3	2010
Indeno[1,2,3- <i>cd</i>]pyrene	2B	2010

Group 1: carcinogenic to humans

Group 2A: probably carcinogenic to humans

Group 2B: possibly carcinogenic to humans

Group 3: not classifiable as to its carcinogenicity to humans

Group 4: probably not carcinogenic to humans

order to be genotoxic. There are three mechanisms leading to the initiation of cancerous activity, epoxidation, particularly in the bay region of the molecule, and may be referred to as *bay*-region diepoxides, quinones, and radical-cation intermediates (Fu et al. 2012). Once activated to their less stable states (epoxides, quinones, and radicals) by Phase I cytochrome P450 enzymes, PAHs may bind to DNA, proteins, or other biologically important compounds. Polycyclic aromatic hydrocarbons are also phototoxic, and thus if present on the skin, can be activated by light through photooxidation forming peroxides and quinones that can cause cytotoxicity and genotoxicity (Fu et al. 2012). Nitro-PAHs are mutagenic and tumorigenic and can be activated similarly to form epoxides and quinones but may undergo additional activation including nitroreduction or subsequent esterification (particularly acetylation), among other combinations of the two with oxidation (Kielhorn et al. 2003). Phase II cytochrome P450 enzymes can detoxify reactive species through conjugation of glutathione, glucuronic acid, or sulfate, therefore highlighting the built-in mechanism to detoxify activated Phase I metabolites, although this does not mean it always occurs.

Polycyclic aromatic hydrocarbons are ubiquitous and potentially carcinogenic contaminants in foods that are consumed on a daily basis, and thus food consumption is an important exposure pathway. Transport to foods primarily occurs through soil or air, while formation in food is attributed to cooking processes. Industries such as coal-burning power plants, oil refining, or transportation contribute significantly to the PAH load in the environment originating from several sources – fossil

Fig. 9 Depiction of the general structures of (a) polychlorinated biphenyls (PCBs), (b) polychlorinated dibenzo-*p*-dioxins (PCDDs), (c) polychlorinated dibenzofurans (PCDFs)

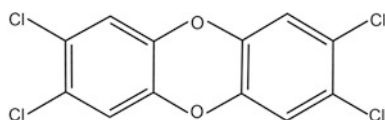
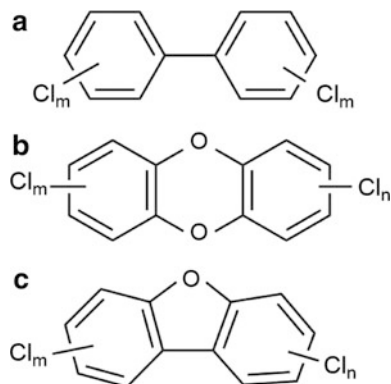


Fig. 10 2,3,7,8-Tetrachlorodibenzodioxin (TCDD), considered one of the most toxic human-generated chemicals

fuels, exhaust, wastewater discharge, and any other sources of incomplete combustion. Polycyclic aromatic hydrocarbons are important food contaminants, and because their formation is from incomplete combustion, they will continue to hold concern in food and the environment.

Polychlorinated Biphenyls and Associated Dioxin and Furan Forms

Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDD or dioxins), and polychlorinated dibenzofurans (PCDF or Furans) (Fig. 9) represent large classes of persistent industrial contaminants and are a large concern for both ecological and human toxicity. For instance, TCDD (2,3,7,8-tetrachlorodibenzodioxin) (Fig. 10) is often labeled as one of the most toxic human-generated chemicals, known for its infamous contamination in the military-used herbicide Agent Orange; contamination in Times Beach, Missouri; and from the Seveso disaster in Italy. Polychlorinated biphenyls and the associated dioxin and furan forms are all complex mixtures of several different congeners listed in both the persistent organic pollutants and persistent, bioaccumulative, and toxic (PBT) categories. Due to their continuance, bioaccumulative abilities, and lipophilic characteristics, exposure to humans through food is particularly common in fish, meat, and dairy.

Polychlorinated biphenyls are a large class of organochlorines containing 209 theoretical congeners, and 12 non-ortho and mono-ortho congeners, which are described as dioxin-like polychlorinated biphenyls due to their toxicological similarity to 2,3,7,8-tetrachlorodibenzodioxin (TCDD) (Fig. 10) (Larsen 2006;

Ross 2004). Of the large number of congeners, 17 are known to be toxic (Larsen 2006). Polychlorinated biphenyls, which are now banned, were produced through catalytic chlorination, using ferric chloride or iron filling, in a mixture of anhydrous chlorine and biphenyls (Urbaniak 2007). Lower chlorination compounds such as the mono- through tetra-chlorination vary in viscosity and range from mobile liquids to oils. Polychlorinated biphenyls with higher degrees of chlorination range from resins to greases to waxes (Urbaniak 2007), have varying degrees of chlorination, and are used in different commercial products. For instance, Arochlor, Clophen, Phenochlor, and Kanechlor are among some of the polychlorinated biphenyl products produced by various companies (Urbaniak 2007). Polychlorinated biphenyls are nonflammable; have high heat capacities, low dielectric currents, and low acute toxicity; are chemically inert; and contain high electric resistance (Ross 2004; Urbaniak 2007). These characteristics made them optimal for a wide range of uses including coolants, flame retardants, hydraulic fluids, dielectric fluids, plasticizers, adhesives, and sealants, among others (Ross 2004; Urbaniak 2007). Unlike polychlorinated biphenyls, the associated dioxin and furan forms are not intentionally produced compounds but are contamination products produced in combustion processes, notably waste incineration (Focant et al. 2004).

Fate and Transport into Food and the Environment

Polychlorinated biphenyls and the associated dioxin and furan forms are lipophilic compounds resistant to degradation and can bioaccumulate within food chains, with meat, dairy, and fish contributing greater than 90 % to human exposure (Domingo and Bocio 2007; Hoffman et al. 2006; Weber et al. 2008). Persistent organic pollutants are generally considered immobile in the environment, due to their high hydrophobicity and affinity for organic matter. However, leachate from landfills can contaminate groundwater as well as other remobilization pathways and thus may contribute to human exposures (Weber et al. 2008). Generally, their solubility in water is low and decreases with an increase in chlorination. Concurrently, volatilization also decreases with increased chlorination (Urbaniak 2007). Although polychlorinated biphenyls are generally present in low concentrations in the atmosphere, through air is the most significant transportation route where lower chlorinated polychlorinated biphenyls tend to be in the gaseous phase and higher chlorinated PCBs partition to particulates and aerosols (Urbaniak 2007). Deposition of particulate and aerosols have a shorter life in the atmosphere due to their susceptibility to washing out wet and dry deposition, while the hydrophobicity of the gaseous low-chlorinated polychlorinated biphenyls are more resistant to wet deposition, and are thus the dominant form present in the atmosphere (Urbaniak 2007). The emissions of the associated dioxin and furan forms of polychlorinated biphenyl are often associated with short-range transport, such as waste incineration, but their emission is also subjected to long-range transport and atmospheric deposition (Weber et al. 2008). The presence of polychlorinated biphenyls and the associated dioxin and furan forms (PCDD/Fs) tend to be low in the organic matter of soils, with greatest contamination and abundance in areas such as those that are close to: contaminated sites from plant discharges, application of sewage sludge to

agricultural land, landfills, improper disposal or storage of chemicals, or areas associated with chemical spills or accidents (Urbaniak 2007; Weber et al. 2008). Discharges of polychlorinated biphenyls and the associated dioxin and furan forms into water result in considerable burdens in sediments, organic matter, suspended particles, and biota, with varying degrees of sorption depending on the degree of chlorination (Urbaniak 2007; Weber et al. 2008).

Food Contamination

The transportation of polychlorinated biphenyls and the associated dioxin and furan forms to food occurs through a variety of routes, many of which are closely associated with their environmental fate. Proximity to point sources and atmospheric deposition are two of the major pathways by which these organochlorines may reach crops and livestock. The ability for these compounds to bioaccumulate in both aquatic and terrestrial systems provides the means for these compounds to detect food. Unless they can be metabolized and excreted, polychlorinated biphenyls will partition into fat and thus become an issue in fish (Ross 2004; Urbaniak 2007; Weber et al. 2008). Fish and other seafood may be one of the most important exposure routes to humans for polychlorinated biphenyls and the associated dioxin and furan forms with an estimated contribution of 55 % and 31 %, respectively, of the total exposure (Domingo and Bocio 2007).

The contamination of polychlorinated biphenyls and the associated dioxin and furan forms in feedstock are also an important route of exposure and bioaccumulation in terrestrial animals. Stricter monitoring of feedstock contamination has been implemented since 200 L of oil contaminated with polychlorinated biphenyl was used for the production of pig and chicken feeds in Belgium (Larsen 2006). Hogs and poultry have less contact with soil since they are often kept indoors; therefore direct contamination from the soil is not a significant route, particularly when compared to any feedstock sources (Hoffman et al. 2006). Alternatively, cows are known to ingest several kilograms of soil as they graze; therefore, the animal may obtain polychlorinated biphenyls and the associated dioxin and furan forms from contaminated grass and soil, contributing to beef, milk, and other dairy product contaminations (Hoffman et al. 2006). Many routes like deposition can contribute to polychlorinated biphenyl presence in soil. It has been noted that sewage sludge applied to agricultural fields has been a source for contamination in eggs, milk, and vegetables (Weber et al. 2008). Anticaking agent like clay material (ball clay) has been traced to contaminated poultry and some fish (Focant et al. 2004; Hoffman et al. 2006; Schoeters and Hoogenbom 2006). A higher contamination risk of the associated dioxin and furan forms and dioxin-like polychlorinated biphenyls has been extensively reviewed in free-range chickens and their eggs, with organic and home-raised chickens being particularly susceptible. However, levels are not necessarily above acceptable limits (Schoeters and Hoogenbom 2006). The review attributed contamination increase to soil contact, either the soil itself or organisms in the soil contaminated with polychlorinated biphenyls and the associated dioxin and furan forms (Schoeters and Hoogenbom 2006).

Analytical Detection

Concentrations of polychlorinated biphenyls and the associated dioxin and furan forms are often described in toxicological equivalents (TEQs) due to the many congeners in terms of the dioxin form of polychlorinated biphenyl, which is the most toxic chemical of the three classes. Extraction of these chemicals from foods and other matrices can be quite extensive and laborious, particularly since they can be present in concentrations as low as pico- or femtogram per gram of matrix (Focant et al. 2004). Automated systems such as solid-phase extraction, pressurized liquid extraction, microwave-assisted extraction, and supercritical fluid extractions have been used to increase detection limits, followed by cleanup and gas chromatography coupled with MS, tandem mass spectrometry (MS/MS), or time-of-flight (TOF) detectors (Focant et al. 2004).

Toxicology

Polychlorinated biphenyls and the associated dioxin and furan forms can yield a string of toxicological end points, including carcinogenicity, immunotoxicity, dermal toxicity, developmental toxicity, reproductive toxicity, and endocrine disruption (Larsen 2006). The aryl hydrocarbon receptor (AhR), common in most animals and tissues, mediates the toxicity, as these compounds bind to the receptor (Larsen 2006; Schoeters and Hoogenbom 2006). Endocrine disruption is primarily linked to dioxin forms, although polychlorinated biphenyls have also been linked, though the consensus is uncertain (Larsen 2006). The International Agency for Research on Cancer has classified the dioxin form of polychlorinated biphenyls as a Group 1 carcinogen, while other dioxin and dioxin-like compounds have been classified as Group 3 carcinogens or not classifiable (Larsen 2006; IARC 2014). Overall, the polychlorinated biphenyls and their associated dioxin and furan forms are common industrial toxicants found in food and thus represent a significant exposure pathway for humans. These compounds have played important roles as ecological and human toxicants for their resistance to degradation, persistence in the environment, and toxicity. Polychlorinated biphenyl contamination has been on the decline since they were banned; thus body burden has declined over the years (Ross 2004). However, these compounds are legacy compounds and their relevance has not vanished and consequently their ecotoxicity and transport to food will be a lasting concern.

Brominated Flame Retardants

Fire safety standards have driven the development and incorporation of flame retardants for use in consumer products. While a variety of different products exist, brominated flame retardants (BFRs) have dominated the market and continue to do so today. Much like other halogenated organic compounds, brominated flame retardants are persistent environmental contaminants found throughout the environment. Antimony oxide (Sb_2O_3) is often mixed with brominated flame retardants and organophosphates, which can also be used as flame retardants, due to their enhancing synergistic effects to reduce flammability (D'Silva et al. 2004;

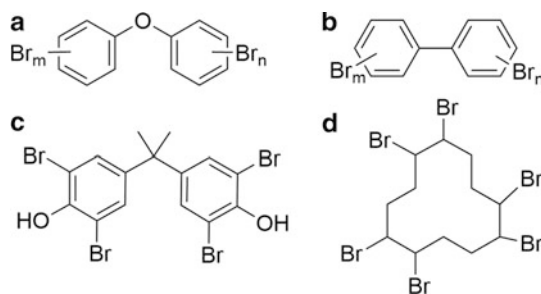


Fig. 11 Structures of common brominated flame retardants: (a) polybrominated diphenyl ethers (PBDEs) and (b) polybrominated biphenyl (PBBs), (c) tetrabromobisphenol A (TBBPA), (d) hexabromocyclododecane (HBCD)

Sjödin et al. 2003). Brominated flame retardants are found in a wide range of products to meet fire safety standards, including building materials, textiles, upholstery, furniture, electronics, and resins. While the content of brominated flame retardants depends on the product, typically polymeric materials contain 5–30 % brominated flame retardants by weight (Sjödin et al. 2003). The abundance of brominated flame retardants in products opens up the opportunity for both point and nonpoint source environmental contamination by many different exposure pathways, including food, dust, mother’s milk, and other consumer products.

Brominated flame retardants fall under two categories, reactive and additive, where reactive flame retardants form covalent bonds within the product’s matrix. Conversely, additive brominated flame retardants do not form covalent bonds within the product matrix and thus are more likely to leach out of the product. Additive brominated flame retardants include polybrominated diphenyl ethers (PBDEs), the most common BFR class, polybrominated biphenyl (PBB), and hexabromocyclododecane (HBCD) (Fig. 11) (D’Silva et al. 2004; Kefeni et al. 2011; Marvin et al. 2011; Sjödin et al. 2003; Yogui and Sericano 2009). Tetrabromobisphenol A (TBBPA) (Fig. 11) is used as both reactive brominated flame retardants in printed circuit boards and additive brominated flame retardants in acrylonitrile–butadiene–styrene (ABS), phenolic, and high-impact styrene resins (D’Silva et al. 2004; Kefeni et al. 2011; Sjödin et al. 2003).

Polybrominated diphenyl ethers are some of the most common brominated flame retardants used and, subsequently, are common environmental contaminants. Polybrominated diphenyl ethers, like polychlorinated and polybrominated biphenyls, have 209 different possible congeners, although they are commercially produced in three degrees of bromination: pentaBDE, octaBDE, and decaBDE (D’Silva et al. 2004; Sjödin et al. 2003; Yogui and Sericano 2009). Each mixture has been used in similar fashions, which include textiles, furniture, appliances, circuit boards, computers, televisions, and other electronics (D’Silva et al. 2004; Kefeni et al. 2011). In 2004, the pentaBDE and octaBDE were banned in the European Union and voluntarily phased out in the United States (Kefeni et al. 2011). Polybrominated biphenyls (PBBs) also have three different commercial mixtures

of bromination – technical hexabromobiphenyl (THBB), technical octabromobiphenyl (TOBB), and technical decabromobiphenyl (TDBB) – although their production has been discontinued (Kefeni et al. 2011). In the United States, THBB was banned in 1977 due to accidental contamination in cattle feed, while TOBB and TDBB were not banned until 1979; however production of TDBB was not discontinued until 2000 in France (Kefeni et al. 2011; Sjödin et al. 2003).

Hexabromocyclododecane (HBCD or HBCDD) has been used as a BFR since the 1960s and is the third most commonly used brominated flame retardant worldwide and second most in Europe (Kefeni et al. 2011). Theoretically, there are 16 stereoisomers of HBCD, with α -, β -, and γ -isomers being the most commonly used forms, but δ - and ϵ -isomers have also been isolated from commercial products (Kefeni et al. 2011; Sjödin et al. 2003; Marvin et al. 2011). Hexabromocyclododecane is primarily used in polystyrene foams, high-impact polystyrene, back coatings of textiles, upholstery, and electronic equipment (Marvin et al. 2011; Sjödin et al. 2003). Due to its persistence and toxicity, the United Nations banned hexabromocyclododecane in 2013 under the Stockholm Convention on Persistent Organic Pollutants. However, the ruling does not go into effect until 2019 to give time for industry and downstream users to switch to other alternatives (Hogue 2013).

Fate and Transport into Food and the Environment

Brominated flame retardants are persistent chemicals in the environment and are capable of undergoing long-range transport and thus are found throughout many food chains and ecosystems. Unlike other persistent organic pollutants which may be considered point source contamination, most brominated flame retardants found in the environment are additive brominated flame retardants that leach out during the products' lifetime and are therefore considered nonpoint source pollutants. However, point sources exist for brominated flame retardants, such as manufacturing sites, electronic waste recycling plants, sewage and wastewater treatment plants, and landfills. Due to their use in building products, cars, furniture, and textiles, brominated flame retardants are common indoor contaminants in air, dust, and particulates, serving as an important route of exposure for humans (Daso et al. 2010; Kefeni et al. 2011).

Generally, brominated flame retardants have low volatility, high lipophilicity, and low water solubility and are resistant to degradation and therefore are very apt to bioaccumulate (D'Silva et al. 2004; Kefeni et al. 2011). Although tetrabromobisphenol A is one of the most widely used flame retardants worldwide, its presence in the environment is lower compared to that of other brominated flame retardants since they are reactive and form covalent bonds within the product's matrix (D'Silva et al. 2004). Although the other brominated flame retardants focused on in this chapter are considered additive, polybrominated diphenyl ethers are often noted with the greatest attention since they have been some of the longest used flame retardants in modern history. As previously mentioned, polybrominated biphenyls were phased out in the United States by the end of the 1970s, while pentaBDEs and octaBDEs were not phased out in the EU till 2004.

Worldwide, brominated flame retardants are often found in soils, sediments, fish, and other biota due to their tendency to partition into hydrophobic media. Concentrations of brominated flame retardants in the environment tend to decrease the further away from urban to rural areas. Nevertheless brominated flame retardants have been found in remote regions, including the Arctic and Antarctica, meaning that their more volatile congeners undergo long-range transport in the atmosphere (Daso et al. 2010; Kefeni et al. 2011; Marvin et al. 2011). Brominated flame retardants are also detected in rivers, lakes, wastewater, and landfill leachate, exemplifying those particular congeners can act outside the general physiochemical properties of these classes (Daso et al. 2010; Kefeni et al. 2011). For polybrominated diphenyl ethers (PBDEs), the pentaBDEs tend to be the more common congeners found in aqueous and atmospheric compartments (Kefeni et al. 2011). Sewage sludge used as fertilizer, atmospheric deposition, and root uptake from contaminated soils has contributed to the brominated flame retardant burden found in plants (Daso et al. 2010; Marvin et al. 2011). Due to their bioaccumulative ability, brominated flame retardants pose an ecotoxicological concern, for instance, they are found in birds and their eggs (Daso et al. 2010; Kefeni et al. 2011; Marvin et al. 2011).

Food Contamination

Food intake is one of the primary exposure route for humans to brominated flame retardants, where they are often found in fish, meat, dairy, oils, and produce, with fat-rich foods having the highest concentrations. Unlike the others, tetrabromobisphenol A (TBBPA) is considered to be nonpersistent and thus does not biomagnify, meaning food intake is not a major pathway for tetrabromobisphenol A; rather inhalation serves as the most prominent human exposure route (Sjödin et al. 2003). Hexabromocyclododecane (HBCD) food contamination has been found with typical levels between <0.01 and 5 ng/g w/w, with meat and fish being the main dietary source (Marvin et al. 2011). A sum of tri- through decapolybrominated diphenyl ethers have been found in poultry at levels between 1.8 and 39 ng/g l.w. in a North American study (Sjödin et al. 2003). A study in Catalonia, Spain, concluded that polybrominated diphenyl ether concentrations were greatest in fish, followed by oils and fats, bakery products, eggs, dairy products, meat and meat products, tubers, cereals, pulses, vegetables, and fruits, respectively (Daso et al. 2010). The highly brominated congeners (hepta- through deca-BDEs) in humans tend to be linked to occupational exposures rather than from food consumption, but alternatively the penta- through hexa-congeners tend to be more prevalent in food (Kefeni et al. 2011). Contrarily, congener-specific contamination for polybrominated biphenyls is not typical (D'Silva et al. 2004). Polybrominated biphenyls have been found in milk fat 0.001–0.053 ng/g of fat (D'Silva et al. 2004). Many studies contradict one another on whether meat or fish is the largest source of contamination for brominated flame retardants, but it should be noted that both categories are the top contributors of brominated flame retardants in the human diet.

Analytical Detection

Chromatographic methods are presently the most commonly used detection methods for determining brominated flame retardant concentrations in food, environmental, and biological samples. A wide variety of extraction, purification, and cleanup procedures have been reported for extraction of brominated flame retardants out of lipid and non-lipid media, primarily through organic solvent partitioning (D'Silva et al. 2004). Gas chromatography–mass spectrometry (GC-MS) has traditionally been used for the analysis of polybrominated diphenyl ethers and polybrominated biphenyls. GC is not suited to separate the hexabromocyclododecane (HBCD) isomers but can be used to determine total HBCD content (D'Silva et al. 2004). LC-MS/MS methods have been described for hexabromocyclododecane and other flame retardants, like tetrabromobisphenol A, although atmospheric pressure photoionization (APPI) or atmospheric pressure chemical ionizations (APCI) sources should be used instead of electrospray ionization (ESI) to prevent ion suppression (Marvin et al. 2011). Brominated organic compounds in general leave a distinctive fingerprint among major mass fragmentation peaks, which allows them to be readily identified when present.

Toxicology

Brominated flame retardants can induce a variety of congener-specific main toxicological endpoints including thyroid and endocrine disruption, neurotoxicity, and carcinogenicity. In addition to the thyroid disruption that can cause changes in metabolism, protein synthesis, and brain development, polybrominated diphenyl ethers may induce reproductive, developmental, and, possibly, immunotoxicity (Kefeni et al. 2011). Polybrominated biphenyls (PBBs) can induce a variety of chemical endpoints similar to the lower polybrominated diphenyl ethers congeners, including cancer and damage of the liver, kidney, thyroid, and nervous, reproductive, and immune systems, and are classified as group 2A carcinogens by the International Agency for Research on Cancer (Kefeni et al. 2011; IARC 2014). Generally, the toxicity of polybrominated biphenyls decreases with the increased number of bromine groups, similar to the trend in the toxicity of polychlorinated biphenyls (D'Silva et al. 2004). Hexabromocyclododecane can also cause thyroid, neurological, developmental, and reproductive toxicity (Marvin et al. 2011). The thyroid is one of hexabromocyclododecane's main targets, causing thyroid hyperplasia and reduced thyroxine circulating concentrations in rats (Marvin et al. 2011). Tetrabromobisphenol A, the nonpersistent compound in the brominated flame retardant class, can undergo photolysis to the pesticide and wood preservative 2,4,6-tribromophenol, which resembles thyroxine, a thyroid hormone, that can potentially act as an endocrine disruptor (Kefeni et al. 2011). Tetrabromobisphenol A can affect thyroid hormones and neurological functions and induce cytotoxicity by disrupting calcium ion homeostasis. It is a strong competitive binder to the thyroid hormone transthyretin and also interferes with thyroxines' role in growth and development (Kefeni et al. 2011).

Because many brominated flame retardants have been banned or phased out due to their persistence, toxicity, and ability to bioaccumulate, the result has been the emergence of NBFRs or novel brominated flame retardant. This class includes decabromodiphenyl ethane (DBDPE), tetrabromobisphenol *A-bis* (2,3-dibromopropylether) (TBBPA-DBPE), bis(2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate (TBPH or BEHTBP), and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB or EHTBB) – to name a few (Covaci et al. 2011). Novel brominated flame retardants are not necessarily less toxic, bioaccumulative, or persistent as traditional brominated flame retardants; rather they are considered novel because they are either new to the market or newly found in the environment (Covaci et al. 2011). Flame retardants will remain an important and significant class of chemicals to prevent easy ignition of electronics, building materials, furniture, textiles, and other products. There is no way to revoke the use of these chemicals without significantly increasing fire hazards, but as industry transitions to novel brominated flame retardants and new classes of flame retardants, industry should venture towards classes that are less toxic and persistent in the environment.

Conclusion and Future Directions

With increased awareness within the scientific community and public regarding adverse health effects of industrial and agricultural chemicals, many (e.g., persistent organic pollutants) have been banned or restricted worldwide to protect human, ecological, and environmental health. Consumers concerned about product content have influenced government response with regulations and laws requiring pre-market or post-market toxicity and/or environmental testing (depending on the law) (e.g., US Toxic Substances Control Act (TSCA), EU Regulation, Evaluation, Authorization, and Restriction of Chemicals (REACH)); however industry should take greater responsibility in testing their products thoroughly for adverse health effects before distributing them. Exposure awareness has led Californians to vote in Proposition 65 in 1986, which required business owners to provide clear and responsible warnings if a listed chemical was present or released in the environment, in their products, or in their home or workplace (OEHHA 2013). The Center for Disease Control (CDC) has developed a National Biomonitoring Program to analyze biofluids (e.g., blood, urine, saliva, etc.) for chemicals or metabolites as an indicator of chemical exposure, aimed to help assess public and environmental health (CDC 2014). However, the presence of trace levels of some chemicals in foods or water or other environmental media should be put in the context of dose–response, i.e., is there an opportunity for exposure at a high enough level to cause harm? If the toxicological effects of the banned compounds (e.g., legacy chemicals) were evaluated prior to use in the environment, they would have been deemed too toxic and their worldwide contamination would have been largely avoided. In addition, emerging contaminants and new technologies such as personal care products and pharmaceuticals (PCPPs) and nanotechnology need to be further

evaluated on not only their toxicity, but also the overall likelihood of exposures as these contaminants transport into foodstuff, drinking and irrigation water, soil, and air. With these lessons learned, full environmental and toxicological awareness needs to be implemented as new industrial chemicals hit the market, including green chemistry. Green chemistry is the practice of developing low-impact and sustainable chemicals in order to reduce the hazard from synthetic chemicals and also reduce hazardous waste generation. Although these chemicals may seem like solutions, their toxicity and environmental effects need to be extensively evaluated before the same cycle is repeated, where either the chemical or their transformation products cause detrimental health effects.

Cross-References

- ▶ [Agricultural Chemical Pollutants](#)
- ▶ [Applications of Nanotechnology in Developing Biosensors for Food Safety](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Heating and Cooking](#)

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Abstract

Plants and/or associated microorganisms produce an enormous variety of bioactive compounds some of which can elicit a wide range of adverse effects ranging from simple gastrointestinal upsets, lingering neurological disease through to death. The toxins can be endogenous to the food plant or an exogenous contaminant introduced from a co-harvested plant or by associated fungi or

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bacteria. More usually, in a balanced, healthy diet, the toxins are well below the threshold for acute and even chronic toxicity. Bulk marketing of many food plants and products has a diluting effect on any localized high concentrations of endogenous and exogenous toxins thereby reducing the risk of related food poisonings. However, many factors can unbalance a diet leading to an increase in the exposure to the potential toxins. Furthermore, under specific circumstances, the food plant can overproduce these toxins leading to localized outbreaks of poisoning. This chapter briefly details a wide range of endogenous toxins that have been identified in some food plants and briefly reviews some examples of detoxifying preparation of food, misidentification of food plants with similar but toxic plants, misuse of potentially toxic food plants, food plant-associated intoxications of uncertain etiology, and the potential for secondary poisoning that may occur following ingestion of food products derived from animals that ingested the toxic plants. Additionally, the potential for otherwise safe food plants to be contaminated with exogenous plant-associated toxins is exemplified by brief discussions of dehydropyrrolizidine alkaloids, corynetoxins, ptaquiloside, and phomopsins, four toxin groups quite different in structure and effects. The wide range of *in vivo* biochemical and physiological interactions potentially involving bioactive plant-associated metabolites, including interactions with therapeutic drugs, reinforces the imperative to continue identification of disease states that could be caused, initiated, or exacerbated by exposure to these toxins. Such exposure can range from short term and high levels through to the more insidious long-term, low-level exposures.

Introduction

It is well established that some food plants included in the human diet contain chemicals of varying complexity and composition that can elicit adverse effects ranging from simple gastrointestinal upsets through to death. These plant-associated natural toxins are differentiated from residues of toxicants such as pesticides that may have been applied to the growing or stored plant material. Some of these plant-associated toxins are endogenous secondary metabolites of the plant food in question, whereas others are exogenous, produced by other plants, fungi, or bacteria that can contaminate the food plant, either in a natural symbiotic situation (e.g., epiphytic and endophytic fungi or colonizing bacteria) or via co-harvesting of toxin-producing plants along with the food plant (e.g., contamination of grains with dehydropyrrolizidine alkaloid-producing plants and plant parts). Some plants are ingested directly by humans – resulting in primary poisoning – while other toxin-containing plants are ingested by livestock that are used to produce animal-derived food. This food is now possibly contaminated with the plant-associated toxins, resulting in secondary poisoning.

As human culture evolved, food plants that could be safely eaten or that required some form of pretreatment to render them safe, or food derived from animals that were primarily exposed to the plant-associated toxins, were identified by trial and

error. This was an easier process when the effects were acute but considerably more difficult when chronically developing or delayed manifestation toxic effects such as neurodegenerative or teratogenic effects, respectively, were present. Once a plant and associated toxins are unambiguously associated with adverse effects, either acute or chronic, the toxins can be considered overt, and food plants, and products derived from them, can be monitored and even regulated to lessen intoxication episodes. By contrast, there is potential for disease states to require or be exacerbated by a food plant-associated toxin but where the toxin-adverse effect association is unclear or not considered. These latent toxins can also be involved in acute or chronic development of disease states.

There are many, easily accessible reviews in journals and books that discuss the potential for food plants to produce chemicals that are toxic to humans or for the food plants to be contaminated with toxin-producing plants or microorganisms. There are also many, easily accessed online documents published by food safety-related agencies that offer advice with respect to food plant-associated poisonings or contraindicated interactions with other bioactives, including therapeutic drugs. The food plant toxins can be either universally toxic (e.g., cyanogenic glycosides) or only harmful to susceptible persons (e.g., allergenic chemicals, contraindicated interactions with medicines, or genetic differences in managing the toxins). International food safety authorities have addressed these issues by commissioning reports and considering expert opinion related to the risk of food plant-associated toxins and the consequent establishment of acceptable exposure levels. This latter regulatory approach can also apply to the prohibition of specific, otherwise naturally occurring, plant-associated toxins being added to foods. Examples include the phenylpropene safrole, a natural component of black pepper, cinnamon, and nutmeg, that is a suspected genotoxic carcinogen; coumarin, a potentially hepatotoxic benzopyrone from *Cinnamomum cassia* (Chinese cinnamon, Chinese cassia); and plant parts and oil from *Acorus calamus* (sweet flag or calamus) that contain the methoxylated phenylpropanoid procarcinogen, β -asarone.

This present chapter will briefly highlight some significant endogenous plant-produced toxins (section “[Endogenous Toxins in Food Plants](#)”). It will then extend to describe some latent toxins, both endogenous and exogenous, that may be produced by plants under specific circumstances, such as phytopathogenic attack, physical damage, or environmental changes; by co-harvested and co-processed plants, such as weeds in a grain crop; and by fungi (endophytes and/or epiphytes) or bacteria colonizing the food plant or the co-harvested weed. This chapter will not address mycological or bacterial toxins in general but rather deal with two classes (sections “[Corynetoxins](#)” and “[Phomopsins](#)”) that are outside the more usual remit of food-related microbiological toxicoses. Additionally, this chapter will exemplify the need for detoxification pretreatment of plant food (section “[Detoxification](#)”), intoxications of uncertain etiology (section “[Uncertain Etiology or Involvement of Putative Toxins](#)”), the potential for unexpected adverse interactions with other bioactives (section “[Potential for Unexpected Effects and Synergistic, Potentiating or Exacerbating Interactions](#)”), the misidentification or misuse of food plants (section “[Misidentification or Misuse of Plants for Food](#)”), and the potential for

plant food-related intoxications to be manifested under local conditions of production that avoid the diluting effect of commodity bulking in the general marketplace (section “[Potential Secondary Poisoning](#)”).

While association of adverse effects to specific food intake is somewhat easier when the effects are acute following ingestion of the food, there is an obvious, intrinsic difficulty in associating a chronically developing or delayed manifestation disease state with a specific dietary intake. A further complication in the efforts to associate human disease with dietary intake of food plant-associated toxins, exemplified in some instances described in this chapter (e.g., section “[Exogenous Toxins in Plant-Derived Food](#)”), the plant-associated toxin was first elucidated because of its acute effects on livestock. However, subsequent research into mechanisms of action, analytical method development, and toxicological profiles can indicate the potential for chronic adverse effects on humans after long-term, low-level dietary exposure to these natural toxins.

Endogenous Toxins in Food Plants

As an evolutionary imperative, plants have developed various means to deter or mitigate herbivory. These can include prolific growth, physical deterrents such as tough leaves or thorns, or chemical deterrents resulting from the biosynthesis of secondary metabolites that have a wide spectrum of bioactivity against insect and mammalian herbivores. Additionally, the biosynthesis and concentrations of toxic metabolites can be dependent upon specific environmental circumstances including infection by plant pathogens, tissue damage, climatic conditions, and soil types. Therefore, it is not surprising that food plants in the human diet can also contain concentrations of secondary metabolites that can manifest adverse effects when ingested at critical amounts (Table 1). Many of the toxins, although effective against insect herbivory, may be present in insufficient concentration to elicit acute toxic effects on humans ingesting moderate and varied diets. However, if the diet is heavily biased toward a particular food plant, then exposure rates and consequences can be greater. Furthermore, little is known about the more latent adverse effects that could result from long-term exposures to such food plant-associated toxins in unbalanced or biased diets.

Antinutritional components of some plant-based foods and food products, while not necessarily toxic per se, can cause undesirable effects by inhibiting the effective absorption of dietary inorganic micronutrients and digestibility of macronutrients. For example, polyphenols (tannins) in some cereal grains and legumes, including red sorghum and red beans, respectively, can inhibit the absorption of nonheme iron and vitamin B₁₂ (thiamin) and also adversely affect digestibility of dietary starches, proteins, and lipids. Similarly, oxalic acid (and oxalates), which can exert renal toxicity at high concentrations, can induce antinutritional effects at lower concentrations by chelating with dietary calcium.

As with all natural chemicals, there can be a large degree of variation in how individuals respond to dietary exposure. As an extreme example, one person's

Table 1 Some examples of well documented endogenous toxins associated with plants in the human food supply

Toxin class (<i>known or suspected</i>)	Toxin examples	Food plant example(s)	Disease/effects
Steroidal glycoalkaloids	α -solanine, α -chaconine	Potato (<i>Solanum tuberosum</i>)	Gastrointestinal; neurological; teratogenic
Cyanogenic glycosides	Amygdalin	Apricot or peach kernels; apple, cherry or pear seeds	Stomach upsets; neurological damage; tachypnea; death; chronic exposure can lead to the syndrome, tropical ataxic neuropathy
	Linamarin, lotaustralin	Cassava (<i>Manihot esculenta</i>) known as yucca, tapioca, gapek, manioc; lima beans (<i>Phaseolus lunatus</i>)	
	Taxiphyllin	Bamboo shoots (<i>e.g., Bambusa vulgaris, Phyllostachys edulis</i>)	
Organic nitriles (<i>cyanides</i>)	β -amino-propionitrile, 3,3'-iminodipropionitrile	Cassava, chickling peas	Neurotoxic: neurodegenerative diseases including Konzo (spastic paraparesis), lathyrism and tropical ataxic neuropathy; osteolathyrism
Non-protein amino acids	Canavanine	Leguminous seeds; alfalfa sprouts (<i>Medicago sativa</i>); sword beans (<i>Canavalia gladiata</i>)	Antinutritional, kidney toxicity, immunocompromising
	β -oxalyl-amino-L-alanine	Chickling peas (<i>Lathyrus sativa</i>)	Chronic neurotoxicity (lathyrism), osteolathyrigenic
	β -methylamino-L-alanine	Cycad seeds (<i>Cycas spp., Zamia spp.</i>)	Neurotoxin: amyotrophic lateral sclerosis/ Parkinsonism–dementia complex
	Indospicine	<i>Indigofera spp.</i>	Hepatotoxic

(continued)

Table 1 (continued)

Toxin class (<i>known or suspected</i>)	Toxin examples	Food plant example(s)	Disease/effects
Furanoterpenoids	Ipomeamarone (<i>ngaione</i>); ipomeanol	Blemished or diseased sweet potato (<i>Ipomoea batatas</i>) known as kumara (NZ), “yam” (USA)	Liver necrosis
Furanocoumarins	Psoralen, bergapten, xanthotoxin	Celery (<i>Apium graveolens</i>), parsnip (<i>Pastinaca sativa</i>), citrus fruits	Phototoxic and photomutagenic; stomach upset; potentially carcinogenic; cutaneous melanomas
Lectins	Phytohemagglutinin	Many legumes: varieties of bean (<i>Phaseolus vulgaris</i>), e.g., red kidney beans (??); white kidney beans, green beans	Hemagglutinins; stomach upset, vomiting, diarrhea
Organic acids	Oxalic acid	Rhubarb, soursob (<i>Oxalis pes-capris</i>), amaranth, spinach, yam, taro, sweet potato, silver beet	Neurological symptoms (muscle twitching, convulsions), bradycardia, bradypnea, vomiting, headache, coma; calcium oxalate nephrolithiasis; antinutritional
	Erucic acid	Canola (rape) oil (<i>Brassica napus</i>)	Hypercholesterolemia, cardiac lipidosis
	Caffeic acid, chlorogenic acid	Wide range of vegetables and fruits	Possible human carcinogen
Steroidal glycosides	Cucurbitacins	Zucchini, cucumber, squash	Gastrointestinal upset with stomach cramps, vomiting, diarrhoea
Aminopyrimidinyl glucosides	Vicine, convicine	Fava beans (<i>Vicia faba</i>) known as broad beans	Favism: a hemolytic response in some people with inherited glucose-6-phosphate dehydrogenase deficiency.

(continued)

Table 1 (continued)

Toxin class (<i>known or suspected</i>)	Toxin examples	Food plant example(s)	Disease/effects
Azoxylglycosides	Cycasin, macrozamin	Cycads (<i>Cycas spp.</i> , <i>Zamia spp.</i>)	Stomach upsets, vomiting, liver disease, possibly mutagenic, carcinogenic and neurodegenerative (amyotrophic lateral sclerosis, Parkinsonism-dementia)
Sterol- β -glucosides	β -sitosterol- β -glucoside, stigmasterol- β -glucoside, campesterol- β -glucoside	Cycads (<i>Cycas spp.</i> , <i>Zamia spp.</i>)	Neurotoxic; possibly neurodegenerative (amyotrophic lateral sclerosis, Parkinsonism-dementia)
Glucosinolates	Sinigrin, progoitrin, epiprogoitrin, glucoraphanin	Soybeans and products such as tofu; Brassica spp. Such as cabbage, Brussels sprouts, turnips.	Goitrogenic via thyroid function suppression
Biogenic amines	Tyramine	Fava beans (<i>Vicia faba</i>) known as broad beans; snow peas	Can induce hypertension with associated organ damage if associated with monoamine oxidase inhibitors; migraines
Monoterpene ketones	β -thujone	Essential oil from sage (<i>Salvia officinalis</i>), tansy (<i>Tanacetum vulgare</i>), wormwood (<i>Artemisia spp.</i>)	Central nervous system effects including convulsions; hyperexcitability, hallucinations; absinthism
Heavy metals	Selenium	Food plants grown in seleniferous soils, e.g., grains	Physical changes in hair and fingernails; neurological disturbances

(continued)

Table 1 (continued)

Toxin class (<i>known or suspected</i>)	Toxin examples	Food plant example(s)	Disease/effects
Enzymatic antivitamin	Thiaminase	Betel nuts, blueberries, black currants, red beets, red cabbage, Brussels sprouts, parts of some ferns,	Beri-beri; anorexia; cardiac enlargement; weight loss; death
Polyphenols (<i>tannins</i>)	Tannic acid, ellagitannin	Red sorghum, red beans, spinach, nuts, berries	Antinutritional; prevents efficient absorption of micronutrients (e.g., Fe) and digestibility of macronutrients (starches, fats, proteins).
Proteinase inhibitors	Inhibitors of trypsin, chymotrypsin, elastase	Soybean (<i>Glycine max</i>), beans (<i>Phaseolus</i> spp.), potato, squash (<i>Cucurbita</i> spp.)	Possible gastrointestinal disturbances; reduced weight gain; potentially, pancreatic cancer
Nortropane alkaloids	Calystegines	Potatoes and potato products; various fruits and vegetables	Glycosidase inhibitors: malnutrition, potential neurological or systemic effects of enzyme inhibition

pleasant snack of peanuts can be a life-threatening anaphylactic shock to another. A meal of fava beans can be readily enjoyed by most people, but those with a genetic deficiency of glucose-6-phosphate dehydrogenase may develop a hemolytic crisis (favism) as a result. Similar to the specific, restricted toxicity of fava beans, there are other instances of plant food toxicity induced by underlying medical conditions, e.g., intolerances toward disaccharides (e.g., sucrose and dextrans), gluten (e.g., in wheat, barley), and phenylalanine (phenylketonuria disease) in food.

Being alert to the potential toxins that have been identified in food plants for humans allows agronomists to monitor new cultivars and thus ensure genetic development does not tip in favor of higher production of the toxins. For example, new celery cultivars need to be checked for unusually high concentrations of the potentially carcinogenic, mutagenic, and/or dermatotoxic furanocoumarins, and a new Lenape cultivar of potatoes was withdrawn from availability as seed stock due to high levels of glycoalkaloids. Furthermore, Esposito et al. (2002) have suggested

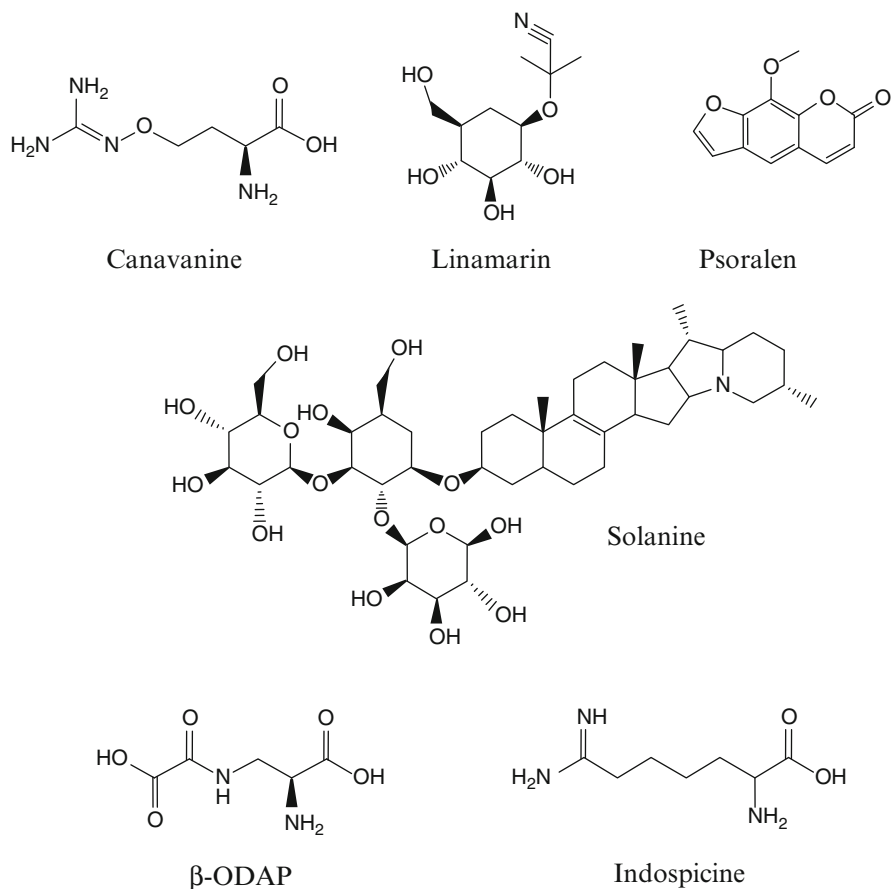


Fig. 1 Structures of select endogenous toxins listed in Table 1

that the glycoalkaloid content of new potato varieties is the most susceptible to changes and should be used to monitor genotypes derived from interspecific hybridizations.

The potentially toxic food plant secondary metabolites belong to many different chemical classes (Table 1, Fig. 1). Some, such as the steroidal glycoalkaloids of green potatoes, have acute effects clearly associated with ingestion of the plant, while others, such as the nonprotein amino acids and sterol- β -glucosides from cycads, are less unequivocally associated with the chronic neurological effects. In addition to the overt acute effects, there is also the potential for latent involvement (e.g., initiation, synergistic, exacerbation, delayed, or progressive effects) of all these toxin classes in the etiology of illness, especially chronically developing disease states. Since most have already been adequately described in the literature, only a few examples involving special circumstances such as processing/detoxification requirements (section “[Detoxification](#)”), uncertain

etiologies (section “[Uncertain Etiology or Involvement of Putative Toxins](#)”), unexpected effects such as contraindications with other bioactive compounds (section “[Potential for Unexpected Effects and Synergistic, Potentiating or Exacerbating Interactions](#)”), and the potential for misidentification (section “[Misidentification or Misuse of Plants for Food](#)”) or secondary poisoning (section “[Potential Secondary Poisoning](#)”) will be discussed with appropriate examples.

Exogenous Toxins in Plant-Derived Food

Exogenous toxins are those that are not biosynthesized by the food plant *per se*. They can be present in the food plant because of contamination by a toxin-producing plant or because of a symbiotic microbial interaction in which the fungi or bacteria actually produce the toxins. As with endogenous toxins, the dietary intake of exogenous toxins can be acutely toxic. Otherwise, the intake can vary from significant (without showing signs of acute poisoning) but intermittent (allowing some period of recovery but possibly initiating a cascade of disease events) through to continual, or intermittent, long-term, low-level exposure leading to chronic disease development.

These exogenous toxins include some classes that have now been incontrovertibly associated with a food-related disease and some that, based upon animal studies and the potential for them to contaminate the human food supply, may represent unrecognized causes or contributors to chronically developing diseases. For example, dietary exposure to the plethora of dehydropyrrolizidine alkaloids and/or their *N*-oxides clearly causes hepatic sinusoidal obstruction syndrome in humans, while intermittent or continuous exposures to lower levels for long periods, based upon effects in research animals, could be contributing or causative factors in the development of pulmonary arterial hypertension and various cancers (Edgar et al. 2011).

While dietary levels of specific toxins may not be sufficient to elicit overt disease in the general public, individual susceptibilities need to be considered. These susceptibilities may arise from genetic differences leading to abnormal metabolism of potential toxins; quantity, quality, or selection choices that lead to a biased diet; geographical location that may impose a dependence on locally grown and contaminated food; and potentiating or exacerbating circumstances peculiar to individuals or groups of people. The latter is exemplified by the alteration of oral pharmacokinetics of felodipine by dietary furanocoumarins (section “[Potential for Unexpected Effects and Synergistic, Potentiating or Exacerbating Interactions](#)”) and the potential for similar interactions, between the toxic dietary component and other dietary components, food supplements, and therapeutic drugs. Additionally the physiological status of the person (e.g., elevated or depressed levels of enzymes, essential vitamins, and biochemical status of minerals such as copper, iron, zinc, and manganese) cannot be ignored. For example, an environmental or genetic predisposition to elevated copper storage may result in an increased risk of poisoning by dietary dehydropyrrolizidine alkaloids relative to people with normal copper loads (Edgar et al. 2011).

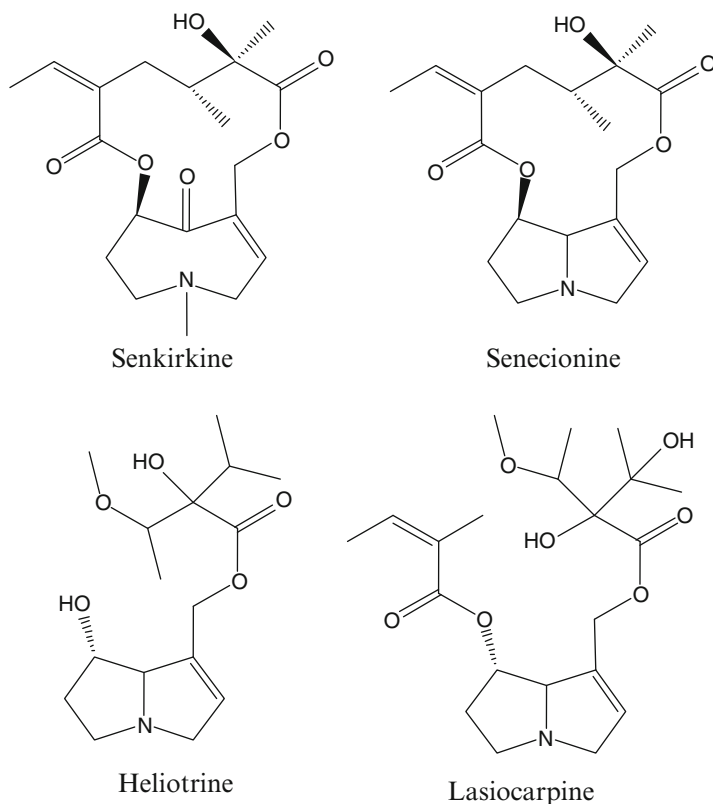


Fig. 2 Examples of common dehydropyrrolizidine alkaloids containing varying necine bases: otonecine (senkirkine), retronecine (senecionine), and heliotridine (heliotrine, lasiocarpine) as well as mono- (heliotrine), di- (lasiocarpine), and macrocyclic diester (senecionine, senkirkine) forms of the alkaloids

Dehydropyrrolizidine Alkaloids

1,2-Dehydropyrrolizidine alkaloids are protoxins that are a subclass of the general family of pyrrolizidine alkaloids (Edgar et al. 2011). The presence of this double bond is critical to the toxic potential of these alkaloids after metabolism by the cytochrome P450 monooxygenases. The dehydropyrrolizidine alkaloid structure also includes one or two esterifying acids giving either mono-, di-, or macrocyclic ester forms of the alkaloids (Fig. 2). Several hundred dehydropyrrolizidine alkaloids exist based on a small variety of necine base structures in combination with a large variety of esterifying acids that differ in composition and/or stereochemistry. In addition to the free-base alkaloid, most dehydropyrrolizidine alkaloids can also exist as their *N*-oxides. While the *N*-oxides are a metabolic detoxification product of the free-base alkaloids, ingested dehydropyrrolizidine alkaloid *N*-oxides are as toxic as the free bases due to *in vivo* reduction and absorption during passage of the gastrointestinal tract (Edgar et al. 2011).

Dehydropyrrolizidine alkaloids have been identified in six plant families with a worldwide distribution, i.e., Apocynaceae, Boraginaceae, Asteraceae (Compositae), Leguminosae (Fabaceae), Ranunculaceae, and Scrophulariaceae. It is because of this widespread geography and distribution among plant genera that the potential toxic effects of dehydropyrrolizidine alkaloids are of such an international concern. The dehydropyrrolizidine alkaloids are known to be hepatotoxic, pneumotoxic, genotoxic, and carcinogenic. Most of the information concerning the acute and chronic toxic effects of dehydropyrrolizidine alkaloids comes from the extensive literature documenting animal poisonings and experimental animal models (Molyneux et al. 2011).

In most cases, the pathway of human exposure and poisoning by dehydropyrrolizidine alkaloids is via contamination of a primary food source such as in the co-harvesting of dehydropyrrolizidine alkaloid (and *N*-oxides)-producing plants with grains used as staple foods. Poisoning can also occur via plants and herbal products used as food supplements or for medicinal purposes. More unknown and sinister is the undefined potential for long-term, low-level primary exposure to dehydropyrrolizidine alkaloids in foods, including pollen and honey (and products made from these) for example, as well as secondary type exposures via eggs, milk, and, perhaps, meat, to initiate or contribute to chronically developing disease states such as pulmonary hypertension and cancers (Edgar et al. 2011).

Intoxications can be acute, subacute, or chronic, and there is the potential for very low-level chronic intoxications that are not easy to associate with exposure to the dehydropyrrolizidine alkaloids or their food plant sources. Documented poisonings have occurred sporadically over the last 100 years in different parts of the world including the former USSR, India, Africa, and Afghanistan. These resulted from the preparation of bread and other staple foods that used grain contaminated with dehydropyrrolizidine alkaloid-containing seeds from various species of plants (Edgar et al. 2011; Molyneux et al. 2011). Most recently, dehydropyrrolizidine alkaloid contamination of grain supplies from the Tigray Region of Ethiopia was the highly suspected cause of veno-occlusive disease, which is also referred to as hepatic sinusoidal obstruction syndrome or, sometimes, simply as “undetermined liver disease”. The veno-occlusive disease outbreaks from that region demonstrated the current and real potential of dehydropyrrolizidine alkaloids to cause serious poisoning episodes even today (Molyneux et al. 2011; CDC 2013). Such extensive outbreaks of intoxication are not typically expected since grain cleaning procedures and bulk storage and distribution methods used in most industrialized countries result in a dilution of any localized high concentrations of the dehydropyrrolizidine alkaloids. However, in circumstances that circumvent these bulk marketing strategies, such as true subsistence and the “grow it yourself, to use it yourself” hobby farming lifestyles, the potential for poisoning is greater. Moreover, given the known mechanism of intoxication based on animal models, the possibility of more chronic effects, including various cancers and pulmonary arterial hypertension, is a concern with long-term, low-level exposures to the dehydropyrrolizidine alkaloids in the diet (Edgar et al. 2011).

Details of the mechanism of dehydropyrrolizidine alkaloid toxicity in mammalian systems have been thoroughly outlined in the literature and will not be

reviewed in detail here. It is simply noted that dehydropyrrolizidine alkaloids require metabolic bioactivation by the cytochrome P450 enzymes to produce the toxic electrophilic dihydropyrrolizine or “pyrrolic” entities that, as bifunctional alkylating agents, can bind to proteins and DNA. While adults of both genders have been poisoned by dehydropyrrolizidine alkaloid-contaminated food, food supplements, or herbal preparations, it is noteworthy that neonates and infants are more susceptible and therefore a prime consideration when setting regulatory levels of dehydropyrrolizidine alkaloids in foods.

The onset of clinical signs may follow days, weeks, or even months of consumption of the contaminated food material. Early symptoms and signs of intoxication include nausea, vomiting, gastric pain, ascites, and hepatic distension. The disease is fully expressed in moderate to severe liver damage and, in some cases, damage to the lungs. The disease has been fatal in many cases. If the liver damage is not extensive, and exposure to the alkaloids is removed, patients may recover. However, there is evidence of delayed onset of clinical signs following a non-acutely toxic dose and a consequent hypothetical basis for recycling of the toxic metabolites (Edgar et al. 2011; Molyneux et al. 2011). In very low-level, long-term chronic cases, the disease might be expressed as cirrhosis and pulmonary arterial hypertension (Edgar et al. 2011). Dehydropyrrolizidine alkaloids have been demonstrated to be carcinogenic in animal models, and thus similar possible chronic effects should be of concern in human exposure.

Diagnosis of dehydropyrrolizidine alkaloid intoxication is often difficult and has historically been a last resort diagnosis after failure to identify other diseases or environmental intoxications as causal factors. The suspected dehydropyrrolizidine alkaloid etiology can then be supported by observing a connection between the onset of symptoms and the presence or use of dehydropyrrolizidine alkaloid-containing plants in the area. Modern analytical techniques, such as high-pressure liquid chromatography-mass spectrometry (HPLC-MS), have added greatly in the detection of dehydropyrrolizidine alkaloids in primary plant material and in contaminated food supplies such as grains and honey (Fig. 3).

Postmortem detection of tissue-bound metabolites has aided diagnosis in animal poisoning cases. More recently, the detection of dehydropyrrolizidine alkaloid metabolites in human serum samples has aided in the diagnosis of dehydropyrrolizidine alkaloid-induced liver disease with the aid of sensitive HPLC-MS/MS instrumentation (Lin et al. 2011).

The documented cases of human intoxications by dehydropyrrolizidine alkaloids have included consumption of contaminated grains, herbal teas, “bush teas,” and food supplements and improper identification and inclusion of dehydropyrrolizidine alkaloid-containing plants as salad components. As alluded to earlier in this section, an additional aspect for concern is other food products and supplements that have not been associated with documented cases of human poisoning or disease caused by the dehydropyrrolizidine alkaloids but that have been demonstrated to contain dehydropyrrolizidine alkaloids and/or known to be toxic through animal studies. These include honey and pollen contaminated via bees visiting dehydropyrrolizidine alkaloid-containing flowering plants (Edgar et al. 2002) and milk, eggs, and meat

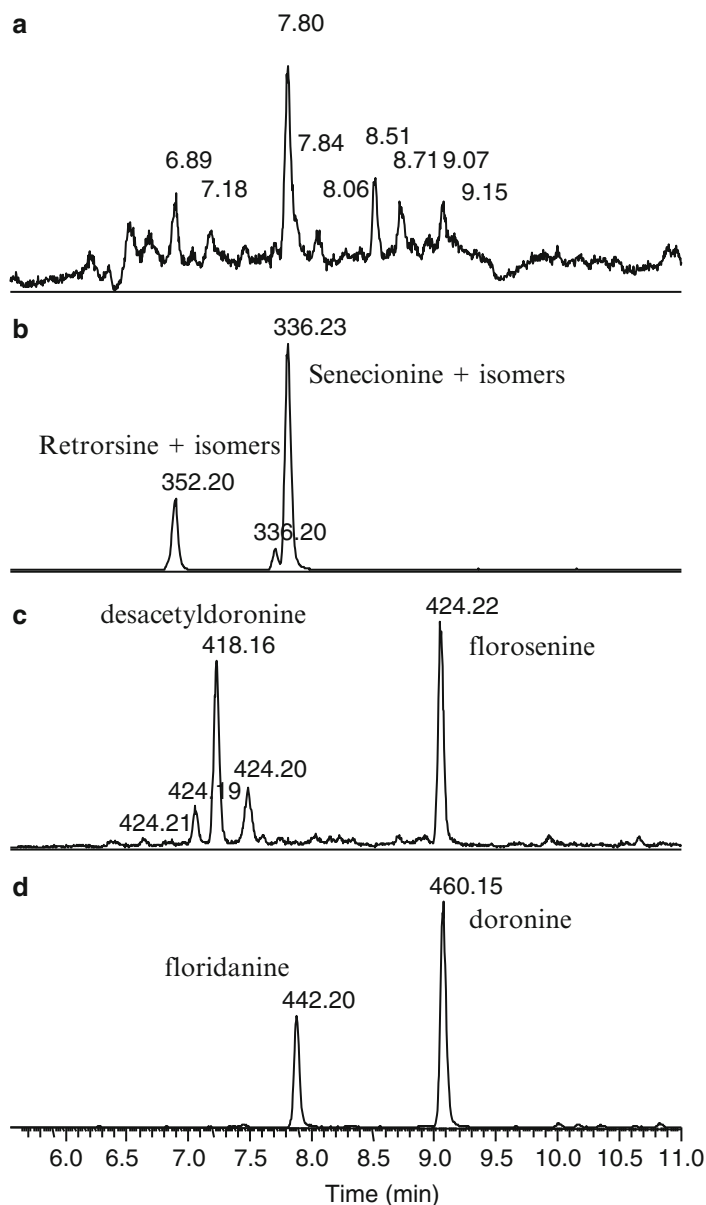
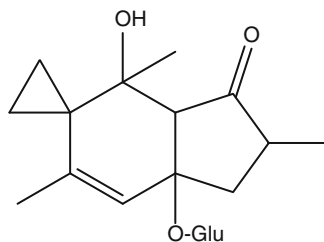


Fig. 3 Example of HPLC-ESI/MS detection of dehydropyrrolizidine alkaloids in contaminated honey. The honey sample was taken from beehives in close proximity to flowering *Senecio madagascariensis* in the Hawaiian Islands: (a) total ion chromatogram followed below with reconstructed ion chromatograms displaying (b) m/z 336, 352; (c) m/z 418, 424; and (d) m/z 442 and 460, which are the masses for the major protonated dehydropyrrolizidine alkaloids detected in the honey

Fig. 4 Ptaquiloside, the carcinogenic sesquiterpene glycoside from *Pteridium* spp. (bracken fern)

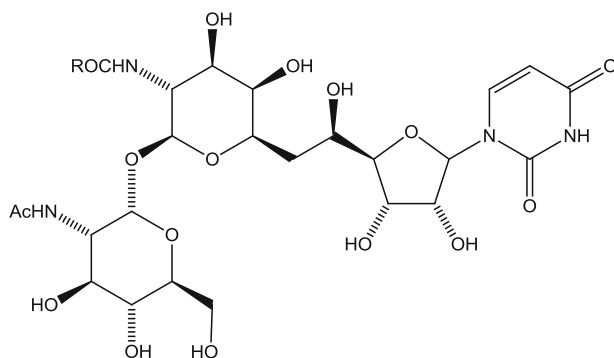


from animals grazing dehydropyrrolizidine alkaloid-containing plants (Prakash et al. 1999; Edgar et al. 2011; Hoogenboom et al. 2011). The concern about dehydropyrrolizidine alkaloid contamination in the food supply on an international basis has prompted the setting of guidelines or regulations in some countries for tolerable levels of toxic dehydropyrrolizidine alkaloids or the outright ban of some products that contain any level of dehydropyrrolizidine alkaloids (Edgar et al. 2011). As with most other toxins that are regulated at very low levels, the challenge is to develop robust analytical procedures that can detect the dehydropyrrolizidine alkaloids and their *N*-oxides. To this end, HPLC-esiMS and MS/MS approaches have been most recently described. However, obtaining pure samples of the many dehydropyrrolizidine alkaloids for analytical standards is a complicating factor.

Ptaquiloside

Ptaquiloside (Fig. 4) is a carcinogenic sesquiterpene glycoside produced by *Pteridium* spp. (bracken fern), a common plant found worldwide. Animals known to graze on bracken fern have developed a number of acute and chronic syndromes including thiamine deficiency, retinal atrophy, bovine enzootic hematuria, epithelial and mesenchymal tumors of the urinary bladder, and numerous tumors of the mouth, esophagus, and forestomachs in cattle (Smith and Seawright 1995).

Humans are directly exposed to ptaquiloside by consumption of the new growth fronds (crosiers) known as fiddleheads. To prepare bracken fern fronds for human consumption, they can be steeped in water treated with ash or sodium bicarbonate to reduce toxicity (Smith 2004). Although direct poisonings of humans have not been documented, epidemiological studies suggest ptaquiloside could be responsible for the higher incidence of gastric malignancies in human populations near dense growths of bracken fern or in people that frequently consume bracken fronds (Smith and Seawright 1995). Some of these instances could also be caused, or contributed to, by the consumption of water into which the ptaquiloside has leached. Clauson-Kaas et al. (2014) have used a sensitive LC-MS/MS method to demonstrate the presence of ptaquiloside in ground and surface waters near bracken fern stands. Concentrations in excess of the estimated threshold for toxicity (carcinogenicity) (1.3–40 pmol/L) were determined in several of the samples analyzed. These authors concluded that a risk exists for people and livestock accessing drinking water from bracken fern-infested catchments.



Where R is a long chain (C₉–C₁₅) fatty acid

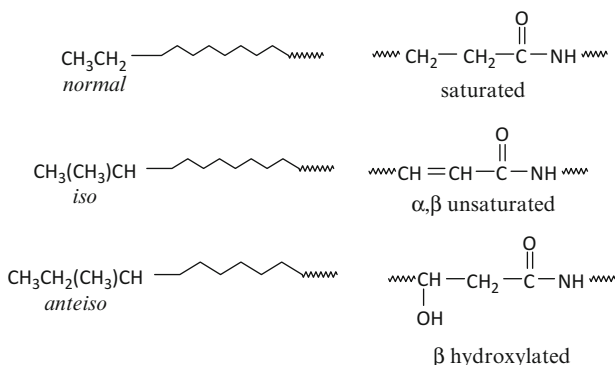


Fig. 5 Corynetoxins: the causative toxins of annual ryegrass toxicity. The common *N*-acetylglucosaminyl-tunicaminylluracil core is modified by the fatty acid substituent, R, differing in the length, terminal branching, and degree of unsaturation or hydroxylation

Corynetoxins

The corynetoxins are a family of about 12 tunicaminylluracil glycolipids that have a common *N*-acetylglucosaminyl-tunicaminylluracil core and are differentiated by the chain length, terminal branching, degree of unsaturation, and hydroxylation of a fatty acid side chain (Fig. 5) (Edgar et al. 1982).

The dietary toxicity of the corynetoxins was first identified in poisonous plant-related livestock studies (Bourke 1994). The severe neurological effects on livestock (sheep, cattle, horses), accompanied by an average 15–20 % but up to 100 % mortality in field outbreaks, were attributed to grazing bacterium-infected annual ryegrass (*Lolium rigidum*). The bacterial colonization of the seed heads of annual ryegrass is an ecological interaction enabled by a nematode (*Anguina funesta*) that first colonizes the seed heads, forming nematode galls, as part of its reproductive cycle. If the nematode carries the bacterium (*Rathayibacter toxicus*) to the seed head, the bacterium can thrive, outcompeting the nematode, and produce a golden-colored “bacterial gall.” The bacterial colony produces the corynetoxins as the plant

begins to senesce. The antibiotic properties of the corynetoxins suggest that the biosynthesis may be part of a survival strategy for *R. toxicus*, eliminating other competitors for the diminishing plant resources during the senescence.

The corynetoxins have an estimated lethal dose of 1–4 mg per kg of body weight for various animal species (Bourke 1994). Moreover, the amphipathic structure of the corynetoxins facilitates membrane binding leading to an accumulative capacity with a cumulative spectrum of effects (Jago and Culvenor 1987). While corynetoxins represent a significant problem in Australia, similar intoxications have been reported in South Africa and the USA. Corynetoxin-like metabolites have also been confirmed in seed samples of *Festuca nigrescens* from New Zealand and the USA (Anderton et al. 2004).

One of the primary biochemical toxic effects of corynetoxins is the potent inhibition of *N*-acetylglucosamine-1-phosphate transferase that is involved in the assembly of *N*-linked glycans before their transfer to nascent proteins in the endoplasmic reticulum. The downstream effects of incomplete protein synthesis are manifested as annual ryegrass toxicity in poisoned livestock. While neurological signs, including hypomotility, incoordination, limb paresis, and cerebral convulsions, are the principal clinical indications of poisoning in livestock, the corynetoxins are not primary neurotoxins. Rather, the impaired synthesis of *N*-linked glycoproteins results in vascular abnormalities in the brain that result in the observed neurological disturbances (Bourke 1994).

In addition to the acute neurological signs and death caused by the corynetoxins, adverse effects on testes (Peterson et al. 1996) and live birth rates (Stewart et al. 2002) have been reported for rats exposed to sublethal doses of tunicamycins, a group of tunicamyluracil compounds closely related (with some common congeners in both toxin groups) and toxicologically equivalent (Colegate et al., unpublished) to the corynetoxins.

Annual ryegrass is utilized as a pasture improvement plant providing valuable feed for livestock. However, and especially with the ecological development of herbicide-resistant annual ryegrass, it can infest and be co-harvested with grain crops destined for human food and hay crops for animal feed thereby providing the potential for contamination of those crops by corynetoxins.

The potential for secondary poisoning of humans that consume meat and milk derived from animals that have been exposed to corynetoxins has been reported to be very low (Allen and Mullan 2011). The practice of bulking grains for marketing has an undoubted diluting effect on the presence of corynetoxins and, thereby, probably avoids cases of overt poisoning of humans that can be directly related to dietary exposure to these toxins. However, there is potential for higher exposures resulting from localized harvesting and utilization of contaminated grain that could lead to poisoning sequelae. Furthermore, the effects on humans who regularly ingest low-level amounts are unknown but could resemble chronic alcoholism. This resemblance may be due to the resultant carbohydrate-deficient transferrin that is one consequence of the corynetoxins blocking carbohydrate modification of nascent proteins (Penno et al. 2012). Highlighting the potential for apparently unrelated or unforeseen effects of sublethal dietary exposure to corynetoxins, it

has been shown that preexposure to sublethal doses of tunicamycins lowers resistance of mice to *Neospora caninum* infections, increasing fetal morbidity and mortality (Cao et al. 2011).

The corynetoxins can be detected in food plant matrices using an indirect competitive enzyme-linked immunosorbent assay (ELISA) or HPLC coupled to a UV or MS detector (Than et al. 2005). The former reports total corynetoxins content, while the latter (Fig. 6) shows individual corynetoxins. However, no regulations so far exist to limit corynetoxins in human food, and consequently neither of these methods is in regulatory use. This is in contrast to industry regulations governing quality control of hay exported from Australia. The regulations are in place to help avoid the clinical or subclinical intoxication of livestock by corynetoxins in the recipient countries. In this case, the risk that the hay poses for livestock is monitored using an ELISA that measures the presence of a water-soluble antigen derived from the corynetoxin-producing bacterium *R. toxicus*.

Phomopsins

Wild lupins (*Lupinus* spp., Fabaceae) are well known to be poisonous to sheep and to produce quinolizidine and piperidine alkaloids, including anagyryne and ammodendrine, which are teratogenic in cattle. However, the teratogenic anagyryne is not produced by the major lupin cultivation species including *L. mutabilis*, *L. albus*, *L. angustifolius*, and *L. luteus*. These particular species have also been agronomically developed to be low in the content of the other alkaloids.

A cultivated lupin-related hepatic disease was recognized in Australia in the early to mid-twentieth century when sheep grazed on lupin stubble after harvesting of the grain (Gardiner 1965). The disease, lupinosis, is a consequence of the antimetabolic effect of hexapeptides, the phomopsins (Fig. 7), produced by the fungus *Diaporthe toxica* (previously *Phomopsis leptostromiformis*) that can infect the lupin seed and stubble (Edgar 1991). The antimetabolic effect is achieved by inhibition of microtubule polymerization and leads to liver disease and to reduced reproductive performance when, for example, ewes are exposed to phomopsins at breeding time (Barnes et al. 1996).

The Australian regulatory authorities initially set a maximum tolerable level of 5 parts per billion of phomopsins in food. Various analytical methods for the detection of phomopsins associated with lupin seed and with other food matrices have subsequently been developed including HPLC-UV, HPLC-esiMS, ELISA, and a combination of HPLC-UV-ELISA (Than et al. 2005). The level of phomopsin contamination in commercial lupin seeds has been investigated using the ELISA-based analytical approach (Than et al. 1994). This study concluded that the highest levels of phomopsin content, up to 4,522 µg/kg seed, were associated with discolored, presumably infected seeds. Within the same study, lupin seed from varieties susceptible to *D. toxica* infection showed the highest levels of phomopsins (ranging from 182–1,413 µg/kg seed). However, even the seed from varieties resistant to *D. toxica*, while on average lower than the susceptible varieties, showed phomopsins up to 588 µg/kg seed. Furthermore, a more recent examination of

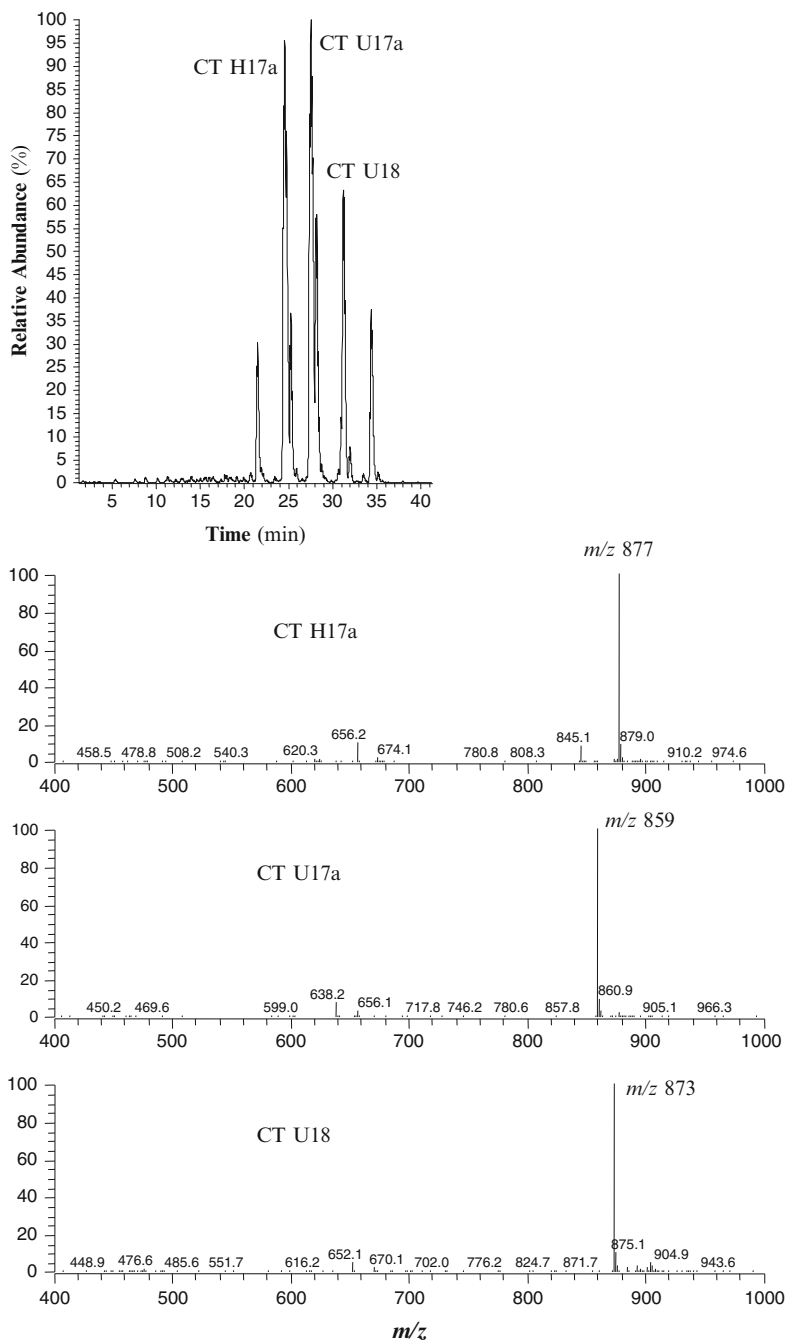


Fig. 6 HPLC-esiMS base ion (m/z 200–1000) chromatogram of purified corynetoxins showing, for example, the mass spectra of three of the major corynetoxins CT H17a, CT U17a, and CT U18

knowledge enabled the safe use of plants that otherwise were toxic to newcomers to the region. For example, the Australian aboriginal people knew, from experience, that the sporocarps of nardoo fern (*Marsilea drummondii*) could be safely eaten after they were soaked in water. However, the early British explorers Robert O'Hara Burke and William John Wills, during the first European crossing of Australia from north to south, apparently starved to death while surrounded by nutritious "bush" food because of the antinutritional thiaminase in the nardoo fern they were subsisting on in the latter part of their journey. Similarly, early pre-European settlement explorers of Australia suffered through bouts of emesis and catharsis after ingesting the seed pulp from zamia palms (*Macrozamia riedlei*) that the aboriginal population rendered safe through soaking in water and extensive drying. More recently, once the toxic entities have been identified, the processing of otherwise toxic plant foods or food supplement products is monitored by validated chemical analysis following Hazard Analysis and Critical Control Point (HACCP) guidelines.

Red Kidney Beans

Kidney beans, varieties of the common bean *Phaseolus vulgaris* (Leguminosae/Fabaceae), are grown and consumed throughout the world. An occasional toxicity of kidney beans is due to the lectin phytohemagglutinin that, because of its polysaccharide structure, binds with and agglutinates red blood cells. The resultant symptoms of poisoning include nausea, vomiting, diarrhea, and occasional abdominal pain within 1–3 h of ingestion (FDA 2012). White kidney beans contain approximately one third of the amount of phytohemagglutinin as red kidney beans (FDA 2012). Other varieties of *Phaseolus vulgaris* such as the string bean, garden bean, and snap bean, with much lower concentrations of phytohemagglutinin, are nontoxic.

Several outbreaks of poisoning due to ingestion of soaked but raw or improperly cooked kidney beans have occurred in the UK, Canada, and Australia between 1976 and 1989 (FDA 2012). Heating presoaked kidney beans for 100 °C for 15 min, at 80 °C for 2 hr, or pressure cooking the beans at 15 psi for 45 min decreases the phytohemagglutinin activity below toxic levels (FDA 2012). In addition to inappropriate precooking treatment of kidney beans, a current major risk of poisoning by kidney beans involves cooking in slow cookers or crock pots at temperatures less than 80 °C such that the phytohemagglutinin concentrations are not adequately reduced.

Reduction of Canavanine Content

Naturally occurring canavanine is a chiral, nonprotein amino acid ((2*S*)-2-amino-4-(((diaminomethylidene)amino)oxy)butanoic acid) (Fig. 1). Since it is a structural analogue of the natural amino acid arginine, it can be incorporated into proteins

instead of arginine thus leading to abnormal proteins and potential dysfunction. Present in many leguminous plants, it can comprise up to about 2.4 % (dry matter) in young alfalfa plants (*Medicago sativa*) and has been tentatively associated with the development of systemic lupus erythematosus (Rosenthal and Nkomo 2000).

In addition to its presence in established foods, canavanine is also present in more underutilized foods that are being promoted for greater use. Sword beans (*Canavalia gladiata*) are one such food that has potential due to its productivity and protein content but is underutilized due to the presence of antinutritional factors, including canavanine. It has been shown (Ekanayake et al. 2007) that mere soaking of sword beans in water is not an effective way to decrease the canavanine content. Though a heating step is required, autoclaving only reduced the canavanine content by 24 %. The best approach involved soaking the beans in excess water overnight followed by boiling in water that led to a 50 % reduction in canavanine content. Roasting of sword beans and sword bean flour (150 °C for 30 min) produced similar results (35 % reduction in canavanine) to the roasting of jack beans (*Canavalia ensiformis*) (Leon et al. 1998). However, roasting jack beans at 164 °C for 26 min resulted in a 95 % reduction in canavanine content, possibly however, at the expense of other nutritional components.

Acute Cassava Poisoning

Ingestion of cassava (*Manihot esculenta*) has been associated with chronic (tropical ataxic neuropathy) and subacute (konzo) neurological disease, possibly involving the intrinsic cyanogenic glycosides (Table 1), exemplified by linamarin (Fig. 1), but as their intact nitriles rather than the free cyanide (Llorens et al. 2011). However, acute poisoning can occur following ingestion due to cyanide released from exposure of the cyanogenic glycosides to various glycosidases present in the plant, intestinal microflora, or liver.

No matter the resultant clinical expression of the toxicity, it seems that decreasing the cyanogenic glycoside content is key to producing a safer food. Given the water solubility of the glycosides, soaking in water forms the basis of efficient detoxification approaches. Similar to the detoxification of sword beans and others (section “[Reduction of Canavanine Content](#)”), soaking followed by boiling in water is more efficient than soaking or boiling alone. Concentrations of cyanogenic glycosides are also efficiently decreased by simple drying, especially when laid out in the sun.

Uncertain Etiology or Involvement of Putative Toxins

Although some food plants have been strongly associated with the development of subacute to chronic disease development in humans, there remains doubt about the actual involvement of putative toxins that have been identified. This aspect is

exemplified here by an endogenous putative toxin and also by the “milk sickness” examples described in the secondary poisoning section (section “[Potential Secondary Poisoning](#)”).

Grass pea/chickling pea/Indian vetch (*Lathyrus sativus* L., Fabaceae), known as shan li dou in China; almorta, muela, tito, and guijo in Spain; khesari or batura in India; arvejas in Venezuela; gilban in Sudan; guaya in Ethiopia; matri in Pakistan; gesette in France; and pisello bretonne in Italy, is grown worldwide and is extensively cultivated in the areas of North Africa, Ethiopia, and west and south Asia (Spencer and Schaumburg 1983; Yan et al. 2006; Barceloux 2009; Woldeamanuel et al. 2012).

Grass pea has agronomic characteristics that include high-yield, high-protein content in seed and nitrogen fixation. It is a resilient crop that is tolerant to drought, floods (water logging), insects and pests, and poor semiarid soils. These characteristics make grass pea an ideal crop and food source, especially in times of food shortage due to drought, floods, and socioeconomic and political conditions (Spencer and Schaumburg 1983; Yan et al. 2006; Barceloux 2009).

Ingestion of grass peas by humans can induce lathyrism, a neurological motor disease involving spastic paraparesis (Spencer and Schaumburg 1983; Yan et al. 2006; Barceloux 2009; Woldeamanuel et al. 2012). The spasticity of the legs can range from an ataxic gait to the inability to walk or stand. Although lathyrism is irreversible, further progression of the disease is abated with removal of grass pea from the diet (Yan et al. 2006; Barceloux 2009; Woldeamanuel et al. 2012). The disease can occur within one month of continued heavy consumption of the seeds but onset is typically in the 3–6 month range. Humans of both genders and of all ages are susceptible; however, young adult males appear to be the most susceptible with heavy labor or physical exertion suggested as another risk factor (Spencer and Schaumburg 1983; Yan et al. 2006; Barceloux 2009; Woldeamanuel et al. 2012).

The neurotoxin β -*N*-oxalyl-L- α , β -diaminopropionic acid (β -ODAP) (Fig. 1) also known as β -*N*-oxalyl-amino-L-alanine (BOAA) was discovered in 1964 and appears to be the toxic agent in grass pea causing lathyrism (Yan et al. 2006; Barceloux 2009; Woldeamanuel et al. 2012). The average concentrations of β -ODAP in mature seeds are generally <1 % but can be <0.1 % in some low-toxin lines and as high as 2.5 % in some wild types (Spencer and Schaumburg 1983; Barceloux 2009; Woldeamanuel et al. 2012). Steeping the dehusked seed in water removes approximately 30 % of the β -ODAP, while boiling the seeds removes 70–80 % of the putative toxin (Yan et al. 2006; Barceloux 2009; Woldeamanuel et al. 2012). However, the shortage of water during drought reduces the likelihood that water will be used to detoxify the seeds (Barceloux 2009).

Although lathyrism is a preventable disease, cases are still reported (Woldeamanuel et al. 2012). Recommendations for prevention or reduction of the risk of lathyrism include avoidance of grass pea and grass pea products, partial detoxification of the seeds through specific preparation and cooking methods, use of low-toxin seed varieties, and a healthy, balanced diet with grass pea as a minor constituent (Woldeamanuel et al. 2012).

Potential for Unexpected Effects and Synergistic, Potentiating, or Exacerbating Interactions

As with all bioactive chemicals, whether natural or synthetic, whether intrinsic to normal food or ingested as a therapeutic or dietary supplement, there is the potential for adverse interactions and consequences. Also, in the context of overt and latent toxicities, there exists the possibility that a known toxin or class of toxins with well-described adverse effects may also be responsible, either directly or indirectly, for other unexpected adverse clinical effects.

The furanocoumarins present in citrus fruits, celery, and parsnip, for example (Table 1), and exemplified by psoralen (Fig. 1), are usually associated with photodynamic dermatitis, a phototoxic reaction resulting in rashes and blistering of the skin that contacts the plant. The compounds are also used therapeutically to treat other skin disorders as a result of their photodynamic cytotoxicity. Furanocoumarins can also cause gastrointestinal upset if ingested in significant quantities. However, highlighting the potential for unexpected adverse effects of some natural toxins in the human diet, it has been hypothesized that increased dietary exposure to furanocoumarins, for example, *via* a preference for orange juice, is linked to an increased incidence of cutaneous melanoma (Sayre and Dowdy 2008).

The presence of furanocoumarins in grapefruit juice and orange juice has been linked to inhibition of cytochrome P450 (CYP3A4) and a consequent increase in the systemic uptake of the calcium channel antagonist felodipine. While consumption of these juices alone has been implicated in a decrease in blood pressure, an unintended consequence of the CYP3A4 inhibition by the furanocoumarins can lead to a larger decrease in blood pressure than desired or intended (Paine et al. 2006).

Other food plants can potentially upregulate or inhibit *in vivo* processing enzymes and thus have adverse interactions with or alter the metabolism of therapeutic drugs. For example:

1. The glucosinolate-producing cruciferous (Brassicaceae) vegetables such as cabbage, Brussels sprouts, and cauliflower that result in *in vivo* formation of isothiocyanates have the potential to inhibit hepatic microsomal P450 activity while enhancing hepatic glutathione S-transferase activity. Among the potential effects are the reduction of oxidative metabolites of acetaminophen (Chen et al. 1996) and the beneficial decrease in acetaminophen-induced hepatotoxicity.
2. Some drugs, such as diuretics, can cause a reduction in the kidney's capacity to remove potassium from the blood. Ingestion of potassium-rich foods such as oranges, green leafy vegetables, and bananas can result in hyperkalemia and consequent effects on the heart.
3. Foods high in fiber (e.g., bran) can decrease the level of therapeutic digoxin used to control heart irregularities.
4. Combination of some lipid-altering drugs, statins, with a high intake of grapefruit juice can raise systemic levels of the statins and increase the chances of adverse side effects.

5. The effect of the anticoagulant warfarin can be muted by the presence of the naphthoquinone-based vitamin K₁ found in the cruciferous vegetables.
6. The antibacterials oxazolidinone and linezolid, and the antidepressant monoamine oxidase inhibitors should not be taken with foods such as some cheeses, preserved meat products, pickled herring, dried fruits, fava beans, etc. that are high in the biogenic amine tyramine since a potentially dangerous increase in blood pressure can occur.

This brief summary is not comprehensive and should only be taken as an indication that such food-drug interactions can occur and that users of therapeutic drugs should consult widely with respect to contraindicated food plants.

Misidentification or Misuse of Plants for Food

Despite the “trial and error” approach to human food plant safety that has, across the millennia, identified potentially poisonous plants and, in some cases, methods for rendering the food prepared from these plants safer for consumption, instances of the misuse or misidentification of food plants still regularly occur. Usually the effects are acute and readily associated with the ingestion of foods that, on investigation, are contaminated with or replaced by known toxic plants. In contrast to these overt toxins, there is also the possibility of more chronic effects that are not so readily associated with the food. Misidentification or contamination of healthy salad plants with similar-looking plants that produce dehydropyrrolizidine alkaloids (section “[Dehydropyrrolizidine Alkaloids](#)”) is just one example of these more latent sources of intoxication (Bundesamt für Risikobewertung 2007).

In 2008, six adult members of a family were admitted to hospital with anticholinergic clinical signs including altered mental state, hallucinations, dizziness, thirst, tachycardia, and mydriasis. After 5 days of hospitalization, all had been released with a final diagnosis of food poisoning-associated altered mental state. A subsequent investigation revealed that jimsonweed (*Datura stramonium*, Solanaceae) was inadvertently included into a stew that the family members ate about an hour or so prior to onset of clinical signs. A qualitative chemical analysis of the stew revealed the presence of the tropane alkaloids, atropine and scopolamine (Fig. 8), expected from some *Datura* species. Additionally the toxic steroidal glycoalkaloids, α -solanine (Fig. 1) and α -chaconine, were found and presumed to have been derived from the potatoes in the stew (Russell et al. 2010).

Another solanaceous source of the tropane alkaloids and potential for misidentification or contamination is deadly nightshade (*Atropa belladonna*). Schneider et al. (1996) reported the atropine concentrations in the plasma and urine of four adults and four children who ingested a pie mistakenly made with deadly nightshade berries. Three of the four adults were delirious and experiencing visual hallucinations. One adult required mechanical ventilation to manage a coma. The children displayed symptoms consistent with a mild peripheral anticholinergic reaction. Also mistaking deadly nightshade berries for blueberries, a woman

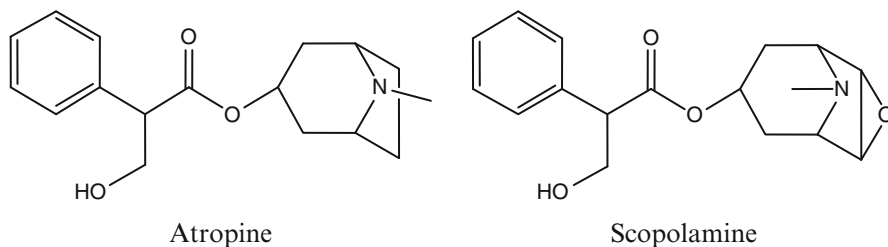


Fig. 8 Atropine and scopolamine: anticholinergic, antimuscarinic tropane alkaloids from Solanaceous plants including *Datura stramonium* and *Atropa belladonna*

ingested six of the fruit with consequent anticholinergic signs including ophthalmic disruptions, loss of balance, agitation, and anxiety (Mateo Montoya et al. 2009). A recent review of poisoning incidents related to tropane alkaloids in food (Adamse et al. 2014) highlighted 24 cases of unintended ingestion of tropane alkaloids in food. Thirteen of these were a result of contamination or mislabeling while 11 were due to mistaken identity. The review also notes that tropane alkaloid contamination has been detected in wasp honey, linseed, soybean, buckwheat, and canned green beans.

Solanum nigra (blackberry nightshade, black nightshade, hound's berry, etc.), along with other closely related species, is somewhat similar to deadly nightshade except that its fruits occur in small bunches rather than individually. The toxins are steroidal glycoalkaloids including α -solanine (Fig. 1) that is associated with *Solanum tuberosum* (potato, Table 1). The berries and leaves of edible varieties (both cultivated and opportune growth) are utilized as food in many countries, but the potential for inclusion of nonedible varieties in the diet, along with concomitant high levels of exposure to toxic steroidal glycoalkaloids, has resulted in it being prohibited for sale as a food product or a component of food in Australia.

While the main food poisoning involving dehydropyrrolizidine alkaloids is associated with exogenous contamination of staple foods (section “[Dehydropyrrolizidine Alkaloids](#)”), it is of note that comfrey leaves (e.g., *Symphytum officinale*, *S. uplandicum*, *S. officinale* x *uplandicum*) that have been and are used in salads are also banned from trade in food products in some countries because of the overt hepatotoxic potential and the latent potential of the dehydropyrrolizidine alkaloids to cause or contribute to other disease states.

Potential Secondary Poisoning

Ingestion of a plant, or products made from that plant, is the most direct route of exposure to food plant-associated toxins (primary poisoning). However, there is also the possibility that toxins ingested by livestock can be toxicodynamically distributed to other tissues destined for the human food supply (secondary poisoning) and include common food items such as eggs, milk, and meat. A prime example of the potential for secondary poisoning involves an indospicine (Fig. 1)-induced hepatic

disease in dogs that were fed meat from horses or camels that had been grazing on *Indigofera linnaei* (Birdsville indigo) (Hegarty et al. 1988).

As previously discussed (section “[Ptaquiloside](#)”), ptaquiloside (found in bracken fern) is carcinogenic. The milk from cows fed bracken fern has been shown to be carcinogenic in mice and rats (Smith 2004). It has been further established that ptaquiloside is present in the milk of animals fed bracken fern (Francesco et al. 2011). While this would not be expected to be an issue with modern bulk marketing and distribution of milk, it could well become a local issue on farms or in areas where locally produced milk was consumed. Similar concerns about localized pockets of relatively higher exposure would apply to situations where the plants are locally grown and consumed or where the plant-associated toxins are being translocated to other food products for local or preferential consumption. Given the associated issues of weed infestation of crops and grazing livestock, this potential for geographically localized intoxications would include dehydropyrrolizidine alkaloids (section “[Dehydropyrrolizidine Alkaloids](#)”) and corynetoxins (section “[Corynetoxins](#)”) among others.

Toxicity issues around white snakeroot (*Ageratina altissima* (L.) King & H. Rob. var. *altissima*, previously *Eupatorium rugosum* Houtt and *Eupatorium urticaefolium* Reichard) are not only an example of secondary poisoning but are also an example of instances where the identity of the toxin or toxins has not been unequivocally identified (section “[Uncertain Etiology or Involvement of Putative Toxins](#)”). A member of the Asteraceae, white snakeroot, is found throughout the eastern half of North America. Livestock that graze the plant can sporadically and unpredictably develop a disease called trembles characterized by weight loss, listlessness, reluctance to move, muscular tremors when forced to stand and move, constipation, and apparent joint stiffness (Burrows and Tyrl 2013). Milk consumed from cows that have grazed white snakeroot, including cows that are asymptomatic, can cause a potentially fatal disease in humans termed milk sickness. Milk sickness was also called sick stomach or the slows because its signs and symptoms were characterized in humans by listlessness, leg pains and cramps, loss of appetite, vomiting, constipation, a white coating on the tongue, and an acetone odor on the breath (Burrows and Tyrl 2013). Many deaths among Midwestern settlers during the 1800s (up to 50 % of overall deaths in some parts of Ohio and Indiana) were caused by milk sickness, sometimes forcing entire settlements to be abandoned (Burrows and Tyrl 2013).

In the late 1920s, a straw-colored oil, believed to be a pure compound, named tremetol, was isolated from white snakeroot and understood to be the toxin in the plant (Couch 1927). Decades later, tremetol was separated into a sterol fraction and a ketone fraction that included tremetone, dehydrotremetone, and hydroxytremetone (Fig. 9) that were all toxic in a goldfish assay (Bowen et al. 1963).

Further work demonstrated that tremetone, but not dehydrotremetone, was cytotoxic in cell cultures (Beier et al. 1993). White snakeroot known to contain tremetone, dehydrotremetone, and other similar compounds was toxic to goats in controlled feeding trials (Lee et al. 2012), but the toxicity has not been conclusively attributed to these compounds individually or in combinations.

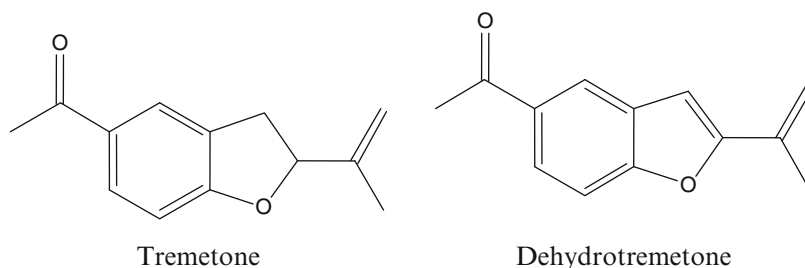


Fig. 9 Benzofuran ketones present in white snakeroot that cause trembles in cattle and milk sickness in humans

Although trembles due to white snakeroot poisoning is still sporadically reported in livestock on a yearly basis, the incidence of milk sickness in humans has been largely eliminated with modern dairy farming practices and the pooling of milk from numerous sources. However, with the current interest in organic, small-scale, and specialty farming in areas with abundant white snakeroot populations, the risk of milk sickness in humans has the potential to increase.

A similar trembles disease occurs in the southwestern USA and northern Mexico when cattle ingest rayless goldenrod (*Isocoma pluriflora* (Torr. & A. Gray) Greene; previously *Isocoma wrightii* (A. Gray) Rydb and *Haplopappus heterophyllus* (A. Gray) S.F. Blake). Similar to white snakeroot, the toxin in rayless goldenrod was initially identified as tremetol (Couch 1930), and later rayless goldenrod was determined to contain tremetone, dehydrotremetone, and other similar compounds (Zalkow et al. 1979) and was toxic to horses (Davis et al. 2013a) and goats in controlled feeding trials (Davis et al. 2013b); however, similarly to the white snakeroot intoxications, the toxin or toxins responsible for the trembles have not been conclusively identified. Although rayless goldenrod-induced trembles is sporadically reported in livestock on a yearly basis, the risk of milk sickness in humans is reduced because small-scale dairy farming is not viable in the southwest USA where rayless goldenrod is prevalent. Nonetheless, localized production and consumption of milk from cows exposed to rayless goldenrod may yet pose a potential problem.

Conclusions and Future Directions

The potential for plant-associated toxins (from the plant per se or from microbial organisms associated with the plant) to enter the human food supply is well known and documented. In many cases the toxins have been unequivocally identified, but there remain many instances of where final conclusive proof of a putative toxin (s) involvement has not been established. Just a cursory examination of the food plants that contain “toxins” (e.g., Table 1) and a personal correlation of those with

the food that one may eat would tend to make a healthy person skeptically wonder about the real hazard that these toxins present to human health. In general terms, providing one is following a diet that is not biased heavily toward a toxin-producing plant, or if none of the potentially exacerbating factors apply, the real risk is obviously minimal. However, in instances where diets are biased due to cultural/traditional influences; droughts, floods, or other environmental influences; poverty; lack of alternative foods; localized production and consumption; or preferential lifestyle choices, then the potential for food poisoning due to plant-associated toxins increases.

Consequently, many foods are regulated with respect to the levels of undesirable, bioactive natural products. While there are many varied methods of analysis for specific food plant-associated toxins, some of which are validated according to international guidelines, an imperative remains to continue to develop and apply efficient analytical methods for monitoring the presence of known (or suspected) toxins in established foods, agronomically or genetically modified foods, and new foods that are being developed.

From a toxicological perspective, it is important to be alert to the possibility that low-level exposures (intermittent or continuous long term) may induce disease in their own right and not necessarily the same as for higher-level exposures with more acute reactions. The low-level exposures may also potentiate adverse effects caused by another bioactive compound, including affecting the bioavailability of certain therapeutic drugs. Therefore, where the data do not exist or are equivocal, then toxicological investigations are required to help define the spectrum of adverse effects that may or may not be rationally expected based upon mechanisms of action and interaction.

To enable toxicological studies, methods for the isolation or preparation of large quantities of the putative toxin(s) are required. Isolation of large quantities of individual toxins can be challenging with a large group of structurally related toxins, e.g., the dehydropyrrolizidine alkaloids (section “[Dehydropyrrolizidine Alkaloids](#)”), that have very similar physicochemical properties. Approaches that are useful on a small scale for structure elucidation studies may not be applicable to larger-scale separations. Other preparation and toxicological considerations include the stability of the putative toxins once they have been extracted from their parent matrix and the possibility of reduced activities of the purified putative toxins because of the loss synergistic effects of co-metabolites (Colegate and Molyneux 2008).

Cross-References

- ▶ [Chemical Composition of Organic Food Products](#)
- ▶ [Chemical Composition of Vegetables and Their Products](#)
- ▶ [Contamination from Industrial Toxicants](#)
- ▶ [Model Fungal Systems for Investigating Food Plant Mycotoxins](#)

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Abstract

One in six people in the USA acquire a foodborne illness each year. *Food poisoning* is a general term used to describe the physiological effects caused by ingestion of contaminated food or water. The effects of ingesting contaminated food range from short-lived symptoms such as vomiting and diarrhea to paralysis and sometimes death. Some foodborne bacterial toxins have enzymatic properties that allow incredibly small quantities of toxin to exert potent physiological effects. Part of the challenge of sustaining a safe food supply is the ability to rapidly detect low levels of these toxins and in particular within a

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biological matrix. Development of useful assays suitable for analysis of complex food matrices is more challenging than traditional laboratory studies of toxin–substrate interactions in a buffer. This chapter surveys the current methods used to detect a variety of bacterial toxins and explores recent research findings that show promise for adaptation to industry. Highlighted here specifically are three genera of gram-positive pathogens that cause foodborne illness through the action of toxins produced while growing in food, namely, *Bacillus*, *Staphylococcus*, and *Clostridium*. Also discussed is what is currently understood about their pathogenesis, the potent toxins they produce, and the efforts to accurately detect these preformed toxins in a biological matrix, specifically food. A special emphasis is placed on botulinum neurotoxins as they are the most lethal foodborne toxins to humans. Since the area of pathogen and neurotoxins detection is a rapidly evolving one, there is also a discussion of a number of important factors one should consider when developing new diagnostics.

Introduction

Food poisoning is a generic term that encompasses two types of disease – one being foodborne infections (where pathogens enter a host and cause a disease state through contaminated food or water) and the second being foodborne intoxications (where preformed toxin molecules enter a host through food and/or water and cause disease by altering the normal functioning of host cells). Pathogens that can cause foodborne illness are bacteria, viruses, fungi, protozoa, and helminths. The degree and severity of pathogenesis caused by foodborne illnesses range from self-limiting diarrhea and vomiting to life-threatening medical emergencies. Although the groups of foodborne pathogens listed above represent diverse biological taxa, they do share a common denominator with regard to their mode of transmission – through contaminated food and water. Food can serve as a favorable matrix for microbial growth, and depending on environmental conditions such as pH and temperature (refrigeration versus room temperature), pathogens can multiply to sufficient numbers to cause illness.

Bacterial pathogens are divided into gram-negative and gram-positive organisms. Gram negatives include *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and other enterobacteriaceae. These gram negatives can cause disease by infecting their hosts, colonizing and damaging the intestinal mucosa, and in some cases producing toxins that may cause acute diseases such as diarrhea and vomiting; however, several of these pathogens such as *E. coli* O157:H7 can also produce hemorrhagic toxins that can cause life-threatening symptoms such as hemolysis and organ failure. This sequence of steps – pathogen enters a host, establishes and colonizes, and then produces toxins – is food infection. On the other hand, foodborne intoxication involves the growth of bacteria and subsequent toxin production in food prior to consumption. Examples here include the toxins produced by the gram-positive *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium* spp. These three pathogens, and in particular their toxins, present the greatest risk of food poisoning in humans (Bennett et al. 2013).

Depending on the health status of the individual, food poisoning can progress from symptoms of self-limiting vomiting and diarrhea to becoming life threatening and in rare cases fatal disease. It has also been suggested that these toxins and their possible harm to humans presents an opportunity for their use in acts of bioterrorism. Techniques for sensitive, accurate, and rapid detection and inactivation of these toxins in food matrices (or any other biological matrix) will be critical to maintaining a safe food and water supply for humans and animals (Gould et al. 2013); some of these same technologies may be extended for use in clinical cases. In addition to these food safety concerns, the study of these bacterial toxins has also spurred interesting biological questions about their evolutionary significance and role in microbial biology and host–pathogen interactions.

A study published in 2013 by the Division of Foodborne, Waterborne, and Environmental Diseases and the Epidemic Intelligence Services of the Centers for Disease Control and Prevention focused on the distinguishing epidemiology and clinical characteristics of three known pathogens that cause foodborne illnesses (Bennett et al. 2013; Gould et al. 2013). Of the estimated 9.4 million foodborne illnesses in the USA each year, the majority of these cases are caused by known pathogens. Of these illnesses, 1.3 million (14 %) are caused by three gram-positive pathogens: *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium perfringens*. These three pathogens synthesize toxins in food prior to its consumption (in the case of *B. cereus* and *S. aureus*) or in vivo after consumption of contaminated food (*C. perfringens*). Criteria for determining the etiology of an illness or outbreak include symptoms, incubation period, duration of the illness, and the suspected contaminated food. From 1998 to 2008, 1,229 foodborne outbreaks caused by *B. cereus*, *C. perfringens*, and *S. aureus* were reported in the United States. Thirty-nine percent of these outbreaks were reported with a confirmed etiology. Vomiting was the key symptom with both *B. cereus* and *S. aureus* illnesses. Meat and poultry were the implicated foods in *C. perfringens* and *S. aureus* cases, and rice dishes were most common in *B. cereus* cases. Problems with food preparation or processing were reported in 93 % of cases. Contamination by a food service worker was common in most *S. aureus* outbreaks (55 %). In the case of *C. botulinum*, in the United States alone, an average of 145 cases are reported each year. Of these, approximately 15 % are foodborne, 65 % are infant botulism, and 20 % are wound associated. Adult intestinal colonization and iatrogenic botulism also occur but rarely. Outbreaks of foodborne botulism involving two or more persons occur most years and are usually caused by toxins present in home-canned foods (Bennett et al. 2013; Gould et al. 2013). Table 1 summarizes important information regarding these organisms and the foodborne illnesses they cause.

For each of these pathogens, major virulence factors are toxin molecules produced by the bacteria while still in unconsumed food, and once ingested, can sicken people. Some of these molecules can be destroyed with heat, but some are heat stable. Since the primary cause of these foodborne illnesses are toxins rather than the presence of the pathogens themselves, their successful detection requires methods beyond standard DNA analysis such as polymerase chain reaction (PCR). Commercial kits are available for detection of many, but not all, of

Table 1 Summary of relevant information about foodborne illnesses caused by toxin-producing gram positive bacteria

Pathogen	Food sources	Incubation	Duration of illness
<i>B. cereus</i>	Variety, rice, leftovers	Diarrhea 6–15 h	24 h
		Emetic 30 min–6 h	
<i>S. aureus</i>	Food made with hand contact and no cooking	1–6 h	24–48 h
<i>C. perfringens</i>	Beef, poultry, gravies	6–24 h	24 h or less
<i>C. botulinum</i>	honey, home-canned low acid foods	Infants: 3–30 days	Variable
		Adults: 12–72 h	

these toxins. The efficacy of these kits varies, and the effects of complex matrices such as food complicate correct identification in many outbreaks. The lack of detection methods has diminished the ability to correctly identify the etiology of some outbreaks of foodborne illness (Khabbaz et al. 2014; Biggerstaff 2014).

The goal of this chapter is to provide a broad overview of foodborne illnesses caused by four toxin-producing pathogens: *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Clostridium botulinum*. The first three organisms cause the most prevalent, toxin-associated foodborne illnesses in the US (Bennett et al. 2013; Gould et al. 2013; Khabbaz et al. 2014). *C. botulinum* is also included in this discussion since it produces the most potent foodborne toxin in humans and the vast number of studies on this toxin has provided an excellent model system for research and development of robust detection reagents against a deadly toxin. The biological properties of their toxins, their role in foodborne illness, and current methods to detect their presence in the environment and in food matrices will be explored. The reader is also encouraged to consult the US Food and Drug Administration's (FDA's) *Foodborne Pathogenic Microorganisms and Natural Toxins Handbook* for further information on these as well as other pathogens and toxins that cause foodborne illnesses.

Development of Detection Assays for Bacterial Toxins in Food: Important Factors to Consider

The development of a robust assay for the detection of any pathogen or biological product of a pathogen (such as toxins) requires consideration of several factors including sensitivity, specificity, matrix effects, and biological activity (Cheng et al. 2012). All of these factors will be discussed below in the context of what have been described using well-established model systems. A particular emphasis is placed on studies describing botulinum toxin detection assays. Many of these studies could be used as a basis for exploring assay development in other toxin systems.

Assay sensitivity is not a simple criterion to define in detecting any pathogen or toxin. Often, the level of sensitivity is determined in part by the method.

For example, the human lethal dose (LD) for oral intoxication with botulinum neurotoxin (BoNT) is estimated at 1 $\mu\text{g}/\text{kg}$ or about 70 μg for a 70 kg adult (Cheng et al. 2012). Assays designed for evaluating food must detect at least this amount in a typical portion. Since portions vary widely between individual foods, assay sensitivity requirements may vary with specific matrices. Foods that typically have large portion sizes would require assays with lower detection limits. Furthermore, the dose to cause illness but not death might be lower. For instance, experience with BoNT exposure in rodents is that a level tenfold lower than the minimal lethal dose falls into this category. Projecting rodent data to humans is risky, but applying the same tenfold lower factor to humans suggests that an assay must have a sensitivity of at least 7 $\mu\text{g}/\text{serving}$, or 0.007 $\mu\text{g}/\text{mL}$ (7 ng/mL) for a 100 mL portion. In contrast, tests designed for analysis of sera or other clinical matrices should be as sensitive as possible to account for low toxin levels. For example, in oral mouse toxicity studies, only a small portion of the ingested BoNT actually survives the harsh conditions in the gut to reach the bloodstream (Cheng et al. 2012; Dunning et al. 2012). Likewise, the lethal toxin intravenous dose varies between 20 and 200 ng in an adult human with approximately 5 L of blood (Cheng et al. 2012; Dunning et al. 2012; Zhang et al. 2012). Taking into account natural degradation and clearance of toxin in sera, the assay sensitivity for diagnostic evaluation must be in the low to sub-pg/mL range.

Many bacterial pathogens can produce multiple toxins depending on their environment, and different isolates within a single genus may produce different toxins. Some of these toxins share significant amino acid identity or similarity. For instance, there are currently seven known serotypes of BoNTs and 32 known subtypes (Kammerer and Benoit 2014). Undoubtedly, new subtypes will be identified in the future. Amino acid sequence differences can vary as much as 70 % among serotypes (Kammerer and Benoit 2014). This level of genetic diversity and variation can prove challenging for both molecular and antibody-based diagnostic methods. False-negative results could be obtained if a gene or protein structure of the toxin differs from established oligonucleotides/PCR primers or if critical antibody binding epitopes are modified. At the very least, assay performance needs to be established using as many toxin sero- and subtypes as practical. Ideally, assays should recognize all known subtypes of each serotype. Such an assay may require incorporating multiple primers for a PCR assay or antibodies for an immunoassay.

In almost all real-world scenarios, toxin samples to be tested are found in a matrix: a food or food product, a clinical sample (serum, sputum, feces, etc.), or an environmental sample (dust, soil, water, etc.) (Cheng et al. 2012; Dunning et al. 2012; Zhang et al. 2012). However, the majority of assay methods are designed, tested, and optimized in buffer conditions, and thus, assay performance, sensitivity, matrix effects, or influence of nearest neighbor compounds may be diminished or altered. For example, a food matrix may contain conditions such as high fat, high protein or salt content, or extreme pH; the presence of active proteases could also interfere with detection sensitivity, increase background signal, and be responsible for false-positive or negative results. Approaches to reduce

matrix interference range from simple sample dilution, pH rebalancing, addition of protease inhibitors, to specific affinity-binding steps prior to detection (Cheng et al. 2012; Dunning et al. 2012; Zhang et al. 2012). Ideally, examination of different matrices containing the target analyte as well as nearest neighbors is necessary to evaluate assay performance. These will help identify methods to circumvent matrix effects on assay performance.

The potent toxicity associated with bacterial toxins can be attributed to their enzymatic properties such as proteolytic (such as BoNT) or ADP ribosylating (such as some toxins produced by *B. cereus*) activity. The differentiation of active versus inactive forms of the toxin is needed for proper risk assessment and should be an important consideration in assay design. However, measuring only enzymatic activity is often insufficient to develop a real measure of activity. For example, measurement of the toxicity of BoNT must consider multiple factors in addition to just the presence of toxin. The toxin must be able to bind host cell receptors; it must translocate across cellular membranes and finally reach the host cell cytosol and cleave its target protein. Thus, the toxicity of BoNT is a multistep process culminating in a proteolytic event. Few assays if any, other than an animal-based bioassay, can measure all aspects of toxin function. Immunoassays (IA) generally do not differentiate between active and inactive toxin and may give false results even when no active toxin is present. An IA for BoNT that requires the presence of both toxin heavy and light peptide chains to obtain a positive signal can detect inactive toxin but a positive response is predictive of a structurally intact toxin (Cheng and Stanker 2013). For botulism, the presence of an intact toxin molecule is necessary for toxicity. Assays measuring endopeptidase activities of BoNTs are available but are not as sensitive and amenable to use in complex matrices. Genomic methods, while sensitive, detect the presence of toxin genes but not that of toxin. Depending on the diagnostic needs, a combination of methods may have to be used to get a more complete profile of the toxin activity.

For the widest application of an assay, it must be user friendly and allow for a timely diagnosis. Laboratory-based assays should use equipment or tools that are readily available and require minimal training to execute. Ideally, the assay should also be field deployable. In the discussion below, basic toxin biology of *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium* spp. are discussed along with what is known about current detection methods for these toxins. In addition to this discussion, the reader needs to know that the FDA sets scientific standards for testing foods for various contaminants. Laboratories, food companies, and government regulators worldwide use these standards to make sure food products are safe to eat and drink. The FDA's Bacteriological Analytical Manual (BAM) describes the agency's preferred laboratory procedures for the detection of pathogens (bacterial, viral, parasitic, as well as yeast and mold) and microbial toxins in food and cosmetics. The complete BAM is available online and is updated as needed (last update was April 2014). The reader is encouraged to consult this informative and excellent online text for detailed methods and criteria for presumptive or confirmative analyses.

***Bacillus cereus* and Its Foodborne Toxins**

Bacillus cereus is a gram-positive, facultatively anaerobic spore-forming bacterium that belongs to the “*Bacillus cereus* group,” also referred to as the *Bacillus cereus sensulato* group. This includes the five closely related species *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, and *Bacillus weihenstephanensis*. These bacteria share a high degree of genetic similarity, and it has been difficult to separate them into different taxa. In fact, there is some debate as to whether they represent variants of a single species. Regardless of these issues associated with taxonomic classification, *B. cereus* is a major foodborne pathogen. Some strains are harmful to humans and produce toxins that cause either diarrhea or an emetic toxin that causes nausea and vomiting (Stenfors et al. 2008). *B. cereus* is also considered an opportunistic pathogen causing bacteremia, meningitis, pneumonia, and gas gangrene–like cutaneous infections, primarily in immune-compromised patients. The United States Centers for Disease Control and Prevention estimates that there are 63,000 annual cases of foodborne illness attributed to *B. cereus* and 20 estimated hospitalizations associated with this pathogen (Bennett et al. 2013; Gould et al. 2013; Khabbaz et al. 2014). Sources of food poisoning by *B. cereus* include a variety of foods such as rice and leftovers as well as food stored at room temperature for prolonged periods of time. *B. cereus* produces one emetic “short incubation” toxin (EME) and three different enterotoxins: nonhemolytic Nhe, HBL (hemolysin B), and cytK (cytotoxin K) (Ramarao and Sanchis 2013; Ceuppens et al. 2011; Senesi and Ghelardi 2010). The large–molecular weight toxins cause diarrheal disease, while emetic disease is caused by a low–molecular weight toxin (1.2 kDa) often associated with rice prepared for a time and temperature insufficient to kill any spores present (Ramarao and Sanchis 2013; Ceuppens et al. 2011; Senesi and Ghelardi 2010). Studies defining the distinct role for each different enterotoxin have revealed that 90 % of the total toxicity can be attributed to the HBL and Nhe enterotoxins. Interestingly, a recent study revealed differential sensitivity to these toxins in different host cell types, suggesting that distinct receptors exist for each toxin (Ramarao and Sanchis 2013).

Biochemical details of HBL, Nhe, and CytK have been revealed through gene cloning and protein expression efforts as well as recent strides in genome sequencing. The emetic toxin, a small–molecular weight molecule (1.2 kDa) also called cereulide, is a heat-stable toxin that is synthesized by the bacteria while it contaminates food (Vangoitsenhoven et al. 2014; Rajkovic et al. 2014). Foodborne intoxication caused by cereulide occurs shortly after ingestion. Cereulide is a potassium ionophore–like compound that destroys the membrane potential of mitochondria and uncouples oxidative phosphorylation through a mechanism similar to the antibiotic valinomycin. Toxicity leads to emetic disease in primates. Chemically, its structure resembles valinomycin and is colorless, tasteless, and odorless. It is a cyclical tripeptide with three repeats of four α -amino and α -hydroxyacids [cyclo (l-O-Val-l-Val-d-O-Leu-d-Ala-)₃]. It is resistant to degradation by heat, acid, and alkali, and the minimal concentration of cereulide that causes disease in humans has yet to be determined accurately, although it has been purported that ingestion of 10 μ g of cereulide per kg body weight may be sufficient to cause emesis.

The synthesis of cereulide occurs during vegetative growth in improperly refrigerated foods. The gene clusters encoding cereulide (called *ces*) are found on a 24 kb gene cluster contained within a megaplasmid called pBCE. Interestingly, the genes are flanked by 5' and 3' sequences that are homologous to the pXO1 toxin plasmid of *B. anthracis*. The toxin is synthesized using nonribosomal peptide synthetases and is difficult to control in food production given the ubiquitous nature of *B. cereus* and environmental resistance of spores. Mechanistically, cereulide acts similarly to valinomycin; it inhibits boar spermatozoa (as low as 1 nM) and causes swelling of their mitochondria, increases conductance across membranes when placed into KCl solutions, and forms adducts with K⁺ ions (Rajkovic et al. 2007). These results were similar when tested on rat liver mitochondria. In HepG2 cells, transcription and cell proliferation were inhibited at 2 nM cereulide. Recently, using pancreatic beta cells, 24 h exposure to 5 ng/mL cereulide was sufficient to cause cell apoptosis, even though the concentration was lower than that associated with systemic disease (Vangoitsenhoven et al. 2014). Early studies utilized monkey-feeding experiments since other suitable models were unavailable (Rajkovic et al. 2014; Heilkenbrinker et al. 2013). Recently, cell culture assays using Vero cell and Hep-2 cells were developed (Rajkovic et al. 2014; Heilkenbrinker et al. 2013).

Past studies utilized the addition of antimicrobial compounds designed to inhibit the production of cereulide in food. The availability of compounds that block the synthesis of cereulide has been lacking. However, a recent study focused on polyphosphates and orthophosphates which are based on their successful use in the dairy and meat industries as emulsifying and stabilizing agents and flavor protectors in addition to their antimicrobial properties. Polyphosphates have been shown to inhibit nonribosomal peptide synthetases as well, including the enzyme that produces cereulide. Using an *in vitro* *B. cereus* growth system and HEp-2 cells, addition of polyphosphates did not block growth of *B. cereus* but did block cereulide gene transcription 70–100 % in reconstituted infant food and oat milk (Frenzel et al. 2011). Whether a similar observation is noted in other foods has yet to be described. Further, the large-scale use of polyphosphates specifically to inhibit synthesis of cereulide and similar toxins shows promise but has yet to be implemented in food-manufacturing settings.

The other toxins produced by *B. cereus* are associated with the diarrheal form of the disease and are referred to as enterotoxins; these are the hemolytic enterotoxin (HBL), the nonhemolytic endotoxin (Nhe), and cytotoxin K (CytK). Both HBL and Nhe are tripartite enterotoxins, while CytK is homologous to b-barrel pore-forming complexes (HBL B/L₁/L₂ = 37.8, 38.5, and 43.2 kDa respectively; and NheA/B/C = 41, 39, 40 kDa, respectively). Collectively, the pathogenesis is likely due to membrane damage of a number of different cells, including intestinal cells. The loss of fluid may also be attributed to activation of host cell adenylate cyclase. Of these three characterized toxins, HBL is the only one that has been shown to play a direct role in diarrhea (Ramarao and Sanchis 2013; Ceuppens et al. 2011; Senesi and Ghelardi 2010; Vangoitsenhoven et al. 2014; Rajkovic et al. 2014).

These toxins are heat labile and are formed 5–16 h post ingestion of contaminated food containing *B. cereus*. Table 2 summarizes information on both the

Table 2 Major emetic and enterotoxins produced by *Bacillus cereus*

Major toxin	Pathology	Molecular structure	Mechanism of action
Cereulide	Emetic food poisoning	Cyclical tripeptide	Ionophore/uncouples electron transport
Non-hemolytic Enterotoxin (Nhe)	Diarrheal food poisoning	Tripartite: NheA, B, C	Pore-forming complex
Hemolysin BL (Hbl)	Diarrheal food poisoning	Tripartite: Hbl B, L ₁ , L ₂	Pore-forming complex
CytK	Diarrheal food poisoning	Single polypeptide	Pore-forming complex

emetic and diarrheal toxins. Other toxins may exist and may play a role in foodborne illness in addition to these noted major toxins.

To further dissect the mechanism of action of these toxins, *in vitro* cell culture models and *in vivo* animal studies have been used. *In vitro* pathology associated with HBL include hemolysis, vascular permeability, degradation of explanted retinal tissue, and necrosis in rabbit skin. *In vivo* models using rabbits have shown ocular necrosis and inflammation and rapid fluid accumulation in a rabbit ileal loop model (which is considered the gold standard test for determining diarrheal activity caused by an enterotoxin). In fact, the potency level of HBL is comparable to cholera toxin, highly suggestive of its role in pathogenesis on the surface of red blood cells, and then hemolytic activity arises through a colloid osmotic mechanism. The equilibration of ions through the pore gives rise to a net influx of ions, an accompanying movement of water, cell swelling, and then lysis. A proposed model for pore formation predicts oligomerization of the B subunit into a heptamer or octamer, and the L1 and L2 subunits help to induce conformational changes in the B subunit to facilitate oligomerization, or to stabilize the oligomer of B (Ramarao and Sanchis 2013; Ceuppens et al. 2011; Senesi and Ghelardi 2010; Vangoitsenhoven et al. 2014; Rajkovic et al. 2014).

The non hemolytic (Nhe) enterotoxin contains three components called NheA, NheB, and NheC. The Nhe complex is toxic to vero cells and supernatants from strains lacking HBL show no toxicity when monoclonal antibodies to NheB are added. The individual components of the complex are synthesized independently and assemble into a ratio of 10:10:1 (NheA:NheB:NheC). NheB is the binding component and the mechanism of action is through pore formation on the membrane and subsequent colloid osmotic lysis. Interestingly, hemolytic activity can be detected in Nhe, although much lower than observed in the HBL complex. Structural comparison shows similar features between NheB and NheC with HBL (Vangoitsenhoven et al. 2014; Rajkovic et al. 2014).

Cytotoxin K is a 34 kDa molecule with enterotoxigenic against intestinal epithelial cells and pore forming capability in planar lipid bilayers. Two forms of CytK have been identified, denoted as CytK1 and CytK2. The role of each form has not yet been deduced. Peptide sequence analyses of CytK reveal homology to *Staphylococcus aureus* leucocidins, γ -hemolysin, and α -hemolysin, *Clostridium perfringens* β -toxin, and *B. cereus* hemolysin II. All of these bacterial proteins

Table 3 A comparison of three currently available test kits for detection of *B. cereus* enterotoxins (Ceuppens et al. 2012)

	BDE VIA™	BCET-RPLA	Duopath™
<i>Bacillus cereus</i> enterotoxin	Nhe-AB	Hbl-L ₂	Nhe-B and Hbl-L2
Minimal sample volume needed (μL)	200	50	150
Sample analyzed	Food	Food, isolate	Food, isolate
Detection limit (ng/mL)			
Manufacturer	1	2	ND
Published studies	2–5	≥0.6	6–20
Time to result (hours)	5	20	0.5

are predicted β -barrel channel forming toxins. Although the mechanism of CytK assembly and function has yet to be clearly described, if CytK follows other similarly beta-barrel toxins, then it would be predicted that they are secreted as water-soluble monomeric proteins and then assemble into beta-barrel-shaped pores/channels through membranes of target cells, causing cell death and lysis. A recent analysis of 616 isolates revealed expression of CytK in 85 % of *B. cereus* and *B. thuriangiensis* isolates (Vangoitsenhoven et al. 2014; Rajkovic et al. 2014).

Characterization of these toxins is critical to better understanding the distribution of isolates with potential of causing foodborne illness. For instance, epidemiological studies have shown that while the presence of *B. cereus* spores in the environment are ubiquitous, the prevalence of emetic-causing *B. cereus* isolates is rare (Stenfors et al. 2008). PCR methods are critical for conformation of cereulide-producing isolates. Stenfors and colleagues (Stenfors et al. 2008) tested a panel of 176 strains including *B. cereus* strains, *B. cereus* group strains, and other *Bacillus* spp. using PCR, immunoassays, and cytotoxicity tests and assessed the consistency of their results. A screening multiplex PCR for the detection of *hbl*, *nhe*, *ces*, and *cytK1* as well as two multiplex PCRs for the differentiation of Hbl genes (*hblC*, *hblD*, *hblA*) and Nhe genes (*nheA*, *nheB*, *nheC*) was applied. They observed a strong correlation for these three independent assays, and no false-negative PCR results were observed for any of the strains that tested positive by immunoassay and using cytotoxicity tests. The three multiplex PCRs proved to be a reliable method for the identification of enterotoxinogenic *B. cereus* isolates (Stenfors et al. 2008).

Given the importance of rapid identification of food contaminated with these toxins, it is necessary to discuss what tests are available to laboratory personnel. Currently, there are three commercial kits available for the detection of *B. cereus* diarrheal enterotoxins. These kits focus on the identification of Nhe and Hbl. A visual immunoassaykit (BDE VIA™ from 3 M Tecra) and two *B. cereus* enterotoxin reversed passive latex agglutination kits (BCET-RPLA from Oxoid) and Duopath™ Cereus Enterotoxins (Merck) (Ceuppens et al. 2012). *B. cereus* might also produce other toxins, but no kits for other toxins are currently available for any of these virulence factors. A comparison of these three kits and their use are shown in Table 3. No detection kit is yet available for cereulide or CytK (Ceuppens et al. 2012).

Genetic exchange among different *Bacillus* spp. complicates determination of the correct etiology in clinical cases. For instance, an isolate of *B. cereus* containing the genes encoding an anthrax-like toxin was probably acquired through lateral gene transfer. This molecule, called certhrax, genetically resembles anthrax and functions as an ADP-ribosylating toxin found similar to what is observed in *B. anthracis* (Visschedyk et al. 2012; Simon et al. 2013; Simon and Barbieri 2014). Certhrax was first identified in *B. cereus* strain G9241. Certhrax shares 31 % sequence identity with anthrax lethal factor from *Bacillus anthracis* that may contribute to pathogenesis. Strain G9241 was isolated from a welder with cutaneous anthrax-like symptoms. Two plasmids encoding the toxin were isolated from G9241. Further investigation of the properties of this unusual toxin will indicate whether an anthrax-like disease, caused by certhrax, could be acquired through a foodborne route. Whether this toxin, or others identified in the future, poses food safety problems has yet to be observed.

The genetic similarity and plasticity among different *Bacillus cereus* group members poses a taxonomic dilemma with many interesting biological questions to be asked. From a food safety standpoint, the high degree of similarity among members, such as *B. cereus* and *B. anthracis*, emphasizes the critical need to identify markers that can allow discrimination among pathogenic and nonpathogenic isolates. Perhaps the development of detection tools that focus on toxins discussed herein may help investigators rapidly identify isolates that pose threats to the food supply.

***Staphylococcus aureus* and Staphylococcal Food Poisoning (SFP)**

Staphylococcus is a gram-positive, catalase-positive, spherical, non-spore forming, nonmotile bacterium that can exist in pairs, short chains, or grape-like clusters (Principato and Qian 2014; Hennekinne et al. 2012; Krakauer and Stiles 2013). They are facultative anaerobes and are ubiquitous in the environment. They can be found in the air, dust, sewage, water, on most surfaces, and on animals and humans. Some staphylococcal species are used in meat and dairy fermentation, and the potential for enterotoxin production by these “food grade” species is inconclusive. Some investigators have demonstrated toxin production, while others have not (Principato and Qian 2014; Hennekinne et al. 2012; Krakauer and Stiles 2013). Strain specificity appears to play a role in whether an isolate does or does not make a toxin that can lead to food poisoning.

Enterotoxigenic strains of *Staphylococcus aureus* are the primary cause of staphylococcal food poisoning (SFP). Twenty-two different enterotoxins have been described to date (as of September 2014) (Hennekinne et al. 2012; Krakauer and Stiles 2013; Omoe et al. 2013; Hait et al. 2014; Jenko et al. 2014). The toxins are single-chain globular proteins with molecular weights of approximately 22–29 kDa. Table 4 provides a summary of the staphylococcal enterotoxins (SE) identified to date. These toxins belong to a family of molecules known as pyrogenic toxin superantigens. Superantigens do not require intracellular processing by antigen-

Table 4 A brief summary of staphylococcal enterotoxins identified to date, their molecular weights, emetic potential, and diagnostic method of detection. *SE* staphylococcal enterotoxin, *SEI* staphylococcal enterotoxin-like (Krakauer and Stiles 2013; Jenko et al. 2014)

Toxin	Molecular weight (kDa)	Causes emesis	Diagnostic method
SEA	27.1	Yes	Commercial kits
SEB	28.4	Yes	Commercial kits
SEC	27.5–27.6	Yes	Commercial kits
SED	26.9	Yes	Commercial kits
SEE	26.4	No	Commercial kits
SEG	27.0	Yes	None
SEH	25.1	Yes	None
SEI	24.9	Weak	None
SE/J	28.5	Nd	None
SE/K	26.0	Yes	None
SE/L	26.0	No	None
SE/M	24.8	Nd	None
SE/N	26.1	Nd	None
SE/O	26.7	Nd	None
SE/P	27.0	Nd	None
SE/Q	25.0	No	None
SER	27.0	Yes	None
SES	26.2	Yes	None
SET	22.6	Weak	None
SE/U	27.1	Nd	None
SE/U2	Nd	Nd	None
(SEW)	Nd	Nd	None
SE/V	Nd	Nd	None

presenting cells before presentation to T cells and can activate T cells by cross-linking MHC class II molecules directly on antigen-presenting cells. This cross-linking can activate many T cells, leading to proliferation and subsequent release of chemokines. These chemokines can potentially cause toxic shock syndrome. SE toxins also can directly interact with epithelial cells and stimulate an inflammatory response. The most widely studied example is toxic shock syndrome (TSS) caused by toxins of *Staphylococcus* spp. (Principato and Qian 2014; Hennekinne et al. 2012; Krakauer and Stiles 2013). Staphylococcal toxins also cause food poisoning, and several SEs have been studied extensively, including SEB, which is a Category B Priority Pathogen as indicated by the National Institutes of Health, NIAID. These foodborne toxins utilize a primary mechanism of action (based on extensive studies using staphylococcal enterotoxin B, denoted as SEB) similar to TSS, an exacerbation of a cell-mediated immune response. In food poisoning, the preformed toxin enters the body, binds receptors, and gains access to immune cells. Specifically, SEB binds directly on major histocompatibility complex II on host cells without intracellular processing. This binding to MHCII then stimulates T-cell binding and activation. In fact, in the case with SEB, it has been reported that one in

five T cells may become activated as a result of SEB superantigen activity. This is in comparison to an estimated 1 in 10,000 T cells stimulated in a typical antigen presentation. Other T-cell populations then become activated in response and stimulate cytokine production and release. Crystal structures of several SEs have revealed similar three-dimensional structures despite differences in primary peptide sequence. The unique folding of these toxins might explain some of their resistant properties. For example, SEs are resistant to proteolytic enzymes, such as trypsin and pepsin, which allows them to remain intact during passage through the digestive tract. These toxins are also resistant to common environmental conditions that would inactivate other enterotoxins – such as freezing, drying, heat treatment, and low pH (Hennekinne et al. 2012; Krakauer and Stiles 2013).

Although a large number of putative enterotoxins have been identified using genomic techniques, the main criterion for categorizing an enterotoxin is whether or not it causes emesis in a primate animal model (Principato and Qian 2014; Hennekinne et al. 2012; Krakauer and Stiles 2013). As shown in the table above, whether all of these toxins causes emesis still remain to be determined, and most of the studies described herein utilized a single serotype. Whether multiple serotypes synergize pathogenesis associated with foodborne illness needs to be addressed. To date, analyses of toxic dosages in food have primarily focused on staphylococcal enterotoxin A (SEA). It has been reported that 0.5 µg of SEA can cause symptoms such as vomiting (Principato and Qian 2014). Others cited a 50 % emetic dose of ~0.2 µg SE per kg of human body weight (Jenko et al. 2014; Attien et al. 2014). Thus, an average adult human would require about 10–20 µg of SE to suffer symptoms. Other authors have argued that dosages as low as <1 µg could cause food poisoning symptoms. Still other studies have shown even lower dosages are sufficient to cause food poisoning (Jenko et al. 2014; Attien et al. 2014). Recovery of *S. aureus* from contaminated food or the detection of SE toxins in food is the primary means of diagnosing staphylococcal food poisoning. If doses lower than those reported can cause illness, detection methods will need to be more robust. *S. aureus* cells are heat sensitive but SE toxins are not, which can complicate diagnosis (Hennekinne et al. 2012).

Bioassays of food extracts in animals and/or superantigen activity in cell cultures can confirm the presence of active toxin. As described before, emetic activity using monkey-feeding or kitten intraperitoneal tests can confirm the presence of active toxin (Jenko et al. 2014; Attien et al. 2014). The house musk shrew is a more recently developed toxicity model (Attien et al. 2014). However, dosages above 2.3 µg are necessary to cause pathology. Since this amount of toxin is much higher than what is needed to cause disease in humans, many investigators do not consider the house musk shrew technique relevant and appropriate (Jenko et al. 2014).

Molecular diagnostic methods for staphylococcal food poisoning include PCR methods detecting enterotoxin genes in contaminated food (Simon and Barbieri 2014). Here, the presence of genes encoding SEs is detected by amplification. The limitation of this method is that it only demonstrates the presence of the SE genes but provides no information on the actual presence of toxin. Therefore, PCR methods measuring enterotoxin genes should not be the only means to determine

that *S. aureus* is the cause of a foodborne outbreak. Newer approaches include the extraction of RNA from contaminated food samples followed by reverse transcription and PCR analysis to detect SE toxin gene expression. Of course, the complex chemistry of food can interfere with the PCR (Jenko et al. 2014; Attien et al. 2014). Additional studies using a variety of food matrices are warranted.

Immunological methods for staphylococcal food poisoning are based on the use of antitoxin polyclonal or monoclonal antibodies (Jenko et al. 2014; Attien et al. 2014). Diagnostic tests are commercially available based on enzyme immunoassay (EIA) including enzyme-linked immunosorbent assay (ELISA) and enzyme-linked fluorescent assay (ELFA). The main challenge using these techniques is the difficulty of detecting the toxin in food matrices. An additional limitation is the lack of availability of antibodies to all the different SE toxins (antibodies only exist for SEA through SEE). Furthermore, some kits have low sensitivity, which can lead to false negatives. Since antisera are available only for SEA through SEE, commercial immunoassays for all SE toxins are not yet possible. Finally, immunoassays generally do not differentiate between active and inactive toxin.

Mass spectrometry (MS)-based methods have been successfully used to identify toxins and are considered the most sensitive of all methods for enterotoxins (Attien et al. 2014). However, sample preparation limits the widespread use of this method. In food matrices, there are many proteins, lipids, and other substances that can interfere with detection of the toxin. In a recent outbreak, MS-based methods were used successfully in the identification of toxins (Attien et al. 2014); however, the costs were less than optimal. The continued development of MS-based methods and production of ELISA-based methods for the other SEs should help investigators quickly identify toxins in food and other biological matrices. Other methods that have been used in the identification of the staphylococcal enterotoxins and may have application in foods are a T-cell proliferation assay and polyacrylamide gel electrophoresis (PAGE) combined with immunoblotting (Omoe et al. 2013; Hait et al. 2014; Jenko et al. 2014; Attien et al. 2014).

AOAC International has approved the microslide double diffusion method as their current standard for evaluating new methods to detect SE toxins (Jenko et al. 2014). Other methods used with food extracts should be at least as sensitive as the microslide method, which requires concentrating 600 mL extracts from a 100 g food sample to about 0.2 mL (Jenko et al. 2014). Less sensitive methods are deemed inadequate. However, radioimmunoassay (RIA), agglutination, and enzyme-linked immunosorbent assay (ELISA) require less or no concentration of food and therefore save time and are considered more sensitive. Latex agglutination is another serological tool for identifying staphylococcal enterotoxins. Several ELISA methods have been proposed for the identification of enterotoxins in foods, but except for a polyvalent ELISA and an enzyme-linked fluorescent immunoassay (ELFA), their specificity has not been studied extensively. Among ELISA methods, the “double antibody sandwich” ELISA is ideal, given that reagents are commercially available in polyvalent and monovalent formats for both toxin screening and serotype-specific identification. An automated enzyme-linked

fluorescent immunoassay has been developed and is commercially available. This method has undergone specificity and sensitivity evaluations and has proven to be an effective serological system for the identification of staphylococcal enterotoxin in a wide variety of foods.

Examining staphylococci isolated from foods for enterotoxin production helps to establish potential sources of enterotoxin in foods. Of the methods developed for laboratory testing for enterotoxin production, culturing cells in semisolid agar is approved by AOAC International (Hait et al. 2014). It is simple to perform and requires minimal, routine laboratory equipment. Another simple approach is the use of pH 5.5 brain-heart infusion (BHI) broth. The major problem with identifying enterotoxins in foods is that minute concentrations are sufficient to cause food poisoning. Pasteurization and thermal processing may render most toxins serologically unreactive. Consequently, false negatives may result if detection methods lack sufficient sensitivity to detect active toxin (Hait et al. 2014; Jenko et al. 2014).

The FDA has now suggested that the VIDAS[®] SET2 be the preferential polyvalent test to ascertain the presence of staphylococcal enterotoxins type A and E (SEA-SEE) in foods. The second-generation antibody optimizes capture and detection by using a combination of SEA-SEE monoclonal anti staphylococcal enterotoxin antibodies. Removal of the Fc region of the antibodies allows for increased specificity by reducing nonspecific binding via the Fc portion of the antibody that may result in false-positive responses. The use of two different polyvalent kits to analyze one possible contaminated sample is also now recommended by the FDA.

The question of whether methicillin-resistant *Staphylococcus aureus* (MRSA) could present possible food safety issues has been discussed in recent years (Doyle et al. 2012; Wendlandt et al. 2013). Isolate CC398 is a lineage of MRSA that is most often associated with asymptomatic carriers in food animals. As of June 2009, only one case of food intoxication due to CC398 MRSA had been identified (Doyle et al. 2012; Wendlandt et al. 2013). Food may be contaminated with CC398, and handling contaminated food may facilitate transmission. Monitoring MRSA that carry toxin genes within the food supply should be a top priority. There is general agreement that more work needs to be done to pinpoint which toxins are directly related to vomiting and/or diarrhea.

***Clostridium perfringens* Produces a Foodborne Toxin**

Clostridium perfringens is an anaerobic, spore-forming gram-positive bacterium that receives most of its attention by causing gas gangrene. However, *C. perfringens* is also the second most prevalent foodborne pathogen (Alves et al. 2014; Stiles et al. 2014; Li et al. 2013). The CDC has estimated that there are one million cases of foodborne *C. perfringens* infections each year (Bennett et al. 2013; Gould et al. 2013). *C. perfringens* is an intestinal bacterium of animals and humans and can be subsequently found in butchered raw meat and poultry (Alves et al. 2014; Stiles et al. 2014; Li et al. 2013). The ingestion and subsequent growth of vegetative cells can accompany toxin production in the intestines of humans. People who are

infected with *C. perfringens* develop abdominal cramping within 6–24 h (typically 8–12 h); vomiting and fever are not symptoms of infection or intoxication. The infective dose is estimated to be 10^6 – 10^7 cells (Stiles et al 2014). Most of the ~ 16 toxins produced by *C. perfringens* are encoded by large plasmids that range in size from ~ 45 kb to ~ 140 kb (Alves et al. 2014; Stiles et al. 2014; Li et al. 2013). These plasmid-encoded toxins are also closely associated with mobile genetic elements. *C. perfringens* strains have been observed to carry up to three different toxin plasmids, with a single plasmid encoding at least three distinct toxin genes (Alves et al. 2014). The five major types of toxins are labeled as (A, B, C, D, and E), theta toxin, and NetB.

Type A *C. perfringens* is found ubiquitously in the environment and can cause gas gangrene in animals and humans (Li et al. 2013; Stiles et al. 2013; Sully et al. 2014). In addition to producing the toxins listed above, it can produce a sporulation-linked enterotoxin (CPE) that causes a major form of food poisoning linked to meat. CPE is a 35 kDa protein, and the *cpe* gene is located on the chromosome or a plasmid (Li et al. 2013). CPE contains a nine-stranded β -sheet and binds claudin receptors located at the tight junctions of intestinal cells. The binding of CPE to these receptors leads to formation of a large complex that is internalized, compromising the function of tight junctions and giving rise to diarrhea. Furthermore, the internalization of the CPE:claudin complexes leads to apoptosis in some cells.

Methods used to determine the presence of *C. perfringens* in biological samples include animal models of infection and intoxication and diagnostic molecular methods (Stiles et al. 2013; Sully et al. 2014). Animal models include the rabbit ileal loop assay and the suckling mouse assay. The rabbit ileal loop assay has also helped to determine the synergistic roles some individual toxins play (Li et al. 2013; Stiles et al. 2013; Sully et al. 2014). Molecular detection methods include PCR, nested-PCR, real-time PCR, and loop-mediated isothermal amplification (LAMP). The majority of isolates from the environment lack the CPE gene. However, *cpe*-positive isolates produce spores with higher levels of resistance and can therefore be selected for in food preparation environments.

During the process of food preparation, clostridial spores can withstand heating to 100 °C for at least 1 h, making it difficult to eliminate them completely. Foods like stews and other slow-cooked meat and poultry dishes are candidates for *C. perfringens* poisoning. Furthermore, improper refrigeration or allowing foods to stand at room temperature are also poor practices that contribute to perfringens food poisoning (Alves et al. 2014; Stiles et al. 2014). Therefore, most of the outbreaks are connected with poor temperature control (also termed *temperature abuse*). The general steps for confirming *C. perfringens* sporulation and enterotoxin production have been established by the FDA and utilize specialized culture media and conditions (to induce sporulation) and a commercially available reversed passive latex agglutination (RPLA) kit for confirming toxin production. Improving our understanding of the basic biology of *C. perfringens* toxins is needed given the large number of different toxins produced and the roles these molecules play in pathogenesis, including foodborne illness.

***Clostridium botulinum*: A Model System to Study Deadly Foodborne Toxins to Humans**

Clostridium botulinum is a gram-positive, anaerobic spore-forming rod that produces botulinum neurotoxin. This is the most potent toxin against humans (Kammerer and Benoit 2014) and can paralyze animals by blocking acetylcholine release by neurons. The botulinum neurotoxin is classified into eight serotypes called A-H, of which A, B, E, and F are known to cause toxicity in humans; however, all serotypes are potentially toxigenic in humans. At least 32 additional subtypes have been described based on differences in both primary peptide sequence as well as structural distinctness. The parenteral lethal dosage for humans is 0.1–1 ng/kg, and the oral dose is 1 µg/kg. A single gram of BoNT released and subsequently inhaled can lead to the deaths of more than one million people (Cheng et al. 2012; Dunning et al. 2012; Zhang et al. 2012; Kammerer and Benoit 2014; Cheng and Stanker 2013).

Isolates categorized into Group I of *C. botulinum* are referred to as “proteolytic” and may produce toxin types A, B, or F. They are widely distributed in the environment and often found in a variety of raw foods. Botulinum toxin can cause symptoms at very low concentrations (possibly as low as 0.005 µg). The onset of symptoms typically takes 12–36 h but usually depends on the amount of toxin ingested and can take much longer. Symptoms include initial diarrhea and vomiting followed by neurological effects including blurred vision, weakness, and difficulty swallowing, talking, and breathing. If not diagnosed early, mortality rates can be as high as 40 %. Modern treatments have reduced this down to less than 10 %. Foods involved in outbreaks are usually incorrectly preserved meat or fish products, but a range of other foods may be implicated, including vegetables preserved in oil and cheese. The botulinum toxins are not heat stable and can be inactivated at cooking temperatures (Cheng et al. 2012; Dunning et al. 2012; Zhang et al. 2012; Kammerer and Benoit 2014; Cheng and Stanker 2013).

Strains in Group II are classified as “nonproteolytic.” They produce toxin types B, E, or F. These isolates are able to grow at temperatures as low as 3 °C and are widespread in the environment. Type E strains are especially common in aquatic environments. There has been much concern that these isolates may produce toxin in refrigerated processed foods without any obvious signs of spoilage. The spores of this group of *C. botulinum* are much less heat resistant than those of Group I strains. Group II toxins are thought to be slightly less potent than Group I types, requiring at least 0.1 µg to cause symptoms. But in terms of other biological properties, they do not differ much. Foods that are involved in outbreaks of Group II botulism include cold-smoked fish and other preserved fish products (Cheng et al. 2012; Dunning et al. 2012; Zhang et al. 2012; Kammerer and Benoit 2014; Cheng and Stanker 2013).

BoNT serotypes can differ from one another by 34–64 % at the amino acid level (Kammerer and Benoit 2014). Genetic variation within each serotype is sometimes significant. Thirty-two toxin subtypes with amino acid sequence differences of 2.6–32 % have been identified thus far, with more likely to be identified in the

future. This extensive serotype and subtype diversity complicates direct antibody and molecular-based assay designs (Kammerer and Benoit 2014). Rarely does one reagent bind all possible serotypes and subtypes. In the bacterium, BoNT is synthesized as a holotoxin of ~150 kDa protein, and it is subsequently processed by a clostridial trypsin-like protease into two polypeptides that are connected by a single disulfide bond. The structure is similar to other known bacterial A-B dimeric toxins. The ~100 kDa fragment, known as the heavy chain (HC), facilitates toxin binding to specific host cell receptors and later in the translocation of the toxin from vesicles into the cell cytosol. The ~50 kDa fragment, known as the light chain (LC), contains the enzymatic domain and is often used for the development of activity-based laboratory assays. A number of different HC and LC specific antibodies have been developed for use in toxin neutralization and toxin detection immunoassays (Cheng et al. 2012; Dunning et al. 2012; Zhang et al. 2012; Kammerer and Benoit 2014; Cheng and Stanker 2013).

A rodent bioassay is considered the gold standard method for detecting BoNTs. Despite much effort to replace the use of animals, it is still the strongest assay to model all aspects of BoNT intoxication: binding, translocation and enzymatic activity, and pathology (Pellett 2013; Dorner et al. 2013; Singh et al. 2013). Studies to replace the mouse bioassay and improve assay time and sensitivity have led to the development of both *in vitro* and *in vivo* systems to detect BoNTs.

The animal assay measures BoNT in minimal lethal dose (MLD) units, which is the lowest dose at which all tested mice die. Mice are usually injected intraperitoneally with 0.5 mL of BoNT sample in a dilution series and then monitored over several days for signs of intoxication and death (Cheng et al. 2012; Pellett 2013; Dorner et al. 2013; Singh et al. 2013). When sufficient sample is available for testing, the identity of the unknown BoNT can be determined using neutralizing antibodies against each of the specific toxin serotypes (A-G). Hence, the serotype can be identified based on which antibody protects respective mice from death. While the mouse bioassay has high sensitivity, it also can detect different serotypes and subtypes as well as measure different aspects of active toxin. The mouse bioassay also is amenable to use in different matrices, one of the major factors discussed earlier. However, the mouse assay is not without drawbacks. Limitations include long assay times, requirement of specialized animal facilities, substantial costs, trained staff, or the use of animals (with death used as an end point). There is also substantial variation in results observed among different research laboratories.

Alternative refined animal assays that do not use death as an end point, such as the mouse phrenic nerve hemidiaphragm assay, have been evaluated (Cheng et al. 2012; Pellett 2013; Dorner et al. 2013). Although sensitive and faster than the use of whole animals, these assays require use of sophisticated equipment and training and are not amenable for use with large samples of complex matrices. A recently developed *in vivo* assay using the toe spread reflex model was tested for the detection of BoNT in buffer, serum, and milk samples (Pellett 2013; Dorner et al. 2013; Singh et al. 2013). This new assay can provide results more quickly than standard mouse bioassays. How well these results can be translated into a deployable kit that is user friendly has yet to be determined.

A number of different nucleic acid methods have been exhaustively developed. The use of the polymerase chain reaction (PCR) to identify the presence of *C. botulinum* DNA was originally used to detect the presence of bacterial spores. The method could detect the presence of as few as 100 spores per reaction mixture for serotypes A, E, and F and only 10 spores per reaction mixture for BoNT/B. Lindström and colleagues developed an enrichment method that could detect as few as 0.01 spores/g of sample for serotypes A, B, and F and 0.1 spores/g of sample for BoNT/E (Pellett 2013; Dorner et al. 2013; Singh et al. 2013). However, one critical drawback of this method is that enrichment often requires 5 days, which is probably too long for some clinical situations. Furthermore, the applicability of the assay for detection of food contamination became obsolete when beef was observed to interfere with the sensitivity of the assay. Also, if contamination were to occur with the actual toxin and not cells, this “traditional PCR” method would not be useful. Multiplex methods have also been tested as a way to analyze unknowns for multiple targets, such as different pathogens and/or associated gene products of those pathogens. This approach, known as multiplex technology, is straightforward for PCR-based approaches. Different combinations or sets of PCR primers, each one highly specific for a gene of interest, can be easily generated, allowing for the amplification of multiple targets in one PCR reaction tube. One such multiplex method was able to discriminate among BoNT serotypes A, B, E, and F, validating mouse bioassay results (Dorner et al. 2013; Singh et al. 2013). Furthermore, Peck and colleagues developed a culture enrichment method that when coupled with multiplex PCR could identify strains of *C. botulinum* that were nonproteolytic (BoNT serotypes B, E, and F) (Dorner et al. 2013; Singh et al. 2013). This method was robust and rapid enough for use with food samples contaminated with *C. botulinum*.

Real-time or quantitative PCR is useful in studies of gene expression: specifically differential expression of genes under different environmental conditions or for comparative studies among different organisms. For detection of clostridia, real-time PCR methods that examine expression of the nontoxic, nonhemagglutinin (NTNH) genes have been developed as well as methods to study toxin gene expression in *C. botulinum* serotypes A, B, E, and F (Pellett 2013; Dorner et al. 2013). In that study, 29 different strains of toxin-producing *C. botulinum* were screened and compared with expression profiles from non-toxin producing clostridia as controls. This assay has a sensitivity of 100-fg/1,000 f. total DNA in the PCR tube (equivalent to approximately 25–250 genomes). Converting this DNA concentration to its equivalent in cells/mL suggested a detection limit of approximately 10^3 – 10^4 cells/mL. Following a 48-h enrichment under anaerobic conditions, these investigators reported the detection of *C. botulinum* serotype A in a naturally contaminated sample of foie gras suspected in a botulism outbreak. Recently, pentaplex methods have been developed to simultaneously identify and discriminate among larger numbers of different serotypes using a wider array of different genes (Dorner et al. 2013; Singh et al. 2013). This technology should prove to be efficient and cost effective.

ELISA is a widely used detection assay format that uses anti-BoNT capture and detector antibodies, usually in a sandwich-type format. The read-out for the assay can be colorimetric, luminescence, or other formats. Most older-generation BoNT

immunoassays are about 10 times less sensitive than the mouse bioassay (Stanker et al. 2013; Stevens et al. 2013). Although not as sensitive, ELISA-based methods are relatively fast, inexpensive, and simple. They are also less subject to inhibitory matrix effects. Amplified enzyme-linked immunosorbent assay (ELISA) for detecting toxins in food matrices has been described. Here, toxins for serotypes A, B, E, and F could be detected in liquid, solid, and semisolid food. Assay performance in a range of foods was evaluated and included broccoli, orange juice, bottled water, cola soft drinks, vanilla extract, oregano, potato salad, apple juice, meats, and dairy items (Wang et al. 2014). Assay sensitivity varied for each botulinum complex serotype, and sensitivities were reported as 60 pg/mL for BoNT/A, 176 pg/mL for BoNT/B, 163 pg/mL for BoNT/E, and 117 pg/mL for BoNT/F. The tests readily detected 2 ng/mL of serotypes A, B, E, and F in a variety of the foods tested. Recently, traditional-format sandwich ELISA assays using highly sensitive mAbs against BoNT/A and BoNT/B have detected as low as 5 pg/mL and 25 pg/mL BoNT/A, in buffer and in a milk matrix, respectively (Stanker et al. 2013; Stevens et al. 2013; Singh et al. 2013); and 100 f. and 39 pg/mL of BoNT/B in buffer and milk matrix, respectively (Pellett 2013; Dorner et al. 2013; Singh et al. 2013; Stanker et al. 2013). These mAbs were used in an electrochemiluminescence ELISA assay using a Meso Scale Discovery (MSD) instrument. Detection sensitivities for BoNT/A using the MSD instrument were similar to traditional ELISAs in the buffer system but offered marked improvement in detection limits and reduction in backgrounds in liquid food matrices (Cheng and Stanker 2013). The higher sensitivity and reduced time required for these new ELISA methods make them possible alternatives or complements for the mouse bioassay.

Multiplex technology discussed before has been applied to the development of methods to analyze multiple epitopes on a single antigen or multiple targets in a single sample (Dorner et al. 2013; Wang et al. 2014; Dunning et al. 2014). This approach uses multiple mAbs as well as polyclonal antibodies to reduce false-positive and false-negative results. The LuminexMAP technology utilizes microsphere beads conjugated with antibodies. The antibody-bead complexes detect multiple epitopes in a single sample; for instance, this technology was used to detect abrin, ricin, botulinum toxins, and staphylococcal enterotoxins in spiked food samples and used paramagnetic beads instead of nonmagnetic polystyrene beads to help in the analysis of food matrices (Dorner et al. 2013; Singh et al. 2013; Wang et al. 2014; Dunning et al. 2014).

Accurate and sensitive detection of contaminated food and other biological samples in the environment is critical. Brunt and colleagues have developed a number of rapid affinity immunochromatography column (AICC) assays for the detection of BoNT serotypes A, B, E, and F in food matrices. These investigators reported a detection limit for BoNT/A of 0.5 ng, twofold more sensitive than earlier reported lateral flow methods. For serotypes B, E, and F, the minimum detection limit ranged from 5 to 50 ng. Although not as sensitive as ELISA or mouse bioassays, immunochromatographic methods generally are rapid assays, requiring only 15–30 min to complete, do not require enrichment steps, and are amenable to use in the field (Wang et al. 2014; Dunning et al. 2014; Sachdeva et al. 2014).

The application of lateral flow methods for detecting toxins has also led to the development of a number of kits for sensitive and rapid testing. Here, capture antibodies are printed on nitrocellulose membranes. Detection antibodies are labeled with materials that can be visualized (e.g., colloidal gold or colored latex beads). The sample is added to a reagent pad containing labeled detection antibodies that bind toxin, wick across the membrane where toxin is retained, thus concentrating the labeled detection antibody. A positive reaction leads to a colorimetric change that is usually detected as a line. These assays are generally qualitative and determine the presence or absence of toxin. Sharma and coworkers tested different commercial lateral flow devices (such as the Bot-Tox-BTA kit) for their capacities to detect toxin in food samples (Dorner et al. 2013; Singh et al. 2013). They were able to detect concentrations of toxin as low as 10 ng/mL for BoNT serotypes A and B and 20 ng/mL for BoNT/E in a variety of liquids such as milk products, soft drinks, and fruit juices. Results by Stanker show sensitivity of 0.5 and 1 ng/mL for BoNT/A in buffer and milk, respectively, in lateral flow devices using the same mAbs described in the ELISA section above (Cheng et al. 2012; Ching et al. 2012). Although simple lateral flow tests have lower sensitivities compared to other methods, they produce rapid results, require no additional reagents or equipment, can be easily interpreted, and have many applications. They can be useful for the rapid screening of samples where the presence of BoNT may be more abundant.

An innovative approach for toxin detection combines antibodies with the amplification power of PCR in an assay called immuno-PCR (I-PCR). Here, instead of a secondary antibody conjugated to the detection enzyme, template DNA is conjugated to the antibody; upon binding of antigen by the antibody, an indirect test for the presence of the BoNT is carried out using PCR. Chao et al. described a sensitive I-PCR method (femtogram amounts, 10^{-15} g) for detection of BoNT/A. These investigators also compared standard ELISA as well as sandwich ELISA methods with the sensitivity of the I-PCR method. Both ELISA methods were sensitive for toxin detection down to 50 fg, and the I-PCR method was between 103 and 105 times more sensitive (Singh et al. 2013; Wang et al. 2014). The use of I-PCR for highly sensitive detection of BoNT in food matrices or other biological backgrounds has yet to be developed.

Rapidly distinguishing between the presence and absence of active versus inactive toxin is critical for intervention (Dorner et al. 2013; Singh et al. 2013). Since BoNTs are zinc metalloproteases, enzyme-substrate assays have been developed using knowledge of the human targets for these enzymes. Activity assays range from mixing toxin with recombinant versions of host targets (such as SNAP-25) and then using immunoblotting to detect cleavage of those substrates, to measuring fluorescence emitted from cleavage of fluorogenic peptide substrates. One such peptide, called SNAPtide, used in an assay with a reverse-phase HPLC with a fluorescence detector, can detect as low as 5 pg/mL of BoNT/A in skim milk (Dorner et al. 2013; Singh et al. 2013). Other peptide substrates, VAMPtide and SYNTAXtide, useful for their cognate BoNTs have been developed. The levels of substrate cleavage correlate well with toxin activity. Other investigators have looked for other indications of substrate cleavage by BoNTs. For instance, Nuss

and colleagues generated antibodies that specifically recognize the full-length version of human SNAP25 but not the cleaved form (Sachdeva et al. 2014; Bagramyan et al. 2013). Use of this antibody to confirm the absence of toxin activity by detecting only the intact, full-length substrate might be useful to confirm the absence of bioactive forms of the toxin.

Cell-based assays measure BoNT receptor binding, translocation, and enzymatic activity and can be viable alternatives to the mouse bioassay (Dorner et al. 2013; Hong et al. 2013; Basavanna et al. 2013). A number of different neuronal and non-neuronal derived cell lines have been generated for use in BoNT assays. These include rat spinal cord cells; chick embryo neuronal cells; neuroblastoma cells N2A, and BE(2)-M17 cells. The read-out for most of the cell-based assays for detection of BoNT/A is the cleavage of SNAP-25. Antibodies for SNAP-25 allow immunoblot detection of cleavage products, specifically detecting a decrease in size of endogenous SNAP-25 protein.

Investigators continue to examine different parameters in order to develop a more robust cell-based assay. The U.S. Food and Drug Administration approved a cell-based assay (developed by Allergan) for use as possible replacement of the mouse bioassay. Details of the assay have yet to be published. Cell-based assays may ultimately prove valuable for toxin detection in food. Detection methods can exploit the power of sensitive antibodies for enrichment or sample preparation as well as the signal amplification ability of enzymatic assays. For instance, the assay with a large immune-sorbent surface area (ALISSA) utilizes a two-step approach; first, an antibody-mediated step concentrates toxin onto a large bead surface. Captured toxin molecules are then used in a SNAPtide protease assay (Bagramyan et al. 2013). When compared to other established methods for toxin detection in food matrices, the ALISSA assay can detect toxin concentrations as low as 50 fg/mL, which is more sensitive than the mouse bioassay or either immunoassay or SNAPtide assay alone. The use of this method to evaluate a number of different food matrices suggests that it may be useful in food contamination studies.

The mass spectrometry-based method, ENDOPEP-MS, uses antibodies to concentrate and extract BoNT serotypes A, B, E, and F from test samples. The concentrated toxins are then subjected to an endopeptidase activity-based assay to generate target cleavage products. Finally, mass spectrometry is used to identify cleavage target products (Dorner et al. 2013; Singh et al. 2013). This approach has been successful in identifying BoNT serotypes A, B, E, and F in a variety of food and clinical sample matrices with sub-mouse bioassay sensitivities. To advance this technique even further, a single, high-affinity mAb (4E17.1) that can simultaneously identify BoNT serotypes A, B, E, and F has been developed (Dorner et al. 2013; Singh et al. 2013). The use of this mAb reduced assay time while maintaining assay sensitivity. The use of mass spectrometry can give fast and definitive results. With the future development of low-cost equipment, this method may be more readily available to investigators.

The analysis of all aspects of the botulinum toxin, from basic science to application, has provided a strong foundation ready to support similar work on other toxins, such as those isolated from the other pathogens discussed herein as well as those which might emerge in the future.

Conclusion and Future Directions

There are almost 10 million reported cases of foodborne illness each year in the USA (the number is actually much higher given the likely number of unreported cases). This large number encompasses all cases caused by bacteria, viruses, fungi, and parasites. Of these, strikingly, almost 14 % are caused by toxin-producing gram-positive bacteria. Furthermore, as pathogens acquire new toxin genes through lateral gene transfer, the emergence of new species, isolates, and serotypes expressing new and different toxins is eminent and unavoidable. Advances in genomic and proteomic analyses will also reveal new toxins in well-studied as well as understudied pathogens. It is an exciting time to be studying any aspect of bacterial toxins, from their basic biology and pathogenesis in foodborne illness to their manipulation as therapeutics for other diseases. Whatever the situation may be, both sensitive and specific methods to rapidly detect and identify these toxins are critical to maintaining a safe food supply and for identifying possible weak links in food preparation and more rapid and effective intervention.

Cross-References

- ▶ [Applications of Nanotechnology in Developing Biosensors for Food Safety](#)
- ▶ [Model Fungal Systems for Investigating Food Plant Mycotoxins](#)

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Abstract

Mycotoxins, e.g., aflatoxins, fumonisins, zearalenone, patulin, ochratoxins, etc., trigger serious health risks to human and animals. Mycotoxin contamination also interferes with the safe production of crops worldwide, resulting in significantly

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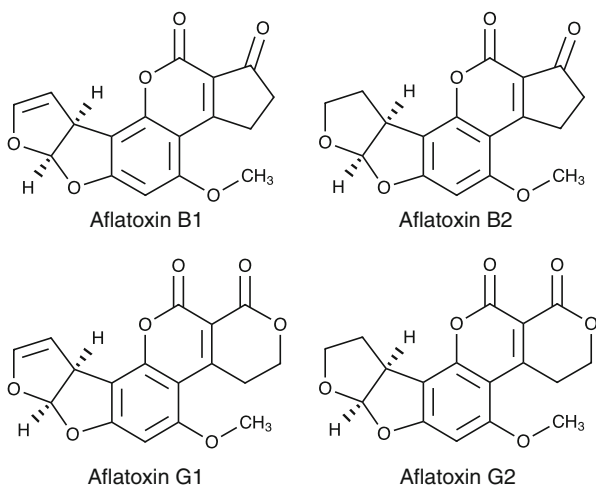
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negative impact on world economy. Aflatoxins are carcinogenic mycotoxins produced mainly by aspergilli, such as *Aspergillus flavus* and *A. parasiticus*. Aflatoxins, when metabolically activated by hepatic cytochrome P450s (CYPs), trigger genotoxicity in mammals through the formation of reactive aflatoxin-8,9-*exo*-epoxide. The resulting 8,9-dihydro-8-(*N*⁷-guanyl)-9-hydroxyaflatoxin adduct causes mutations (frameshift, GC to TA transversions) or DNA damage (chromosomal breaks, aberrations) and thus negatively affects human or animal health. The yeast *Saccharomyces cerevisiae* is a useful system for investigating the mechanisms of toxicity of aflatoxins, where mammalian CYPs can be functionally expressed to metabolically activate aflatoxins. Using recombinant *S. cerevisiae*, the consequences of CYP polymorphisms on the differential aflatoxin toxicity, effects of CYP-inhibitory phytochemicals on aflatoxin activation, transcriptional responses of cells to aflatoxins, etc., have been investigated. Of note, the aflatoxicosis could be ameliorated by *S. cerevisiae* cell walls. Aflatoxins interact with *S. cerevisiae* cell wall components, such as β -D-glucans, thus adsorbing/removing aflatoxins from the contaminated sources. Lastly, *S. cerevisiae* strains, such as gene deletion mutants, could also serve as useful genetic tools for identifying molecular targets or mode of actions of antifungal agents. This facilitates expedited development of new, safe antifungal drugs/fungicides, resulting in the effective control of aflatoxin-producing aspergilli.

Introduction

Aflatoxins (AFs) are carcinogenic difuranocoumarins, a group of polyketide mycotoxins, which include the structurally related AFB₁, AFB₂, AFG₁, and AFG₂ (Fig. 1). Aflatoxins are produced as secondary metabolites by certain ascomycetous fungi in the genus *Aspergillus* (Roze et al. 2013). *A. flavus* Link and *A. parasiticus*

Fig. 1 Structures of aflatoxins B₁, B₂, G₁, and G₂



Speare are the most common species associated with aflatoxin production. Contamination of food, feed, or other agro-products by aflatoxins triggers a food safety issue, imposing serious health concerns, such as hepatocarcinogenesis, suppression of immune systems, inhibition of embryo and fetal development, etc. (Wangikar et al. 2005; Molyneux et al. 2007; Shephard et al. 2008). Considering the increasingly strict regulations worldwide, there is an urgent demand to develop methods for preventing aflatoxin contamination in agricultural commodities.

The model yeast *Saccharomyces cerevisiae* is a useful tool for examining mechanisms of toxicities of aflatoxins or for screening antifungal agents with the identification of gene/molecular targets in view that the entire genome of *S. cerevisiae* has been sequenced and well annotated. Many genes in *S. cerevisiae* are also orthologous to those in other eukaryotes. For example, structural homology of genes involved in stress-activated signal transduction/antioxidant system has been confirmed between *S. cerevisiae* and the aflatoxigenic *A. flavus* (Kim et al. 2005). Of note, gene deletion mutants of *S. cerevisiae* have been very effective tools for identifying the mode of action of newly developed antifungal agents (Parsons et al. 2004). There are approximately 6,000 strains of yeast deletion mutants available, which are constructed based upon the number of genes/open reading frames (ORF) identified in the yeast genome-sequencing project ([Saccharomyces Genome Database](#)).

S. cerevisiae has also been used as an aflatoxin decontaminating agent, where aflatoxins physically interact with the cell wall components of yeasts. *S. cerevisiae* is one of the few organisms for which the biogenesis and structure of the cell wall components are well characterized (Orlean 2012), thus allowing effective decontamination of aflatoxins. In this chapter, recent progress in the use of the yeast *S. cerevisiae* for effective control of toxic aflatoxins and fungal pathogens is discussed.

An Overview of Food Plant Mycotoxins

Mycotoxins are toxic secondary metabolites produced by certain fungi, which pose serious health/food safety risks to human/animals and/or disrupt the quality of cereal grains worldwide (McCormick 2013). Mycotoxins, including aflatoxins, fumonisins, zearalenone, patulin, ochratoxins, etc. (Fig. 2), damage around 25 % of global food and feed crop production, where filamentous fungi, such as *Aspergillus*, *Fusarium*, *Penicillium*, etc., are the main producers of these toxic compounds (Moretti et al. 2013 and references therein). Mycotoxins interfere with the functions or integrity of various cellular components in target organisms. For example, fumonisins produced by *Fusarium* sp. disrupt sphingolipid metabolism in human/animals, resulting in liver cancer, equine leukoencephalomalacia or porcine edema, etc. (see Table 1 and references therein). Zearalenone, also produced by *Fusarium* sp., exerts estrogenic activity, causing hyperestrogenism or lowered fertility in animals (Table 1). Consequently, mycotoxin contamination in food/feed crops triggers significantly negative impact on the world economy (e.g., losses in crop yields/animal or food industries). Therefore, methods are urgently needed for effective control of mycotoxin production or contamination.

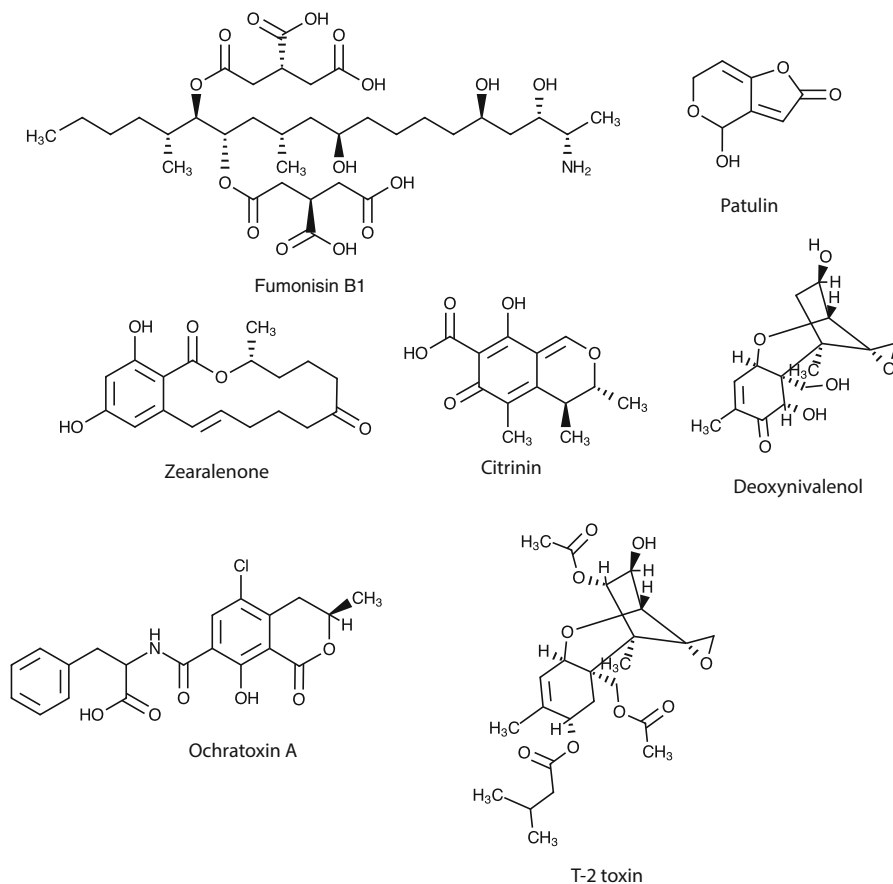


Fig. 2 Structures of mycotoxins (fumonisin B₁, zearalenone, patulin, ochratoxin A, citrinin, deoxynivalenol, T-2 toxin)

***Saccharomyces cerevisiae*: A Model Organism for Aflatoxin Toxicity Studies**

The molecular mechanisms of toxicity of certain mycotoxins to humans or environments are still not well understood. Recently, molecular biological/genetic advances in the model yeast *S. cerevisiae* make it possible to identify the mode of action and/or cellular targets of several mycotoxins, including aflatoxins (Carberry et al. 2012; Chang and Ehrlich 2013; Bin-Umer et al. 2014). *S. cerevisiae* could serve as a heterologous gene expression system, in which cellular responses, e.g., DNA recombination/repair, mutations, etc., after exposure to aflatoxins can be determined.

Table 1 Characteristics of food plant mycotoxins

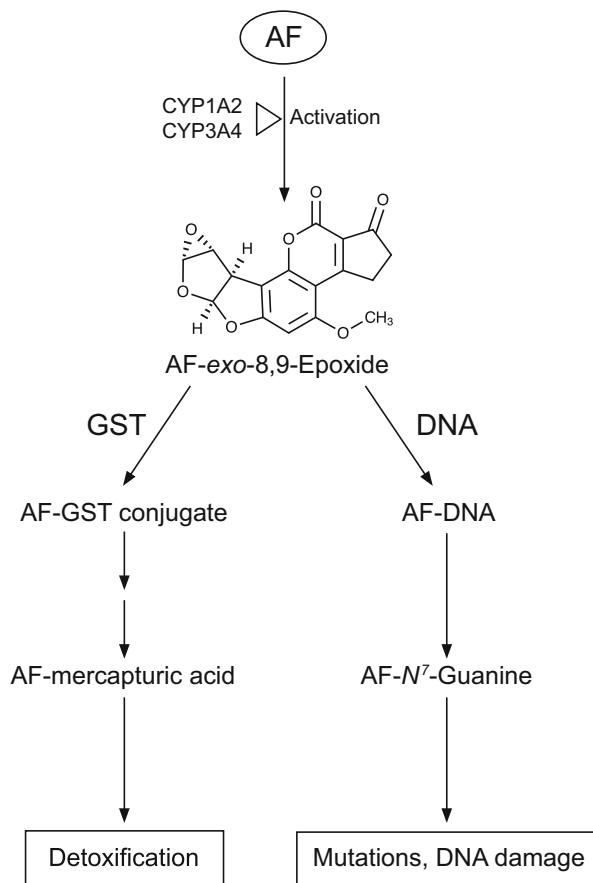
Mycotoxins	Producers	Impact on food safety/ health	Economic impact	Control strategies	References
Fumonisin	<i>Fusarium verticillioides</i> , <i>F. proliferatum</i>	Disruption of sphingolipid metabolism, liver cancer, equine leukoencephalomalacia, porcine edema	Losses in crop yields	Fungicides, use of carboxylesterase from the fumonisin-degrading bacterium <i>Sphingopyxis</i> sp. as a feed additive	Merrill et al. (1993), McCormick (2013), Cruz et al. (2014)
Zearalenone	<i>Fusarium</i> sp.	Estrogenic activity, oxidative stress, hyperestrogenism in swine, lowered fertility in cows	Losses in animal industries	Fungicides, use of <i>Bacillus subtilis</i> to ameliorate zearalenone toxicosis	McCormick (2013), Lautert et al. (2014), Zhao et al. (2014)
Trichothecenes (deoxynivalenol, T-2 toxin, etc.)	<i>Fusarium</i> sp., <i>Trichoderma</i> , <i>Trichothecium</i> , <i>Stachybotrys</i> , <i>Myrothecium</i> , <i>Spicellum</i>	Inhibition of eukaryotic protein synthesis	Losses in agricultural commodities	Fungicides, Use of the yeast <i>Trichomonascus</i> clade, use of aerobic/anaerobic bacteria as biocontrol agents	McCormick et al. (2012), McCormick (2013), Shi et al. (2014)

(continued)

Table 1 (continued)

Mycotoxins	Producers	Impact on food safety/ health	Economic impact	Control strategies	References
Patulin	<i>Penicillium expansum</i> , <i>Aspergillus</i> , <i>Byssoschlamys</i>	Neurotoxic, immunotoxic, teratogenic, carcinogenic	Losses in food industry	Fungicides, use of biocontrol yeast <i>Rhodospiridium</i> <i>kratochvilovae</i>	Moake et al. (2005), Castoria et al. (2011), McCormick (2013)
Citrinin	<i>Aspergillus</i> , <i>Penicillium</i> , <i>Monascus</i>	Nephrotoxic	Losses in poultry, grain, and food industries	Fungicides, use of biocontrol bacterium <i>Moraxella</i> sp.	Devi et al. (2006), McCormick (2013)
Ochratoxin	<i>A. ochraceus</i> , <i>A. ostianus</i> , <i>Penicillium</i> <i>verrucosum</i>	Nephrotoxic, Balkan endemic nephropathy	Losses in animal industry, losses in agricultural commodities (cereals, grapes, coffee, etc.)	Fungicides, use of biocontrol yeasts (<i>Saccharomyces</i> , <i>Rhodotorula</i> , <i>Cryptococcus</i> , <i>Trichosporon</i> <i>mycotoxinivorans</i>), use of biocontrol bacterium <i>Phenyllobacterium</i> <i>immobile</i>	McCormick (2013), Pfohl-Leszkowicz et al. (2014)

Fig. 3 Metabolism of aflatoxins following CYP activation. *GST*, glutathione-*S*-transferase (see Abrar et al. 2013 and references therein)



Roles of Cytochrome P450s (CYPs) in Aflatoxin Toxicity

Cytochrome P450s (CYPs) are enzymes, which play important roles during the metabolic activation and detoxification of toxic chemicals or dietary carcinogens such as aflatoxins (Nebert and Dalton 2006). Aflatoxins are activated in the liver, forming a very reactive aflatoxin-*exo*-8,9-epoxide. In humans (Fig. 3), hCYP1A2 and hCYP3A4 are the primary CYPs catalyzing the activation of aflatoxins (Sengstag et al. 1999; Guo et al. 2006; Fasullo et al. 2010 and references therein; Fasullo et al. 2014 and references therein). Aflatoxins are primarily detoxified via glutathione-*S*-transferase (*GST*)-mediated conjugation between glutathione (*GSH*) and aflatoxin-*exo*-8,9-epoxide. However, the aflatoxin-*exo*-8,9-epoxide that escaped from *GST* conjugation (or other detoxification) specifically reacts with the *N*⁷ guanine residue of DNA, resulting in the formation of 8,9-dihydro-8-(*N*⁷-guanyl)-9-hydroxyaflatoxin (aflatoxin-*N*⁷-Guanine) adduct

(Fasullo et al. 2010 and references therein). The binding of aflatoxin-*exo*-8,9-epoxide to N^7 guanine residue of DNA causes mutations (frameshift, GC to TA transversions) and/or DNA damage, such as chromosomal strand breaks or aberrations and micronuclei formation (Guo et al. 2006 and references therein). Therefore, the aflatoxin- N^7 -Guanine adducts need to be repaired by nucleotide excision repair (NER), double-strand break, and X-ray repair systems (Fasullo et al. 2010 and references therein). Aflatoxin-*exo*-8,9-epoxide also modifies RNA and protein by forming covalent adducts (Guo et al. 2006 and references therein).

Heterologous Expression of Mammalian CYPs in *S. cerevisiae*

S. cerevisiae cells per se do not activate aflatoxins and thus do not show sensitivity after exposure to aflatoxins. However, heterologous expression of human or animal CYPs in *S. cerevisiae* enables the yeast cells to activate aflatoxins, making it feasible to investigate the mechanisms of toxicity of aflatoxins in *S. cerevisiae* (see also Table 2). For example, recombinant *S. cerevisiae* strains expressing mouse liver CYP2A (mCYP2A) enzymes were constructed in early 1990s (Pelkonen et al. 1994). Results showed: (a) the differential AFB₁-activating capacities of three mCYP2A enzymes (mCYP2A4, mCYP2A5, P4507 alpha) and (b) the direct relationship between the levels of CYP activity and cellular viability when treated with AFB₁. Thus, the study demonstrated that the CYP-expressing yeasts could serve as inexpensive, effective tools to examine aflatoxin toxicity.

Investigation of Aflatoxin-Induced Recombination and DNA Repair

Mitotic recombination events can result in gene conversion (nonreciprocal exchange), inversions, deletions, or translocations (reciprocal exchange), where *S. cerevisiae* could serve as a monitoring system for quantifying recombinational activity of DNA-damaging agents (Sengstag et al. 1996 and references therein). For example, CYP-expressing *S. cerevisiae* strains have been used for the studies of “recombinational” activity of aflatoxins, where DNA repair or aflatoxin-induced global gene expression pattern has been characterized. (1) Mitotic recombination is one of the triggers for loss of heterozygosity, a phenomenon by which the second alleles of genes, such as tumor suppressor genes (e.g., *TP53*), are lost (Sengstag and Würigler 1994 and references therein). Human CYP genes (hCYP1A1, hCYP1A2), along with NADPH-CYP oxidoreductase, expressed in *S. cerevisiae* activated AFB₁. This activation triggered the induction of significant mitotic recombination in cells and thus explained the possible mechanisms of AFB₁-mediated hepatocarcinogenesis (Sengstag and Würigler 1994). (2) Yeast cells expressing hCYP1A2 revealed the involvement of homologous recombination and nucleotide excision repair, post-replication repair, etc. in the tolerance or repair of AFB₁-induced DNA damage (N^7 -guanine DNA adducts) (Guo et al. 2006). (3) The global gene expression pattern of hCYP1A2-expressing yeast cells exposed to AFB₁ was analyzed by microarray (Keller-Seitz et al. 2004), where the functions of the AFB₁-induced genes belong to (a) the maintenance of DNA integrity, such as DNA repair; (b) control of cell

Table 2 Use of CYP-expressing *S. cerevisiae* strains for the studies of AF toxicities

Study performed	Gene(s) used/ involved	Results/effects	References
Activation of AFB ₁ by hCYP1A2 polymorphisms	hCYP1A2	Differential carcinogen genotoxicity in different CYP1A2 alleles	Fasullo et al. (2014)
Genetics of DNA repair of AFB ₁ -DNA adducts	hCYP1A2	Stimulation of Rad51 foci by AFB ₁ -DNA adducts	Fasullo et al. (2010)
Identification of downstream effectors for genotoxic events associated with AFB ₁	hCYP3A4, <i>MEC1</i> , <i>RAD53</i> , <i>DUN1</i>	Requirement of <i>MEC1</i> , <i>RAD53</i> , and <i>DUN1</i> in response to AFB ₁ -DNA adducts	Fasullo et al. (2008)
Identification of hCYP1A2-inhibitory phytochemicals	hCYP1A2	Inhibition of hCYP1A2-mediated mutagenicity by phytochemicals	Peterson et al. (2006)
Analysis of cellular responses to AFB ₁ using cDNA microarrays	hCYP1A2	Activation of DNA damage response genes by AFB ₁	Guo et al. (2006)
Elucidation of transcriptional response of yeast to AFB ₁	hCYP1A2, <i>RAD51</i> , <i>RAD1</i>	The genotoxic properties of AFB ₁	Keller-Seitz et al. (2004)
Measurement of in vitro AFB ₁ -induced <i>p53</i> mutations	<i>TP53</i> (Human tumor suppressor gene)	Enhancement of cytosine methylation by AFB ₁ -induced guanine mutations at CpG	Chan et al. (2003)
Testing if the codon 249 hot spot of <i>TP53</i> is related to a particular susceptibility to AFB ₁	hCYP1A2, P450 reductase, <i>ADE2</i> , <i>TP53</i>	Codon 249 of <i>TP53</i> tumor suppressor gene is not hot spot for AFB ₁ in <i>S. cerevisiae</i>	Sengstag et al. (1999)
Testing mitotic recombination by AFB ₁	hCYP1A1, hCYP1A2	Induction of significant level of mitotic recombination in <i>S. cerevisiae</i>	Sengstag and Würgler (1994)
Testing differential activity of three mouse liver CYP2A enzymes to activate AFB ₁	mCYP2A	Differential activation of AFB ₁ by mouse CYP2A enzymes in a different manner	Pelkonen et al. (1994)

growth/division, such as NER or recombinational repair; (c) participation in recombination (*RAD51*, *RAD1*), etc. Collectively, AFB₁ upregulates “recombinational” repair pathway in cells that involves *RAD51* and *RAD1* genes.

More detailed information regarding the downstream effectors for genotoxic events associated with AFB₁ is currently available, which has been determined in hCYP1A2-expressing *S. cerevisiae* (Fasullo et al. 2008). AFB₁ induced the expression of DNA damage-inducible genes, such as *RAD51* and ribonucleotide reductase (*RNR*)-encoding genes, through a *MEC1* (ataxia telangiectasia and Rad3-related, ATR homolog)-dependent pathway. *MEC1* is required for both AFB₁-mediated

recombination and mutation, where AFB₁-DNA adducts are common substrates for both recombination and mutagenesis. AFB₁ also triggered Rad53 activation in hCYP1A2-expressing yeast cells (Fasullo et al. 2010). Rad53 activation correlated with cell-cycle delay in yeast and the subsequent formation of Rad51 foci. *RAD51* facilitated DNA replication after replication fork stalling or collapse in AFB₁-exposed cells.

Impacts of CYP Polymorphisms on Differential Aflatoxin Toxicity

Human susceptibility to AFB₁ is highly variable and affected by multiple genetic factors, such as CYP gene polymorphisms in human populations. To investigate whether a single CYP polymorphism confers higher levels of AFB₁ genotoxicity, *S. cerevisiae* was engineered to express individual human CYP genes having genetic polymorphisms (Fasullo et al. 2014). There was significantly less AFB₁-associated genotoxicity in yeast expressing hCYP1A2 (I386F), while that expressing hCYP1A2 (C406Y) exhibited intermediate levels of genotoxicity compared to yeast expressing hCYP1A2 (D348N) or wild type. Therefore, different levels of AFB₁ genotoxicity exist depending on types of CYP1A2 alleles expressed in cells. The results of this investigation, in parallel to epidemiological studies, could provide the basis for identifying particular individuals susceptible to aflatoxins.

Identification of CYP-Inhibiting Phytochemicals

Of note, the CYP-expressing *S. cerevisiae* could also be used for screening beneficial/healthy compounds that inhibit the activity of CYPs, thus preventing aflatoxin activation in the liver. For instance, phytochemicals inhibiting hCYP1A2-mediated carcinogen activation were identified using the recombinant *S. cerevisiae* (Peterson et al. 2006). The pretreatment of yeasts with psoralen or other compounds, found in apiaceous vegetables, significantly improved cell survival with reduced hCYP1A2-mediated mutagenicity after aflatoxin treatment. Apigenin from similar sources also significantly decreased mutagenicity. Studies indicated that dietary constituents could modulate biotransformation enzymes, such as CYPs. Therefore, CYP-expressing yeasts can be useful tools for understanding the impacts of food components on biotransformation systems in cells.

Deviation of Yeast-Based Results from Mammalian Studies

Aflatoxin studies on the “tumor suppressor” gene *TP53*, expressed in *S. cerevisiae*, showed certain deviation from the phenomenon observed in mammals. Human primary liver cancers, linked to AFB₁ exposure, indicated a specific hot spot mutation at codon 249 of *TP53*. To investigate whether the codon 249 hot spot is particularly susceptible to AFB₁ or whether it is the resulting phenotype of the codon 249 mutation, a recombinant *S. cerevisiae* was constructed with hCYP1A2 and P450 reductase (Sengstag et al. 1999). Only few cases had a mutation in *TP53* gene, while other mutations involved a recombination event (a typical aflatoxin-associated event in cells). Thus, codon 249 was not a hot spot if the *TP53* was expressed in the heterologous system, *S. cerevisiae*.

Similar results were obtained in a later study, where the impact of AFB₁ on cytosine methylation was examined using the *TP53* cDNA template (Chan et al. 2003). Results indicated the enhancement of AFB₁-induced *TP53* mutations at certain CpG sites after methylation in CpG, where the dominant mutation type was GC to TA transversions. This is the same type of mutation induced by the AFB₁-mediated adduct formation. However, no *TP53* mutation was detected at the codon 249 hot spot. Therefore, although the majority of cellular genotoxic events associated with aflatoxin in *S. cerevisiae* are similar to that detected in mammals, codon 249 of *TP53* is not a hot spot in *S. cerevisiae*.

Decontamination/Detoxification of Aflatoxins Using *S. cerevisiae* Cell Walls

S. cerevisiae, either as intact cells or as cell wall components, decontaminates aflatoxins. The α -D-mannan and β -D-glucan, the main polysaccharides constituting the *S. cerevisiae* cell wall, act as effective aflatoxin adsorbents (Shetty and Jespersen 2006; Kogan and Kocher 2007). Hydroxyl, ketone, and lactone groups are involved in the formation of both hydrogen bonds and van der Waals interactions between mycotoxins and cell wall components, such as β -D-glucans (Yiannikouris et al. 2006).

Use of Total Cells of *S. cerevisiae* for Aflatoxin Decontamination/Detoxification

The *S. cerevisiae* cell-mediated aflatoxin detoxification in mammals was investigated in the early 1990s (see Table 3). For example, commercial broiler chicks receiving aflatoxin-contaminated feed had suppressed body weight, while it was significantly improved with the inclusion of 0.1 % *S. cerevisiae* cells (Stanley et al. 1993). The relative weights of liver, heart, and proventriculus or the serum concentrations of albumin and total protein could be restored with the dietary inclusion of *S. cerevisiae* cells. Later studies showed that *S. cerevisiae* cells could also reduce the micronucleated normochromatic erythrocytes (MNNE) rate, a cellular indicator of genotoxicity, in mice fed AFB₁-contaminated corn (Madrigal-Santillán et al. 2006). The “*S. cerevisiae* cell wall-aflatoxins” complexes effectively pass through the gastrointestinal (GI) tract of mammals and thus can prevent the aflatoxin absorption by humans and livestock (Bueno et al. 2007; Pizzolitto et al. 2012 and references therein). For example, viable *S. cerevisiae* strains examined under ruminant GI conditions (e.g., different pHs) successfully survived and adsorbed AFB₁ (Dogi et al. 2011).

Effect of Immobilized *S. cerevisiae*

S. cerevisiae cells could also be used in the industrial platform, such as immobilized cells to decontaminate aflatoxins present in agricultural commodities. The binding

Table 3 Use of *S. cerevisiae* cell walls for decontamination/detoxification of AFs or other mycotoxins

Mycotoxin detoxifying agents	Target mycotoxins	Results/effects	References
Commercial yeast cell walls (mannans, β -glucans)	AFB ₁ , ZEA (Zearalenone)	Adsorption of AFB ₁ and ZEA by mannans and β -glucans. Inhibitory effect of the corn matrix for AFB ₁ adsorption	Pereyra et al. (2013)
<i>S. cerevisiae</i> 01, 08	AFB ₁	AFB ₁ removal by <i>S. cerevisiae</i> isolated from broiler feces. Strain and mycotoxin-concentration dependency of the AFB ₁ decontamination	Pizzolitto et al. (2012)
<i>S. cerevisiae</i> RC008, RC016	AFB ₁	Survivability and AFB ₁ adsorption of yeasts under GI conditions. Helping cellulolytic bacteria in ruminal fluid and reducing AFB ₁	Dogi et al. (2011)
<i>S. cerevisiae</i> cell walls	AFB ₁ , OTA (Ochratoxin A)	Protection of monogastric animals against selected mycotoxins by yeast cell wall	Firmin et al. (2010)
<i>S. cerevisiae</i> immobilization	AFB ₁	Enhancement of surface binding of AFB ₁ by acid and heat treatments in the exponential phase	Rahaie et al. (2010)
<i>S. cerevisiae</i> CECT 1891, lactic acid bacteria (LAB)	AFB ₁	Physical adsorption model for AFB ₁ binding to <i>S. cerevisiae</i> , useful for selecting the most efficient microorganism to remove AFB ₁	Bueno et al. (2007)
<i>S. cerevisiae</i>	AFB ₁	Isolation of AFB ₁ binding <i>S. cerevisiae</i> from indigenous fermented foods. Determining AFB ₁ binding is a physical phenomenon	Shetty et al. (2007)
<i>S. cerevisiae</i>	AFB ₁	Reduction of micronucleated normochromatic erythrocytes (MNNE) rate in mice fed AFB ₁ contaminated corn by <i>S. cerevisiae</i>	Madrigal-Santillán et al. (2006)
β -D-Glucans	AFB ₁ , patulin, deoxynivalenol, OTA	Involvement of hydroxyl, ketone, and lactone groups in the formation of both hydrogen bonds and van der Waals interactions between mycotoxins and β -D-glucans	Yiannikouris et al. (2006)
<i>S. cerevisiae</i>	AFs	Anti-aflatoxicosis effect of <i>S. cerevisiae</i> in broiler chicks	Stanley et al. (1993)

of the immobilized *S. cerevisiae* cells to aflatoxin contaminating pistachio nuts is an example (Rahaie et al. 2010). After immobilization, *S. cerevisiae* achieved aflatoxin surface binding of 40–70 % in the exponential growth phase. Acid and heat treatments increased the binding ability to approximately 55–75 %, depending on types of treatment and aflatoxin concentrations. Aflatoxin binding was a physical phenomenon, where saturation could be reached within 2–3 h of the decontamination process. Thus, immobilization of intact yeasts also ameliorated the aflatoxin contamination in highly susceptible foods, such as tree nuts.

Selection of Optimal *S. cerevisiae* Strains for Aflatoxin Decontamination

Since both the stability and concentrations of “*S. cerevisiae* cell wall-aflatoxins” complexes formed are critical for effective aflatoxin decontamination, selection of optimal yeast types is necessary (Bueno et al. 2007; Pizzolitto et al. 2012 and references therein). To facilitate the selection/identification of the optimal *S. cerevisiae*, a physical adsorption model has been established for the binding of AFB₁ to yeasts (Bueno et al. 2007). The model allows the estimation of the number of binding sites/cells and the reaction equilibrium constant involved. These parameters are useful for evaluating the adsorption efficiency of test microorganisms, therefore enabling determination of the most efficient yeasts for decontaminating AFB₁. Examples include *S. cerevisiae* strains isolated from broiler feces. Yeast strains identified were tested for the AFB₁ removal capacity, for their tolerance to salivary and GI conditions, etc., where the amount of AFB₁ removed was dependent on types of strains employed and AFB₁ concentrations (Pizzolitto et al. 2012). A theoretical model was applied in the study based on simulated salivary and GI conditions, which allowed the selection of the most efficient *S. cerevisiae* strain for AFB₁ removal or decontamination.

S. cerevisiae strains with AFB₁ binding capabilities were also isolated from fermented foods (Shetty et al. 2007). AFB₁ binding was strain specific, where the highest binding capacity was observed with cells collected at the exponential growth phase, while the binding reduced toward the stationary phase. AFB₁ binding was not affected by the cells grown at 20–37 °C, while it was significantly reduced at 15 °C. Therefore, these properties, such as growth phase, temperatures, etc., are critical parameters in selecting starter cultures for fermented foods where high aflatoxin level leads to a potential health risk.

Use of Yeast Cell Wall Components for Aflatoxin Decontamination/ Detoxification

Along with the entire cell of *S. cerevisiae*, the cell wall components of yeasts, such as β -D-glucans, could also be used as mycotoxin adsorbents. For example, the in vitro kinetics of the interaction between three different mycotoxins, i.e., AFB₁, deoxynivalenol, and ochratoxin A (OTA), and β -D-glucans (cell wall component)

from several sources at three pH values (mimicking the digestive tract) has been investigated (Yiannikouris et al. 2006). Acid and neutral pHs gave the highest affinity rates for mycotoxins, where the order of the affinity (high to low) was AFB₁ > deoxynivalenol > OTA. Mycotoxin adsorption involved both the (1 → 3)-β-D-glucans and the (1 → 6)-β-D-glucans. Differences in the binding capacity of the mycotoxins depended upon specific physical and chemical characteristics of cell wall components. The in vivo data also supported the differences in the adsorption capacity of mycotoxins by yeast cell wall. Results from rats after oral administration of AFB₁ and OTA indicated that the decontamination of OTA was less efficient compared to AFB₁ (Firmin et al. 2010).

Meanwhile, the influence of the corn matrix on the adsorption levels of mycotoxins, i.e., AFB₁ and zearalenone (ZEA), by two commercial yeast cell walls was evaluated in vitro (Pereyra et al. 2013). Although both types of yeast cell walls adsorbed AFB₁ and ZEA, they could not adsorb AFB₁ in the presence of corn matrix, while they could adsorb ZEA in the presence of matrix. Therefore, the corn matrix influences the adsorption of mycotoxins by yeast cell walls. Collectively, studies indicated that the environments of mycotoxin removal process, such as culture conditions, existence of food matrix, etc., are also important factors for the optimal mycotoxin decontamination.

***S. cerevisiae* as a Molecular Tool for Identifying Antifungal/Antimycotoxigenic Compounds and Their Mechanisms of Action**

Antifungal agents currently in use have not kept up with the current medical or agricultural demands to cure/prevent fungal infection or contamination. Considering the emergence of fungal resistance and the high toxicity of certain antifungal agents, new antifungal drugs/fungicides are urgently needed, especially for mycotoxin producers. The yeast *S. cerevisiae* is a useful model fungal system for identifying (1) new antifungal/antimycotoxigenic agents and (2) their molecular targets or modes of action (Table 4).

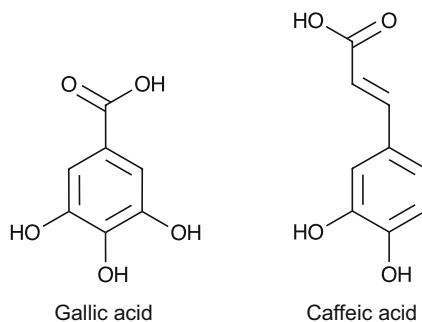
Control of Mycotoxigenic Fungi by Targeting Antioxidant Systems

Antioxidant systems of fungi have been targeted with natural phenolic compounds or their structural derivatives. Natural phenolics are potent redox cyclers, thus inhibiting fungal growth through disruption of cellular redox homeostasis (Kim et al. 2011 and references therein). For example, redox-active benzaldehydes were examined against aflatoxigenic *A. flavus*, where *S. cerevisiae* served as a model system for identifying gene targets of benzaldehydes (Kim et al. 2011). Benzaldehydes, which target cellular antioxidant components of fungi, effectively inhibited the growth of fungal pathogens. These compounds also possess chemosensitizing capabilities, thus significantly enhancing antifungal efficacy of conventional drugs/fungicides when co-applied (see also Kim et al. 2004).

Table 4 Use of *S. cerevisiae* in the antifungal/antiaflatoxigenic screening targeting *Aspergillus* and/or other fungal pathogens

Antifungal/antiaflatoxigenic agents	<i>S. cerevisiae</i> system	Compared fungi	Cellular activity involved	Results	References
Benzaldehydes	<i>S. cerevisiae</i> gene deletion mutants	<i>Aspergillus</i> , <i>Penicillium</i>	Antioxidant, signaling kinases	Inhibition of fungi by natural benzaldehydes targeting cellular antioxidant system	Kim et al. (2011)
Small molecule collection	<i>S. cerevisiae</i> reporter bioassay	<i>Aspergillus</i> , <i>Candida</i> , etc.	Antioxidant, heavy metal stress	Identification of potent, broad-spectrum antifungal drug leads	Tebbetts et al. (2012)
Macrotretrolides	<i>S. cerevisiae</i> reporter bioassay	<i>Aspergillus</i> , <i>Candida</i> , etc.	Histidine kinase, ion transport	Potent fungistatic activity of the macrotretrolides against biofilms	Tebbetts et al. (2013)
6-Nonadecynoic acid	Microarray, gene deletion mutants	<i>Aspergillus</i> , <i>Candida</i> , etc.	Fatty acid homeostasis	Elicitation of fatty acid stress by 6-NDA in the presence of oleate. Fungicidal effect	Xu et al. (2012)
Caspofungin (CAS), Staurosporine	<i>S. cerevisiae</i> mutant collection	<i>Aspergillus</i>	Chitin synthesis, transport, etc.	Synergistic or synergistic-to-additive activities when staurosporine was combined with CAS	Markovich et al. (2004)
Sampangine	Microarray, gene deletion mutants	<i>Aspergillus</i> , <i>Candida</i> , etc.	Heme metabolism	Hypoxia by sampangine. Perturbations in the biosynthesis or metabolism of heme	Agarwal et al. (2008)
Azoles	<i>S. cerevisiae</i> <i>erg11/cyp51</i>	<i>Aspergillus</i>	Cytochrome P450 (CYP51A, 51B)	Complementation of <i>S. cerevisiae</i> <i>erg11/cyp51</i> by <i>A. fumigatus</i> CYP51A, 51B	Martel et al. (2010)
Natural phenolic derivatives	<i>S. cerevisiae</i> gene deletion mutants	<i>Aspergillus</i>	Antioxidant, signaling kinases	Synergy between cinnamic acid and strobilurin fungicide	Kim et al. (2004)
Hydrogen peroxide (H ₂ O ₂)	<i>S. cerevisiae</i> gene deletion mutants	<i>Aspergillus</i>	Mn-superoxide dismutase	Functional complementation of <i>A. flavus</i> Mn-superoxide dismutase in <i>S. cerevisiae</i> <i>sod2Δ</i>	Kim et al. (2005)
Blasticidin A (BcA) from <i>Streptomyces</i>	<i>S. cerevisiae</i> wild type	<i>Aspergillus</i>	Protein synthesis	BcA inhibition of AF production with a decrease in AF biosynthetic enzymes	Yoshinari et al. (2010)
Caffeic acid, organic peroxide	<i>S. cerevisiae</i> gene deletion mutants	<i>Aspergillus</i>	Alkyl hydroperoxide reductases	Caffeic acid inhibition of AF gene expression in <i>A. flavus</i>	Kim et al. (2008)

Fig. 4 Structures of gallic and caffeic acids



Control of Mycotoxin Production by Modulating Antioxidant Systems or Protein Biosynthesis in Fungi

Studies have shown that application of certain compounds derived from natural sources, such as the bacterium *Streptomyces* sp., or modulation of oxidative stress in the mycotoxigenic *Aspergillus* strains disrupts the secondary metabolism/production of aflatoxins (Yoshinari et al. 2007; Grintzalis et al. 2014).

Antioxidants

Compounds such as gallic acid (Fig. 4) or its derivatives have been known as potent inhibitors of aflatoxin biosynthesis in *A. flavus*. Responses of particular mutants of *S. cerevisiae* indicated that they were potent antioxidants (Kim et al. 2005). For instance, a functional complementation of the *A. flavus* mitochondrial superoxide dismutase (Mn-SOD) gene in a *S. cerevisiae sod2Δ* (Mn-SOD gene deletion) mutant demonstrated the potential of *S. cerevisiae* to serve as a model system for the functional genomics of the aflatoxigenic *A. flavus*.

Caffeic acid (Fig. 4) could also reduce aflatoxin production by *A. flavus* (Kim et al. 2008). Microarray analysis indicated expression of most of the genes in the aflatoxin biosynthetic pathway was downregulated in the caffeic acid-treated *A. flavus*. Notable upregulated genes were that of four orthologs of the *S. cerevisiae* alkyl hydroperoxide reductase (*AHP1*) gene family. *S. cerevisiae AHP1* encodes the enzyme detoxifying organic peroxides, indicating caffeic acid may induce these genes in *A. flavus*. In *S. cerevisiae* bioassays, caffeic acid worked as a potent antioxidant against organic peroxides. Therefore, induction of *AHP1* genes protects the *A. flavus* from organic peroxides, such as lipoperoxides, encountered during host-plant (e.g., tree nuts) infection. It is suggested that this detoxification attenuates the signals inducing aflatoxigenesis in *A. flavus*.

Protein Biosynthesis Inhibitors

Blasticidin A and/or S (BcA, BcS) (Fig. 5), antibiotics produced by *Streptomyces*, inhibit aflatoxin production. A decrease in the level of ribosomal proteins in *S. cerevisiae* was observed after exposure to BcA (Yoshinari et al. 2010). BcA inhibited protein synthesis in *S. cerevisiae*, similar to BcS. BcS also inhibited

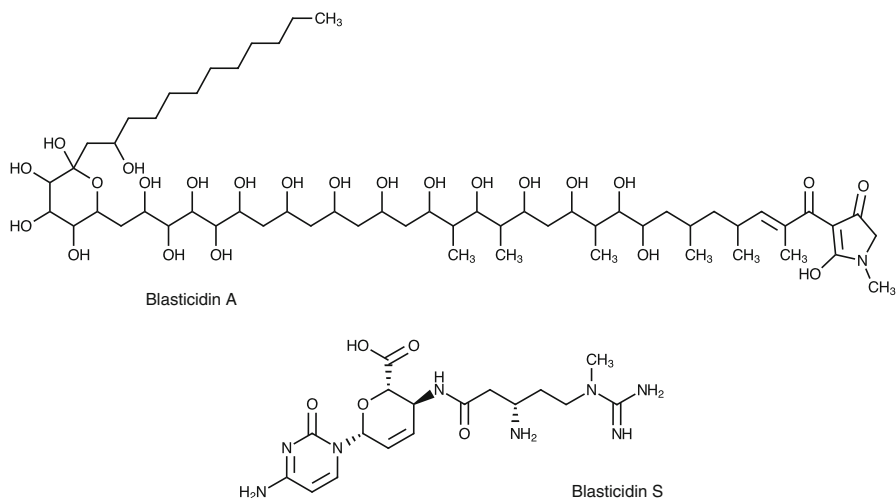


Fig. 5 Structures of blasticidin A and S

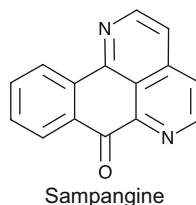
aflatoxin production in *A. parasiticus*. A decrease in the level of aflatoxin biosynthetic enzymes was also detected in *A. flavus*, thus indicating inhibition of protein synthesis would be a useful way to control aflatoxin production.

Identification of Antifungal Agents or Their Mechanisms of Action Using *S. cerevisiae*

S. cerevisiae has been used to investigate the mechanisms of action of antifungal natural products, such as 6-nonadecyanoic acid (6-NDA) (Xu et al. 2012). 6-NDA, a plant-derived acetylenic acid, possesses potent antifungal activity against fungal pathogens including *A. fumigatus*. Antifungal activity of 6-NDA, examined in *S. cerevisiae*, was via the disruption of cellular fatty acid homeostasis. The genetic resources, such as gene deletion mutants, for *A. flavus* or *A. parasiticus* available are few in number. Therefore, *A. fumigatus*, a human fungal pathogen for which genetic/genomic tools are vastly available, could also serve as a model strain for investigating comparative genetics/genomics or mechanisms of antifungal responses in other aspergilli, such as *A. flavus* or *A. parasiticus*.

Sampangine (Fig. 6), a plant-derived alkaloid, showed antifungal activity against fungal pathogens, including the filamentous fungi *A. fumigatus* (Agarwal et al. 2008). *S. cerevisiae* was used for (1) transcriptional profiling analysis and (2) elucidation of the mechanism of action of the compound. Sampangine triggered a transcriptional response, which is indicative of hypoxia. *S. cerevisiae hem1Δ* mutant, lacking the first enzyme in the heme biosynthetic pathway, exhibited increased sensitivity to the compound, suggesting the antifungal activity of sampangine is via the perturbations in the heme metabolism.

Fig. 6 Structure of sampangine



Antifungal small molecules could also be screened in a *S. cerevisiae* reporter bioassay (Tebbetts et al. 2012), such as the *S. cerevisiae* heterologously expressing the *HIK1* gene. *HIK1* encodes a group III hybrid histidine kinase (HHK) derived from *Magnaporthe grisea*. The “group III HHKs” are conserved in fungi, while they do not exist in mammals. Therefore, HHKs could be unique fungal drug targets by the screened antifungal agents. Two compounds possessing broad-spectrum antifungal activity against filamentous and yeast pathogens (including drug-resistant strains) have been identified. When combined, these compounds showed a synergy with fluconazole, an azole drug targeting fungal CYP51 enzyme 14 α -demethylase. In a subsequent study, similar “group III HHK” bioassay was applied to screen microbial natural products for antifungal agents (Tebbetts et al. 2013). Macrotetrolides, a group of ionophores, were identified from the screening.

S. cerevisiae mutant collection (individual gene knockout mutants; [Saccharomyces Genome Database](#)) was screened for identifying genes affecting susceptibility of yeasts to caspofungin (CAS), a disrupter for glucan/cell wall synthesis (Markovich et al. 2004). The gene functions of CAS-sensitive mutants are: (1) cell wall and membrane integrity, chitin and mannan biosynthesis, and ergosterol biosynthesis and (2) vacuole and transport, transcriptional control, etc.

Functional Complementation of *S. cerevisiae* Mutants with *Aspergillus* Genes

Characteristics of certain genes/alleles, for which the functions have not been identified, can be examined in *S. cerevisiae*. For example, the sterol 14 α -demethylase (CYP51) of fungi is the target of azoles. Genome data indicated that the human pathogen *A. fumigatus* possesses two genes, *cyp51A* and *cyp51B*, that encode the sterol 14 α -demethylase-like proteins. To precisely determine the functions of *A. fumigatus cyp51A* and *cyp51B*, *S. cerevisiae erg11/cyp51* (sterol 14 α -demethylase) mutant was used as a host to heterologously express *A. fumigatus* CYP51 genes (Martel et al. 2010). The yeast transformants expressing *A. fumigatus* CYP51 genes exhibited azole sensitivity, thus indicating the functional complementation for ergosterol biosynthesis in *S. cerevisiae* mutant by *A. fumigatus* CYPs (see also Alcazar-Fuoli et al. 2011).

Galactomannan is one of the major components of fungal cell wall. The α 1,6-linked mannans constitute essential component of the *A. fumigatus* galactomannan

(Lambou et al. 2010 and references therein). In *S. cerevisiae*, the *ScOCH1* gene encodes α 1,6-mannosyltransferase, which is responsible for the synthesis of the α 1,6-linked mannan. The four orthologous genes of *ScOCH1* gene were identified in the *A. fumigatus* genome and were heterologously expressed in *S. cerevisiae* *Scoch1* Δ mutant (Lambou et al. 2010). Out of the four *A. fumigatus* orthologs, only *AfOCH1* functionally complemented the *S. cerevisiae* *Scoch1* Δ mutant phenotype. Collectively, studies demonstrated the effectiveness of *S. cerevisiae* mutants for the functional characterization of genes from other fungi, such as *Aspergillus*.

Conclusion and Future Directions

The yeast *S. cerevisiae* is an effective, inexpensive model system for the studies of mycotoxins, including aflatoxins. Recombinant *S. cerevisiae* strains expressing mammalian CYP genes provide useful information concerning aflatoxin toxicity. They can elucidate the mechanisms of aflatoxin toxicity and the outcome of CYP gene polymorphisms under aflatoxin exposure, while they function as the system for screening CYP-inhibitory beneficial compounds. Identifying the mechanisms of certain deviation of yeast-based aflatoxin results, such as the codon 249 hot spot mutation in *TP53*, compared to the mammalian studies, warrants future study. The intact cells of *S. cerevisiae* or its cell wall components could decontaminate aflatoxins (or other types of mycotoxins). Properties such as sources of yeast strains, culture conditions, cell wall compositions, existence of (corn) matrix, etc., affected the aflatoxin binding efficacy. Establishment of models for the selection of the most efficient *S. cerevisiae* cells/cell wall components would facilitate the optimal decontamination/detoxification of aflatoxins. *S. cerevisiae* can also serve as a molecular tool for identifying antifungal and/or antimycotoxigenic compounds, such as safe, natural products, their cellular targets, as well as modes of action. Antioxidant systems, protein biosynthesis, cell wall or membrane biosynthesis, etc., have been proven to be the targets of the identified compounds. Functional complementation of *S. cerevisiae* mutants with *Aspergillus* genes can also identify the functional gene orthologs, which then can be targeted effectively by drugs/fungicides. Currently, genome sequence data is available for the aflatoxigenic *A. flavus* (but not for *A. parasiticus*). However, comparing to other *Aspergillus* species, such as *A. fumigatus*, *A. nidulans*, *A. niger*, *A. oryzae*, etc., the progress in the generation of core genetic resources (e.g., gene knockout mutants) or the functional characterization of genes identified in the *A. flavus* genome is relatively slow (see [Aspergillus Genome Database](#); [Aspergillus Comparative Database](#)). Many of the characterized genes belong to the aflatoxin biosynthesis pathway genes, while gene candidates for the antifungal treatment/targets are yet to be explored. Therefore, the genetically well-characterized *S. cerevisiae* could serve as an effective genetic/genomic tool, especially for control of aflatoxigenic *Aspergillus* in the future.

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Cross-References

► Chemical Composition of Vegetables and Their Products

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Part V

Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Processing

Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Drying

26

Nathamol Chindapan, Chalida Niamnuy, and Sakamon Devahastin

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Abstract

Evolutions of the moisture content and temperature that take place during drying typically lead to various changes of foods, which may or may not be desirable. Understanding of such changes is of importance for an effective design and operation of a drying process to yield dried products of desirable quality.

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In this chapter, some important changes that take place during drying of foods are reviewed. These include nutritional changes of various classes of foods, including fruits and vegetables, meat and seafood products, as well as grains and legumes; the changes are discussed in terms of such important constituents as phenolic compounds, flavonoids, carotenoids, vitamins, pigments, flavor and aroma compounds, as well as amino acids and lipids. Toxicological changes are also discussed in terms of the ability of drying to help reduce antinutritional factors, mycotoxins, and pesticide residues in selected foods undergoing drying.

Introduction

Drying is a process that transforms liquids, semisolids, or solids into a final product of a solid form via the application of heat to evaporate liquids, which are in almost all cases water; either complete or partial removal of liquids can be performed. One exception to the aforementioned definition may be applicable to freeze-drying, which is a process that takes place below the triple point of a liquid being removed. In such a case, drying takes place through sublimation of solids (e.g., ice) into vapors without passing through the liquid phase. A true drying process must involve the following two basic requirements: (1) phase transformation within a material and (2) a solid product at the end of the process. For this reason, drying is a different process from evaporation, which involves the transformation of liquids into a final product of a more concentrated but yet a liquid form. Drying, which can sometimes be called thermal dewatering, is also different from such mechanical dewatering operations as filtration, centrifuge, and sedimentation. Drying is generally performed to extend the storage life of a product. This is particularly true in the case of foods since drying is a process that reduces the moisture (water) content of a material, leading to inactivation of spoilage microorganisms and reduced rates of most deleterious reactions.

Despite the ability to extend the shelf life of a product, changes of the moisture content and temperature that take place during drying typically lead to various chemical changes, including lipid oxidation reactions, enzymatic browning reactions, as well as nonenzymatic browning reactions (such as the Maillard reaction, which is a reaction between amino acids and reducing sugars). Degradation of various compounds, e.g., pigments, vitamins, and proteins, is also a common phenomenon that may take place during drying. These chemical reactions lead both to desirable (e.g., development of desirable flavor and aroma) and undesirable (e.g., browning, losses of nutrients, and even formation of toxins) changes of a product. Understanding of the various changes that may take place during drying is of paramount importance for an effective design and operation of a drying process to yield a dried product of desirable quality for consumers. This understanding should also lead to an ability to utilize an appropriate drying process (and condition) as a tool to enhance the safety of a dried product as well.

Nutritional Changes of Foods During Drying

Nutritional Changes of Fruits and Vegetables During Drying

Fruits and vegetables are a rich source of nutrients, including phenolic compounds, flavonoids, carotenoids, vitamin C, as well as a variety of pigment and volatile compounds. These nutrients provide an array of health benefits to humans, including antioxidant and anticancer activities. While drying is one of the most widely used methods for the preservation and processing of fruits and vegetables, the process leads to many changes, some are desirable but most of the time undesirable, of the nutrients. The changes nevertheless depend on the types and conditions of the utilized drying process as well as the types of fruits and vegetables undergoing drying.

Changes of Phenolic Compounds During Drying

Phenolic compounds (or phenolics or polyphenols) are chemical substances containing aromatic rings bearing one or more hydroxyl groups. These compounds exhibit many health-promoting effects such as the ability to reduce blood pressure and lower the incidences of cancer and cardiovascular diseases. Phenolic compounds can be classified into phenolic acids, coumarins, flavonoids and stilbenoids, lignans and lignins, suberins and cutins, tocopherols and tocotrienols, as well as tannins (Shahidi and Naczk 2004). Changes of phenolic compounds in fruits and vegetables during drying can be represented either in terms of the total phenolics content (TPC), which can simply be evaluated using a spectrophotometric method and is usually expressed as gallic acid equivalent, or in terms of individual phenolics, which are usually evaluated via a chromatographic method.

Hot air-drying is probably the most common method of drying for fruits and vegetables. Most fruits and vegetables subjected to hot air-drying, however, suffer significant losses of phenolic compounds. Nevertheless, as mentioned earlier, degradation levels of phenolic compounds depend on the drying conditions as well as the existence of a pretreatment step prior to drying. Normally, a higher drying temperature would cause an increasing probability for the degradation of phenolic compounds, which is due both to thermal and oxidative degradation. Note that oxidative degradation is due to the action of hydrolytic enzymes, including polyphenol oxidase (PPO), peroxidase (POD), and phenolase (Kuljarachanan et al. 2009; Vega-Gálvez et al. 2012; Mrad et al. 2012; Santos-Sánchez et al. 2012). For this reason, thermal and/or chemical pretreatments are generally performed to inactivate these deleterious enzymes prior to drying. Kuljarachanan et al. (2009), for example, reported that although hot-water blanching, which was performed to inactivate hydrolytic enzymes, caused some losses of phenolic compounds, dried blanched lime residues still exhibited higher TPC than the dried unblanched residues.

On the other hand, some studies have reported that fruits and vegetables subjected to hot air-drying, especially at higher air temperatures, exhibited higher TPC than the fresh ones (Lou et al. 2015). In such a case, higher air temperatures (110–150 °C) might cause the release of bound phenolics from plant cell walls,

resulting in the higher analyzable contents of phenolics. Igual et al. (2012) also noted an increase in TPC, which was calculated from the summation of the contents of individual phenolics (gallic acid, caffeic acid, chlorogenic acid, catechin, epicatechin, and kaempferol) of dried apricots after hot air-drying at 40 °C and 60 °C; the increase was noted despite the decreased contents of gallic acid and caffeic acid. This was related to an increase in chlorogenic acid, catechin, and epicatechin of the dried apricots.

Another commonly used technique for drying fruits and vegetables is freeze-drying, which is recognized for its superior ability to preserve heat-sensitive bioactive compounds in dried fruits and vegetables (Ratti 2008). The porosity of a freeze-dried product is also generally reported to be higher than that of an air-dried product. Materska (2014) indeed reported that the content of phenolic compounds in a freeze-dried pericarp of peppers, which were extracted from the sample by the use of an ethanol-water mixture, increased by approximately 55 % in comparison to that in the fresh pericarp. This is probably due to the higher extraction efficiency of phenolic compounds as a result of the increased tissue porosity. Slight decrease of phenolic compounds upon freeze-drying has also nevertheless been reported (Vashisth et al. 2011; Rudy et al. 2015). Despite its advantages, freeze-drying is a rather expensive process and may not be applicable to all types of fruits and vegetables.

To minimize phenolic compound (and other bioactive compounds) degradation and at the same time improve the economics of the drying process, a number of alternative drying techniques have been proposed. Among the available methods, vacuum drying is one of the most popular. Owing to the operation at a lower pressure (and hence a lower water vaporization temperature and oxygen content), vacuum drying is capable of maintaining the amounts of phenolic compounds at a higher level (Suvarnakuta et al. 2011; Vashisth et al. 2011). At the same drying temperature, vacuum drying is expectedly more rapid than hot air-drying at an atmospheric pressure; this naturally leads to less thermal degradation of phenolic compounds. In addition, a lower level of oxygen implies that oxidative degradation would occur less extensively during vacuum drying. An improved version of vacuum drying, viz., the so-called low-pressure superheated steam drying (LPSSD), which involves drying under the superheated steam environment at a lower pressure, has also more recently been proposed. LPSSD has noted to be especially effective for oxygen-sensitive compounds as virtually no oxygen is present in the drying chamber; no oxidation reactions are therefore possible (Devahastin and Mujumdar 2014).

Besides the aforementioned processes, a number of other alternatives have been tested for their ability to alleviate the losses of phenolic compounds. Use of microwave, either as a stand-alone source of energy for drying or in combination with other more conventional processes, e.g., hot air tray drying, fluidized bed drying, spouted bed drying, or even vacuum drying, has, for example, proved to help reduce the losses of phenolic compounds in fruits and vegetables (Igual et al. 2012; Fujita et al. 2013). More traditional processes, e.g., sun and solar drying, on the other hand, generally result in higher losses of phenolic compounds

due to the longer required drying time of these processes (Sun et al. 2015). For fruit and vegetable juices or extracts, spray drying is a typically preferred choice. Due to a very short required drying time, spray drying could normally maintain phenolic compounds in the juices or extracts at a satisfactory level (Shofinita and Langrish 2014).

A summary of selected works on drying and retention of phenolic compounds in fruits and vegetables is given in Table 1.

Changes of Flavonoids During Drying

Flavonoids are diphenylpropanes belonging to the polyphenolic class that includes a C6–C3–C6 carbon framework. More than 4,000 flavonoids in fruits and vegetables have been identified. The common family members of flavonoids include flavones, isoflavones, flavanones, anthocyanins, flavonols, and flavanols (Charles 2013). Flavonoids have been claimed to have many health-promoting effects, including antioxidant, antiallergic, anticancer, anti-inflammatory, and antiviral activities. Since flavonoids are sensitive to heat, oxygen, and light, the compounds can easily degrade during drying.

Freeze-drying has again noted to generally be the most effective method to retain flavonoids in fruits and vegetables. In some cases, however, freeze-drying may not be the best drying method to preserve flavonoids. For instance, García-Salas et al. (2013) who studied the changes in the amounts of flavonoids in whole lemons, which were chopped, triturated, and dried via freeze-drying and vacuum drying at 35 °C for 48 h, reported that among flavonoids found in lemons, eriocitrin, neohesperidin, vicenin-2, and diosmetin 6,8-di-C-B-glucoside had the highest concentration values. Interestingly, the amounts of these flavonoids in lemons dried by vacuum drying were significantly higher than those dried by freeze-drying. Higher temperature of vacuum drying might probably break the glycosidic bonds of phenolic compounds, giving rise to the aglycone molecules, which were detected flavonoids.

Hot air-drying, as expected, generally results in lower flavonoid retention. However, in some cases, at higher drying air temperatures, tissue structure of the plants may be altered, leading to higher flavonoid contents than those observed at lower drying temperatures (Lou et al. 2015).

A number of studies have been made to compare different drying methods for their ability to retain flavonoids in fruits and vegetables; some of them are listed in Table 1.

Changes of Carotenoids During Drying

Carotenoids are fat-soluble yellow, orange, or red pigments containing 3–13 conjugated double bonds and, in certain cases, 6 carbon hydroxylated ring structures at one or both ends of the molecule. Carotenoids can be classified into two classes, i.e., carotenes, which contain carbon and hydrogen atoms, and xanthophylls (oxycarotenoids), which contain carbon, hydrogen, and at least one oxygen atoms. The important carotenoids in fruits and vegetables, in particular, the orange and yellow fruits and vegetables, as well as green leafy vegetables, are lycopene,

Table 1 Selected studies on drying and some nutritional compounds in fruits and vegetables

Nutrition compound	Type of food	Drying method	Condition	References
Phenolics and vitamin C	Red pepper	Hot air-drying	Drying temp.: 50 °C, 60 °C, 70 °C, 80 °C, and 90 °C Air velocity: 2.0 ms ⁻¹ Sample size: 4.0 mm in thickness Final moisture content: equilibrium condition	Vega-Gálvez et al. (2009)
Phenolics and vitamin C	Cabbage (outer leaves)	Hot air-drying	Drying temp.: 70 °C, 80 °C, and 90 °C Air velocity: 2.0 m s ⁻¹ Sample size: 0.5 × 5 cm Final moisture content: 0.1 kg/kg (dry basis) Pretreatment: blanching in hot water at 93 °C for 2 min	Nilnakara et al. (2009)
Phenolics and vitamin C	Lime residue	Hot air-drying	Drying temp.: 60 °C, 80 °C, 100 °C, and 120 °C Drying time: 3–10 h Pretreatment: blanching in hot water at 95 °C for 5 min	Kuljarachanan et al. (2009)
Phenolics	Apple	Hot air-drying	Drying temp.: 40 °C, 60 °C, and 80 °C Air velocity: 0.5, 1.0, and 1.5 m s ⁻¹ Sample size: 5.0 mm in thickness Final moisture content: equilibrium condition	Vega-Gálvez et al. (2012)
Phenolics and vitamin C	Pear	Hot air-drying	Drying temp.: 30 °C, 40 °C, 50 °C, 60 °C, and 70 °C Air velocity: 1.5 m s ⁻¹ Sample size: 8 × 8 × 8 mm Final moisture content: 0.02 kg/kg (dry basis)	Mrad et al. (2012)
Phenolics, lycopene, and vitamin C	Tomato	Hot air-drying (rotating tray dryer)	Drying temp.: 45 °C, 50 °C, and 60 °C Air velocity: 0.6 and 1.2 m s ⁻¹ Sample size: 6 mm in thickness Final moisture content: 10 g/100 g (wet basis) Pretreatment: soaking in 10 g/L sodium metabisulfite for 10 min	Santos-Sánchez et al. (2012)
Phenolics and flavonoids	Kumquat	Hot air-drying	Drying condition: Set 1: 110 °C and 130 °C for 0.5, 1.0, 1.5, and 2.0 h Set 2: 150 °C for 1.5 h	Lou et al. (2015)

(continued)

Table 1 (continued)

Nutrition compound	Type of food	Drying method	Condition	References
			Sample size: 0.5 cm in thickness	
Phenolics and vitamin C	Apricot	Hot air-drying	Drying temp.: 40 °C and 60 °C	Iguar et al. (2012)
		Microwave drying	Drying condition: 40 °C, 100 W	
		Hot air-microwave combined drying	Drying condition: 100 W, 40 °C with microwave power until 40 % moisture content, and 40 °C with hot air until the end of the process Sample size: cut into halves Final moisture content: 20–25 g/100 g (dry basis) Pretreatment: soaking in sodium metabisulfite (1.5 g/L) for 1 h	
Phenolics, flavonoids, vitamin C, and anthocyanins	Cranberries	Freeze-drying	Freezing temp.: –30 °C Drying condition: 30 °C, 50 °C, and 70 °C, 52 Pa Sample size: 10 mm in diameter Final moisture: 50 g/kg sample	Rudy et al. (2015)
Phenolics	Muscadine pomace	Hot air-drying	Drying temp.: 70 °C and 80 °C Air velocity: 0.2, 0.4, and 0.6 m s ⁻¹ Drying time: 180 and 240 min	Vashisth et al. (2011)
		Freeze-drying	Drying condition: 30 °C for 14–16 h	
		Vacuum belt drying	Drying temp.: Zone 1: 60 °C, 70 °C, 80 °C, and 90 °C Zone 2: 80 °C, 90 °C, 95 °C, 100 °C, and 105 °C Zone 3: 100 °C, 110 °C, and 120 °C Zone 4: 100 °C, 110 °C, and 120 °C Drying time: 60 and 90 min Pressure: 3–5 kPa Sample size: 8.9 cm in diameter, 2 and 4 mm in thickness Final moisture content: and 1.8 g/100 g	

(continued)

Table 1 (continued)

Nutrition compound	Type of food	Drying method	Condition	References
Phenolics (xanthones)	Mangosteen rinds	Hot air-drying	Drying temp.: 60 °C, 75 °C, and 90 °C	Suvarnakuta et al. (2011)
		Vacuum drying	Drying temp.: 60 °C, 75 °C, and 90 °C Absolute pressure: 7 kPa	
		LPSSD	Drying temp.: 60 °C, 75 °C, and 90 °C Absolute pressure: 7 kPa Sample size: 1 mm in thickness Final moisture content: 0.10 kg/kg (dry basis)	
Phenolics, vitamin C, and anthocyanidins	Camu-camu	Spouted bed drying	Drying temp.: 60 °C, 80 °C, 95 °C, and 110 °C Air velocity: 1.8 m s ⁻¹	Fujita et al. (2013)
		Freeze-drying	Drying condition: -80 °C and 100 mTorr for 120 h	
Phenolics and flavonoids	Un-matured citrus	Sun drying	Drying temp.: 20–25 °C for 3 days Relative humidity: 60 %	Sun et al. (2015)
		Hot air-drying	Drying temp.: 60 °C for 10 h Air velocity: 2 m s ⁻¹ Relative humidity: 60 %	
		Freeze-drying	Drying condition: -50 °C, 12 Pa for 12 h Sample size: 0.5 cm in thickness Final moisture content: 10 g/100 g (dry basis)	
Phenolics	Orange peel extracts	Spray drying	Inlet air temp.: 100–200 °C Atomization air flow rate: 1,052 L/h Liquid feed pump rate: 4 mL/min Aspirator rate: 38 m ³ h ⁻¹ Outlet air temp.: 43–79 °C Sample preparation: extraction of phenolics from orange peels using microwave	Shofinita and Langrish (2014)
Flavonoids and anthocyanins	Red onion	Freeze-drying	Freezing condition: immersion in carbon dioxide snow for 3 min and storage at -20 °C Drying condition: -70 °C, 4.2 Pa for 24 h	Pérez-Gregorio et al. (2011)
Flavonoids, anthocyanins,	Grape skin	Freeze-drying	Freezing condition: -78 °C for 12 h Drying condition: -49 °C,	De Torres et al. (2010)

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Table 1 (continued)

Nutrition compound	Type of food	Drying method	Condition	References
and volatile compounds			2.4×10^{-2} mBar for 24 h Final moisture content: 5.1 % and 5.5 %	
Flavonoids	Apple	Air-drying	Drying condition: 50 °C for 14 h Initial surface area of apple slices: 39.9 cm ²	Schulze et al. (2014)
		Freeze-drying	Drying condition: freezing using liquid nitrogen and freeze-dried for 72 h	
		Microwave vacuum drying	Drying condition: Period 1: 500 W, 100 hPa for 25 min Period 2: 1,000 W, 100 hPa for 60 s Period 3: 80 W, 100 hPa	
Carotenoids	Mango	Hot air-drying	Drying condition: Treatment A: 60 °C for 20 h Treatment B: soaking in 1 % sodium hydrogen sulfite for 30 min prior to hot air-drying Treatment C: soaking in 1 % ascorbic acid for 30 min prior to hot air-drying	Chen et al. (2007)
		Freeze-drying	Treatment D: 53 °C, 0.06 torr for 32 h Treatment E: soaking in 1 % sodium hydrogen sulfite for 30 min prior to freezing drying Treatment F: soaking in 1 % ascorbic acid for 30 min prior to freeze-drying Sample size: 3 × 9 cm Final moisture content: 18 %	
Phenolics and carotenoids	Papaya	Hot air-drying	Drying temp.: 50 °C, 60 °C, 70 °C, and 80 °C Absolute humidity: 10 and 25 g water/kg dry air Air velocity: 0.2, 0.5, 0.7 ms ⁻¹ Sample size: 20 × 30 × 20 mm Final moisture content: 13.5 g/100 g (dry basis) Pretreatment: soaking in 2.5 g/100 g calcium lactate for 1 h, blanching at 60 °C for	Udomkun et al. (2015)

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Table 1 (continued)

Nutrition compound	Type of food	Drying method	Condition	References
			1 min and immersion in 30 °Brix sucrose at 60 °C for 6 h	
Carotenoids	Carrot	Hot air-drying	Drying temp.: 60 °C, 70 °C, and 80 °C Air velocity: 0.8 m/s	Hiranvarachat et al. (2008)
		LPSSD	Drying temp.: 60 °C, 70 °C, and 80 °C Absolute pressure: 7 kPa	
		Vacuum drying	Drying temp.: 60 °C, 70 °C, and 80 °C Absolute pressure: 7 kPa Final moisture content: 0.1 kg/kg (dry basis)	
Carotenoids	Paprika	Refractance Window™	Drying condition: 94 °C for 3 min Belt velocity: 0.45–0.58 mmin ⁻¹	Topuz et al. (2011)
		Freeze-drying	Drying condition: –70 °C, 40 mmHg for 8 days	
		Hot air-drying	Drying condition: 60 °C for 7 h Air velocity: 0.76 m s ⁻¹	
		Natural convective drying	Drying condition: under gloomy condition at room temperature for 8–10 days Preparation: ground into fine puree using a blender	
Carotenoids	Gac	Hot air-drying	Drying temp.: 40 °C, 50 °C, 60 °C, 70 °C, and 80 °C Air velocity: 0.2 m/s	Mai et al. (2013)
		Vacuum drying	Drying temp.: 40 °C, 50 °C, 60 °C, 70 °C, and 80 °C Vacuum pressure: 9,000 Pa	
		Freeze-drying	Drying condition: –20 °C, 250 Pa Sample size: 1–5 mm in thickness Final moisture content: 15–18 % (dry basis)	
Vitamin C, carotenoids, and anthocyanins	Cherry, nectarine, apricot, red bell pepper, peach, plum	Freeze-drying	Drying condition: freezing using liquid nitrogen and freeze-dried for at least 48 h Sample size: 5 mm in thickness	Leong and Oey (2012)
Vitamin C	Onion	Hot air-drying	Drying temp.: 30 °C, 50 °C, and 60 °C Air velocity: 0.35 ms ⁻¹	Mota et al. (2010)

(continued)

Table 1 (continued)

Nutrition compound	Type of food	Drying method	Condition	References
			Sample size: 2.5 cm in diameter, 3–4 mm in thickness Final moisture content: 20 % or below	
Vitamin C	Kiwifruit	Hot air-drying	Drying temp.: 25 °C, 35 °C, 45 °C, 55 °C, and 65 °C Air velocity: 0.3, 0.6, and 0.9 m/s Relative humidity: 40 %, 55 %, 70 %, and 85 % Sample size: 7 mm in thickness Final moisture content: equilibrium condition	Kaya et al. (2010)
Vitamin C	Papaya	Hot air-drying	Drying temp.: 40 °C, 50 °C, 60 °C, and 70 °C Air velocity: 1.0 and 1.32 ms ⁻¹ Sample size: 20 × 20 × 20 mm	Kurozawa et al. (2014)
Chlorophylls	Basil	Hot air-drying	Drying condition: 40 °C for 26 h Dry matter: 77.05 %	Bušić et al. (2014)
		CO ₂ drying	Drying condition: 40 °C for 2–4 h Pressure: 80–100 bar CO ₂ flow rate: 1,000 L/h Dry matter: 91.89–94.59 %	
		Freeze-drying	Drying condition: –20 °C, 0.005 bar for 4 days Dry matter: 93.95 %	
Chlorophylls and carotenoids	Coriander foliage	Hot air-drying	Drying condition: 45 °C until constant weight (~24 h)	Divya et al. (2012)
		Microwave drying	Microwave power: 850, 600, 450, 300, and 180 W Drying time: 30, 60, 90, 120, and 150 s	

β -carotene, lutein, zeaxanthin, β -cryptoxanthin, and astaxanthin. Carotenoids are photo-, thermal-, and oxygen-labile components and hence can easily degrade during drying; both thermal and oxidative degradation and isomerization into other conformational forms can occur (Suvarnakuta et al. 2005; Hiranvarachat et al. 2008).

Hot air-drying is again known to cause a significant reduction in carotenoids in fruits and vegetables. Nevertheless, suitable pretreatment methods, when applied prior to drying, can help reduce the losses of these compounds (Chen et al. 2007; Udomkun et al. 2015).

Selected works on drying and the changes of carotenoids in fruits and vegetables are listed in Table 1.

Changes of Vitamin C During Drying

Vitamin C or L-ascorbic acid is one of the major water-soluble free radical scavengers found in fruits, especially citrus fruits, cherry, kiwi, melon, and vegetables like tomato, leafy greens, cauliflower, broccoli, cabbages, and Brussels sprouts. Unfortunately, vitamin C can be easily degraded, depending on many variables such as temperature, pH, light, time, presence of enzymes, oxygen, and metal-based catalysts (Santos and Silva 2008; Kurozawa et al. 2014). Vitamin C stability in dried fruits and vegetables is nevertheless mainly related to the employed methods and conditions of drying. In general, sun, solar, and hot air-drying expectedly cause significant losses of vitamin C in dried fruits and vegetables. Freeze-drying is again a preferred alternative to retain ascorbic acid in dehydrated fruits and vegetables due to the use of a lower drying temperature.

Different variables, especially the combined effect of drying temperature and time (e.g., high-temperature short time vs. low-temperature long time) as well as the presence or absence of oxygen, also affect the vitamin C retention. Pretreatment step (e.g., washing, peel removal, blanching, and slicing) prior to drying also exerts a negative effect on the retention of vitamin C in the subsequent dried fruits and vegetables (Santos and Silva 2008).

The effects of selected drying techniques, including solar drying, hot air-drying, microwave drying, osmotic dehydration, freeze-drying, and alternative drying (foam mat drying, drying under modified atmosphere, superheated steam drying, impingement drying, Refractance Window[®] drying), and conditions on vitamin C contents in dehydrated fruits and vegetables are given by Santos and Silva (2008).

Some studies on the degradation of vitamin C in fruits and vegetables subjected to different drying methods and conditions are listed in Table 1.

Changes of Pigment Compounds During Drying

Fruits and vegetables are a major source of natural pigments, viz., anthocyanins, betalains, chlorophylls, and carotenoids (Marquez and Sinnecker 2008). These pigments are responsible for the variety of colors of fruits and vegetables. Color changes of fruits and vegetables during drying are indeed due to the losses and/or alterations of these compounds. With the increasing trend toward the use of (and hence the need to produce) natural colorants from plant materials, it is important to prevent or alleviate the losses and/or alterations of these pigments before they are extracted from their sources. It is noted that extraction is in many cases done on dried plant materials since it is not convenient or economical to store and then extract fresh plant materials.

Anthocyanins are compounds contributing to the red, blue, and purple pigments in plant tissues. The major anthocyanins found in fruits are cyanidin, peonidin, delphinidin, petunidin, malvidin, and pelargonidin (Leong and Oey 2012). The effects of an array of drying methods on anthocyanins stability have been reported; some of the works are listed in Table 1. Drying generally results in a lower amount of anthocyanins in dried fruits and vegetables because anthocyanins can be rapidly oxidized by oxygen (Leong and Oey 2012) and degraded by heat (Rudy et al. 2015). Some pretreatments prior to drying such as acid pretreatment also pose a negative effect on the retention of anthocyanins (Rudy et al. 2015).

Chlorophylls are the most abundant natural pigments and are the source of the green color in plants (Marquez and Sinnecker 2008). Different drying methods differently result in the losses of chlorophylls in fruits and vegetables. Hot air-drying is again reported to cause a more extensive degradation of chlorophylls in comparison with freeze-drying, microwave drying, or other advanced drying methods, e.g., supercritical CO₂ drying (Divya et al. 2012; Bušić et al. 2014). This is because the heat during drying can result in the isomerization and degradation of the chlorophyll molecules. In addition, the magnesium atom in a chlorophyll molecule can be easily displaced by a hydrogen ion, resulting in the conversion of bright-green chlorophylls to olive-brown pheophytins. Chlorophyll stability can nevertheless be improved by the addition of chloride salt of sodium, magnesium, or calcium or by the formation of metal complexes of pheophytins with zinc or copper. Suitable pretreatments such as blanching to inhibit hydrolytic enzyme or alkaline treatment to retard the loss of the magnesium atom prior to drying can also help improve the pigment stability (Von Elbe and Schwartz 1996).

Some works on drying and chlorophyll stability are listed in Table 1.

Changes of Volatile Compounds During Drying

Aroma and flavor of fruits and vegetables result from a complex mixture of a large number of volatile compounds coming from carbohydrates, amino acids, and fatty acid metabolisms (Pérez and Sanz 2008). Among the most important volatile compounds in fruits, one may cite terpenoids, also known as isoprenoids or terpenes, furanones, ketones, alcohols, aldehydes, and ester (Pérez and Sanz 2008), while those in vegetables include terpenoids, glucosinolates, alk(en)yl cysteine sulfoxides, and phenolic compounds (Jones 2008). Removal of water during drying causes either degradation or evaporation (or both) of such compounds.

De Torres et al. (2010) studied the changes of the volatile compositions of two grape skin varieties (Carménère and Cabernet Sauvignon) subjected to hot air-drying at 60 °C and freeze-drying. A total of 97 volatile compounds were identified in the two grape varieties. The total amount of terpenes, sesquiterpenes, norisoprenoids, alcohols and aldehyde families, acids, esters, and benzene derivatives significantly decreased upon hot air-drying, but were better retained by freeze-drying. On the other hand, the total amount of the derivative compounds of furan, pyran, and lactones significantly increased upon hot air-drying; in particular, furfural increased sixfolds upon drying. The total amount of these compounds did not change upon freeze-drying, however.

Nutritional Changes of Meats and Seafood Products During Drying

Meat and seafood products are an important source of protein, fat, essential amino acids, minerals, as well as some vitamins and other micronutrients (Zhang et al. 2010). Since fresh meat and seafood are rich in nutrients, but contain a relatively large amount of water, they are perishable and exhibit short shelf life. Drying is one of the common preservation methods that can be used to prolong the shelf life of meat and seafood products. Upon drying, however, changes of various nutritional parameters of meats and seafood cannot be avoided.

Changes of Protein and Amino Acids During Drying

Protein is a major nutrient in meats and seafood, and it is the level and quality of protein that give the value of these products in the market. The proteins of meats and seafood are more complete than vegetable proteins because meat- and seafood-based proteins contain essential amino acids that are required for human body but are otherwise absent in plant-based foods (Higgs and Mulvihill 2002). However, meat and seafood proteins suffer changes when being processed.

Different drying methods are noted to affect the quantity and quality of meat and seafood proteins; the changes can be either desirable or undesirable. Deng et al. (2015), for example, reported that squid undergone freeze-drying, hot air-drying, and heat pump drying experienced an increase in the total essential amino acid (leucine, isoleucine, and valine) contents when compared with that in the raw squid. On the other hand, hot air and heat pump drying led to a lower content of the total nonessential amino acids (histidine, arginine, and glycine). This may be the result of the change in the compositions of nitrogenous compounds by heat. Although a significant decrease in the total nonessential amino acid content was observed, the contents of glutamic acid and aspartic acid, which contribute to the stronger umami taste of the dried squid, increased. When considering the electrophoretic pattern of myosin, it was found that myosin extracted from the raw squid mainly consisted of myosin heavy chain (250 kDa), heavy meromyosin (170 kDa), light meromyosin (100 kDa), and tropomyosin (43 kDa). Myosin extracted from the freeze-dried squid was similar to that from the raw squid, but myosin heavy chain disappeared. This is probably because the myosin heavy chain degraded into heavy meromyosin and light meromyosin during freeze-drying. Almost all myosin extracted from the hot air-dried squid degraded into tropomyosin, while myosin extracted from the heat pump-dried squid was still rich in light meromyosin. These results suggested that hot air-drying caused more damage to squid myosin structure than heat pump drying, while freeze-drying most effectively retained the myosin integrity.

Different results and conclusions may be applicable when considering different other products. Mardiah et al. (2012), for example, studied the effect of drying on amino acid contents and quality of fish flake dough made from stingray meat, which was dried by hot air at 60 °C. The protein quality was assessed in terms of the amino acid score and essential amino acid index. The results showed that drying slightly decreased the contents of some amino acids such as isoleucine, threonine, and

phenylalanine in the fish flake dough as compared with those in the dough prior to drying; a slight decrease in the amino acid score and essential amino acid index was also observed. In this case, drying did not significantly affect the amino acid composition and their quality.

Changes of Lipids During Drying

Lipids are a group of naturally occurring molecules that include triglycerides, sterols, and phospholipids. The major lipids of meats and seafood are triacylglycerol (or triglyceride), which composes of three fatty acids esterified to a glyceride backbone. The fatty acids of which triglycerides are composed vary in length of carbon chain as well as degree of saturation. The long-chain highly unsaturated fatty acids are readily oxidized (Pigott and Tucker 1990) and hence are sensitive to degradation during drying, especially when being conducted under an oxygen-rich environment such as in the case of hot air-drying. A method that provides an oxygen-depleted (or even oxygen-free) condition and/or rapid drying (e.g., vacuum drying, superheated steam drying, microwave vacuum drying) expectedly leads to less changes of lipids (Fu et al. 2015). Sun and solar drying, on the other hand, generally leads to significant changes of lipids since direct exposure to UV and visible light during sun drying leads to lipid oxidation (Hernández-Becerra et al. 2014).

Changes of Volatile Compounds During Drying

Important flavor and aroma compounds in meats and seafood include aldehydes, ketones, hydrocarbons, pyrazines, acids, esters, alcohols, nitrogen- and sulfur-containing compounds, as well as other heterocyclic compounds. Due to their differences in the chemical structure, the flavor and aroma characteristics of different compounds are quite different (Ba et al. 2012) and also respond differently to the drying process. Total volatile basic nitrogen (TVBN) content of seafood, for example, generally increases upon drying (Deng et al. 2014); the increase is associated with the formation of volatile basic components such as trimethylamine and ammonia as well as the enzymatic degradation and bacterial spoilage. Higher temperature and oxygen content again result in a higher level of TVBN.

Drying is noted to result in a decrease in the number of volatile compounds, but in an increase in their total concentration in a dried sample (Deng et al. 2015). The decrease in the number of volatile compounds results from the evaporation of such compounds during drying. On the other hand, formation of volatile compounds via the Maillard reaction and lipid oxidation, which are accelerated by heat, leads to the higher total concentration of the volatile compounds.

Different drying methods may also result in different groups of volatile compounds in a dried sample. Deng et al. (2015), for example, reported that the major volatile compounds of raw squid are alcohols and ketones, while those of the heat pump-dried squid are aliphatic hydrocarbons and aromatic hydrocarbons, those of air-dried squid are aldehydes and nitrogenous compounds, and those of freeze-dried squids are phenols and sulfocompounds. This may be the result of the difference in the utilized drying temperature, which affects the mechanisms and pathways of

volatile formation. Generally, higher temperature produces higher concentrations of aliphatic aldehydes, benzenoids, polysulfides, heterocyclic compounds, and lipid-derived volatiles.

Nutritional Changes of Grains and Legumes During Drying

Grains and legumes are important diets for the world's population because they serve as a good source of carbohydrate and protein. Important grains for human consumption include maize, wheat, barley, rye, sorghum, millet, rice, and oat, while legumes include alfalfa, clover, lupine, mesquite, soybean, chickpea, cowpea, and lentil. Several legumes (e.g., rapeseed, soybean, and peanut) exhibit high oil content, while some grains and legumes are also rich in nutraceuticals, especially phenolic compounds. Nevertheless, legumes often contain a number of antinutritional factors, which can limit their nutritional quality; this important aspect will be discussed later in the chapter.

Drying has widely been used for grains and legumes processing, primarily for improving the palatability, stability, and safety of the products. The effect of drying on nutritional changes of grains and legumes is briefly described below.

Changes of Phenolic Compounds During Drying

The major phenolic compounds presenting in grains are phenolic acids, flavonoids, and tannins. These phenolics may exist either in a free, esterified/etherified, or insoluble bound form (Shahidi and Nacz 2004). Different results regarding the losses of phenolic compounds upon drying have been reported. Martin-Cabrejas et al. (2009), for example, investigated the effect of drying on TPC of legume flours (from chickpea, lentil, white bean, or pink-mottled cream bean). Each legume was soaked in tap water for 16 h and boiled for different durations. The soaked-cooked seeds were dried at 75 °C for 6 h. The results showed that drying did not cause a decrease in TPC of all legumes, except for lentil, as compared with that of the seeds after cooking. On the other hand, Miranda et al. (2010) who investigated the change in TPC of quinoa seeds during convective drying at 40–80 °C for 150–420 min reported that TPC of quinoa seeds decreased with an increase in the drying temperature, especially at higher temperatures of 60 °C, 70 °C, and 80 °C. The losses may be the result of the binding of phenolic compounds with other compounds such as protein or the alterations in the phenolic structure, which then became more difficult to extract and determined by standard analytical methods. Similar results are reported by Tsantili et al. (2011) who investigated the changes in the total phenolic and total flavonoid contents of pistachio nuts dried at 45 °C for 34 h.

Rumruaytum et al. (2014) later monitored the changes in phenolics in Thai native rice varieties such as Sung Yod Phattalung and Nauykaur subjected to superheated steam-fluidized bed drying at 170 °C for 2.5, 3, and 4 min. The results showed that higher drying temperatures and longer time caused a significant decrease in TPC of Sung Yod Phattalung, but an increase in TPC of Nauykaur.

This is because Sung Yod Phattalung cultivar contains more heat-sensitive phenolics such as cyaniding-3-*o*-*b*-glucoside than Nauykaur cultivar. The increase in TPC of Nauykaur cultivar might, on the other hand, resulted from the release of bound phenolics from the plant cell walls at the higher drying temperatures.

Changes of Protein and Amino Acids During Drying

Grain and legume proteins are recognized for their essential amino acids. Several proteins and peptides from grains and legume seeds can even be categorized as nutraceuticals or functional components; these include, for example, storage proteins 7S and 11S globulins, prolamins, and glutelins from legume seeds (soybean, lupine) and cereal grains (wheat) (Carbonaro et al. 2014).

Miranda et al. (2010) investigated the change in the protein content of quinoa seeds during convective drying at 40–80 °C for 150–420 min. Drying led to 10 % reduction in the protein content. The loss of protein was thought to be due either to denaturation or change in the protein solubility during drying. Moreover, the reduction might result from the release of amino acids from the protein after denaturation; amino acids could then react with other chemical compounds such as reducing sugars to produce melanoidins via the Maillard reaction.

Changes of Lipids During Drying

Lipids contained in grains and legumes are mostly polar lipids, including phospholipids, glycolipids, and triacylglycerols. Grains and legumes can accumulate a large amount of polar lipids in their endosperms. Soybean may contain up to 8 % phospholipids, while whole oat grain lipids include 8–17 % glycolipids and 10–20 % phospholipids. Triacylglycerols are the main reserve material in oil seeds containing edible and industrial oils such as soybean, sunflower, safflower, rapeseed, canola, and flax (Nawar 1996).

Miranda et al. (2010) investigated the change of the lipid content of quinoa seeds during convective drying at 40–80 °C for 150–420 min. Drying was noted to lead to around 12 % reduction in the lipid content of the seeds, due either to enzymatic hydrolysis during the first drying period when hydrolytic enzyme was still activated or to lipid oxidation due to the oxygen-rich nature of the drying medium (air).

Toxicological Changes of Foods During Drying

Antinutritional Factors in Vegetables, Grains, and Related Products

Substances generated either in natural feeds by the normal metabolic activities of such species or by other different mechanisms, which reduce the nutrient utilization or exhibit contrary to optimum nutrition for humans and animals, are termed antinutritional factors (ANFs) (Kumar 1991; Soetan and Oyewole 2009). ANFs mainly present in vegetables, herbs, grains, and tuber crops. Different types of natural substances may exhibit different toxicities to different individuals. Degradation of ANFs by drying depends on the types of ANFs and foods as well as the

methods and conditions of drying. Some important ANFs and the effect of drying on the changes of selected ANFs are briefly described below.

Protease Inhibitors

Many plants contain protease inhibitors, which depress the availability of biologically active enzymes essential to the utilization of nutrition by both humans and animals. Protease inhibitors may be classified by the type of proteases they inhibit such as trypsin inhibitors, sulfhydryl proteinase inhibitors, acid proteinase inhibitors, metalloproteinase inhibitors, peptidase inhibitors, carboxypeptidase inhibitors, and α -amylase inhibitors. Protease inhibitors are widely presented in soybean, barley, maize, wheat, avocado, peach, plum, tomato, and potato. Protease inhibitors can reduce the growth rate of humans and animals by inhibiting the proteolysis of dietary protein (Spencer and Berman 2003).

Several works exist on the use of drying and other appropriate thermal processing to reduce the amount of protease inhibitors. Prachayawarakorn et al. (2004), for example, studied the use of superheated steam-fluidized bed drying to eliminate trypsin inhibitor in soybean. Urease activity measurement was used as an indirect indicator of the inactivation of trypsin inhibitor. Inlet steam temperatures of 120 °C, 135 °C, and 150 °C and initial soybean moisture contents of 13.5 %, 19.5 %, and 16.0 % (dry basis) were tested. The results showed that the rate of urease inactivation was enhanced at increased drying temperature. Temperature played an important role in accelerating the urease inactivation for soybean having low to moderate initial moisture contents. To be able to inactivate urease while maintaining soybean quality, in terms of protein solubility, lysine, and color, inlet steam temperature should not exceed 135 °C. Adamidou et al. (2011) investigated the influences of extrusion preconditioning and drying temperature on trypsin inhibitor contents in field pea (*Pisum sativum*), chickpea (*Cicer arietinum*), and faba bean (*Vicia faba*). The tested extrusion temperatures were 70 °C for the low-temperature, 90 °C for the middle-temperature, and 100 °C for the high-temperature preconditioning. The tested temperatures for conveying drying (after preconditioning) were 90–120 °C for the low-temperature and 120–150 °C for high-temperature drying. Extrusion preconditioning followed by drying could reduce the content of trypsin inhibitors in all types of the test legumes; different conditions were required for different types of legumes, however. The highest reduction of trypsin inhibitors reached 59 % for field pea, 92 % for chickpea, and 44 % for faba bean.

Cyanogenic Glycosides

Cyanogenic glycosides are the water-soluble substances occurring in various plant species such as lima bean, sorghum, bitter almond, choke cherry seeds, bamboo shoots, corn, chickpea, cashew nut, and cassava. Hydrolysis of these glycosides results in the highly toxic hydrogen cyanide; this occurs when plant tissues are disturbed as during processing. Hydrolysis is generally accelerated by β -glucosidase and other enzymes naturally present in the plant tissues. Cyanide

shows toxicity by inactivating heme enzymes, resulting in acute, life-threatening anoxia. It also leads to psychoactive symptoms, euphoria, hallucination, abdominal pain, depression, and stupor (Coulombe 2001; Altug 2003; Shibamoto and Bjeldanes 2009).

Eruvbetine et al. (2003) studied the effect of drying methods and conditions on the changes of hydrogen cyanide in cassava leaves and tubers. Oven-drying (at 40–60 °C) and sun drying were evaluated and found to be capable of reducing the hydrogen cyanide content in both the leaves and tubers. Oven-dried samples exhibited a significantly higher level of hydrogen cyanide compared to the sun-dried samples. Cooke et al. (1981) and Fish and Trim (1993) also observed similar observations. Interestingly, mixing the leaves and tubers together prior to drying led to a more extensive reduction in the hydrogen cyanide content than doing so after drying.

Phytates

Phytates are the principal storage forms of phosphorus in plant tissues and can be found in several plants such as nuts, legumes, germ and bran of grains, green beans, carrot, broccoli, potato, artichoke, blackberry, strawberry, and fig. Phytates act as antimineral compounds as they can bind important minerals like zinc, cobalt, iron, calcium, magnesium, and copper; these metals then become unavailable for absorption (Altug 2003; Brimer 2011).

Bhuiyan et al. (2012) studied the effect of drying on the changes of phytate-P in maize. The tested drying methods were sun drying and hot air-drying (at 105 °C for 30 min and 24 h). Drying was noted to be capable of reducing the content of phytate-P in maize. Hot air-drying for 24 h resulted in the largest reduction in the phytate-P content; this was followed by hot air-drying for 30 min and sun drying. Pontoppidan et al. (2007) reported that phytates in cereals were fairly destroyed at 90 °C. This indicates that phytates are partially heat labile and the degradation reduction is not enzymatic in nature.

Mimosine

Mimosine is a toxic nonprotein amino acid, which is mainly present in the legumes *Leucaena* and *Mimosa*. Mimosine has noted to result in acute and chronic toxicosis in livestock fed with forages containing those legumes. Mimosine reversibly blocks cell cycle progression in mammalian cells (Kulp and Vulliet 1996) and causes poor growth, alopecia and reproductive problems, hair loss, goiter, reproductive disorders, and epithelial damage (Poonam and Pushpa 1995; Akande et al. 2010).

Benjakul et al. (2012) investigated the effect of drying methods on mimosine content in lead (*Leucaena leucocephala*) seed extract. The tested drying methods were oven-drying (at 60 °C for 12 h) and freeze-drying. Oven-drying, as expected, resulted in a lower mimosine content of the extract when compared to freeze-drying. This indicates that heating or drying can help destroy mimosine. Budi et al. (2006) indeed reported that the optimum degradation rate of mimosine occurs at a temperature around 70 °C.

Oxalate

Oxalate is another antimineral compound found in the leaves, stems, and roots of several plants including rhubarb, spinach, beets, potato, tea, coffee, peas, berries, carrot, lettuce, turnip, and cocoa (Omaye 2004). Oxalate binds calcium, potassium, and other needed trace minerals and makes them unavailable for absorption. High oxalate level can be the cause of irritation of the digestive system and blood calcium chelating, resulting in hypocalcemia, which leads to the decrease of bone growth. Accumulation of insoluble calcium oxalate crystals in kidney may cause kidney stones. Increased oxalate intake can also result in diarrhea, blood-clotting problem, and convulsion (Altug 2003; Pussa 2008).

Mbah et al. (2012), among others, studied the effect of drying on the changing level of oxalate in *Moringa oleifera* leaves. The leaves were obtained fresh, cleaned, and washed prior to drying (sun drying, shade drying, and oven-drying). The results revealed that all the tested drying methods led to the decreased oxalate content of the leaves.

Tannins

Tannins are the water-soluble phenolic compounds, which can be found in tea, coffee, chocolate, banana, sorghum, spinach, and raisin. Tannins can be classified into two groups, namely, proanthocyanidins (or condensed tannins) and hydrolyzable tannins. Tannins have the ability to bind and precipitate proteins and cause liver damage. In addition, they can strongly bind metals such as iron, copper, and zinc and cause reduction in the gastrointestinal absorption of these metals. Tannins also inhibit digestive enzymes and reduce the bioavailability of vitamin B₁. These toxicological effects show a larger impact when tannins are present in the bloodstream (Coulombe 2001; Altug 2003).

Palmer et al. (2000) studied the effect of drying on condensed tannin content in the leaves of *Calliandra calothyrsus*. Free condensed tannin content slightly increased with an increase in the drying temperature under anaerobic conditions, whereas there was a linear decrease in the content under aerobic drying conditions; similar trend was observed for the total condensed tannin content. The samples freeze-dried at lower temperatures (25 °C and 45 °C) exhibited the same trends as those hot air-dried. Aerobic drying might have led to oxidative reaction of tannins, resulting in a portion of tannins being irreversibly bound to the sample structure and that could not be extracted by the employed analytical procedures.

Selected works on drying and antinutritional factors in vegetables, grains, and related products are listed in Table 2.

Mycotoxins in Food Products and Their Changes During Drying

Mycotoxins are highly toxic compounds that can contaminate foods and agricultural produces. Mycotoxins are produced by at least 120 different filamentous fungi. Any food on which fungi have grown can contain mycotoxins, which cannot be

Table 2 Selected studies on drying and antinutritional factors in vegetables, grains, and related products

ANF	Type of food	Drying method	Condition	References
Protease inhibitors (Trypsin inhibitor)	Soybean	Hot air-spouted bed drying	Drying temp.: 120 °C, 130 °C, 140 °C, and 150 °C Initial moisture content: 26 %, 28 %, and 30 % (dry basis)	Wiriyumpaiwong et al. (2003)
		Hot air-fluidized bed drying/superheated steam-fluidized bed drying	Drying temp.: 120 °C, 135 °C, and 150 °C Initial moisture content: 135, 195, and 360 (g/kg dry basis)	Prachayawarakorn et al. (2006)
Cyanogenic glycosides	Cassava leaves	Combined infrared radiation and fluidized bed drying	Infrared radiation power: 4, 6, and 8 kW	Domdee et al. (2011)
		Sun drying	Drying temp.: 25 °C Relative humidity: 71 %	Ravindran et al. (1987)
		Hot air-drying	Drying temp.: 60 °C Preparation: chopped and unchopped Wilting time: 0, 1, 2, and 3 days	
		Hot air-drying	Drying temp.: 60 °C, 70 °C, 80 °C, and 90 °C for 10 h pretreatment Soaking in water for 2, 3, and 4 days Blanching in boiling water for 3, 6, 9, and 12 min	Udensi and Ukozor (2005)
		Sun drying	Preparation: boiling in distilled water for 5 and 10 min	Musa and Ogbadoyi (2012)
Phytates	Roselle (<i>Hibiscus sabdariffa</i>) Legumes	Hot air-drying	Drying temp.: 60 °C overnight Pretreatment: Soaking in distilled water for 4 h Cooking in an autoclave at 121 °C for 10, 20, 40, 60, and 90 min and 128 °C for 20 min	Rehman and Shah (2005)
		Hot air-drying	Drying temp.: 70 °C, 90 °C, and 100 °C Pretreatment:	Adamidou et al. (2011)

(continued)

Table 2 (continued)

ANF	Type of food	Drying method	Condition	References
	Common bean and chickpea	Hot air-drying Microwave drying	Extrusion at 380 rpm, feed rate of 107–116 g/min, temp. of 90–120 °C and 120–150 °C Hot air-drying at 60 °C, 70 °C, and 80 °C Microwave drying at 350 and 700 W Pretreatment: Soaking in distilled water for 12 h Cooking with hot plate, autoclave, and microwaves	Bilgiçli (2009)
	White sorghum seeds	Hot air-drying	Soaking for 20 h in distilled water at grain to water ratio of 1:5 (w/v) and drying at 45 ± 5 °C	Afiy et al. (2011)
	<i>Moringa oleifera</i> (leaves)	Sun drying Shade drying Hot air-drying	Direct sunlight for 4 days and brought indoor at night Natural current of air for 6 days Drying temp.: 60 °C	Mbah et al. (2012)
Mimosine	<i>Leucaena leucocephala</i> (leaves)	Hot air-drying	Pretreatment: M1 = fresh sample M2 = M1 + drying at 60 °C for 24 h M3 = M1 + autoclaving at 121 °C for 20 min + drying at 60 °C for 24 h M4 = M2 + autoclaving at 121 °C for 20 min + drying at 60 °C for 24 h M5 = M2 + drying at 80 °C for 24 h M6 = M2 + drying at 80 °C for 48 h M7 = M2 + drying at 80 °C for 72 h M8 = M2 + soaking in water 24 h + drying at 60 °C for 48 h M9 = M2 + soaking in water 48 h + drying at 60 °C for 48 h M10 = M2 + soaking in water 72 h + drying at 60 °C for 48 h	Chanchay and Poosaran (2009)
Oxalate	Leaves and petiole of taro	Hot air-drying Sun drying	Exp. 1: drying at 65 °C for 48 h Exp.2:	Hang and Preston (2010)

			<p>Drying at 65 °C for 48 h Boiling in water for 15 min and drying at 65 °C for 24 h Sunlight for 2 days with temp. ~ 34 °C and drying at 65 °C for 24 h Soaking in water for 3 h and drying at 65 °C for 24 h Ensling with 3 % molasses and packed in airtight plastic bags for 21 days and drying at 65 °C for 24 h Drying temp.: 65 °C for 48 h</p>	Hang and Binh (2013)
	Roselle (<i>Hibiscus sabdariffa</i>)	Sun drying	<p>Pretreatment: Boiling in distilled water for 5 and 10 min Drying temp.: 25 °C Relative humidity: 71 % Drying temp.: 60 °C Preparation: Chopped and unchopped Wilting time: 0, 1, 2, and 3 days</p>	Musa and Ogbadoyi (2012) Ravindran et al. (1987)
Tannins	Cassava leaves	Hot air-drying	<p>Freeze-drying Hot air-drying</p>	Ferreira et al. (2004)
	Pigeon pea seeds	Hot air-drying	<p>Pretreatment: Soaking in distilled water for 4 h Cooking in an autoclave at 121 °C for 10, 20, 40, 60, and 90 min and 128 °C for 20 min</p>	Rehman and Shah (2005)
	Legumes	Hot air-drying	<p>Pretreatment: M1 = fresh sample M2 = M1 + drying at 60 °C for 24 h M3 = M1 + autoclaving at 121 °C for 20 min + drying at 60 °C for 24 h</p>	Chanchay and Poosaran (2009)

(continued)

Table 2 (continued)

ANF	Type of food	Drying method	Condition	References
			M4 = M2 + autoclaving at 121 °C for 20 min + drying at 60 °C for 24 h M5 = M2 + drying at 80 °C for 24 h M6 = M2 + drying at 80 °C for 48 h M7 = M2 + drying at 80 °C for 72 h M8 = M2 + soaking in water 24 h + drying at 60 °C for 48 h M9 = M2 + soaking in water 48 h + drying at 60 °C for 48 h M10 = M2 + soaking in water 72 h + drying at 60 °C for 48 h	
	<i>Moringa oleifera</i> (leaves)	Sun drying Shade drying Hot air-drying	Direct sunlight for 4 days and brought indoor at night Natural current of air for 6 days Drying temp.: 60 °C	Mbah et al. (2012)

detected by appearance, taste, or smell. Mycotoxin production depends on weather, moisture, and temperature and may persist long after the actual fungi have disappeared from the products (Altug 2003; D'Mello 2003; Omaye 2004). Some mycotoxins are unstable to cooking conditions or food processing procedures, and drying can help reduce the contents of those mycotoxins.

Aflatoxins

Aflatoxins are probably the most important mycotoxin in foods. Aflatoxins are toxins produced by microfungi, mostly belonging to the species *Aspergillus flavus* and *Aspergillus parasiticus*. The main aflatoxins, namely, B₁, B₂, G₁, and G₂, can be found in foods that have not been sufficiently dried and stored at warmer condition. Aflatoxins are usually found in several food raw materials such as peanuts, cereals, cottonseeds, fig, soybean, almond, barley, cassava, corn, peas, millet, rice, sesame seeds, sorghum, sweet potato, wheat, paprika, and spices. The main concerns about aflatoxins are their acute toxicity and ability to produce cancers, especially, liver cancer. Acute toxicity of aflatoxins can indeed lead to the death of humans within 72 h due to liver damage and hemorrhaging in the intestinal tract and peritoneal cavity (Omaye 2004).

Several studies have indicated that drying can cause degradation of some aflatoxins in foods. Gowda et al. (2007), for example, studied the effect of drying on the reduction of aflatoxins in animal feeds containing crushed yellow maize, wheat bran, peanut meal, mineral mixture, and salt. The tested drying methods and conditions were hot air-drying at 80 °C for 6 h and natural sunlight for 2 days (27–35 °C for 14 h). Hot air-drying helped reduce aflatoxins content by 57.6 %, whereas sunlight drying led to 83.7 % reduction in the aflatoxin content. The extent of aflatoxin reduction depends on the drying method, temperature, duration of drying, and thickness of a sample (or sample layer).

Ochratoxins

Ochratoxins are the toxins produced by the microfungi *Aspergillus ochraceus*, *Penicillium viridicatum*, *Penicillium verrucosum*, and *Penicillium cyclopium*. Ochratoxins can contaminate barley, pork, corn, wheat, oat, rye, green coffee beans, peanut, grape juice, wine, cocoa, dried fruits, and spices. Ochratoxin A has been noted to cause proximal tubular lesion of kidney and hepatic degeneration in test animals. In addition, ochratoxin A can cause the reduction of gluconeogenesis and disturb anion transport, leading to intercellular alkalization (Altug 2003; Pussa 2008).

Kouadio et al. (2012) monitored the effect of sun drying on the ochratoxin A (OTA) content in coffee cherries. Six lot sizes of coffee cherries without OTA were put out for drying on the day of harvest on a 3 m² area of concrete until the moisture content of coffee cherries reached 11–12 %. The quantity of coffee cherries put on the drying area was noted to significantly affect the OTA production. OTA contamination increased with an increase in the quantity of coffee cherries per square meter of the drying area (in the range of 30–60 kg/m²). On the other hand,

the samples from the 10 and 20 kg/m² lots were free of OTA. This is because the larger thickness of coffee cherry layer led to less exposure to air and sunlight. This unfavorable condition subsequently led to fungi contamination and the production of OTA on the coffee cherries.

Fumonisin

Fumonisin are the mycotoxins produced by microfungi, of which *Fusarium moniliforme* and *Fusarium proliferatum* are the most important. Fumonisin B₁, B₂, and B₃ are most well known and usually contaminate corn crops. Fumonisin exhibit a wide range of toxicity effects, including leukoencephalomalacia; hepatic and renal toxicoses in horses, hogs, and rats; as well as porcine pulmonary edema and hepatic cancer in rats. Increased esophageal cancer risk associated with the higher levels of fumonisin in corns has been noted in China, South Africa, Italy, Iran, and the United States (Altug 2003; Pussa 2008; Shibamoto and Bjeldanes 2009).

Yilmaz and Tuncel (2010) investigated the effect of drying on the changing level of fumonisin contamination in corn. Corn kernels were dried using hot air-drying, and the results were compared with those of the kernels subjected to infrared drying and combined infrared-hot air-drying at a temperature of 45 °C. Drying insignificantly affected fumonisin concentration due probably to the use of the low drying temperature. The results are in agreement with those of Alberts et al. (1990) who reported that the changing levels of fumonisin were not observed when drying was conducted at 60 °C. Fumonisin are heat stable, so higher drying temperature is required for fumonisin decontamination.

Trichothecenes

Trichothecenes are the mycotoxins produced by microfungi, of which *Fusarium graminearum*, *Fusarium poae*, *Fusarium tricinctum*, *Fusarium nivale*, *Fusarium solani*, and *Fusarium culmorum* are the most important. These fungi usually infect cereal grains such as wheat, rice, barley, oat, sorghum, rye, corn, sunflower seeds, and certain forage grasses. More than 20 compounds of trichothecenes have been reported to be produced, including T-2 toxin, neosolaniol, diacetylvalenol (DNIV), deoxynivalenol (DON), HT-2 toxin, and fusarenon. Trichothecenes can cause acute inflammation, hemorrhage, and leukopenia. They are involved in a human disease known as alimentary toxic aleukia (ATA), which causes atrophy of bone marrow, agranulocytosis, necrotic angina, sepsis, and even death (Altug 2003; Omaye 2004; Pussa 2008).

Keblys et al. (2000) studied the changes of type B trichothecene as DON after drying of grains. Wheat and barley drying was conducted by keeping the grains in a ventilated oven at 40–50 °C for 4–6 days and then grinding and subjecting the products to air at 70 °C for 24 h to completely dry the products. As high as 96 % of the DON remained in the grains after drying, indicating that only slight decomposition of DON occurred upon drying.

Selected works on drying and mycotoxins in food products are listed in Table 3.

Table 3 Selected studies on drying and some mycotoxins in food products

Compound	Type of food	Drying method	Condition	References
Aflatoxins	Red chili	Double-pass solar drying (DPSD) Cabinet drying (CD) Open-air sun drying	Drying temp.: 53.76 ± 7.6 °C Relative humidity: 23.73 ± 9.5 % Drying temp.: 46.44 ± 6.4 °C Relative humidity: 35.86 ± 10.6 % Drying temp.: 34.00 ± 1.8 °C Relative humidity: 56.33 ± 5.6 %	Banout et al. (2011)
	Cottonseed meal and corn flour	Hot air-drying	Drying temp.: 80 °C for 1 h Preparation: Extrusion with three heating zones (100, 110 °C, and the one adjusted according to the experimental design)	Reyes-Jáquez et al. (2012)
	Peanut meal	Spray drying	Cocurrent drying at airflow rate of $35 \text{ m}^3 \text{ h}^{-1}$ T_{in} : 185 °C T_{out} : 90 °C Nitrogen gas flow rate of 660 L h^{-1}	Oakes et al. (2013)
		Cabinet drying	Drying temp.: 80 °C for 12 h	White et al. (2013)
Ochratoxins	Coffee cherries	Sun drying	Drying time between Dec 1997 and Feb 1998 Drying temp.: 26.4–27 °C Relative humidity: 77–82 %	Bucheli et al. (2000)
	Green coffee	1st step: sun drying 2nd step: mechanical drying	Relative humidity: 25 % for 5 days Drying temp.: max. 55 °C for 2–3 days	Paulino de Moraes and Luchese (2003)
	Coffee cherries	Sun drying	Preparation: Mixing 2 times/day Mixing 4 times/day Mixing 6 times/day Mixing 8 times/day Mixing 10 times/day	Kouadio et al. (2006)
	Coffee	Sun drying	Drying temp.: 17–36 °C for 7–8.5 h Relative humidity: 90 %	Velmourougane et al. (2011)
Fumonisin	Corn kernels	Circulating air-drying	Drying temp.: 70 ± 2 °C	Ono et al. (2002)

Pesticides Contaminated in Food Products and Their Changes During Drying

Pesticides are one of the main chemical inputs that are used to increase the agricultural productivity. Pesticide residues after harvesting need serious concern as they pose hazard to consumer health. Normally, a waiting period prior to harvesting is needed to reduce the residues to a safe level for consumption; if plants are harvested before the completion of the waiting period, consumers are at risk of consuming pesticide residues. Several processes such as washing, cleaning, peeling, blanching, cooking, milling, baking, and pasteurization have been applied after harvesting to further alleviate the problem related to pesticide residues. Drying has also been reported to be capable of reducing the pesticide residue level due to evaporation, degradation, and co-distillation of the residues (Athanasopoulos et al. 2005). The effect of drying on pesticide residues has indeed been reviewed by Kaushik et al. (2009) as well as Bajwa and Sandhu (2014).

Nath et al. (1975) tested the capability of a drying process to remove some pesticides from okra. The employed drying condition was hot air temperature of 68 °C for 8 h. Malathion was applied at 0.05 % and 0.1 %. Ninety two percent of malathion residues could be removed via drying at both dosage levels. Carbaryl was also applied at 0.25 % and 0.5 % and drying was noted to remove 79 % and 77 % of the residues, respectively. Endosulfan was applied at 0.1 % and 0.2 %, and 58 % and 57 % of the residues could be eliminated, respectively.

Cabras et al. (1998a) studied the effect of drying on the decomposition of several pesticide residues in grape during the raisin production process. Different drying methods led to the different effects on the different pesticides. This is because some pesticides decompose due to heat, while others decompose due to co-distillation or the combined action of heat and co-distillation. In the case of sun drying, the residue levels of benalaxyl, metalaxyl, and phosalone in raisin were the same as those in fresh grape, whereas the levels were higher for iprodione and lower for vinclozolin and dimethoate. Oven-drying resulted in about 57 % and 41 % decrease in the iprodione and procymidone contents, respectively, whereas no decrease was observed in all of the other pesticides.

Cabras et al. (1998b) also investigated the changes in the pesticide residues during prune drying processes. The studied pesticides were diazinon, bitertanol, iprodione, phosalone, and procymidone. Drying led to the lower contents of procymidone, iprodione, and bitertanol, but to the same content of phosalone compared with the contents of these compounds in fresh prune. Cabras and Angioni (2000) later investigated the effect of drying on the changing levels of several pesticide residues in grape. The residue levels of benalaxyl, phosalone, metalaxyl, and procymidone in sun-dried grape were the same as those in the fresh grape, whereas the levels were higher for iprodione and lower for vinclozolin and dimethoate. In the case of hot air-drying, the levels of benalaxyl, metalaxyl, and vinclozolin were similar in both the fresh and dried fruits, whereas iprodione and procymidone residues were lower in raisin compared to those in the fresh grape.

Lee (2001) studied the effect of drying on the reduction of chlorpyrifos and fenitrothion that were artificially contaminated red pepper. Sun drying or hot air-drying could eliminate 20–30 % of these pesticide residues.

Conclusion and Future Directions

Nutritional and toxicological changes of foods during drying are complex phenomena that depend not only on drying methods and conditions but also on the unique characteristics of raw materials and the existence of a pretreatment step prior to drying. Although drying in most cases adversely affects the nutrients of dried products, the degradation (or retention) of the nutrients can be improved by appropriate selection of conditions of traditional drying methods or via the use of alternative drying technologies. Drying, on the other hand, generally leads to a desirable toxicological effect on dried food products since drying can help eliminate antinutritional factors, mycotoxins, and pesticide residues. Due to its counteracting effect, the use of appropriate drying techniques at optimum conditions to reduce various toxicological substances while at the same time maintaining the relevant nutrients in dried food products needs to be investigated.

Cross-References

- ▶ [Agricultural Chemical Pollutants](#)
- ▶ [Chemical Composition of Organic Food Products](#)
- ▶ [General Properties of Major Food Components](#)
- ▶ [Model Fungal Systems for Investigating Food Plant Mycotoxins](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Heating and Cooking](#)
- ▶ [Plant-Associated Natural Food Toxins](#)

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Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Freezing

27

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Abstract

Freezing is one of the easiest, quickest, most versatile, trustworthy, and convenient methods of preserving foods. Proper methods of pre-freezing treatment, freezing process, keeping in frozen state, and thawing guarantee minimal quality and nutrient losses and formation of toxic compounds during this cycle. In this chapter principles and methods of freeze/thaw process are discussed in connection with nutritional and toxicological aspects of freezing of foods of both plant (fruits and vegetables) and animal (meat and milk) origin. To retain better the

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texture, flavor, and most of the nutrients, fast freezing of the food raw material is usually recommended. To guarantee the possibly minimal formation of toxic compounds as well as minimize microbial growth, temperatures below $-18\text{ }^{\circ}\text{C}$ ($0\text{ }^{\circ}\text{F}$) are advised for long-term frozen storage of food items. Dairy products are seldom stored in frozen state.

Introduction

Some Principles of Food Freezing

Every preparation process reduces the amount of nutrients in food. In particular, processes that expose foods to higher temperatures, light, and/or oxygen cause the greatest nutrient loss. It is well known that heating (cooking, frying, etc.) destroys foods by changing their textural and chemical structure. Proteins are coagulated and their amino acid monomers are broken down, thus making them unusable as amino acids by the organism. Carbohydrates (starches and sugars) become partially or wholly caramelized, though this is not readily detectable in the earlier stages of cooking. Caramelized sugars are indigestible, hence toxic to the body. Minerals are converted to their unusable and poisonous inorganic state, and vitamins are largely, if not wholly, ruined. Nutrients can also be “washed out” from foods by fluids that are introduced during a cooking process. For example, boiling a potato can cause part of the potato’s B and C vitamins to migrate to the boiling water. Similar losses also occur during broiling, roasting, or frying in oil, if drain off the drippings occurs. Typical maximum losses of vitamins and minerals as compared to raw food occur during cooking (25–70 %, folates being the highest, loss of vitamin C about 50 %) followed by drying (mostly 0–50 %, vitamin C even by 80 %) (USDA 2003). The real losses will depend on many different factors, including type of food and cooking time and temperature. On the other hand, heating at sufficiently high temperatures helps provide safe food due to killing of pathogenic and other adverse bacteria.

The question arises whether the opposite extreme, freezing, likewise alters the chemical and textural structure of foods. The data, published in USDA 2003, show that the maximal nutrient loss during freezing is mostly in the interval of 0–10 %, except again in vitamin C with about 30 % (Table 1).

During freezing the temperature of a food is reduced below its freezing point (temperature), and a part of the water undergoes a change in aggregate state to form ice crystals. This process is partially reversed during the thawing resulting in the formation of exudates; this increase in the concentration of cell contents can create various undesirable products. Freezing point of food is defined as the temperature at which the first ice crystal appears and the liquid is in equilibrium with the solid phase. Freezing point of pure water is $0\text{ }^{\circ}\text{C}$ ($32\text{ }^{\circ}\text{F}$; $273\text{ }^{\circ}\text{K}$). However, when food systems are frozen, the process becomes more complex due to the existence of both free and bound water. Strongly bound water does not freeze even at very low temperatures. Unfrozen water contains soluble substances that cause a decrease in

Table 1 Typical maximum nutrient losses of selected vitamins and minerals (%) as compared to raw food (Data from USDA 2003)

Vitamins or minerals	Freezing	Drying	Cooking	Cooking + draining
Vitamins				
Vitamin A	5	50	25	35
Vitamin C	30	80	50	75
Thiamine	5	30	55	70
Riboflavin	0	10	25	45
Niacin	0	10	40	55
Vitamin B ₆	0	10	50	65
Folate	5	50	70	75
Vitamin B ₁₂	0	0	45	50
Minerals				
Calcium	5	0	20	25
Iron	0	0	35	40
Magnesium	0	0	25	40
Phosphorus	0	0	25	35
Potassium	10	0	30	70
Sodium	0	0	25	55
Zinc	0	0	25	25
Copper	10	0	40	45

the freezing point of water lower than 0 °C (freezing point depression–cryoscopic effect). During the freezing process, the concentration of soluble compounds increases in the unfrozen water, resulting in a continuous variation in freezing temperature. There are empirical equations in literature that help calculate the temperature of certain foods as a function of their moisture content (Guignon et al. 2008).

Freezing point of food, also called cryoscopic point, depends on the concentration of soluble substances in the cellular fluid and on the average is −0.6 °C to 1.2 °C for meat, −0.55 °C for milk, −0.5 °C for eggs, and −0.6 °C to −2 °C for fish. During further cooling, temperatures are usually reduced from −18 °C to −25 °C and sometimes to −50 °C or −60 °C (−76 °F) and even below. In the last case almost all the water in the products gets frozen.

Freezing time is defined as time required to reduce product temperature from its initial temperature to a given temperature at its thermal center. Since the temperature distribution within the product varies during freezing process, the thermal center is generally taken as reference. Thus, when the geometrical center of the product reaches the given final temperature, this ensures the average product temperature has been reduced to a storage value. Freezing time depends on the following most important factors such as dimensions and shape of the product particularly thickness, initial and final temperatures, temperature of refrigerating system, surface heat transfer coefficient of product, change in enthalpy, and thermal conductivity of product (Persson and Löhndal 1993; FAO 2005).

Freezing is one of the oldest and most widely used methods of food preservation, which potentially delivers a high degree of safety, nutritional value, and sensory quality and prevents, or at least minimizes, both microbial and chemical changes. Almost any food can be frozen, although some foods require special treatment before being frozen safely. If defrosted correctly, frozen foods are generally as safe as their original condition. In vegetables, some enzymes remain active at freezing temperature and continue to catalyze reactions leading to plant spoilage unless denatured with mild and fast cooking (blanching). Most types of meat, fish, and fruits do not need to be blanched before freezing, because their enzymes do not affect them in the same way when frozen.

The frozen state of food combines the beneficial effects of low temperatures and reduction of water activity, at which microorganisms cannot grow, chemical reactions are slowed down, and cellular metabolic reactions are delayed (Delgado and Sun 2000). Clarence Birdseye, an American inventor in the early twentieth century, was the first to develop a freezing process that preserved both taste and appearance, in addition to keeping the product safe from spoilage. In 1922, he founded Birdseye Seafoods Company from the observations on how Inuits in the Arctic preserved fish by quick freezing in the environmental conditions and how the fish tasted fresh when thawed and eaten. Birdseye continued to develop freezing technology until 1929, when the first frozen foods became commercially available (Archer 2004). Today, freezing is the only large-scale method that makes movement of large quantities of food over long distances possible (Persson and Löhndal 1993).

Food freezing consists of three main stages. The first stage is cooling the product to its freezing point (pre-cooling or chilling stage) until appearance of the first crystal called nucleus. Respective process of producing this seed is defined as nucleation. Once the first crystal appears in the solution, the second, phase transition change occurs from liquid to solid with further crystal growth. Finally, cooling the product to the storage temperature (tempering stage) will follow. The transition stage that involves the conversion of water to ice through the crystallization process of intra- and extracellular water is the key step in determining the efficiency of the process and the quality of the frozen product. The freezing process results in ice crystals formed from intra- and extracellular water and subsequent crystal growth. If the material frozen is pure water, the freezing temperature will be 0 °C, and up to this temperature, there will be a subcooling until ice formation begins. In the case of foods, during this stage, the temperature decreases to below freezing temperature and, with the formation of the first ice crystal, increases to freezing temperature. The freezing speed directly influences the nucleation and ice crystal size. Decreased growth of the initially formed ice crystals is a result of a high heat removal rate and causes an increased rate of nucleation. The second stage is the freezing period; a phase change occurs, transforming water into ice. For pure water, temperature at this stage is constant; however, it decreases slightly in foods, due to the increasing concentration of solutes in the unfrozen water portion. The third stage starts when the product temperature reaches the point where most freezable water has been converted to ice and ends when the temperature is reduced to the planned storage temperature (Persson and Löhndal 1993).

It is important to control the whole freezing process, including the pre-freezing preparation and post-freezing storage as well as defrosting of the product, in order to achieve high-quality products.

In the freezing of tissue foods, formation of large ice crystals which are mostly extracellular results in significant damages to the cells and tissues. On the other hand, formation of fine crystals that are evenly distributed both inside and outside the cells guarantees better preservation of the quality of the product due to lesser damages to the tissue. Rapid freezing is advantageous for freezing of many foods; however, some products are susceptible to cracking when exposed to extremely low temperature for long periods. Several mechanisms, including volume expansion, contraction and expansion, and building of internal pressure, are proposed in the literature explaining the mechanisms of product damage during freezing (FAO 2005). However, in some processes such as freeze-drying (lyophilization) and freeze concentration, large crystals are more desired. Therefore, the control, understanding, and prediction of the crystallization process and related phenomena in regard to the crystal characteristics are essential for the improvement of freezing processes (Kiani and Sun 2011).

Freezing reduces water activity that together with low temperature in turn inhibits microbial growth and slows down (per)oxidation and other reactions. However, not all water present in the food forms water crystals as a result of the freezing. In food, there exists a fraction of the water called bound water that can be found in strongly bound molecular structures, which does not freeze up to $-30\text{ }^{\circ}\text{C}$. This water forms a monolayer that is attached to polar groups of food components such as amino (NH_2) and carboxyl (COOH) groups of proteins and OH groups of starches, among others. Bound water that is very difficult to extract constitutes between 5 % and 10 % of the total mass of water contained in food.

When a food is frozen, its water expands, since density of ice crystals is lower than density of water (0.92 and 1.00 kg/cm^3 , respectively, at $0\text{ }^{\circ}\text{C}$). This causes two immediately destructive processes:

1. The cell walls burst and the cell contents are spilled; hence, the cell's integrity and life are lost.
2. Oxidation occurs where air reaches the frozen foodstuff; hence, nutrients may be lost or degraded and often toxic compounds are formed.

In addition, two other important events follow:

1. When the cells burst, certain part of their organelles, lysosomes, release self-destructing enzymes called lysozymes. While these enzymes are not active during freezing and some are even denatured, those which remain intact will speedily decompose the cell contents upon thawing. Lysozymes are in the cells for the purpose of self-destructing of dead cells.
2. Oxidation of the burst cells is the foremost cause of food deterioration during frozen storage and thawing. Frozen foods never taste as good as their fresh counterparts, even if no additives and pre-freezing treatments are employed.

Carbohydrates and proteins may undergo limited oxidation. Fats are susceptible to peroxidation or rancidification, whereby polyunsaturated fatty acids are particularly prone to oxidation. Different products, both nonvolatile and volatile, are formed giving rise to the aroma and taste characterized as “rancid.” Vitamins are particularly susceptible to oxidative damage. Vitamin E is damaged during freezing and storage. A survey of frozen meals analyzed after frozen storage reported losses of up to 85 % of thiamine, 55 % of vitamin A, 33 % of vitamin E, and 25 % of niacin and pyridoxine. Storage at temperatures below $-40\text{ }^{\circ}\text{C}$ stops food degradation reactions and prevents development of microorganisms, substantially prolonging shelf life of different foods. Whether oxidized or decayed by its own lysozymes, dead cells become an excellent soil for bacteria and fungi when the temperature becomes favorable again during thawing. While microorganisms such as bacteria are also inert during freezing, they become active just as soon as they are thawed. Hence, frozen foods, once removed from the freezer, decompose much more rapidly than fresh foods do. However, it is notable that freezing does not affect foods of little water content – nuts, seeds, dried legumes, and dried fruits lose nothing by freezing. In nature, seeds and nuts remain fertile no matter how cold it gets. In principle, the more water a food contains, the more the food can be adversely affected by freezing.

Methods of Food Freezing

Freezing of food can be accomplished by either mechanical or cryogenic (flash) freezing. In each case, the freezing process and the results are fundamentally different. The freezing kinetics is important to preserve the food quality and texture. Mechanical freezers were the first to be used in the food industry and are still used in the vast majority of freezing lines. They function by circulating a refrigerant, normally ammonia, around the system, which withdraws heat energy from the food product. This heat is then transferred to a condenser and dissipated into air or water. The refrigerant itself, now at high pressure, hot liquid, is directed into an evaporator. As it passes through an expansion valve, it is cooled and then vaporizes into a gaseous state.

In the case of cryogenic (flash) freezing, the objects are quickly frozen by subjecting them well below water’s melting/freezing point to cryogenic temperatures either by direct contact with dry (carbon dioxide) ice and ethanol at $-78.5\text{ }^{\circ}\text{C}$ or even in liquid nitrogen at $-196\text{ }^{\circ}\text{C}$. Flash freezing is also used in the food industry to quickly freeze perishable food items. During cryogenic freezing, the ice crystals remain small, avoiding damage to the cell walls. As a result, the defrosted product is fresh and appetizing.

In theory, either freezing method can be applied to any type of food. In practice, however, the food processor must carefully balance the capital cost, operating cost, regulatory compliance cost, and product quality to determine the best method for the company and the consumer (Hung 2001).

The efficiency of a freezing process is defined via the mean freezing rate (given in cm/h) by the physically correct formula which is $w = d_0/z_0$, where d_0 corresponds to the shortest distance from the core of the product to its surface and z_0 is the time it takes the product core to cool from 0 °C to -10 °C. Freezers can be classified on the basis of this definition: a domestic freezer, for example, has a mean freezing rate of 0.1 cm/h, which is relatively slow freezing. Freezer chambers offer 0.1–0.5 cm/h. Freezing is more rapid in a cold-air fluidized bed (0.5–5 cm/h). Rates of 5 cm/h and above can be achieved with cryogenic freezing methods. In order to freeze at rates of 5 cm/h or above, however, more than just a high temperature gradient is required. The nitrogen must boil directly on the surface of the food or sublimate the CO₂ snow. Ideally, a food 5 cm (2-in.) thick should freeze completely in about 2 h.

The freezing rate in °C/h for a product or package can be defined as the ratio of difference between initial and final temperature of product to freezing time. At a particular location within the product, a local freezing rate can be defined as the ratio of the difference between the initial temperature and desired temperature to the time elapsed in reaching the given final temperature (Persson and Löhdal 1993).

How quickly the food freezes also depends on the amount of solutes (soluble salts, proteins, and carbohydrates) which affect the temperature at which ice crystals are formed. The higher the level of solutes, the lower the temperature must be for the food to freeze. Comparison of different methods of food freezing is published by Estrada-Flores (2002).

For more deep acquaintance with various technological aspects of food freezing including packaging, a recently published textbook edited by Sun (2012) can be recommended.

Effect of Freezing on Quality of Frozen Products

The quality of frozen products and consumer acceptance can be enhanced by optimizing process conditions such as rate of freezing, quality of raw materials, and storage conditions details. However, important factors can be grouped in a less confusing way based on conclusions drawn from the aspects related to quality deterioration. These groups are sensory quality, including the physical and chemical aspects of quality deterioration, microbiological quality, and nutritional quality of frozen products.

Sensory Quality

The main components of the overall sensation of flavor are taste and aroma. The receptors on the tongue are responsible of perceiving flavors, while aroma generally contributes to total flavor. The analysis used to determine the effects of freezing process, frozen storage, and thawing on product flavor is largely based on the changes produced in chemical compounds. Sensory quality of frozen products is commonly determined based on texture, which includes both the properties

perceived by sensation in the mouth and appearance. Therefore, a good understanding of the physical aspects of freezing will help improve the product's quality retention during the freezing process.

Texture

Most fruits and vegetables are over 90 % water of total weight. The water and dissolved solutes inside the rigid plant cell walls give support to the plant structure and texture to the fruit or vegetable tissue. In the process of freezing, when water in the cells freezes, an expansion occurs and ice crystals cause the cell walls to rupture. Consequently, the texture of the produce is generally much softer after thawing when compared to nonfrozen produce. This textural difference is especially noticeable in products normally consumed raw, as in the case of fruits. It is usually recommended that frozen fruits be served before they are completely thawed, since in the partially thawed state, the effect of freezing on the fruit tissue is less noticeable. On the other hand, due to the fact that cooking also softens cell walls, textural changes caused by freezing are not significantly noticeable in products cooked before eating, as in the case of most vegetables.

High-pressure freezing promotes uniform and rapid ice nucleation and growth through the whole sample. Dehydro-freezing has been successfully used in freezing of vegetables and fruits with the advantage of less damage to plant texture because of partial water removal before freezing. Studies have been carried out for the biotechnological use of antifreeze and ice nucleation proteins because of their uniqueness in directly improving freezing processes. Thawing under pressure can be achieved at lower temperature than that at atmospheric pressures (Li and Sun 2002).

Freezer Burn

One of the most common forms of quality degradation due to moisture migration in frozen foods is freezer burn, a condition defined as the glassy appearance in some frozen products produced by ice crystals evaporating on the surface area of a product. The grainy, brownish spots occurring on the product cause the tissue to become dry and tough and to develop off-flavors. This quality defect can be prevented by using heavyweight, moisture-proof packaging during the freezing process.

Nutritional Quality

Nutrients are substances found in food: carbohydrates, fats/oils, proteins, vitamins, and minerals. Dietary fiber and some phytochemicals of plant foods, although not nutrients as such, are needed for good health. What food does in the body does through the nutrients it contains. The body utilizes these nutrients to grow and keep healthy and strong. All nutrients needed by the body are available through food. No food by itself (except for breast milk which is adequate for babies up to 6 months of age) has all the nutrients needed for full growth and health. Food therefore must be balanced. A variety of foods is therefore needed to get all the nutrients the body needs. Specific nutrients do their best work in the body when present with other nutrients. Nutrients are therefore mutually supportive. Minimizing nutritional losses should be a goal of processing that is strived for

whenever possible. For any type of food preservation method, the retention of nutritional components is a concern, but freezing is probably the least destructive when properly done.

Microbiological Quality

Generally, total numbers of various microorganisms such as bacteria, fungi, and protozoa as well as viruses associated with raw food decrease slightly during freezing, but bacterial spores are practically unaffected by freezing. Most vegetative gram-positive bacteria are more resistant; gram-negative bacteria show higher sensitivity to freezing. Still, it is extremely rare for a foodborne illness to be traced back to a frozen food. Freezing preserves food by either stopping microbes from multiplying or halting the food's own enzyme activity that would otherwise cause the food to rot. Most pathogens do not multiply at freezer temperature and many of them perish because their enzymes do not work properly to maintain normal cell activity. Also, pathogens need water to grow, and freezing turns the available water into solid ice crystals.

However, it should be borne in mind that freezing does not kill the pathogens and parasites and make food sterile. The best way to kill microorganisms is still to cook food thoroughly. Although pathogens do not multiply in the freezer, certain species can survive the freezing process by going into “hibernation” or forming resistant cells (e.g., spores) and up to 70 % may come to life again as the food begins to thaw. For example, *Salmonella* have been known to survive for 7 years at -23°C in ice cream, and *Campylobacter* can survive freezing if the initial contamination levels are high. Also, freezing does not affect toxins left by certain bacteria (e.g., staphylococcal enterotoxin and botulinum toxins).

Slow freezing (lowering the temperature by $1^{\circ}\text{C}/\text{min}$) kills some pathogens as ice formation draws up the usable water which makes the solutes more concentrated. Microbes cannot control their water loss, and they dehydrate by osmosis. Large ice crystals and shards damage the cell walls of both the pathogens and the food. Fast freezing that is recommended for preservation of food cell membranes or walls causes less damage to the structure of the food, but on the other hand also kills fewer pathogens.

Approximately 75 % of ice in frozen food forms at temperatures of 0.5°C and -5°C , and during fast freezing, this temperature range is quickly surpassed. Unfortunately, this also means there is less osmotic pressure on pathogens so they more tend to survive. The freezer must be operating effectively at -18°C or below since in various meats frozen at slightly warmer temperatures than this, “psychrotrophic” molds (black spot, white spot, or feathery molds) can grow, causing the food to spoil (Foodsmart 2007).

Cryoprotection

Cryoprotection has been a matter of concern for the food industry in its search for efficient ways of improving the quality of frozen-thawed products. Sometimes,

special additives are used to protect food macromolecules against denaturation. For example, the cryoprotective effects of maltose ($w = 0\text{--}10\%$) on washed chicken meat (WCM) were investigated. WCM produced from broiler meat was frozen and stored for 360 days on $-30\text{ }^{\circ}\text{C}$. Myofibrillar protein functional stability was monitored by salt extractable protein (SEP) and differential scanning calorimetry (DSC). SEP showed that the addition of maltose caused smaller decrease of protein solubility during frozen storage. The peak transition temperatures (T_p) and denaturation enthalpies (ΔH) of myosin and actin of WCM samples increase during frozen storage with the increase of mass fraction of maltose. Since the value of denaturation enthalpy is directly related to amount of native proteins, higher values of ΔH indicate to the higher cryoprotective effects of maltose (Kovachevich and Mastanjevich 2012).

Spinach samples were treated with a combination of pulsed electric field (PEF) and vacuum impregnation (VI) prior to flash freezing in liquid nitrogen and thawing at room temperature. VI was used to impregnate trehalose (40 % w/w solution) as cryoprotectant, and PEF was used to distribute the cryoprotectant in the extra- and intracellular spaces of the tissue. Strawberries were treated with VI prior to freezing in liquid nitrogen and thawing at room temperature. VI was used to impregnate a combination of trehalose (12 % w/w) and cold acclimated winter wheat extract (AWWE, 0.02 % w/w) containing antifreeze protein. Results were evaluated by assessing the maintenance of the texture of the tissue as well as by cell viability analysis using fluorescence microscopy. Results showed that impregnating fruit and vegetable tissues using VI alone or in combination with PEF with the tested cryoprotectants improves drastically the cryoprotection of the treated tissues. Cryoprotection was proved through the maintenance of cell viability and texture after one freezing/thawing cycle (Goméz Galindo et al. 2011).

Innovation on freezing of fruit and vegetables, aiming at preserving cell vitality after freezing and thawing cycles, should be based on knowledge of the natural cryoprotection mechanisms at the cellular level. Among these mechanisms, certain plant tissues accumulate osmotically active substances in the cytoplasm as well as antifreeze proteins (AFPs). The protective role of these substances such as some amino acids (e.g., proline), quaternary ammonium compounds (e.g., betaine), numerous sugars (e.g., sucrose and trehalose), and several other substances has been widely studied. There is a wealth of information on their stabilizing effects on biological molecules, cells, and organisms, which indicates their functional role in the stress tolerance of many, although not all, organisms. Apart from decreasing the chemical potential of water and the freezing point in the cytosol, the hydrophilic nature of sugars is well suited for stabilizing the cell membrane through hydrogen bonding between the hydroxyl groups on the sugar and the polar residues in phospholipids, preventing dehydration effects in membranes. At high AFP concentrations (μM), minimal crystal growth occurs, forming very small, stable hexagonal bipyramids. AFPs are also strong inhibitors of recrystallization (Goméz Galindo et al. 2011).

Beneficial and Detrimental Nutritional Aspects of Freezing

Foods of Plant Origin

Freezing is recognized worldwide as one of the best methods available in the food industry for preserving food products of high quality, such as vegetables. The decrease of temperature inhibits metabolic processes occurring in vegetables after harvesting as well as slows down the rate of microbiological growth that may compromise the material quality. In addition, the short heat treatment that precedes freezing (blanching) enhances color and texture of vegetables extending their shelf life (Paciulli et al. 2015).

Fruits and vegetables are highly perishable foods subject to rapid deterioration by microorganisms, enzymes, or oxidation reactions. The use of freezing technologies allows the retention of freshness of fruits and vegetables for long periods, extending their availability well beyond the normal season of most horticultural crops. The rate of freezing for plant tissues is extremely important due to the effect of freezing rate on the size of ice crystals, cell hydration, and damage to cell walls (FAO 2005). It is advised that most frozen fruits will maintain a high quality for 8–12 months, while unsweetened fruits will lose quality faster than those packed in sugar or sugar syrups. Most vegetables will maintain a high quality for 12–18 months at -18°C or lower (FAO 2005).

Fruits and vegetables are important sources of vitamin C, folate, and minerals; colored fruits and vegetables are also a source of antioxidant polyphenols and carotenoids. The freezing process itself has no effect on nutrients, but during blanching (prior to freezing) water-soluble nutrients may be leached out during the process.

However, in the case of fruits, physical changes such as ice recrystallization during storage at subzero temperatures and thawing may result in textural changes leading to deleterious textural changes (softening) reversing the advantage of fast freezing. Moreover, the fruit structure can collapse during thawing resulting in loss of the cells' water-holding capacity and leading to drip loss. Use of cryoprotectors can help to reduce this kind of deterioration of fruits during freeze/thaw process (Gómez Galindo et al. 2011).

Retention of Nutrients During Pre-freezing Treatments

Attempts have been made to improve the resistance of fruit and vegetables to freezing damage such as reducing the freezable water content by osmotic dehydration prior to freezing, reinforcing cell–cell adhesion by calcium chloride application with or without low methoxyl pectin, or improving the textural quality and drip loss of frozen-thawed fruits by application of high fructose corn syrup and high methoxyl pectin. Vacuum infusion is one of the preferred techniques for infusing the fruits with external solutions, filling the porous fractions of the tissue, and therefore, the infused solution is present in the apoplast. However, these methods cannot avoid the freeze-induced damage to cell membranes, turgor loss, and the

consequent loss of cell vitality, all deteriorating the fresh-like characteristics of the product (Goméz Galindo et al. 2011).

Chemical changes that can cause spoilage and deterioration of fresh fruits and vegetables will start directly after harvesting. In most cases, blanching just before freezing is essential for producing quality frozen vegetables (Rickman et al. 2007a, b). In vegetables, some enzymes remain active even at freezing temperature and continue to spoil them unless stopped with mild and quick cooking (blanching). Blanching also helps destroy microorganisms on the surface of the produce. Development of rancid oxidative flavors through contact of the frozen product with air (oxidation) is another group of chemical changes that can occur in frozen products. This problem can be controlled by excluding oxygen through proper packaging. It is also advisable to remove as much air as possible from the freezer bag or container to reduce the amount of air in contact with the product.

Most types of meat, fish, and fruits do not need to be blanched before freezing because their enzymes do not affect them in the same way when frozen. Fruits, opposite to vegetables, are usually not blanched prior to freezing owing to their delicate nature that favors undesirable texture changes and inherent acidity (Rickman et al. 2007a). Enzymes in frozen fruits can be controlled by using chemical compounds, which interfere with deteriorative chemical reactions. Ascorbic acid is an example that may be used either in its pure form or in commercial mixtures with sugars for inhibition of enzymes in fruits. However, fruits may sometimes be blanched under mild (low temperature) conditions prior to freezing (Reyes de Corcuera et al. 2004).

Blanching is a cooking process prior to freezing, canning, or drying in which mostly vegetables are heated for the purpose of inactivating enzymes; modifying texture; preserving color, flavor, and nutritional value; and removing trapped air, for example, in broccoli florets, and gases within vegetable cells and replaces them with water, forming a semi-continuous water phase that favors a more uniform crystals growth during freezing. Gas removal is the main benefit of blanching before canning because it allows easier can fill, reduces strain on can during heating, improves thermal conductivity, and reduces can corrosion. Although, in this case, enzyme inactivation also takes place, it is not relevant because any remaining activity is destroyed on retorting (Reyes de Corcuera et al. 2004).

Hot water and steam are the most commonly used heating media for blanching in industry, but microwave and hot gas blanching have also been studied. Mostly, the food substance is plunged into hot water at temperatures between 75 °C and 95 °C, removed after a brief interval (usually 1–10 min depending on vegetable), and immediately plunged into iced water or placed under cold running water (shocking or refreshing) that often serves to transport them to the next part of the process and to stop the cooking process. Blowing cold dry air has also been used.

The meaning of *blanch* is “to whiten,” but this is not always the purpose of blanching in cooking. Food is blanched also to soften it, or to partly or fully cook it, or to remove a strong taste (e.g., of cabbage or onions), or to prevent development of off-flavors and off-colors during frozen storage. However, sometimes in case of

onions, leeks, and peppers, blanching is omitted to keep the intrinsic flavor and color of these vegetables. Blanching stops the food's enzyme action and destroys microorganisms present on the vegetables surface substantially reducing the risk of food poisoning. Processing conditions are usually set up to inactivate enzymes, but other quality parameters, such as color and texture, are commonly monitored. Blanching facilitates peeling and dicing and is also accompanied by microbial load reduction.

Blanching indirectly and directly affects the off-flavor of polyunsaturated fatty acid toxic oxidation end products (different aldehydes and ketones) by inactivation of enzymes such as lipoxygenase (LOX) responsible for off-flavor development through oxidation of polyunsaturated fatty acids (PUFA). Sometimes, blanching increases flavor retention, and sometimes, it removes undesirable bitter flavors from the product.

Blanching can result in undesirable softening of vegetable tissues. However, calcium can be added to reduce this undesirable process. Texture assessment of the effects of blanching includes sensory characterization of firmness, crispness, and crunchiness and instrumental measurements such as cutting energy and maximum shear force.

Blanching can have both direct and indirect effects on color. The former is exemplified by the destruction of pigments, such as chlorophyll, by heat. A good example of an indirect effect is in potato processing, in which the reducing sugar content can be adjusted via water blanching, affecting color development during later more intensive heating steps where the Maillard reaction takes place.

Blanching process together with washing and peeling can often be associated with substantial nutrient loss, particularly of water-soluble substances. Nutrients leach out from the product, especially during water blanching. In addition, vitamins are degraded by heat. About 25 % of the vitamin C (ascorbic acid isomers) and a greater percentage of folate are lost during the blanching process that occurs before foods are frozen probably because of their high solubility and heat susceptibility. About 10 % of thiamine (vitamin B₁) is also lost during blanching. Vitamin C is the most common conservative indicator, but also vitamins B₁ and B₂, carotenes, and dietary fibers have been assayed as indicators of nutrient retention (Reyes de Corcuera et al. 2004).

It is important to remember that these percentages of nutrient loss are very general and can be different with different foods. Nevertheless, as one can see from the numbers above, the freezing-connected loss of these water-soluble vitamins never comes close to outweighing the amount of vitamins retained.

Blanching may decrease the amount of carbohydrate, fat, and water-soluble protein in certain vegetables. Early investigation in the 1970s and 1980s found these nutrients decreased in several vegetables such as one 1976 study that found that blanching decreased the albumin (a kind of protein) and amino acids (the building blocks of protein) in green peas. A study on the effect of blanching and freezing on the nutrition of over 20 vegetables found that blanching had either no effect on the dietary fiber content of vegetables or, in some cases, slightly increased available amounts (Rickman et al. 2007b).

It has been shown by Rickman et al. (2007b) that the mineral and dietary fiber content of vegetables tend to be more resilient against loss from processing than vitamins. Approximately 78–91 % of minerals are retained after blanching. When performing blanching in hard water, the uptake of calcium, potassium, and sodium from the water far exceeds the potential mineral loss from the processing. According to Puupponen-Pimiä et al. (2003), contents of dietary fiber components were not affected or increased slightly; minerals in general were also stable, except some losses of soluble minerals by leaching.

Blanching and freezing of peas decreases content of vitamin C and riboflavin as well as potassium, but not other nutrients, such as thiamine, carotene, dietary fiber, calcium, magnesium, sodium, iron, and zinc (Weaver et al. 2014).

Rickman and coauthors (Rickman et al. 2007a) have shown that blanching prevents the degradation of phenolic antioxidants from oxidation during storage and increases bioavailability of those antioxidants. However, an extensive study of the effects of blanching/freezing and long-time freezer storage of various bioactive compounds of more than 20 vegetables showed strong plant dependence of these effects (Puupponen-Pimiä et al. 2003) and significant losses (20–30 %) of both antioxidant activity and total phenolics in many vegetables. The same study also found that blanching produced no degradation of plant sterols and carotenoids, but significant loss of vitamin C (up to one-third) and especially of folic acid was observed.

Retention of Nutrients During Freezing and Frozen Storage

When fresh fruit goes into a home freezer or travels through an industrial freezing process, ripening and decay processes slow dramatically. Because regional delicacies are no longer confined to a narrow growing season or a small area, fruits become more widely available. Shipping flash-frozen fruits is also easier and less expensive for growers and processing companies, putting fruit within reach of a wider economic range.

Many frozen fruits retain most of their fresh flavor, but some varieties inevitably lose something already in their trip from the field to the freezer. Fruits with high water content such as watermelon and oranges can become mealy or pulpy when defrosted. As the water within them freezes, the ice crystals punch through cell walls and turn fruit soft. Some fruits also lose their color when frozen, darkening or browning even though they remain safe and pleasant to eat.

It has been shown that vitamins are rather stable during freezing and frozen storage of different fruits. For example, in frozen strawberries the total and biologically active ascorbic acid remained the same for a year or longer if the food was stored below -18°C . However, conversion of ascorbic acid to dehydroascorbic acid (partially active) and 2,3-diketogulonic acid (totally inactive) increases with increasing storage temperatures, and nearly complete conversion occurs in 8 months at -10°C and less than 2 months at -2°C . These findings were decisive to establish -18°C as the upper limit for frozen food storage and for the use of biologically active ascorbic acid as an indicator of deterioration in storage (Kyureghian et al. 2010).

Retention of vitamin C in homogenized raw fruits and vegetables stored under routine conditions was investigated. Raw collard greens (*Brassica oleracea* var. *viridis*), clementines (*Citrus clementina* hort. ex Tanaka), and potatoes (*Solanum tuberosum*) were studied having different expected stability of ascorbic acid. Samples were homogenized in liquid nitrogen, assayed immediately, then stored at -60°C , and analyzed at time points up to 49 weeks. Vitamin C concentrations were stable in clementines and in the orange juice but decreased in collards and potatoes (14.7 % and 30.4 %), respectively, after 49 weeks. Significant losses had occurred already after 12 weeks (Phillips et al. 2010).

Freezing fruits as well as vegetables also does not cause any substantial loss of vitamin A and β -carotene. The B-group vitamins also remain unaffected. According to Shofian et al. (2011), freezing and even more drastic freeze-drying processes have little effect on some antioxidants in fresh fruits but could markedly affect others. It was established that the ascorbic acid content of freeze-dried tropical fruits and melons was not affected greatly, but beta-carotene levels decreased in some samples.

The B-group vitamins, including riboflavin, thiamine, niacin, and folate, readily dissolve in water. Ascorbic acid is also water soluble. When foods rich in these vitamins are boiled or soaked, some of the foods' nutritional content ends up in the water. In case of flash freezing of fruits, water-soluble vitamins like B vitamins and vitamin C are retained.

Many of the minerals as well as phytonutrients like polyphenols keep fairly well in frozen foods. For example, excellent antioxidants anthocyanin flavonoids can be well preserved during freezing of fruits. Even though they are rather delicate, there was no significant reduction found in the levels of anthocyanins in blueberries expressed as cyanidin 3-rutinoside equivalents after 3 months of freezing. At the same time, fruit drying resulted in reduction of the total anthocyanin level by 41 %. However, when drying was preceded with osmotic dehydration, 49 % of anthocyanins, the most labile group of plant polyphenols, were lost (Lohachoompol et al. 2004).

Carbohydrates and proteins may undergo limited oxidation during frozen storage. Fats are susceptible to rancidification, and polyunsaturated fatty acids are particularly prone to oxidation. Different products, both nonvolatile and volatile, are formed that give rise to the aroma and taste characterized as "rancid."

Contents of nitrates, nitrites, and oxalates in the leafy part of dill and in whole plants (leaves with petioles and stems) harvested at the 25 cm stage of growth were studied by Kmiecik et al. (2004). In relation to whole plants (leaves, petioles, and stems), the leaves alone of fresh dill were characterized by a much lower content of nitrates (54 %), a higher content (though below 1 mg/1000 g) of nitrites, and also a higher content of oxalates (26 %). The blanching induced considerable reductions of the contents of nitrates, nitrites, and oxalates. This concerned whole plants to a greater degree than leaves, oxalates being an exception. Freezing and keeping of both blanched and non-blanched samples at -20°C and -30°C during up to 12 months did not affect the level of the analyzed compounds in frozen products, with the exception of non-blanched whole plants in which the level of nitrites

significantly rose in relation to the raw material. Irrespectively of the applied temperatures during refrigerated storage, the level of analyzed compounds was slightly changed in relation to that recorded in the material directly after freezing (Kmiecik et al. 2004).

In a study by Prasad and Chetty (2008), the effects of freezing at -20°C on the nitrate-N content of the four selected vegetables were studied over a period of 7 days. When foods are stored for a short period of time under deep freezing, microbial action would not be expected to proceed. The nitrate-N content only slightly fluctuated from the original, i.e., fresh nitrate-N values over the 7-day period. In terms of nitrate-N content, a declining trend was evident although not anticipated in all the samples of four vegetables. The loss of nitrate-N over the 7-day period in the vegetables studied was 2.0 % for Chinese cabbage, 8.3 % for celery, 1.4 % for lettuce, and 10.9 % for English cabbage. The minor loss of nitrate-N content may be attributed to a microbial action taking place during the period when the samples are removed from freezing and thawed.

Meat and Meat Products

Retention of Nutrients During Pre-freezing Treatments

Different pre-freezing treatments can be used for meat products that generally result in a change in storage time. Heating prior to freezing can result in a 50 % longer practical storage life (PSL) for sausages. However, the heating process could be critical since muscles cooked to higher temperatures are most susceptible to oxidative and other damage during storage and thawing. Heat treatments such as frying tend to produce short storage lives. Breaded products are often fried, and although breading alone may have a protective effect on a product, the addition of oil may have an opposite effect.

Mincing has been found to significantly reduce PSL of the product, probably due to the induced heating and the increased surface area that results. Addition of fat to mince can further lower storage life unless a high-grade wrapping material, which has the ability to exclude oxygen, is used to wrap the product. Smoking is generally advantageous owing to the antioxidant properties of the smoke. Smoked broilers and ham store well for over a year without remarkable quality and nutrients loss. However, smoking may provide meat with carcinogenic products of incomplete burning of wood such as polyaromatic hydrocarbons (PAH).

Additives, such as spices, seasonings, antioxidants, and protein concentrates, can substantially prolong PSL. The use of various vegetable and fruit extracts has been shown to help control rancidity in different meat products. However, an addition of sodium chloride may reduce the storage life because of increased rancidity. Mechanically deboned (recovered) meat used in a range of comminuted meat products can cause accelerated rancidity owing to its higher fat content, higher surface area, and iron content (James and James 2002).

Retention of Nutrients During Freezing and Frozen Storage

As global trade increases and the distance between producer and consumer expands, the need to freeze meat for transportation increases. Beef, lamb/mutton, and chicken are the meat products that are produced worldwide in the greatest quantities, and hence, the majority of research to date in the meat science discipline has focused on these species.

Freezing is one of the most important preservation methods for meat and meat products since compared with other methods, it leads to a minimal loss of quality during long-term storage. Frozen meat products may be prepared from raw or cooked meat. Products are quick frozen at $-20\text{ }^{\circ}\text{C}$ to $-40\text{ }^{\circ}\text{C}$ by blast freezing (a variant of flash freezing) and vacuum packaged to prevent development of rancidity. Still, freezing and frozen storage can cause chemical and structural changes in meat such as water-holding capacity, color, and texture, depending on species and storage conditions (temperature, duration, temperature fluctuations, etc.).

Frozen storage is the most common long-term preservation technology in muscle foods because it inhibits microbiological spoilage and retards undesirable autolytic reactions. However, there is still some cell disruption and destruction of muscle fiber due to the formation of ice crystals. Therefore, during frozen storage at relatively high temperatures, many reactions can still occur between different meat components. Ice crystal size and distribution in the intra- or extracellular spaces of frozen meat vary with freezing rate, while the total amount of ice formed depends on the temperature achieved during freezing. Generally, total numbers of microorganisms decrease slightly during freezing. However, bacterial spores are practically unaffected by freezing, and oxidative reactions remain active during frozen storage of muscle foods. The oxidation of macromolecules leads to irreversible chemical changes and impaired quality traits in meat and meat products (Utrera and Estévez 2013).

Currently, lipid and protein oxidation is one of the biggest economic problems in the meat industry. It compromises the nutritional quality, limits shelf life, increases toxicity, and decreases the market value of meat and meat products. For example, fish and poultry meats are susceptible to oxidative reactions due to their high concentrations of oxidation catalysts such as myoglobin and iron. However, the rate and extent of oxidation can be retarded, reduced, or prevented through the application of natural antioxidants (Falowo et al. 2014). Oxidative reactions in meat are the most important factor in quality losses, including flavor, texture, nutritive value, and color. For example, breakdown of phospholipids by lipases has been observed in frozen meat. These reactions occur at various freezing temperatures and storage periods. Lipid oxidation is induced by oxyradical and/or lipid-free radical generation and results in the generation of several toxic compounds that will be considered in the section on lipid oxidation. Hence, freezing rate and frozen storage temperature substantially influence both the structure and sensory qualities of frozen meat (Soyer et al. 2010).

A temperature of $-55\text{ }^{\circ}\text{C}$ ($-67\text{ }^{\circ}\text{F}$) has been suggested as ideal storage temperature for meat to completely prevent quality changes including loss of nutrients (Hansen et al. 2004). At these low temperatures, enzymatic reactions, oxidative rancidity, and ice recrystallization are likely to be minimal, and thus, only few deteriorative changes will occur during long-term storage (Zhou et al. 2010).

Cryogenic freezing offers shorter freezing times compared with conventional air freezing because of the large temperature differences between the cryogen and the meat product and the high rate of surface heat transfer resulting from the boiling of the cryogen. However, there may be some distortion of the shape of the product caused by the cryogenic process that might impact on the commercial application. Furthermore, the cost of cryogenic liquid is relatively high and therefore may limit its commercial application (Zhou et al. 2010).

Lipids and proteins may undergo limited oxidation during frozen storage. Fats are susceptible to rancidification, and polyunsaturated fatty acids are particularly prone to oxidation. Different products, both nonvolatile and volatile, are formed giving rise to the aroma and taste characterized as “rancid.”

Lipid oxidation can be carried over to proteins; direct reaction of proteins with reactive oxygen species (ROS) is also possible. Oxidation causes changes in physical and chemical properties of proteins, such as structure, conformation, solubility, resistance to proteolysis, and enzymatic activity. These changes may alter the quality and processing properties of fresh meat. Both side chains of amino acids and amino acidic “backbone” of a protein are oxidizable. Both cross-links and molecular fragments are formed. Sulfur-containing cysteine and methionine are the most sensitive. In the side chain oxidation of thiol group, hydroxylation of aromatic groups and formation of carbonylic group can occur. In the course of oxidation of backbone, the secondary and tertiary structure of the protein molecule can be substantially changed and backbone fragmented. Intramolecular S-S, dityrosine, and other bridges are formed; the protein can aggregate or polymerize. As a result, protein digestibility and amino acid availability, i.e., nutritional value of meat, are reduced.

Protein carbonylation during oxidation of meat is reviewed by Estévez (2011). Recent studies have demonstrated that meat proteins undergo carbonylation during frozen storage of pork, beef, poultry, turkey, and rhea meat. Mostly, this process is directly linked to oxidation of lipids and affected by the type of muscle (Estévez et al. 2011; Soyer et al. 2010), freezing temperature (Soyer et al. 2010), the packaging conditions, and previous operation such as pre-mincing (Estévez et al. 2011). In addition to these external factors, the susceptibility of meat to suffer protein oxidation is closely related to endogenous factors such as the composition of proteins and lipids, iron content, metabolism of the muscle, and antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase–reductase system (Estévez et al. 2011). The knowledge on the effect of the aforementioned endogenous factors on the occurrence, extent, and consequences of protein oxidation during frozen storage of meat is still limited. Owing to the high content of iron, the main promoter of protein carbonylation in meat systems (Estévez et al. 2011), oxidative muscles may have a lower oxidative stability than

glycolytic muscles. In this regard, a previous study devoted to porcine meat subjected to frozen storage linked the high susceptibility to carbonylation of oxidative muscles with their large iron content. However, there are other factors depending on the metabolic profile of muscles that may be influential such as the lipid content and characteristics, the content of reducing sugars, and the presence of antioxidant enzymes. Additionally, further understanding of the consequences of protein oxidation during frozen storage of meat would clarify the actual technological relevance of such reactions and would urge meat scientists to develop antioxidant strategies to control the potential negative impact of oxidative protein damage on muscle food quality (Utrera et al. 2014b).

The effect of three frozen storage temperatures ($-8\text{ }^{\circ}\text{C}$, $-18\text{ }^{\circ}\text{C}$, and $-80\text{ }^{\circ}\text{C}$) on protein oxidation in beef patties was studied using novel oxidation markers. Additionally, the connection between lipid and protein oxidation and the impact of the latter on particular quality traits (water-holding capacity, color, and texture) of subsequently processed beef patties (cooking/cold stored) were investigated. Protein oxidation was measured as the loss of tryptophan fluorescence and formation of diverse lysine oxidation products such as α -aminoadipic semialdehyde, α -aminoadipic acid, and Schiff bases. A significant effect of storage temperature on protein oxidation was detected. Frozen storage increased the susceptibility of meat proteins to undergo further oxidation during processing. Timely interactions were found between lipid and protein oxidation. Plausible mechanisms by which oxidative damage to proteins may have an impact in particular quality traits are thoroughly discussed in Utrera et al. (2014a).

Soyer et al. (2010) have studied the impact of freezing at $-7\text{ }^{\circ}\text{C}$, $-12\text{ }^{\circ}\text{C}$, and $-18\text{ }^{\circ}\text{C}$ and frozen storage at $-18\text{ }^{\circ}\text{C}$ for a period of 6 months on lipid and protein oxidations in chicken leg and breast meats. Results confirm a number of previous studies that protein oxidation, measured as carbonyl content and sulfhydryl content, is linked to lipid oxidation, as previously reported for fish and turkey meat. The authors reported significant increases of the total amount of protein carbonyls during 6 months of frozen storage at $-18\text{ }^{\circ}\text{C}$ (from 1.8 to 2.9 nmol/mg protein). It can be concluded that the frozen storage has strong effects on the susceptibility of chicken muscle lipid and protein to oxidative reactions due to high content of polyunsaturated fatty acid. Results of this study clearly showed that the mechanisms and reaction pathways for the oxidation of lipids and proteins were directly linked to prooxidative and antioxidative factors in chicken leg and breast meat. Moreover, chicken leg meat frozen at $-7\text{ }^{\circ}\text{C}$ suffered more oxidative damage to proteins than breast meat frozen at the same temperature. Therefore, decreasing the freezing temperature would reduce the oxidation of proteins in leg meat during frozen storage. On the other hand, the freezing and storage temperatures tested did not minimize lipid and protein oxidation, which seemed to occur simultaneously (Soyer et al. 2010).

Xia et al. (2009) found a slight but significant increase of total protein carbonyls in porcine longissimus dorsi subjected to five freeze/thaw cycles (from 1.09 to 1.16 nmol/mg protein). By analyzing specific protein carbonyls, Estévez et al. (2011) found a significant increase of α -aminoadipic semialdehyde (AAS) and γ -glutamic

semialdehydes (GGS) during the first 2 months of frozen storage at -18°C , while a significant decrease was detected by the end of the freeze storage (4 months).

Leygonie et al. (2012) have reviewed the possibilities for mitigation of the undesirable effects of freezing and thawing on meat quality by using novel methods that increase the rate of phase transition. However, these methods are generally more expensive than their conventional counterparts. One such a method is high-pressure freezing, which results in instantaneous and homogenous ice crystal formation throughout the product due to the high supercooling effect achieved on pressure release. Increased pressure causes a shift in the type of ice crystals that are formed from type I (lower density than liquid water) to type IV ice crystals. Type IV ice crystals are smaller and denser than water and do not cause the product swelling that occurs with type I crystals. Hence, with type IV ice crystals, there is less mechanical damage to the cell structures, which results in a superior quality product. Another novel method is high-pressure thawing that has received less attention than high-pressure freezing. In the former case, it has been noted that the phase transition time can be reduced by ca. 50–60 % compared to the traditional atmospheric thawing practices. This causes lower microbial spoilage, a firmer product, and less thaw drip losses. However, the drawbacks of this method such as loss in color, a decrease in water-binding capacity, and protein denaturation have also been reported.

The addition of antifreeze proteins can control the structure and size of ice crystals in frozen foods. These proteins decrease the temperature at which freezing is initiated and retard recrystallization during frozen storage. The major drawbacks of this method are the high cost and lack of knowledge of concerned consumer as well as their acceptance of these additives.

The *antemortem* supplementation of vitamin E has been shown to reduce the rate of oxidation in the meat *postmortem*. Vitamin E is partitioned into the cellular membranes where it acts as an antioxidant, protecting the phospholipids from free radicals and thereby decreasing the rate of lipid and pigment oxidation. Therefore, as freezing and thawing increase the rate of oxidation post thawing, the supplementation *antemortem* increases the antioxidant levels in the meat and retards the oxidation. This simple and relatively inexpensive means of mitigating the deleterious quality effects of freezing and thawing on meat quality has proven successful in lamb and beef.

Studies on vitamin E supplementation of fresh meat have been successful for chicken, turkey, and pork, but further studies are required to confirm the effectiveness of such treatments in relation to freezing and thawing.

Pietrasik and Janz (2009) have evaluated the use of brine injection to reduce the exudate loss upon thawing of beef. They found that the purge loss was significantly lower in injected samples compared to the noninjected control, but the tenderness and color index (CIE a^*) of the former samples were significantly decreased. In this study it was reported that the injection of beef with brine solutions prior to freezing increased the consumer's purchase intent and degree of liking of the products. Therefore, this mitigation method appears to be an inexpensive and commercially applicable solution to decreasing purge losses upon thawing.

The commercial application of these novel processes is still disputed, however, even though scientific research indicates that they lead to an increase in the quality of meat. *Antemortem* supplementation with antifreeze proteins and vitamin E appears promising in reducing the effects of freeze/thaw on meat quality, especially vitamin E in retarding myoglobin, lipid, and protein oxidation during long-term frozen storage. The use of brine injection has also been shown to decrease moisture losses in frozen/thawed meat, likely due to the fact that the salts in the brine aid in improved binding of water. Similarly, modified atmosphere packing (MAP) could mitigate the color deterioration of frozen/thawed meat, but a balance is necessary to minimize the rate of oxidation envisaged with such treatments (Leygonie et al. 2012).

Shellfish are low in fat, especially in saturated fatty acids (SFA), but contain healthy omega-3 fatty acids and are excellent sources of protein. The content of total fat and omega-3 fatty acids found in different species of fish and shellfish can vary depending on a number of factors including the diet of each species, the season and location of the catch, the age and physiological status of the individual organism, and reproductive cycles. In most cases the content of omega-3 fatty acids is related to the total fat content of the species. The American Heart Association recommends eating a variety of fish and shellfish at least twice a week because they are excellent sources of protein, good sources of omega-3 fatty acids, and are low in saturated fatty acids.

To preserve their high quality, seafood should be stored at a temperature of ca. -30°C . However, freezing causes deterioration of sensory and functional properties of meat, mainly as a result of diminished solubility of proteins and water absorbability. Stability of frozen food products depends on their quality, the method used to prevent oxidation and water evaporation, as well as on temperature of storage.

The nutritive value of protein from tissues of invertebrates is similar to that of egg and milk proteins and higher than that of meat from mammals. This is determined by the morphology of muscle tissue. Owing to its specific structure, protein of the invertebrates is better digestible, and their meat requires shorter heat treatment. During storage of frozen seafood, many changes are likely to occur in the structure of nutrients, which has a significant impact on, e.g., texture of the finished product. It may also slightly affect the content of nutrients (Czech et al. 2015).

Milk and Dairy Products

Effect on Nutrients During Freezing

Milk (dairy) products are seldom frozen for storage, an exception are ice creams. Nevertheless, freezing can be used to preserve some dairy foods. However, for milk and other fluid dairy products, freezing is not recommended. While freezing has little impact on the nutritional value of milk, it does decrease milk's quality by affecting delicate texture of milk. Instead, the dairy industry is investigating new

technologies, for example, use of carbon dioxide gas or bacteriocins like nisin, produced by lactic acid bacteria, to extend the shelf life of dairy foods (Miller et al. 2007).

In principle, freezing is a safe and acceptable way to store milk, and the changes in nutritional value would be negligible. This would be the case for any type of milk, including a lactose-free product. In some cases, there will be a few minor changes in the way the milk appears and tastes. However, freezing can break down the fat globules causing destruction of the even distribution of the fat in milk. This will not be an issue with nonfat milks, but with low-fat, reduced-fat, or whole milk.

Depending on the speed at which the milk is frozen, there can be slight changes in taste and color. In addition, a small amount of sediment may develop. These changes are negligible and do not reflect any change in the wholesomeness of the milk. A good rule of thumb is again the faster the freeze, the smaller the damage. One can facilitate a quicker freeze by placing the container next to the freezer wall or on a metal shelf.

It should be remembered, however, that because milk is a rich source of nutrients, it provides an ideal food for bacteria and other microorganisms. Pasteurization helps destroy most of the bacteria that are present at bottling, but not all. Freezing does not destroy microorganisms; it just suspends or slows their growth. The quality of the defrosted milk will be no better than that of the milk at the time it was frozen. It is always best to put the milk in well-sealed containers (Uexpress 2013).

Hard or semihard cheeses can be frozen. Frozen cheese will be crumbly and a little dry and will not slice as well, but the flavor will be just as good as fresh cheese. Cheese is recommended to freeze in small pieces – no more than 200 g per chunk – and sealed in foil, freezer wrap, plastic film, or bag. Cottage cheese and ricotta cheese can be frozen for a month. Creamed cheese can be frozen for later use in cooking, dips, or as icing. All of the cultured, soured dairy products lose their smooth texture when frozen. They become grainy and sometimes separate out their water. They can still be used for cooking. Flavored yogurts may be more stable because of the fruit and sugar. It may taste more acidic when thawed.

Toxicological Aspects of Food Freezing

Lipid Oxidation as a Source of Versatile Toxicants

Lipid oxidation is one of the most important detrimental processes occurring during food, particularly meat processing and storage. Lipid oxidation is most probably at the second position in food deterioration after microbial spoilage of meat, but somewhat underrated so far. While microbial spoilage causes mainly acute responses, lipid oxidation may cause chronic toxic responses.

Meat oxidation begins with oxidation and decomposition of heme of myoglobin molecule and liberation of Fe^{3+} ions that further catalyze oxidation of other meat constituents, not only polyunsaturated fatty acids (PUFA) and cholesterol but also

proteins. Oxidation has detrimental effects on both meat quality (change of color and texture, appearance of unpleasant smell, reduction of water-holding capacity due to formation of aldehydes (32), carbonyl derivatives of proteins, denaturation products of heme) and healthiness. Healthiness involves formation of endocrine disruptors such as leukotoxin diols and other oxylipins followed by formation of mutagenic as well as genotoxic substances such as malonic dialdehyde (MDA) or “killers” of normal cells like 4-hydroxy-2-nonenal (4-HNE). Also, microbes, both pathogenic and of putrefaction, can cause meat oxidation. Oxidation degree of meat lipids (fats) depends on:

- Fatty acid composition of meat
- Content of prooxidants that initiate oxidation, such as ions of Fe^{3+} , Cu^{2+} , and heavy metals
- Content of meat intrinsic antioxidants, such as vitamin E (tocopherols + tocotrienols)

Especially easily get oxidated polyunsaturated fatty acids (PUFAs), such as linoleic, arachidonic, linolenic acids that are liberated by hydrolysis from complex lipids such as triglycerides, or phospholipids or glycolipids either by enzymatic or nonenzymatic mechanism. Respective lipolytic enzymes, catalyzing fatty acid oxidation, can be either endogenous or originate from psychrotrophic microorganisms during long-term cold storage of meat.

(Per)oxidation of unsaturated fatty acids can be divided into two main phases:

Phase 1. Formation of oxylipins (OL) – peroxides, hydroperoxides, epoxy-acids, and peroxyacids at double bonds of the hydrocarbon skeleton. The hydrocarbon skeleton of a fatty acid molecule remains unchanged, and oxygen-containing side chains (hydroxy, epoxy, etc.) are linked to double $\text{C}=\text{C}$ bonds. No obvious effect on meat sensoric quality is still observed. OL are found in all aerobic organisms and are divided into endogenous and exogenous. Non-problematic endogenous OL, acting as signal transducers (secondary messengers), are synthesized in a cell from free PUFAs, mostly from linoleic (LA, 18:2n-6) and arachidonic (AA, 20:4n-6) acids that typically are not stored in tissues, but enzymatically (phospholipases, glucolipases etc.) liberated when necessary for the cell. On the other hand, problematic exogenous OL are mostly formed from linoleic acid and reach organism with food, particularly with rancidified meat. Absorbed exogenous OL may overstimulate signal transduction for Ca^{2+} -mediated phosphorylation of proteins that can lead to carcinogenesis, chemotaxis, and apoptosis (programmed cell death).

Potentially highly toxic linoleic acid oxylipins are leukotoxin diol (LTX-diol; 9,10-dihydroxy-12-octadecenoic acid; 9,10-DiHOME) and isoleukotoxin diol (iso-LTX-diol; 12,13-dihydroxy-9-octadecenoic acid; 12,13-DiHOME). Exogenous LTX-diols are endocrine disruptors (hormonal system disturbers) of female rats and mitogens, stimulating in vitro proliferation of human breast cancer cells (Markaverich et al. 2007). Content of leukotoxin diols may rise to hazardously

high numbers during production and storage of comminuted meat products such as minced meat or mechanically deboned/separated/recovered meat (MDM), mostly of chicken or turkey (Püssa et al. 2009).

Phase 2. Disintegration of oxylipins into aldehydes, ketones, alcohols, acids, and other low molecular substances, which have substantial effects on meat sensoric quality and safety. These secondary products of fatty acid oxidation that cause rancid, fatty, pungent, and other off-flavors are generally quantified by the thiobarbituric acid reactive substances (TBARS) method that actually measures concentration of malonic dialdehyde (MDA). MDA is mutagenic both in bacterial and human cells, forming different adducts with DNA molecule. By complexing with glutathione, MDA significantly reduces content of this essential antioxidant in cells. However, new data show that the most hazardous aldehyde formed is 4-hydroxy-2-nonenal (4-HNE) that acts as an intercellular signal carrier, modulating gene expression, cell proliferation, differentiation, and apoptosis.

Vieira et al. (2009) found that TBARS of fresh meat were significantly lower than of meat stored for 90 days at -20°C . Consequently, frozen storage is not necessarily sufficient to fully stop oxidation. Although peroxidation was not measured in the aforementioned study, it would be expected that primary lipid oxidation would cease at such low temperatures by 90 days, and secondary lipid oxidation detectable by TBARS method would commence. As a result, freezing and thawing of muscle tissue caused accelerated TBARS accumulation that can be attributed to the damage of cell membranes by ice crystals and the subsequent release of prooxidants, especially the heme iron. Thanonkaew et al. (2006) found that lipid oxidation occurs in both lean and fatty meats. Consequently, fatty acid oxidation takes place primarily at the cell membrane level and not in the triglyceride fraction.

It has been shown that frozen storage can have a significant temperature-dependent effect on the formation of malonic aldehyde and also of hexanal (Utrera et al. 2014a). The lowest temperature used (-80°C) totally inhibited lipid oxidation of beef patties, while patties frozen at -8°C and -18°C underwent significant lipid oxidation. Furthermore, the extent of TBARS formation was significantly higher in samples stored at -8°C than in those stored at -18°C . These results are comparable with those previously reported for formation of α -amino adipic semialdehyde (AAS) reflecting the timely coincidence between the occurrence of lipid and protein carbonylation during frozen storage of meat. The evolution of the TBARS during the subsequent processing was again consistent with the progress of protein carbonylation. The loss of TBARS during cooking may respond to the involvement of MDA and other aldehydes in further reactions induced by high temperatures, such as the attachment of aforementioned lipid oxidation secondary products to meat proteins. During the subsequent cold storage, the amount of TBARS increased once again as a result of onset of further oxidative reactions, previously described for the formation of AAS (Utrera et al. 2014a).

Free radicals formed during fatty acid oxidation are in turn able to oxidize meat cholesterol into oxy-, keto-, and epoxycholesterols that have demonstrated proatherogenic (atherosclerosis-promoting) activity in the composition of blood

low-density lipoproteins (LDL). Fried and processed food, particularly fast food, contains high amounts of oxysterols that are also found, for example, in eggs and in milk powder.

Circumstance that concentration of α -oxysterol in human blood correlates well with its content in his/her food and that α -oxysterol was not found in the blood of humans, whose food contained only normal unoxidized cholesterol, supports the hypothesis that just oxidized cholesterol consumed with food is the source of harmful for blood vessel walls effects of cholesterol and not fats in general but specifically oxidated fats in LDL are responsible in negative effect of LDL on human coronary heart system.

Plat et al. (2014) have reported that oxysterol and chemically related oxidized plant sterols (oxyphytosterols) increase the proportion of severe atherosclerotic lesions in female LDL receptor ($^{+/-}$) mice. Oxyphytosterols, although in low concentrations, have been identified, for example, in coffee beans, French fries, heated vegetable oils, infant milk formulas, parenteral nutrition, and potato chips.

Conchillo et al. (2005) studied the intensity of lipid oxidation and formation of cholesterol oxidation products (COP) in raw and cooked chicken breasts stored at -18°C for 3 months under aerobic and vacuum conditions. Raw samples showed low COP levels (4.6–7.4 $\mu\text{g/g}$ fat) under both conditions. Cooked samples showed total COP levels in grilled and roasted samples of 28.9 and 39.3 $\mu\text{g/g}$ fat in aerobic packaging and 4.9 and 20.2 $\mu\text{g/g}$ fat in vacuum packaging, respectively. Significant correlations were found between the lipid oxidation parameters and cholesterol oxidation indices. Hence, vacuum packaging was particularly efficient in slowing down the oxidation process of cholesterol during frozen storage of cooked samples.

A healthy diet rich in antioxidants may block the oxidation process of cholesterol. Good sources of antioxidants include fruits, vegetables, and certain herbs and spices. Healthy alternatives to fast food, which also boosts oxysterol, include whole grains, fresh fruits and vegetables, seeds, and nuts.

Formation of Other Toxic Compounds in Food Products

Biogenic Vasoactive Amines

Histamine [2-(4'-aminoethyl)imidazole] and other toxic biogenic vasoactive amines such as cadaverine, putrescine, tyramine, spermine, and so forth are formed from respective amino acids (histidine and others) by the action of bacterial decarboxylases during processing and storage of fish, cheese (most of all Swiss cheese), meat, and other fermented foods (Püssa 2014). Histamine is factually included into the normal physiology of mammals; it can be found, for example, in the stem cells and basophils. The most common symptoms of histamine intoxication are connected with the cardiovascular system; an excess of histamine causes pathological broadening of peripheral blood vessels resulting in nettle rash, hypotonia, flush, and pulsating headache. Several other symptoms may also occur. Histamine is also involved in generating the so-called scombroid fish poisoning

that emerges after eating of either rotten or bacterially contaminated fish. Intoxication develops rapidly – either practically immediately or during half an hour – and lasts usually for 3 h, but may last for several days too. Histamine is also a potent allergen. The key measure for the control of histamine production in fish is rapid chilling as soon as possible after death to a temperature as close to the freezing point as possible, particularly where the fish has been exposed to warm water. This will inhibit the formation of bacterial histidine decarboxylase. However, even rapid chilling to 4.4 °C or less may only give a safe shelf life of 5–7 days. Once frozen, the fish can be stored safely for extended periods and further histidine decarboxylase will not be formed.

Biogenic amine accumulation was also studied in spontaneously fermented sausages (Fuet) manufactured from unfrozen fresh meat (U-sausages) and frozen-thawed meat (F-sausages). The aim was to investigate whether the frozen storage of raw materials affects the microbial composition and its aminogenic activity during sausage fermentation. Tyramine was the major biogenic amine in all sausages. Although the final levels were similar, tyramine accumulated more rapidly in F-sausages, which contained putrescine as the second amine. By contrast, U-sausages accumulated much more cadaverine than putrescine. F-sausages showed a slightly lower pH and free amino acid content as well as higher counts of technological flora (lactic acid and gram-positive catalase-positive bacteria) and lower counts of enterobacteria. Therefore, to freeze the meat raw materials for few days before sausage manufacture could be a useful practice, because it helps to reduce enterobacteria development and cadaverine production (Bover-Cid et al. 2006).

Most decarboxylases are unstable to freezing; for example, histidine decarboxylase becomes inactive after 8–15 days at –20 °C. Although dependent on conditions, the effect of freezing and frozen storage on microbial growth and enzymatic activity does not generally favor the formation of biogenic amines. The existing concentrations must, therefore, come mainly from the fresh product and will be strongly influenced by pre-freezing treatment and storage conditions. It was found that the proportion of biogenic amines remained stable in pork stored for 12 days at –18 °C. Increase of cadaverine and putrescine and a decrease of histamine, spermidine, and spermine after 8 days at –20 °C were also detected (Ruiz-Capillas and Jimenez-Colmenero 2004).

Maillard Reaction Products

Freezing as well as drying, roasting, or baking may accelerate different unwanted chemical reactions like Maillard reaction (nonenzymatic browning) between compounds containing an amino group such as amino acids and reduced saccharides (aldoses and ketoses). This reaction leads to the formation of mutagenic furans, aminocarbonyls, pyrazines, and other promelanoid secondary amines, products of Amadori and Heyns that presumably inhibit the growth and hinder multiplication, damage the liver, cause allergies, and so forth (Püssa 2014, p. 292). The compounds formed may be either sweet or ill smelling. The highest reactivity in the Maillard reactions expresses pentoses (e.g., ribose). Among amino acids, lysine is the best generator of the dark color; that is why the milk proteins containing much lysine are

brown most intensively. Formation of melanoids can be hindered by the removal of one reactant – either saccharide or amino compound from the mixture. The Maillard reaction occurs most intensively at water activities a_w between 0.5 and 0.8, corresponding to the temperatures between $-3\text{ }^\circ\text{C}$ and $-20\text{ }^\circ\text{C}$. Recent overviews of the Maillard reaction and food connections have been published by Wang et al. (2011) and Tessier and Birlouez-Aragon (2012).

Conclusion and Future Directions

Freezing methods for food are convenient and easily applied. Since invention, freezing is one of the few methods which allows the preservation of food attributes such as taste, texture, and safety while maintaining the nutritional value to a great extent. Frozen products are similar to the original fresh products, particularly if good handling and safety practices are utilized both before and after freezing. Retention of quality and safety are better achieved when rapidly frozen foods are kept at temperatures of $-18\text{ }^\circ\text{C}$ or even lower. At these temperatures microorganisms cannot grow, and any deteriorative reactions take place at very low rates. Frozen agricultural products can retain their quality over long storage periods if the correct procedures are applied.

Future challenge is study and application of new methods of freezing, enabling better control, understanding, and prediction of the physical aspects of freezing like crystallization process of water. Expanded use of cryoprotectors could reduce deterioration of fruits and vegetables during freeze–thaw process. Additional knowledge in the chemistry of oxidation processes of food constituents, particularly lipids and proteins, could help reduce formation of a variety of toxic compounds during freezing and thawing of various food items, particularly of meat.

Cross-References

- ▶ [Bioactive Substances of Plant Origin](#)
- ▶ [Chemical Composition of Fish and Fishery Products](#)
- ▶ [Chemical Composition of Meat and Meat Products](#)
- ▶ [Chemical Composition of Vegetables and Their Products](#)

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Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Heating and Cooking

28

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Abstract

Heating and cooking is one of the oldest methods of food treatment that can be used to increase the shelf life and improve the palatability, aroma, taste, appearance, and texture of food. It also provides safe food to the consumer by inactivation of pathogenic organisms/microorganisms, toxins, or enzymes and production of additional antimicrobial substances or enzymes. However, heating of foods may also have undesirable consequences, e.g., the loss of nutrients such as vitamins, essential amino acids, unsaturated fatty acids, and minerals. Heat treatments also affect the allergenicity of food, either reducing or increasing it. Moreover, thermal treatment of food also produces harmful substances and toxic compounds like Maillard reaction products, furan, heterocyclic aromatic amines, acrylamide, acrolein, and *trans* fatty acids. These toxic compounds are considered as carcinogenic, mutagenic, genotoxic, and teratogenic properties and are a concern from a human health risk point of view.

Introduction

Heating is one of the oldest methods of food treatment. The various thermal treatments (like boiling, drying, grilling, frying, and roasting) affect quality of food in many ways. This treatment can alter the aroma, taste, color, appearance, and texture and can also increase the shelf life of food thus contributing decisively to acceptance of foods. The shelf life of food can be improved by inactivation of pathogenic organisms/microorganisms, toxins, or enzymes and production of additional antimicrobial substances or enzymes. The thermal processing either activates or inactivates the food allergens depending upon types of foods, presence of ingredients, and type of heating. Heating of foods often is a condition for intended consumption. The foods like raw vegetables, potatoes, and rice need to be cooked to render them palatable while thermal processing can be applied to produce edible foods like bread and bakery products. Heating of foods may also have undesirable consequences, e.g., the loss of nutrients such as vitamins, essential amino acids, and unsaturated fatty acids. Moreover, thermal treatment of food also produces harmful substances and toxic compounds like furan, heterocyclic aromatic amines, and acrylamide (Gerhard et al. 2007).

Acrylamide may be formed by baking, frying, and roasting of foods, particularly of cereal products (e.g., roasted cereals, bread, and bakery products), potato products (e.g., chips, fried potatoes, and crisps), coffee, and cocoa. It is formed on heating of foods above 120 °C by the Maillard reaction, preferentially by condensation of the asparagine (amino acid) with fructose and glucose (reducing sugars). Acrylamide is considered a probable human carcinogen, implying that its metabolite epoxypropanamide (glycidamide) is the actual active genotoxic agent. In animal experiments with rodents, glycidamide-DNA adducts were found in all tissues investigated at high dosage. Furthermore, animal experiments have shown that acrylamide exhibits toxic effects to the nervous system and the reproductive organs (Gerhard et al. 2007).

Heterocyclic aromatic amines (HCAs/HAAs) are formed in varying concentrations by frying or grilling or by comparable methods of heating meat and fish, depending on the type of protein, the temperature, and the heating time. Two main types of HCAs are formed in varying amounts, depending on the temperature and precursor compounds: isoquinoline (so-called IQ compounds) and carboline derivatives. Many HAAs show a mutagenic potential in bacterial and mammalian cell-based test systems and are carcinogenic in animal experiments. In humans, bioactivation of HAAs to carcinogenic metabolites varies greatly between individuals. Furan is a volatile compound that has been detected in heated food products like coffee, bread, vegetable, and meat preserves, as well as in baby food. Its presence in widely different types of foods suggests that it is formed by different routes. Oral application of furan to rats and mice led to different tumor patterns. 3-Monochloro-1,2-propanediol (3-MCPD) is regarded as a reference substance for a series of undesirable compounds known as chloropropanols. 3-MCPD may be produced in the ppb ($\mu\text{g}/\text{kg}$) range during manufacture of seasoning sauces from hydrolyzed vegetable protein as well as by baking or toasting of bread, cereals, and coffee beans. 3-MCPD is formed at temperatures significantly above 100 °C by various formation mechanisms. More recent studies show that certain foods may contain not only chloropropanols but also their esters in quantities that can be 50 to 150 times higher than those of 3-MCPD. 3-MCPD has caused tumors in animal experiments when applied at high dosage (Gerhard et al. 2007).

Effect of Various Nutrients During Cooking and Heating of Foodstuffs

Cooking, an important food processing, increases the flavor, appearance, texture, stability, and digestibility of foods as well as destroys pathogens and deactivates toxic substances. The heat-induced chemical changes in amino acids, proteins, sugars, carbohydrates, lipids, minerals vitamins, and other food components reduce nutritive values and even the formation of some toxic chemical compounds (discussed under subsequent section).

Food is essential to life. Food is a mixture of chemical components, and nutrients account for more than 99.9 % of the food. Nutrients are the building blocks of the human body needed for growth, to maintain and repair the body tissues, to regulate body processes, to furnish energy for the body's functions, as well as reproduction of living organisms. The main classes of nutrients are carbohydrates, proteins, fats, vitamins, and minerals. The macronutrients (e.g., carbohydrates, proteins, fats) are the major sources of energy and building materials for the living cells. The micronutrients (vitamins and minerals) are equally very important but needed in small amounts (Karmas and Harris 1988). Food also contains some antinutritive substances. Antinutritives are mainly found in plant material. These antinutritive substances induce toxic effects indirectly, by interference with the functioning and utilization of nutrients or causing nutritional deficiencies. There are mainly three types of antinutritives like antiproteins (e.g., protease inhibitors and lectins),

antiminerals (e.g., phytic and oxalic acid), and antivitamins (e.g., ascorbic acid oxidase and antithiamine). The antiproteins and antiminerals interfere with the digestion, absorption, or metabolic utilization of protein (amino acids) and minerals respectively whereas antivitamins inactivate or destroy vitamins.

The food processing techniques like heating (pasteurization, sterilization) and cooking (boiling, baking, broiling, microwaving, roasting, frying, steaming, stewing, and smoking) are considered as practice to transform raw foods into ready for consumption. The heating or cooking is employed in food to ensure safe, nutritive, high quality of food products to the consumers. The primary objective of cooking is to increase the palatability of the food. Heating helps in better food digestibility and enhances the bioavailability of bioactive food components through changes in the physicochemical structure of food matrix (Burri et al. 2009), while blanching, pasteurization, and sterilization are meant to increase storage life of the foodstuff and to minimize foodborne diseases. The major nutritional benefits of heating of foods include formation of color, aroma, and taste active compounds as well as antioxidants; improving the palatability of the raw materials; and elimination or reduction of microbial load, natural toxins, and enzyme inhibitors. The formation of taste, aroma, and color via the Maillard reaction in foods and beverages (e.g., bread, breakfast cereals, roasted meat, soy sauce, malted beverages, and coffee) is a typical peculiarity of these products. Melanoidins formed during final stage of the Maillard reaction contribute brown color to the product. The various Maillard reaction products have shown beneficial chemoprotective properties in both in vitro and in vivo studies. The number of factors will influence the nutritional content of the food. The level of nutrient in food will depend on various conditions like oxygen, pH, heat, and light which can be prevailed during food processing (Burri et al. 2009). Depending on the nature of food being processed and the amount of nutrient presence in the food, retention of nutrient content varies. For example, sensitivity of vitamin C to heat varies with pH. The vitamin and macronutrient contents of foods are more likely to be affected by processing.

Blanching is a heat process frequently applied to tissue systems prior to freezing, drying, or canning. It is a mild heat treatment and important step in the processing of fruits and vegetables. Blanching is used to (i) inactivate enzymes which would contribute to undesirable changes in color, flavor, or nutritive value during storage; (ii) wilt the tissue to facilitate packing, removing tissue gases prior to container filling, increasing the temperature of the tissue prior to container closing, and inactivating or activating enzymes; and (iii) reduce microbial loads prior to further processing (Karmas and Harris 1988). Blanching in hot water results in large losses of water-soluble vitamins by leaching thermally labile nutrients and bioactive compounds. However, inactivation of oxidative and other degradative enzymes (e.g., lipoxygenase for carotenoids, polyphenol oxidase for flavonoids, ascorbic acid oxidase for vitamin C) during blanching will prevent further and greater losses by enzyme-catalyzed degradation during processing and storage. Due to heat treatment, considerable losses of carotenoids and flavonoids have been found in fruits and vegetables juices. Heat treatment, on the other hand, also increases

bioavailability of carotenoids presumably by softening or breaking cell walls and membranes. Heating conditions (time-temperature combination) should therefore be optimized so that bioavailability is increased, but without provoking significant degradation.

The term “nutritional losses in food processing” is generally taken to refer to all nutrients; the most sensitive by far are vitamin C and to a lesser extent B₁ – the other nutrients are much more stable, and very little is lost in most processes. Where losses of nutrients take place, the relative importance of the food in question as a source of the particular nutrient must be taken into account. The losses from the poorer sources of the nutrient in question are of little significance, e.g., vitamin C of milk is largely destroyed during pasteurization, but milk, even if consumed untreated, is not an important source of this vitamin. Biological measures of protein quality will show a fall only when the limiting amino acid is affected, e.g., milk, meat, and fish are limited by methionine plus cystine (sulfur-containing amino acids) and contain a relative surplus of lysine. If the amount of available lysine is reduced, there will be no change in the biological measure until the damage is severe enough to make lysine limiting (Bender 1978).

Most legumes contain a number of toxins and other substances that inhibit digestive enzymes. Destruction of them increases the nutritional value of the proteins. Legumes contain toxins like goitrogens and phytohaemagglutinins (agglutinate the red blood cells) that lead to death of animals if fed raw beans. The raw kidney beans cause nutritional muscular dystrophy in pregnant ewes due to a heat-labile vitamin E antagonist. Cottonseed meal contains gossypol which is both toxic and combines with lysine so reducing the nutritional value of the protein. When these toxins are themselves proteins, they are inactivated by heat. Raw egg white contains mucoprotein and avidin that combine with the vitamin, biotin, and render it unavailable. Some types of fish contain thiaminase which can destroy thiamin if the fish is eaten raw. Heat treatment destroys them. Many cereals contain a bound form of niacin which is not biologically available but can be liberated during baking/heating and under alkaline conditions. Phytate present in cereal bran and in mature legumes can reduce the absorption of calcium, phosphate, and iron by forming insoluble salts which can be reduced during processing. The heating of cereal grains increases feed conversion efficiency and palatability due to the destruction of peroxidase, lipoxygenase, and esterase. The Maillard reaction is responsible for a small nutritional loss in return for the desirable flavors and color produced in foods like roasted meat, crust of bread, and biscuits (Bender 1978).

There are mainly two kinds of nutrient losses in meat, viz, loss of juices containing protein and vitamin B in solution and reduced availability of amino acids and partial destruction of thiamin. There is difference between the temperature of the oven, outer parts, and inner parts of a piece of meat. The extent of the losses will vary with temperature, time, type of cooking, size of the portion, and content of connective tissue (Bender 1978). Many processed foods are enriched by vitamins and minerals as nutrients. The stability of naturally present nutrients may differ from those added nutrients. This may be due to difference in chemical form of added nutrient as well as physical protection afforded to the natural nutrients by the

other food ingredients. During enrichment, the stabilized forms of vitamins are added which may be more stable than those naturally present. Consequently, added nutrients can be more stable or less stable than the natural ones. In protein-enriched soya, added methionine (a sulfur-containing amino acid) was more sensitive than the naturally present protein (Bender 1978).

There are number of books and papers available on nutrient aspects. Readers are advised to refer some of the excellent books such as Bender (1978), Karmas and Harris (1988), Gerhard et al. (2007), Burri et al. (2009) for further information.

Changes in Proteins and Amino Acids

The essential building blocks for proteins are the amino acids which can be stabilized by various forces and bonds involved in primary, secondary, tertiary, and quaternary structures. Among the 22 amino acids that make up most proteins, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are considered as essential amino acids. The nutritional value of proteins is dependent on the quantity, digestibility, and availability of essential amino acids. Proteins have complex molecular structure that can undergo various changes during thermal treatments. During thermal processing, both positive and negative effects occur in proteins. It improves the digestibility and availability by denaturation of protein structure. Various enzymes (proteinous in nature) such as lipases, lipoxygenases, and hydrolases as well as trypsin inhibitors have abilities to destabilize the foods or decrease the digestibility of food items. The denaturation of proteins during thermal processing deactivates these enzymes as well as the inhibitors and improves the quality of food products. Upon denaturation, peptide bonds of the protein are more readily available for hydrolysis by proteolytic enzymes. The excessive heat applied to the outside of foods of the puffing exploding type of processing used in popcorn or breakfast cereals causes severe protein damage. The amino acids are completely decomposed or produce cross-linkages forming polyamino acids leading to destruction of amino acids. Protein denaturation, on the other hand, also imposes a greater risk of rapid subsequent microbial contamination under poor hygienic or storage conditions, respectively, imposing the risk of food poisoning. Immunological properties of β -lactoglobulin (whey protein) may be altered by heat denaturation which can be less efficiently transported than the native form (Gerhard et al. 2007).

The Maillard reaction product like *N*- α -acetyl-carboxymethyl-L-lysine/pronyl-L-lysine exhibits an antioxidative capacity fivefold higher than that of ascorbic acid. The amino acid reacts with reducing sugar in the Maillard reaction and reduces the protein quality. The lysine, as one of the essential amino acids, is involved in this reaction, and bioavailability is lost (Burri et al. 2009). The transformation of the side chains of protein-bound amino acids in basic conditions leads to formation of cross-linked amino acids during thermal treatments of foods. A dehydroalanine residue may be formed. The ϵ -amino group of lysine may react to give a secondary amine, which is normally indicated with the trivial name of lysinoalanine (LAL).

The nutritional consequences of LAL formation in foods include adverse effects on growth, protein digestibility, protein quality, and mineral bioavailability and utilization. LAL has been shown to provoke lesions in rat kidney cells causing nephrocitomegaly.

There is little (in juice) or no damage to protein content when meat is heated. During higher-temperature roasting, only outer parts get damaged and reduce the availability of some of the amino acids through Maillard-type reactions in case of meat products. Fish proteins are limited by the sulfur amino acids and contain a relative surplus of lysine so that damage to the latter may have no effect on net protein utilization. There is loss of available lysine and tryptophan in the loaf of bread (Bender 1978). Thermal treatment changes the tertiary structure of proteins that may lead to a change in epitopes responsible for allergenicity of certain foods. However, not all allergens can be inactivated by heat treatment (Gerhard et al. 2007).

Changes in Carbohydrates

Carbohydrates account for major energy intake in a balanced diet. Carbohydrates are generally regarded as being stable to processing. However, there is some loss both by leaching into processing water and by breakdown. Losses by leaching will be governed by the factors like particle size, time, and volume of water. During cooking of foods, carbohydrates are lost by breaking. The amount of starch decreases and sugars increase during preliminary soaking of beans (Bender 1978); during wet heat treatment of vegetables and fruits, a more considerable loss of low-molecular weight carbohydrates (i.e., mono- and disaccharides) than that of sucrose into the processing water takes place. The loss of glucose and fructose at boiling was higher than that of sucrose. No leaching of dietary fiber into the processing water has been reported with blanching and boiling. Glycosidic linkages in the dietary fiber polysaccharides may be broken during heat treatment. A decreased association between fiber molecules, and/or a depolymerization of the fiber, results in a solubilization. Heat treatment can also change hydration properties; e.g., boiling increased the water-binding capacity slightly in wheat bran and apple fiber products, whereas autoclaving, steaming, and roasting had no significant effects.

Simple sugars are easily digested in the raw form by digestive enzymes and converted into monosaccharides. However, in certain raw vegetables like potato, starch is encapsulated within the starch granules which cannot be digested by digestive enzymes. Upon cooking of potatoes, the starch granules are gelatinized, and the starch becomes digestible (Burri et al. 2009). Gelatinization refers to the irreversible loss of the crystalline regions in starch granules that occurs upon heating in the presence of water. Gelatinized starch is not in thermodynamic equilibrium and a progressive reassociation of the starch molecules upon aging. This recrystallization (retrogradation) may reduce the digestibility of the starch. The retrogradation of the amylopectin component is a long-term phenomenon occurring gradually upon storage of starchy foods. Amylose, however, reassociates

more quickly. The crystallinity of retrograded amylopectin is lost following reheating to approximately 70 °C, whereas temperatures above 145 °C are required to remove crystallinity of retrograded amylose. This is a temperature well above the range used for processing of starchy foods. This implies that retrograded amylose, once formed, will retain its crystallinity following reheating of the food. The parboiling (i.e., a pretreatment involving heating and drying) of rice reduces the stickiness of the rice, possibly by allowing leached amylose to retrograde and/or form inclusion complexes with polar lipids on the kernel surface which affect the final cooking qualities of the rice. In pasta products, gluten forms a viscoelastic network that surrounds the starch granules, which restricts swelling and leaching during boiling. Nonenzymatic browning reactions occur between reducing sugars and amino groups in foods during heat treatment and storage.

With retrogradation, resistant starches are produced which are poorly degraded by α -amylase and thus generally reach the large intestine, where microbial fermentation takes place. Resistant starches thus may affect the microbial flora composition and the microbial metabolism which may have consequences for gut health by changing, for example, the short-chain fatty acid patterns. Short-chain fatty acids (SCFA) and particularly butyrate are considered to be important substrates for colonic epithelial cells, and relations between butyrate concentration and the risk for colorectal carcinogenesis have been observed. Thus, foods which lead to a higher production of butyrate in the colon may have beneficial effects. Therefore, the generation of resistant starches by thermal treatment of starchy foods may also be associated with beneficial nutritional and health effects. The amount of resistant starches in a food influence the glycemic index (GI). High amounts of resistant starch in a food generally are associated with a low GI, and this property may be nutritionally beneficial. The low-GI diets are responsible to decrease risk of type-2 diabetes and coronary heart disease (CHD) and improve in various metabolic risk factors. Thermal treatment of starchy foods thus favorably changes the nutritional properties of these foods (Gerhard et al. 2007).

Changes in Lipids

Lipids provide 9 kcal/g energy which is highest among other constituents and carriers of fat-soluble vitamins (A, D, E, K) and carotenoids. Lipids undergo various degradative changes which have effects on palatability rather than nutritional value. The nutritional value of fats is limited to the energy content of the triglycerides and to their content of essential fatty acids as well as fat-soluble vitamins. The damage of these vitamins and essential fatty acids is considered as loss of nutritional value. Changes in the fat content of the food itself must be included under nutritional changes – some foods may gain fat and so increase their energy content, and possibly their vitamin and essential fatty acid content; others may lose fat in the process. There is no significant loss of nutritional value of fatty foods such as dairy products, eggs, and meat fat during normal heat processing (Bender 1978). The physicochemical properties of fats are highly affected by

temperature. Unsaturated fatty acids (monounsaturated fatty acids: MUFA; polyunsaturated fatty acids: PUFA) and cholesterol are rapidly oxidized in the presence of oxygen. Furthermore, MUFA and PUFA may undergo isomerization with heat treatment. Fat oxidation compounds in foods are nutritionally undesirable and have not been shown to have any beneficial effects; however, these compounds are of toxicological relevance (Gerhard et al. 2007). At extremes of baking conditions, linoleic acid and possibly other fatty acids may be converted (due to lipoxygenase activity) to unstable hydroperoxides which may affect both the lipid and vitamin (oxidation of fat-soluble vitamins) of the product (Karmas and Harris 1988).

The fats/oils undergo various food preparation techniques, and one of them is deep fat frying. The oils are used as a heat exchange medium to cook the food product that can improve palatability, quality of food product, and provide crispness to, e.g., french fries. But acrylamides are formed in fried products which have toxic effects on living organisms. The frying temperatures range from 135 ° C to 190 ° C depending on the food product (Burri et al. 2009). During frying, fats/oils undergo many desirable and/or detrimental chemical reactions. These reactions include thermal oxidation, polymerization, hydrolysis, flavor changes, and darkening. Excessive heating will cause reaction of carbonyl components resulting from the decomposition of unsaturated fatty acids which may lead to reactions similar to those involved in nonenzymatic browning. During thermal heating of fats/oils, small amounts of *trans*-fatty acid are also produced.

Frying has little or no impact on the protein or mineral content of fried food, whereas the dietary fiber content of potatoes is increased after frying due to the formation of resistant starch. Moreover, the high temperature and short transit time of the frying process cause less loss of heat-labile vitamins than other types of cooking. For example, vitamin C concentrations of French-fried potatoes are as high as in raw potatoes, and thiamine is well retained in fried potato products as well as in fried pork meat. Although some unsaturated fatty acids and antioxidants are lost due to oxidation, fried foods are generally good sources of vitamin E. It is true that some fat is inevitably taken up by the food being fried, contributing to an increased energy density (Fillion and Henry 1998). Frying improves the digestibility. Pan frying and deep frying all resulted in an apparent loss of cholesterol in low-fat fish probably due to its leaching out into the frying oil. However, when plant foods are fried in animal fat, cholesterol is taken up by the product. The protein digestibility of foods such as hake, beef, pork, swordfish, meatballs, and fish balls is not affected by frying. However, presence of reducing substances (glucose and wheat flour) reduces the nutritive utilization of proteins. The amount of available lysine in white fish dropped from 20 % to 30 % after deep fat frying in various fresh vegetable oils. This is due to the formation of bonds between the amino groups of the protein and oxidation products of the fat. Fish proteins contain a relative surplus of lysine; small damage to this amino acid should not alter the net protein utilization. Deep fat frying significantly increased the percentage of resistant starch due to the formation of amylose-lipid complexes. Although frying decreases the amount of digestible starch in potato, dietary fiber content is increased. Mineral contents are very little affected by deep fat frying in both potatoes and fish.

Changes in Vitamins

Heat treatment may lead to a decrease in essential nutrients (like various vitamins) and consequently reduce the nutritional value of certain foods. Vitamins are organic components in food that are needed in very small amounts for growth and for maintaining good health. There are mainly two types of vitamins, viz., fat-soluble (e.g., A, D, E, and K) and water-soluble (e.g., folate, vitamin B₁₂, biotin, vitamin B₆, niacin, thiamin, riboflavin, pantothenic acid, and vitamin C). The water-soluble vitamins are heat sensitive whereas fat-soluble vitamins are relatively heat stable. The transformations lead to loss of vitamins and proteins during the Maillard reaction (Burri et al. 2009). Vitamins are unstable in foods. The cooking/heating conditions cause vitamin loss. These losses are varied depending upon severity of temperature and length of exposure, presence of oxygen, light, and moisture. Vitamin A is stable under an inert atmosphere; however, it rapidly loses its activity when heated in the presence of oxygen, especially at higher temperatures. Carotenoids have highly unsaturated structure making them highly susceptible to degradation in presence of heat, oxygen, and light. Vitamin D is vulnerable to alkaline pH range, light, and heat. Retention of vitamin D varied in the range of 60–90 % during culinary treatment of meat and fish. Vitamin E is lost during roasting and broiling and is unstable in presence of reducing agents (light, peroxides, oxygen). During culinary treatments of various types of meat, 44–95 % of vitamin E is retained and 60–93 % in case of legumes. Vitamin K is relatively stable during culinary treatment (Emilla et al. 2006; Mahesh and Uday 2013). Thiamine is highly unstable at alkaline pH, but thermal degradation occurs even under slightly acid conditions. Riboflavin is stable to heat but sensitive to light upon high heat treatment. Folate is lost in food during cooking because it breaks down under heat and leaches into cooking water. Folic acid is one of the heat-labile vitamins and is rapidly destroyed with increasing temperature. Folic acid deficiency is associated with the incidence of neural tube defects and with plasma homocysteine concentrations, an independent risk factor for cardiovascular disease. The bioavailability of folates from natural sources is highly variable and may depend on the food matrix structure and stabilizing factors in the food to reduce folate degradation, for example, by thermal treatment (Gerhard et al. 2007). Pantothenic acid is the most stable vitamin during thermal processing with pH levels of 5–7. Niacin is the most stable water-soluble vitamin. Vitamin B₆ is resistant to heat, acid, and alkalis but sensitive to light in neutral and alkaline solutions. Vitamin B₁₂ is generally considered to be stable; it undergoes large losses through leaching into the cooking water. Vitamin B₁₂ is normally stable during pasteurization of milk, but sterilization is responsible for 20 % loss of it. Biotin is labile when heated in alkaline solutions (Emilla et al. 2006; Mahesh and Uday 2013).

Thiamin is by far the most heat-labile of the vitamins in meat whereas niacin and riboflavin are relatively stable. Most white fish are relatively poor sources of vitamins, and so losses are of lesser importance. The vitamin D in fatty tissue of fish is relatively more heat stable. High-temperature treatment of cereals leads to destruction of thiamin and loss of available lysine. Baking destroys 15–30 % of the

thiamin of bread with no loss of riboflavin or niacin (Bender 1978). During sterilization of milk, pyridoxal (Vitamin B₆) converts into amine, and on subsequent storage the pyridoxamine complexes with sulfur compounds leading to the formation of bis-4-pyridoxyl disulfide which has much lower biological activity (in rats). The most sensitive nutrients are vitamins C and B whereas vitamins A and D are not affected when milk is subjected to pasteurization. However, there is considerable destruction of vitamins C, B₁₂, B₆, folate, and thiamin, but losses can be reduced by increasing the temperature and decreasing the time (Ultrahigh temperature – UHT) (Bender 1978).

Changes in Minerals

Minerals are the inorganic elements that are essential to the functioning of the human body. The minerals contribute to flavor, texture, and when digested can provide the cofactors for enzymes that influence nutrition. Minerals that remain in food after processing are known as chemically available while minerals which upon consumption become biologically functional to the organism are known as biologically available. Minerals are relatively not affected and lost during food processing, but minerals combine with nutrients or nonfood components during heating. They may become unavailable for digestion due to these interactions. However, destruction of phytates (known as binding ligands) increases the bioavailability of minerals (Mahesh and Uday 2013). The reaction products of depolymerization processes under high temperature might change the chemical form of iron, and it becomes more soluble, which increases its availability. Partially or completely ionized salts of oxalic acid (oxalates) form water-insoluble complexes with Ca⁺², Fe⁺², and Mg⁺². The oxalates are considered as antinutrients by virtue of their disruption of mineral absorption. Foods rich in oxalates potentially can decrease important minerals absorbed into the system. Cooking removes oxalates present in food such as tea leaves, spinach, or cocoa. Blanching and cooking of spinach leaves improves HCl-extractable iron, calcium, and zinc, which is similar to the acidic environment of the stomach. However, calcium, iron, and zinc are lost by heat treatments. During kitchen cooking, minerals are leached out into the hot water. The compounds formed during chemical reaction between proteins and sugars bind minerals. During heating, ascorbic acid is destroyed and in so doing eliminates a key component that facilitates iron absorption.

Baking is not likely to affect the content of minerals in foods. Heat treatment may profoundly affect the absorption and utilization of minerals, through cleavage of complexes, which otherwise renders these minerals less absorbable. Phytate can undergo hydrolysis during the bread-making process that improves the absorption of the phosphorus. During the baking process, some fiber components like hemicelluloses may undergo transformation which may affect mineral absorption. Iron may undergo oxidation or reduction during the baking process, and this might affect its absorbability or biological value (BV) (Karmas and Harris 1988).

Changes in Non-Nutrients and Other Components

Non-nutrients are those bioactive compounds in food that do not seem to be essential for avoiding a specific disease or a clinical condition associated with a deficiency for a specific substance, e.g., a nutrient. However, non-nutrients may have important nutritional and biochemical functions by acting, for example, as antioxidants or having antimicrobial or anticarcinogenic properties, respectively. The bioactive non-nutrients, specifically polyphenols/phytochemicals, are important from a human physiological point of view. Phytochemicals are secondary plant metabolites which are present in small and varying amounts in plants that include carotenoids, flavonoids, isoflavonoids, phenolic acids, glucosinolates, monoterpenes, phytosterins, and saponins. Different polyphenols react differently to thermal treatment. Virgin olive oils contain lignans, hydroxytyrosol, and tyrosol-like substances. The lignans are less affected by heating to 180 °C for 25 h, microwave heating for 10 min, or boiling in a pressure cooker for 30 min than hydroxytyrosol and tyrosol-like substances. Thus, thermal treatment differentially affects bioactive compounds present in the same food and thus very likely changes the nutritional characteristics of that food considerably. Anthocyanins are water-soluble plant pigments present in different fruits and vegetables. The bioavailability of anthocyanins does not seem to be affected by thermal processing. Carotenoids are also a large group of plant pigments, e.g., α - and β -carotene, lycopene, lutein, and zeaxanthin. During thermal processing of tomato, isomerization of lycopene from *all-trans*-lycopene to *cis*-lycopene isomers occurs depending on the temperature and the time of heating. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity; despite a decrease in vitamin C content with increased duration of processing at 88 °C, lycopene content and total antioxidant activity increased, respectively. The bioavailability of lycopene has been shown to be much higher from processed tomato products (tomato paste) as compared to fresh tomato in a human dietary intervention study (Gerhard et al. 2007).

Food allergies can be defined as adverse, immune-mediated reactions to foods that occur in certain individuals. Food allergy is an abnormal *immunological* response to a food or food component; food allergens are almost always proteins. Food allergic disorders include acute, possibly life-threatening allergic reactions, as well as chronic debilitating diseases. The allergenicity of certain food allergens may be affected by thermal processing. Besides the allergens themselves, however, other modifying factors present in food may play an important role with respect to thermal treatment and heat inactivation of allergens. Heat treatments affect the allergenicity of food, either reducing or increasing it. Processing may destroy existing epitopes on a protein or may generate new ones (neoallergen formation) as a result of changes involving protein conformation. The thermal processing of proteins can form oligomers, become denatured, degraded, aggregated, cross-linked, fragmented, and reassembled, and these changes can alter the overall IgE binding profiles of food allergens. During the Maillard reaction, the interaction with sugars may modify the tertiary structure of the proteins and thus modify their

conformational epitopes, creating novel IgE binding sites, masking the allergenic structure, or exposing previously unavailable sites. The dominating major allergen in codfish is Gad c1, which seems to be extremely stable to thermal treatment. The thermal processing changes the peanut allergens. Roasting of peanuts increased the biological activity 90 times compared to an extract of raw peanuts. The increase in allergenicity may be caused by a Maillard reaction.

Nucleic acids as well as the low molecular weight compounds such as nucleotides, nucleosides, and purine and pyrimidine bases are ubiquitous in food. In fresh unprocessed food, the main part of the nucleo compounds – more than 95 % – is present as RNA and DNA. Heating processes change the distribution of the nucleo compounds in comparison to the raw material. During cooking and frying, the proportions of the low molecular weight nucleo compounds as nucleotides, nucleosides, and free bases expand. Furthermore, it is well known that the individual components increase the content of uric acid in human serum in a different manner which is important for patients suffering from gout. The microwaving heating process reduces the formation of low molecular weight nucleo components in vegetables in comparison to normal cooking and pressure cooking (Isabelle and Alfred 2007).

Toxicological Aspects of the Chemical Changes of Food Components During Heating and Cooking

Heat processing is one of the means to preserve foods and maintain food supplies for longer periods of time than their seasonal availability. The development of various types of thermal processing leads to improvement in palatability of foods, reduction in deterioration of food during storage, as well as improving the quality and safety of food products. The exposure of food to heat can be considered the most used processing step, and hence heating is one of the inevitable steps in a majority of food preparations. The wide range of heat treatments like baking, grilling, frying, toasting, roasting, broiling, microwaving, stoves, pasteurization, and sterilization are applied to various foods depending on their end use that can improve product quality as well as consumer acceptance. However, during processing, various toxic compounds are generated from interactions between various food components as well as added ingredients/additives. The chemical changes in food components, including amino acids, proteins, sugars, carbohydrates, vitamins, and lipids produce some toxic chemical compounds. During thermal processing, the toxic compound produced is generally designated as HEATOX, i.e., heat-generated food toxicants, and considered as carcinogens, genotoxins, neurotoxins, as well as antinutrient effects. Lineback and Stadler (2009) have defined “processing toxicants (process-induced toxicants, process-formed toxicants) as those substances present in food as a result of food processing/preparation that are considered to exert adverse physiological (toxicological) effects in humans, i.e., substances that create a potential or real risk to

human health. Food in this definition also includes beverages and nonalcoholic drinks such as coffee and tea, and thus both parts of the diet are included.”

During heating and cooking, a number of chemical reactions occur simultaneously, and one of the key reactions is the Maillard reaction. It is a very complex reaction involving reducing sugars and amino acids. The Maillard reaction is known to produce more than 550 volatile compounds that contribute to the flavors and aromas while nonvolatile products such as the melanoidins contribute to the browning colors in cooked foods. However, compounds formed during Maillard reactions have adverse physiological effects or potential health risks (Lineback and Stadler 2009). There are various books and papers available on this topic but readers are advised to read one of the excellent books edited by Stadler and Lineback (2009) for further details.

Heterocyclic aromatic amines, acrylamide, acrolein, hydroxymethylfurfural, furans, chloropropanols, and chloroesters, Maillard reaction products, and nitrates as potential toxicants are produced during heating/cooking of foods as well as aspect on *trans*-fatty acids in heat-treated oils that can be discussed in subsequent sections.

Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural (5-hydroxymethyl – 2-furaldehyde, HMF) is formed naturally during heating and is an intermediate in the Maillard reaction (MR). The molecular formula of HMF is $C_6H_6O_3$ having molecular weight 126.11 g/mol, and melting point varies from 32 °C to 34 °C, which is shown in Fig. 1.

HMF is considered as one of the direct indicators for assessing the quality of heat-treated food. When carbohydrate-rich products undergo heat treatment and subsequent storage, HMF is produced.

Formation and Occurrence of HMF in Food

The formation of HMF depends on presence of precursors like glucose, fructose, amino acids, as well as the temperature, pH, and duration of storage. The acidic foods can form high amounts of HMF. The food products which can be stored at longer time contribute to high HMF. Sugars decompose into furfural compounds by the Maillard reaction, caramelization, or pyrolysis of either reducing moieties of disaccharides or free monosaccharides (Morales 2009). The mechanism for formation of HMF is shown in Fig. 2.

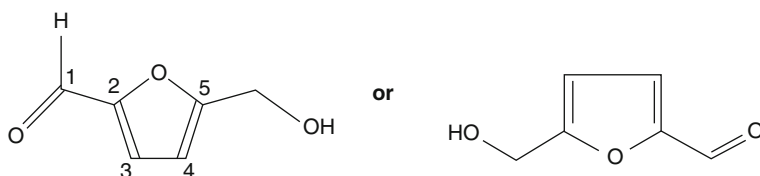


Fig. 1 5-hydroxymethyl – 2-furaldehyde, HMF

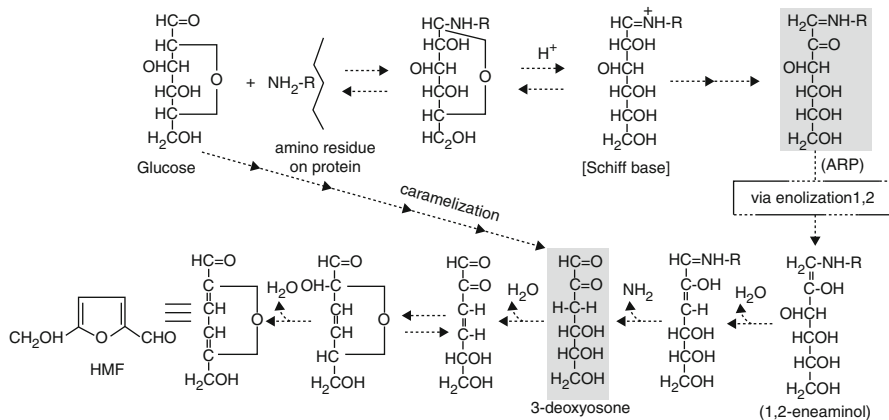


Fig. 2 Formation of HMF during heat treatment of foods (Morales 2009; Copyright © 2009 by John Wiley & Sons, Inc. Reprinted with permission)

The Maillard reaction is a complex reaction that has occurred in heated, dried, or stored foods. This reaction is considered as nonenzymatic browning or amino-sugar browning. During heating of food, the Maillard reaction occurs, which results in formation of characteristic color and flavor in the products. The Maillard reaction can be divided into three (i.e., early, advanced, and final) stages. In the early stage, the condensation of a carbonyl group (e.g., glucose) with a free amino group (ϵ -amino group of lysine) which forms glycosylamines subsequently undergoes series of reactions producing unstable Schiff bases to more stable amadori rearrangement product (ARP). Under advanced stages of the Maillard reactions, the ARP undergoes several degradation reactions during severe heat treatment or prolonged storage, leading to production of 1,2-dicarbonyls. ARP is degraded to form reductones and furfurals that can react further to produce colored, high molecular mass products and melanoidins in the final stage. Dicarbonyl compounds can lead to the formation of Strecker aldehydes, pyrazines, thiophenes, and furans which contribute to flavor. Moreover, the dicarbonyl fragments can act as precursors of acrylamide (Morales 2009). HMF is formed by degradation of the ARP via 1,2-enolization pathways. The positively charged amino group shifts to enol forms in which a hydroxyl group is eliminated to yield 2,3-enol. The 2,3-enol subsequently hydrolyzed a Schiff base to glycosulose-3-ene, which is an unsaturated dicarbonyl compound and undergoes cyclodehydration to form HMF (Morales 2009).

Caramelization involves the degradation of reducing sugars without the condensation step. When sugars are heated above their melting points under alkaline or acidic conditions, they darken to form brown color polymers. HMF arises from monosaccharides through an acid-catalyzed dehydration and cyclization mechanism. HMF is formed from both the degradation of hexoses and an intermediate in the Maillard reaction. The formation of HMF is dependent on the temperature, water activity, pH, acidity, presence of bivalent metals, organic or inorganic acids,

Table 1 HMF content in selected food products

Food products	HMF content (mg/kg)
Baby food (cereal-based)	0–57.18
Baby food (milk-based)	0.18–0.25
Barley	100–1,200
Bottled tomato puree	3.95–9.94
Bread	3.4–87.7
Breakfast cereals	6.59–240.51
Caramel products	110–9,500
Chicory	200–22,500
Coffee (instant)	400–4,100
Cookies	0.5–74.5
Dried fruits	1–780
Dried plums	1,100–2,200
Fruit juices	2–22
Jam	5.5–37.7
Malt	100–6,300
Must (<i>arrove</i>) syrup	3,500–11,000
Soluble coffee clusters	691–4,023
Sugarcane syrups	100–300

Compiled from Morales (2009) and Kowalski et al. (2013)

or salts. The reducing carbohydrates directly undergo 1, 2 enolization, dehydration, and cyclization reactions during caramelization. Caramelization requires higher temperatures than the Maillard reaction to develop HMF, and different sugars have a different impact on the formation of HMF (Morales 2009). HMF undergoes decarboxylation, oxidation, dehydration, and polycondensation reactions during further processing and storage. The various products like levulinic acids, formic acids, 5-hydroxymethyl-2-furan carboxylic acid, 5-hydroxymethylfuroic acid, furan-2,5-dicarboxylic acid, 2-(2'-hydroxyacetyl)-furan, and pyranone are degraded products from HMF.

The various thermal treatments like baking, roasting, and sterilization applied in preparation of food products like breakfast cereals, coffee, bread, as well as pasteurized juices or pulps is responsible for formation of HMF. In roasted products like coffee and roasted chicory are found appreciable amounts of HMF. In fresh and untreated fruit juices, HMF is not present. However, when the fruit juices undergo heat treatment and prolonged storage, HMF is produced. HMF is formed in milk upon treatment at temperatures above 120 °C. Infant formulas are fortified with ascorbic acid, lactose, and iron, which are relatively more susceptible to the formation of HMF. The HMF contents found in selected food products are shown in Table 1.

Toxicity of HMF

There are possibilities of colon cancer, tumors in kidney, skin papillomas, induction of chromosomal aberrations, as well as an irritant to the eyes, upper respiratory

tract, skin, and mucous membranes by HMF and related substances that have been reported in the literature. However, it is not confirmed whether human exposure to HMF represents a potential health risk, and the data from epidemiological studies or case reports on potential association of HMF with cancer risk in humans are not available. The *in vitro* and *in vivo* data available raise some concern with respect to genotoxicity (Morales 2009; Kowalski et al. 2013). Studies on rat and mice, however, have indicated potential carcinogenic properties of HMF. HMF can initiate and promote the growth of aberrant crypt foci in rat colons. Furfural and 5-hydroxymethyl-2-furfural induced a significant number of chromosome aberrations. The oral acute toxicity for HMF by oral or gavage administration to rodents is reported as LD50 ranging between 2.5 and 5.0 g/kg bw. Human diet contains abundant amounts of HMF, and humans may ingest up to 150 mg HMF/day, equating to 2.5 mg bw/day for a 60 kg person (Ulbricht et al. 1984).

Maillard Reaction Products

The Maillard reaction is also known as the “nonenzymatic browning reaction.” A French scientist, Louis Camille Maillard, studied the reaction in the period 1912–1917. As mentioned earlier, the Maillard reaction is aminosugar type of browning in which reactions between a free amino group of amino acids, peptides, and proteins and a carbonyl group of reducing sugars occur during the preparation or the subsequent storage of foods. The Maillard reaction also takes place *in vivo*, and hence, to differentiate nonenzymatic glycosylation (in food) with the enzymatic glycosylation of proteins (in living cells); the word “glycation” is used. Proteins get modified *in vivo* during diabetes and aging that produces various products known as advanced glycation end products (AGEs) through various chemical pathways as described for heated foods. The AGEs are involved in several diseases (i.e., pathophysiological processes). The glycotoxins are also used for dietary glycation compounds in heated foods (Henle 2009).

Formation and Occurrence of Maillard Reaction Products in Food

Maillard reactions occur in mainly early, intermediate, and final (or advanced) stages, which is described in Fig. 3.

During early stages of the Maillard reaction, the major reaction products are the lysine derivatives such as *N*- ϵ -lactulosyllysine, *N*- ϵ -fructosyllysine, or *N*- ϵ -maltulosyllysine. Moreover, in infant formula, the Amadori products are *N*- α -lactulosylvaline, or *N*- α -lactulosylleucine is also formed. The 1,2-dicarbonyl compounds (glyoxal, methylglyoxal, 3-deoxyglucosulose) are produced during intermediate stage. The dicarbonyl compounds represent the direct precursors for the formation of AGE as it is produced in advanced stages of the Maillard reaction (Henle 2009). The various AGEs are quantified in foods, viz., *N*- ϵ -carboxymethyllysine (CML), pyrraline, pronyl-lysine, pentosidine, glyoxal-lysine-dimer, methylglyoxal-lysine-dimer, and 3-deoxyglucosulose-lysine-dimer. The chemical structures of CML and pyrraline are shown in Fig. 4.

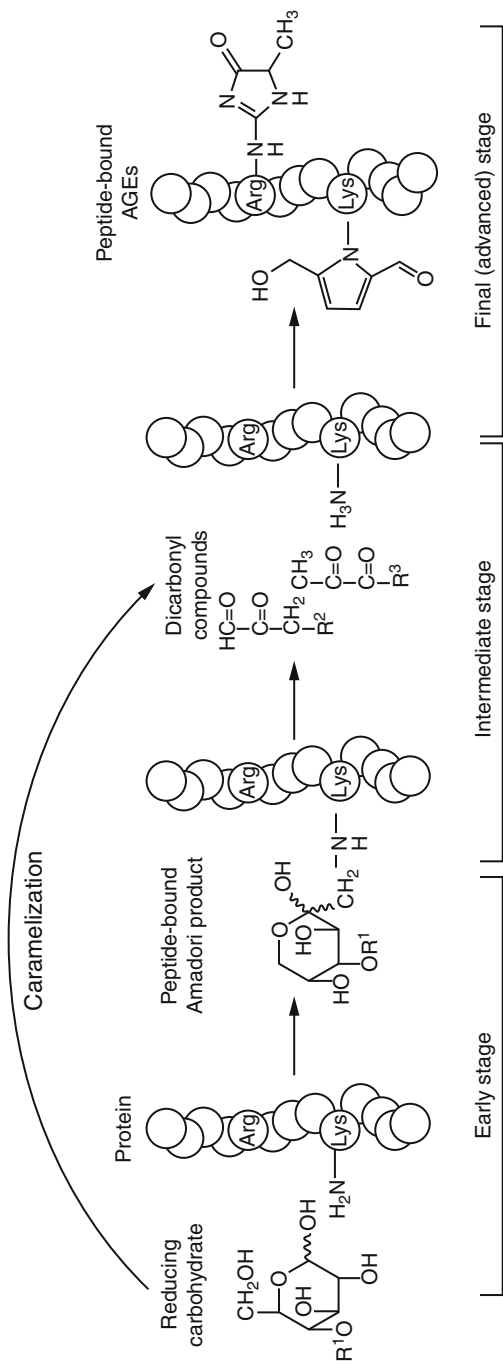


Fig. 3 Stages of Maillard reactions in food. R^1 : H in case of glucose, a β -galactosyl or α -glucosyl moiety in the case of maltose or lactose respectively; R^2 : 3-deoxyglucosulose R^3 : 1-deoxyglucodiulose (Henle 2009; Copyright © 2009 by John Wiley & Sons, Inc. Reprinted with permission)

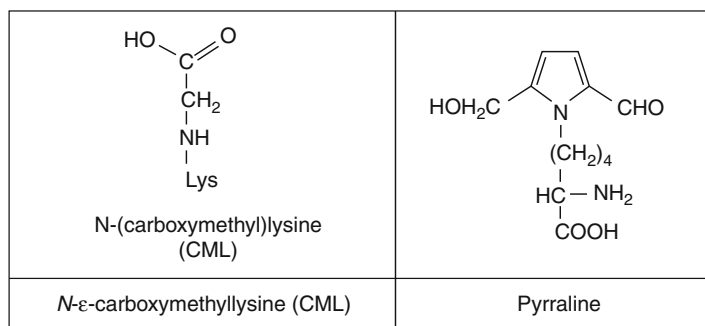


Fig. 4 Chemical structure of CML and pyrraline

The pyrraline is heterocyclic AGEs formed by reaction of the ε-amino group of lysine with 3-desoxyglucosulose. Pyrraline is a suitable indicator for “advanced stages” of the Maillard reaction. The *N*-ε-carboxymethyllysine (CML) was the first amino acid derivative of the advanced Maillard reaction formed from oxidative cleavage of Amadori products. It accounts for 3–10 % of the Amadori products (Büser and Erbersdobler 1986). The Maillard reaction products in foods are shown in Table 2.

The heat treatment of milk and subsequent storage leads to 10–20 % modification in lysine which can extend up to 70 % in case of lactose-hydrolyzed whey. The lysine derivatives like *N*-ε-fructoselysine and *N*-ε-maltoselysine are found in bakery products. Pyrraline is also found in milk products, enteral formula, pasta, and bakery products. The pyrraline concentrations found ranged from 150 mg/kg protein in sterilized milk up to 3,700 mg/kg protein in bread crusts. The concentration of pentosidine was up to 35 mg/kg protein found in roasted coffee and some bakery products.

Toxicity of Maillard Reaction Products

Protein-containing foods that undergo early Maillard reactions contain Amadori compounds which represent the major form of glycation compounds. However, the concentration of advanced glycation compounds is significantly lower and divided up in several individual compounds of varying concentrations. The glycation reaction occurring in vivo was linked to the pathophysiology of diabetes and corresponding biological disorders such as cataract, joint stiffening, or diabetic nephropathy. AGEs represent an important class of uremic toxins because uremic patients were found to accumulate glycation compounds such as pentosidine or CML in the plasma and tissues. However, debate is going on about the toxicological effect of a dietary glycation compound. Food rich in excess AGE may contribute to oxidation and inflammation which cause health effects. The formation of AGEs in the bodies of healthy people is a slow process, but progression of this process is favorable in presence of dietary Maillard reaction products. We usually consume a large quantity of food having AGEs in our dietary life. Therefore, the safety of food AGEs is a problem of concern (Chuyen et al. 2005; Sebekova and Somoza 2007).

Table 2 Maillard reaction products in foods

	Amadori compounds mmol/mol lysine	CML mmol/mol lysine	Pyrroline mmol/mol lysine	Pronyllysine mmol/mol lysine	Pentosidine mmol/mol arginine	Foods	1,2-dicarbonyl compounds	
							Glyoxal	Methylglyoxal
Foods							ppm	ppm
Milk products	–	nd–10	nd–25	–	–	Cheese	4–6	4–11
Pastured milk	1–2	–	–	–	–	Yogurt	0.6–0.9	0.6–1.3
UHT	2–5	–	–	–	nd–0.01	Wine, sherry	0.6–0.9	0.7–1.8
Sterilized milk	up to 50	–	–	–	–	Cocoa	0.9–3.4	0.02
Milk/whey powder	50–200	–	–	–	–	Roasted beans (Coffee)	20–130	20–220
Lactose-hydrolyzed Whey powder	up to 700	–	–	–	–	Brew (Coffee)	–	23–47
Bakery products							–	–
Bread crumb	up to 200	nd–20	1–10	–	nd–0.4	–	–	–
Bread crust	up to 800	–	–	0.01	–	–	–	–
Pasta	up to 400	–	up to 180	0.1	–	–	–	–
Roasted meat	1–10	up to 0.1	nd–13	–	–	–	–	–
			nd	–	–	–	–	–

Adopted from Henle (2009) with modification

nd not detected

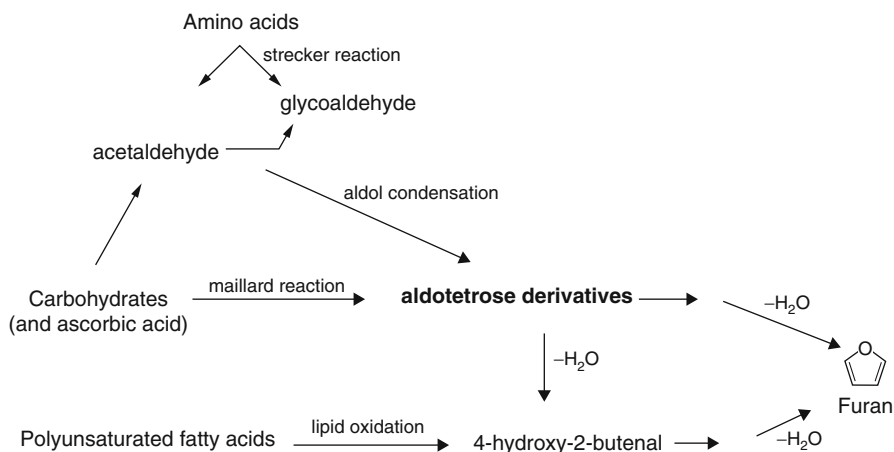


Fig. 5 Formation of furan from amino acids, carbohydrates and polyunsaturated fatty acids

Hazards of Furan

Furan is a colorless chemical (C_4H_4O) having a low molecular weight of 68, a high volatility with boiling point of $31\text{ }^\circ\text{C}$, and water insoluble. Furans are a major class of compounds forming during the Maillard reactions in foods (Maga 1979). The metabolite of furan, *cis*-2-butene-1,4-dial, plays an important role in furan-induced toxicity, including carcinogenesis, probably attributable to a genotoxic mechanism.

Formation and Occurrence of Furan in Foods

Furan is formed from multiple sources, viz., thermal degradation of reducing sugar (glucose, lactose, and fructose) alone or in the presence of amino acids (Maillard reaction); thermal degradation of certain amino acids; thermal oxidation of ascorbic acid, polyunsaturated fatty acids, and carotenoids (Maga 1979; Perez and Yaylayan 2004; Vranová and Ciesarová 2009). According to the US FDA (2004b), a variety of carbohydrate/amino acid mixtures or protein model systems (e.g., alanine, cysteine, casein) and vitamins (ascorbic acid, dehydroascorbic acid, thiamin) have been used to generate furan in food. Becalski and Seaman (2005) reported that furan can be formed through the oxidation of polyunsaturated fatty acids (PUFAs) at elevated temperatures while the addition of commercially available antioxidants (such as tocopherol acetate) reduced the formation of furan up to 70%. The formation of furan (Vranová and Ciesarová 2009) is mentioned in Fig. 5.

US Food and Drug Administration (US FDA) published a report on the occurrence of furan in a number of foods that undergo thermal treatment, especially canned and jarred foods (US FDA 2004a). Parent furan was identified in a small number of heat-treated foods, such as coffee, canned meat, bread, cooked chicken, sodium caseinate, hazelnuts, soy protein isolate, hydrolyzed soy protein, rapeseed protein, fish protein concentrate, and caramel back in the 1960s and 1970s (Maga 1979). The levels of furan in foods are mentioned in Table 3.

Table 3 Furan content in selected foods

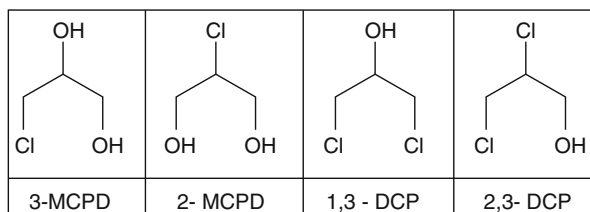
Foods	Furan ($\mu\text{g}/\text{kg}$)
Infant foods	1.3–87.3
Coffee beans/powder	239–5,050
Baby foods	1–112
Fruit preserves	0.9–37
Coffee brewed	3–125
Bread, toasted	18–30
Crisp breads/crackers	4.2–18.6
Fish	1.5–8
Meats	1.7–39
Sauces	3.3–46
Soups	3–125
Vegetables (in cans and jars)	0.8–48
Candy and chocolate	0.5–10.3
Baked beans	23.3–122

Source: EFSA (2004a, b) and FDA (2004)

The analytical method used had a limit of quantification of about 5 $\mu\text{g}/\text{kg}$ for most foods and 2 $\mu\text{g}/\text{kg}$ for most liquids including coffee. The FDA (2004) found that many heat-treated foods contained detectable furan, including almost the entire baby foods sold in jars and many of those sold in cans. The highest levels were for vegetables, particularly beans, squash, and sweet potatoes, packed in jars or cans (Crews and Castle 2007). Substantial levels of furan (20–200 $\mu\text{g}/\text{kg}$) have been reported in foods not cooked in closed containers, including potato crisps, crackers and crisp breads, and toasted bread (Hoenicke et al. 2004). High levels of furan are found in roasted coffee beans, probably on account of the roasting process where the high temperatures exceed most other food-processing procedures (Crews and Castle 2007).

Toxicity of Furan

Furan induces tumors in animal assays; the most remarkable is the induction of hepatic cholangiocarcinomas in rats and mice (Vranová and Ciesarová 2009). IARC (1994) has classified furan as possibly carcinogenic to humans (Group 2B). The European Food Safety Authority (EFSA) has expressed the opinion that “furan is clearly carcinogenic in rats and mice” and that “the weight of evidence indicates that furan-induced carcinogenicity is probably attributable to a genotoxic mechanism” (EFSA 2004a). Furan is absorbed quickly into the body but is also excreted with high efficiency. Furan administered to 50 mice at 8 or 15 mg/kg bw 5 days per week for 2 years causes loss of body weight at 15 mg/kg bw and significantly increases in hepatocellular adenomas and carcinomas (Crews and Castle 2007). It is likely that furan or *cis*-2-butene-1,4-dial reacts with DNA in target cells and can play a role in furan-induced tumors (Peterson et al. 2000). EFSA (2004a) estimated exposure to furan based on the limited data available. Baby food was of particular interest as a high proportion of samples sold in jars and cans contained furan and such foods may form the sole diet of many babies. The estimated intake based on

Fig. 6 Major substances of chloropropanol

consumption of baby food from glass jars was $<0.2\text{--}26\ \mu\text{g}$ furan per day or $<0.03\text{--}3.5\ \mu\text{g}/\text{kg}$ bw per day for a 6-month-old baby weighing 7.5 kg. The daily intake from coffee based on data from 45 samples was $2.4\text{--}116\ \mu\text{g}/\text{person}$, making coffee the major dietary source for adults (Crews and Castle 2007).

Chloropropanols and Chloroesters

Chloropropanols and their fatty acid esters (chloroesters) are formed during the processing and manufacture of certain foods and ingredients (Colin and Peter 2009). The chloropropanols and chloroesters could be formed in hydrolyzed vegetable proteins (HVP) produced by hydrolysis of proteinaceous by-products from edible oil extraction (such as soybean meal, rapeseed meal, and maize gluten) by hydrochloric acid (Velíšek et al. 1979). The main chloropropanol found in HVP was 3-chloropropane-1,2-diol (3-MCPD) together with lesser amounts of 2-chloropropane-1,3-diol (2-MCPD), 1,3-dichloropropanol (1,3-DCP), 2,3-dichloropropanol (2,3-DCP), and 3-chloropropan-1-ol (Colin and Peter 2009), which are shown in Fig. 6. The chloroesters were intermediates in the formation of dichloropropanols (DCPs) and monochloropropanediols (MCPDs) which formed from lipids (Collier et al. 1991).

Formation and Occurrence of Chloropropanols and Chloroesters in Food

The free glycerol was shown to be the major precursor of MCPDs in leavened dough in preparation of bread (Hamlet 2004; Hamlet et al. 2004). During proving, yeast produced the glycerol that reacted with added chloride during baking accounting for approximately 70 % of the MCPDs formed. Addition of glucose promoted MCPD in dough due to removal of potential amino inhibitors (e.g., amino acids) via the Maillard reaction (Breitling-Utzmann et al. 2003; Hamlet and Sadd 2005). The formation of MCPDs from glycerol via the intermediate glycidol (Hamlet et al. 2004) is shown in Fig. 7.

Minor precursors of MCPDs (together with chloride) in dough were found to be monoacylglycerols, lysophospholipids, and phosphatidylglycerols present in white flour used in bread making. These compounds could account quantitatively for the remaining contribution (30 %) to MCPD in bread (Hamlet et al. 2004; Colin and Peter 2009).

Fig. 7 Formation of MCPDs from glycerol via the intermediate glycidol (Hamlet et al. 2004)

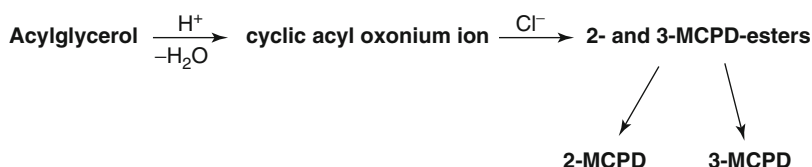
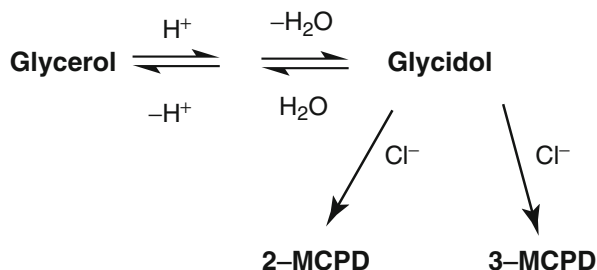


Fig. 8 Proposed mechanism of formation of MCPD-ester from partial acylglycerols

The chloroester formation was correlated with MCPD generation which was increased on heating (Hamlet et al. 2004; Hamlet and Sadd 2004). The presence of low levels of chloroesters in bread crumb due to $<100^\circ\text{C}$ temperature indicates that partial acylglycerols (i.e., mono- and diacylglycerols) may form these compounds. The cyclic acyl oxonium ion formed during this temperature and subsequently the ring structure is opened by chloride ion that can generate chloroesters as intermediate product of MCPD (Colin and Peter 2009), which is shown in Fig. 8.

The chloropropanol (3-MCPD) has been found in cereal, coffee, cheese, licorice, fish, and meat. Thermally processed products (e.g., cereals) account for the greatest incidence of 3-MCPD with some of the highest amounts found in products attaining high temperatures (e.g., bread crust and toasted bread). Processed garlic accounted for the highest incidence and concentration of 3-MCPD which varies from 5 to 690 $\mu\text{g}/\text{kg}$ (Colin and Peter 2009). The 3-MCPD present in various foods is mentioned in Table 4.

The formation of 3-MCPD-esters (monoesters and diesters with higher fatty acids) may be widespread in processed foods derived from cereals, potatoes, meat, fish, nuts, and oils (Hamlet et al. 2004; Hamlet and Sadd 2004; Svejtkovská et al. 2004). Hamlet *et al.* (2004) and Hamlet and Sadd (2004) measured 3-MCPD-esters in bread and toast. The highest amounts were found in regions of the bread that attained the highest temperature (i.e., the crust), and concentrations increased from 60 to 160 $\mu\text{g}/\text{kg}$ when the bread was toasted over 40–120 s. The highest level of 3-MCPD-esters (6,100 $\mu\text{g}/\text{kg}$) was found in a sample of French fries (Svejtkovská et al. 2004). The 3-MCPD-esters present in various foods are mentioned in Table 5.

Table 4 3-MCPD in selected foodstuff

Foodstuff	3-MCPD ($\mu\text{g}/\text{kg}$)
Breads	<10–76
Crust on bread	24–275
Cake, fruit	<10–210
Crackers/toasts	<10–134
Doughnuts	11–24
Cheese	<10–95
Smoked fish	<10–191
Licorice	20–23
Bacon	<5–22
Beef burger/hamburger	7–71
Smoked meats (Bacon)	<10–47

Compiled from Food Standards Agency (2001), Crews et al. (2001), EC (2004), Colin and Peter (2009)

Table 5 3-MCPD-esters in selected foodstuff

Foodstuff	3-MCPD-esters ($\mu\text{g}/\text{kg}$)
Breads (toast)	60–160
Coffee	100–390
Nuts, roasted	433–500
Virgin seed oils	100–300
Virgin germ oils	100–300
Refined seed oils	300–1,234
Refined olive oils	300–2,462

Compiled from Hamlet et al. (2004), Hamlet and Sadd (2004), Colin and Peter (2009)

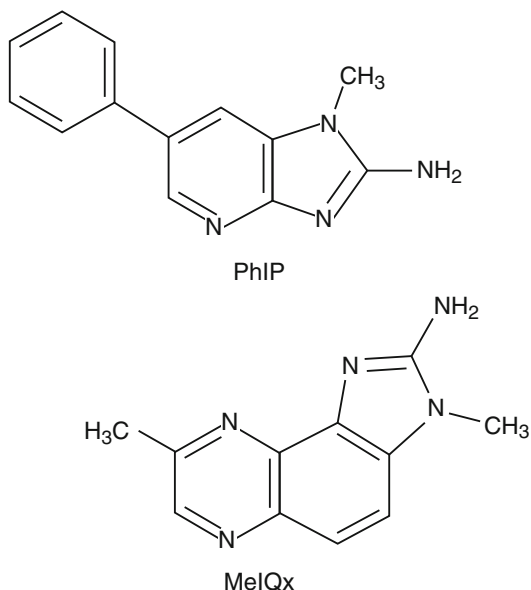
Toxicity of Chloropropanols and Chloroesters

The EU Scientific Committee concluded that the 3-MCPD should be regarded as a genotoxic carcinogen and since a safe threshold dose could not be determined, residues in foods should be undetectable by the most sensitive analytical method (EC 1997). In rats and mice, the kidney was the main target organ for toxicity with effects also observed on male fertility. The 1,3-DCP was hepatotoxic, induced a variety of tumors in various organs in the rat, and was genotoxic in vitro (Schlatter et al. 2002a, b). JECFA (2006) concluded that a representative mean intake of 1,3-DCP for the general population was of 0.051 $\mu\text{g}/\text{kg}$ body weight per day and an estimated high-level intake (young children included) was 0.136 $\mu\text{g}/\text{kg}$ body weight per day.

Heterocyclic Aromatic Amines

Sugimura, a Japanese scientist, first discovered carcinogenic and/or mutagenic heterocyclic aromatic amines (HCAs/HAAs) in fish and meat cooked at temperatures over 150 °C in 1977, and more than 25 HCAs have been isolated and identified in cooked meat products (Nagao et al. 1977; Sanz et al. 2008). Heterocyclic

Fig. 9 Heterocyclic aromatic amines



aromatic amines are potent mutagens at ng/g levels in cooked foods (Sugimura 2002). Some of the HAAs like 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4-dimethyl-imidazo[4,5-f]quinoline (MeIQ) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) reported as possible human carcinogens follow in class 2B as well as probable human carcinogens (2-amino-3-methylimidazo[4,5-f] quinoline (IQ), class 2A) by International Agency for Research on Cancer (IARC 1993; Kizil et al. 2011). Some of the chemical structures of HAAs are shown in Fig. 9.

Formation and Occurrence of Heterocyclic Aromatic Amines in Food

HCAAs can be classified into two major groups called as aminoimidazoazoarenes (AIAs) [or imidazoquinoline (IQ)-type compounds or thermic HCAAs] and aminocarboline [or non-IQ-type HCAAs]. The AIAs are the most important class in cooked foods formed by heat-induced nonenzymatic browning known as Maillard reaction which involves the reaction of free amino acids creatine, creatinine, and hexoses during cooking of foods at conventional cooking temperatures (150–300 °C). They have an imidazo group linked to a quinoline, a quinoxaline, or a pyridine. The aminocarboline are mainly formed by pyrolysis of amino acids and proteins at higher temperatures above 300 °C (Jagerstad et al. 1983; Sugimura and Adamson 2000; Busquets et al. 2004; Kizil et al. 2011). Some of these carboline contain a 2-aminopyridine moiety as a common structure. The formation of them highly depends on method, time, temperature of cooking; type of meat, fat, and moisture content (Minako and Sugimura 1995; Sugimura and Adamson 2000); pH, sugar, free amino acid, and creatinine content of meat; lipid oxidation; and

Table 6 The HCAs levels in some of the cooked foods

Processed foods	PhIP (ng/g)	MeIQx (ng/g)
Chicken, fried	0–70	0–3
Meat extract	0–4	0–80
Beef burger, fried	0–32	0–7
Beef burger, pan residue	0–13	0–6
Beef stock cube	0–0.3	0–0.6
Salmon, fried	0–23	0–5
Fish	0–10	0–2
Poultry	0–330	0–3
Red meat	0–35	0–10

Compiled from Skog et al. (1998) and Busquets et al. (2004)

presence of antioxidants (Pais et al. 1999; Kizil et al. 2011). The HCA levels in some of the cooked foods are shown in Table 6.

Toxicity of Heterocyclic Aromatic Amines in Food

The mutagenicity in *Salmonella* varied more than 160,000 times between the strongest and the weakest HCAs, affected by number and positions of exocyclic substituents, especially the 2-amino-group of the imidazo part of the molecular structure present in most HCAs (Nagao et al. 1977). HCAs have been found to be potent carcinogens, which induce a variety of histological types of tumors in multiple organs following long-term oral administration (Eisenbrand and Tang 1993; Sugimura et al. 1993). It is notable that some HCAs induced tumors of the colon (PhIP, IQ, MeIQ), mammary gland (PhIP, MeIQ), and prostate (PhIP), which are common cancers in Western countries and have been associated with Western life style, i.e., high fat/meat consumption. There is also good epidemiological evidence correlating a high intake of HCAs with colon cancer although this correlation is not consistent (Margaretha and Kerstin 2005). Human exposure to HCAs has been estimated to range from a few ng/day to some µg/day. The major source of human exposure to HAAs is through consumption of household-cooked meats and fish. The range of HAAs detected in foods is highly variable and dependent on cooking preferences, i.e., pan-frying or barbecuing of meats at high temperature produces the greatest amounts of HAAs (Robert 2009).

Acrylamide

Chemically, acrylamide ($\text{CH}_2 = \text{CH-CO-NH}_2$; 2-propenamide) is a water-soluble low-molecular-weight compound (molecular weight 71.08) built up of a reactive ethylenic double bond linked with a carboxamide group (IARC 1994) which is shown in Fig. 10. It is white crystalline solid with melting point $84.5 \pm 0.3^\circ\text{C}$ and a high boiling point (136°C at 3.3 kPa/25 mmHg). Acrylamide is a difunctional monomer, containing a reactive electrophilic double bond and an amide group (Habermann, 1991).

Table 7 Acrylamide levels in different processed foods

Food	Acrylamide (ppb)
Baked products: bagels, breads, cakes, cookies, pretzels	70–430
Beer, malt, and whey drinks	30–70
Biscuits, crackers, Bakery products and biscuits	18–3,200
Bottled prune juice	53–267
Breakfast cereals	30–1,649
Canned baby foods	10–121
Chips/French fries	59–5,200
Chocolate powder	10–909
Chocolate products	2–826
Coffee powder	170–1,188
Crisp bread	800–1,200
Fish products	30–39
Gingerbread	90–7,834
Infant biscuits	10–1,060
Potato crisps	117–4,215
Potato fritters	42–2,779

(e.g., potatoes) varies greatly; there is very wide variability of acrylamide levels even within one food category. Acrylamide is formed when carbohydrate-rich foods are fried, baked, or roasted at high temperatures above 120 °C (INFOSAN 2005). The acrylamide concentrations in different foods are listed in Table 7.

High levels of acrylamide are found in processed foods like bread crust, crisp bread, potato chips, French fries, tortilla chips, different baked goods, coffee, and cereal formulations. However, due to variations in processing, parameters like temperature, time, nature of frying oil, and nature of food matrix are responsible for wide variations in levels of acrylamide in the foods (Margaretha and Kerstin 2005).

Toxicity of Acrylamide

FAO/WHO (2002) estimated an intake of acrylamide to be in the range of 0.3–0.8 µg/kg bodyweight per day for an adult corresponding to 21–56 µg/day for a person weighing 70 kg. Rice (2005) has reviewed the carcinogenicity of acrylamide. The mutagenic and carcinogenic properties of acrylamide are assumed to depend on the epoxy metabolite, glycidamide. Glycidamide induces mutation in bacteria. Acrylamide has a potential to cause a spectrum of toxic effects (IARC, 1994; European Union Risk Assessment Report 2002), including neurotoxic effects as has been observed in humans. IARC (1994) reported that acrylamide and its metabolite glycidamide form covalent adducts with DNA and hemoglobin in mice and rats; acrylamide induces gene mutations, chromosomal aberrations, and cell transformation in mice and rodents, which makes an overall evaluation that acrylamide is probably carcinogenic to humans.

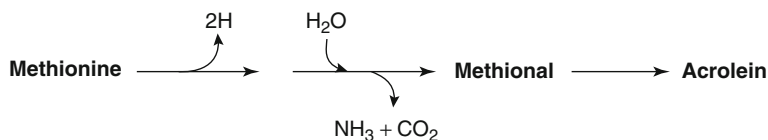


Fig. 12 Formation of acrolein from methionine

Acrolein

In 1839, Berzelius had characterized aldehydes from a thermal degradation product of glycerin, who named it acrolein (2-propenal). Acrolein is ubiquitously present in (cooked) foods and in the environment. The presence of acrolein was reported, in early 1960s, in samples obtained from pyrolysis of fats. Acrolein was reported as a volatile aroma constituent in various foods. It is formed from carbohydrates, vegetable oils and animal fats, and amino acids during heating of foods. Chemical reactions responsible for release of acrolein include heat-induced dehydration of glycerol, retroaldol cleavage of dehydrated carbohydrates, lipid peroxidation of polyunsaturated fatty acids, and Strecker degradation of methionine and threonine.

Formation and Occurrence of Acrolein in Food

Esterbauer et al. (1991) stated that frying of foods in oils produces acrolein. Acrolein reported to form during food processing (neutral pH and 100 ° C) from various amino acids and polyamines. The 3-substituted propanals produced from decarboxylation and deamination of amino acids (e.g., methionine, cystathionine, homocysteine, and homoserine) can readily decompose to yield acrolein (Shibamoto 2009). It was hypothesized that via Strecker degradation, methional formed from methionine and further oxidation of the methional formed acrolein, which is shown in Fig. 12.

Maillard reactions are responsible for generation of cooked flavor in various heated food products as a result of reaction between amino acids and carbonyls. The cooking or dietary oils, as a source of carbonyl reactant, contributed to formation of volatile acrolein. Moreover, processing of lipid-rich foods as well as cooking practices like deep-fat frying are responsible for generation of acrolein (Beauchamp et al. 1985; Shibamoto 2009), especially in the air. The concentrations of acrolein in the air 15 cm above the surface of heated oil were found in the range of 2.5–30 mg/m³ (Shibamoto 2009). The heated lipids are the major source of acrolein in foods. Upon high temperature, acrolein was proposed to form from the dehydration of glycerol (Izard and Libermann 1978); it may also form from oxidative degradation of various fatty acids via radical chain mechanism involving hemolytic fission of R-O bonds more likely to occur at high temperature (Frankel 1982). The formation of acrolein from triglycerides in food is illustrated in Fig. 13.

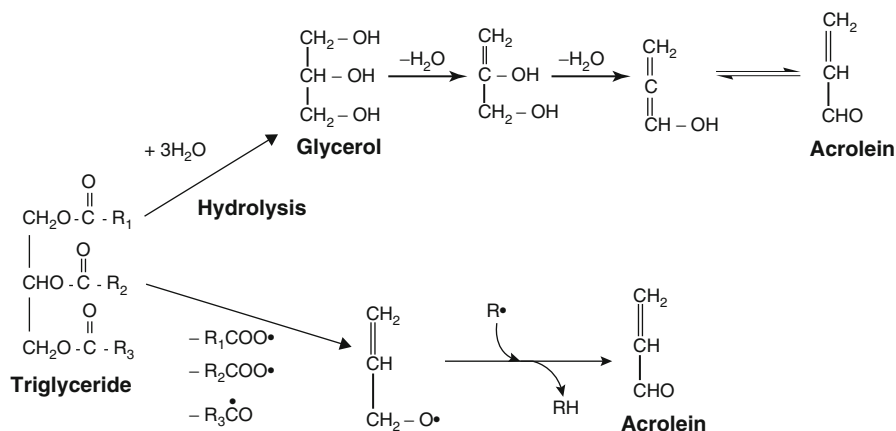


Fig. 13 Formation of Acrolein from triglyceride

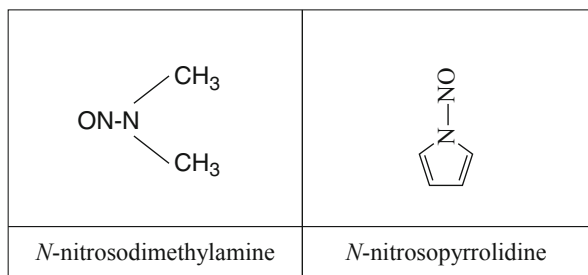
Toxicity of Acrolein

There have been many reports on the toxicity of acrolein. Acrolein is commonly present in the outdoor air as well as indoor air pollution as it forms from lipids such as cooking oils, lipid-rich foods such as beef and pork which undergo at high-temperature processing, and acrolein is mainly concerned with inhalation toxicity. A clinical study was carried out on human volunteers and found that the average threshold of sensation ranged from 0.09 (eye irritation) to 0.30 mg/kg (respiration rate, throat irritation), and nasal irritation occurred at 0.15 mg/kg (0.35 mg/m³) (Weber-Tschopp et al. 1977). A typical acute oral LD₅₀ ranging from 10.3 to 33 mg/kg in Sprague–Dawley rats has been reported (Bioassay Systems Corporation 1981). The ocular and nasal irritation, growth depression, histopathological changes of the respiratory tract, (Feron et al. 1978), as well as Alzheimer's disease (Calingasan et al. 1999) were also found.

Nitrates, Nitrites, and Nitrosamines

Nitrates and nitrites, used as food additives in preserved meats, prevent the growth of botulinum toxin-producing organism. However, nitrates and nitrites have been shown to have adverse effects, responsible for formation of nitrosamines. Nitrites react with secondary amines to form a variety of nitrosamines. Sodium nitrite is used to preserve herring meal which was found to be a source of nitrosamines in food. Methylamines in the fish meal reacted with sodium nitrite to form dimethyl-nitrosamine. Nitrosamines are detected in cured meats, smoked fish, and soy protein foods dried by direct flame. The secondary amines reacting with nitrous acid is considered as principal chemical reaction for formation of *N*-nitrosamines. The former are present at low concentrations in proteinaceous foods. The nitrous acid can be formed from enzymatic reduction of nitrate, which is commonly present in

Fig. 14 Common nitrosamines produced in cooked and heat-processed food



small quantities in water and foods or from sodium nitrite used as a preservative (James and Kenneth, 1975; Richard and Phillip 1975).

Formation and Occurrence of Nitrosamines in Foods

N-nitroso compounds are divided into *N*-nitrosamines, which are stable, and *N*-nitrosamides, which are unstable groups. In acidic conditions, nitrous acid is generated from nitrite and can be reacted with amine which undergoes nitrosation reactions. All nitrosamines have N–N = O as functional group. Nitrosation reaction can occur during the frying of nitrite-cured bacon. The amines necessary for the nitrosation reaction occur widely in many foods. The *N*-nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPYR) are two nitrosamines commonly reported in cooked foods, which are shown in Fig. 14.

Formation of nitrosamines through various processes during manufacture, cooking, and/or storage leads to their presence in food. Major significance for nitrosamine formation in the food is the presence of *N*-nitrosatable amines and of nitrosating agents. A large group of *N*-nitrosoamines, viz., NDMA (*N*-nitrosodimethylamine), NDEA (*N*-nitrosodiethylamine), NPYR (*N*-nitrosopyrrolidine), and NPIP (*N*-nitrosopiperidine) present in food are volatile carcinogenic. However, the main forms of *N*-nitroso compounds in food are nonvolatile (e.g., proteins containing *N*-nitrosated peptide linkages, such as *N*-nitrosoproline) which are noncarcinogenic or nonmutagenic but might act as precursors to volatile carcinogenic nitrosamines (Margaretha and Kerstin 2005). When bacon or smoked belly of pork is fried in which nitrite is added as preservative, *N*-nitrosoproline (NPRO) is produced through nitrosation of the proline, which subsequently decarboxylates to NPYR. High temperature and long frying time increase the amounts of NPYR formed (Margaretha and Kerstin 2005). However, as much as 90 % of the volatile nitrosamines produced during cooking are vaporized (Walker 1990; Tricker and Preussmann 1991; Tricker and Kubachi 1992).

The smoking of fish or meat and direct-fired drying of milk can be accompanied by the formation of oxides of nitrogen leading to the deposition of nitrate in the foods. The formation of *N*-nitrosamines in meat and meat products can occur as a result of various processing techniques such as smoking, salting, and/or curing. NPYR in cured meat is mainly formed by heat-induced decarboxylation of nitrosoproline. In addition to the generation of NPYR by thermal decarboxylation

Table 8 Different *N*-nitrosamines in heat treated food

Foods	Concentration, $\mu\text{g}/\text{kg}$	Types of <i>N</i> -nitrosamine
Meat products	nd-14	NDMA, NPYR, NDEA
Fish products	nd-131	NDEA, NPYR
Beer	<0.2-68	NDMA
Malt beverages	up to 1,080	NDMA
Milk powder	0.07-1.05	NDMA
Instant coffee, infant formula, cocoa, powdered egg and instant soup	<1	NDMA
Dried chillies, dried chili powder	6.1-16	NPYR, NDMA

of NPRO, *N*-terminal NPRO-containing peptides might also act as progenitors. The different *N*-nitrosamines in heat-treated food are shown in Table 8.

Toxicity of Nitrates, Nitrites, and Nitrosamines

NDMA is the most common *N*-nitrosamine found in food that contributes primarily to the total human exposure with volatile *N*-nitrosamines. For most industrialized countries, the estimated average present-day intake of volatile *N*-nitrosamines is approximately 0.2–0.3 $\mu\text{g}/\text{person}$, respectively 3.3–5 ng/kg body weight. Beer, meat products, and fish are considered the main sources of exposure (Michael and Gerhard 2009). Barnes and Magee (1954) reported liver damage and liver tumors in rats after the administration of dimethylnitrosamine. Since then, many researchers worked on biological effects of *N*-nitroso compounds and found them to be carcinogenic, mutagenic, and teratogenic. *N*-nitrosamines, potent carcinogens, found mainly in protein-rich foods include *N*-nitroso-piperidine, *N*-nitroso-diethylamine, *N*-nitroso-pyrrolidine, *N*-nitroso-dimethylamine. These compounds are likely to increase the risk of rectum, bladder, colon, pancreas, and stomach cancers. Preformed and endogenously formed *N*-nitrosamines are well absorbed from the gastrointestinal tract. The rate of absorption varies for different *N*-nitrosamines. *N*-nitrosamines are distributed by the bloodstream and are rapidly metabolized in the liver, particularly at low concentrations. Most of the *N*-nitrosamines are precarcinogens and subject to metabolic activation. The DNA-damaging effect is generally accepted to be the causative factor for the carcinogenicity of *N*-nitrosamines (Michael and Gerhard 2009).

General Aspect on *Trans* Fatty Acids

Saturated and unsaturated fatty acids are present in lipid-containing foods. Most naturally occurring unsaturated fatty acids are found in the *cis* form. *Trans* fatty acids are the *trans* isomers of unsaturated fatty acids. The *cis* and *trans* forms refer to the position of the hydrogen around the double bonds on the fatty acid chain.

When the atoms are on the same geometric side of the chain, they are referred to as being in the *cis* position; those on opposite sides of the chain are in the *trans* position. A high level of *trans* acids will pack together to form hard or crystal fats. The *trans* fatty acids (TFAs) are generally defined as unsaturated fatty acids that contain nonconjugated carbon–carbon double bonds in the *trans* configuration.

Foods containing partially hydrogenated edible oils are major sources of *trans* fatty acids in the diet. A small amount of TFAs in edible oils is produced during the cooking and frying processes. Thermal treatments of fats and oils such as deodorization, cooking, and frying generate TFA isomers. Heating mainly induces *trans*-18:2 and *trans*-18:3 formations. The degrees of TFA formation during frying depends on the frying condition and on the frying materials. When partially hydrogenated fats are used, the formation of TFA is generally lower, but high initial contents of these acids result in a larger concentration of *trans* isomers in fried food. The quantity of *trans* isomers formed at elevated temperature indicates that a specific amount of energy is required to transfer double bonds from *cis* to *trans* configuration. Activation energy for isomerization decreases when the number of *cis* double bonds increases. TFA accumulation in edible oils by heating has been associated with the thermal oxidative deterioration of unsaturated FAs.

Singlet oxygen–induced *trans* fat formation and free radical–induced isomerization are the two mechanisms leading to formation of *trans* fat during heat treatment. Singlet oxygen reacts with *cis* double bonds and alters *cis* double bonds into *trans* configuration. In addition, a free radical can be added reversibly to a double bond to form a radical adduct. When a double bond is reconstructed, *trans* configuration is favored because a *trans* double bond is more thermodynamically stable. Singlet oxygen and free radicals are known as the key initiators in lipid oxidations. During thermal treatment, both lipid oxidation and *trans* fat formation occur simultaneously; however, *trans* fat formation has never been reported along with the lipid oxidation (Tsuzuki 2011; Vu and Siwarutt 2013).

A number of substances like free fatty acids, phospholipids, carbohydrates, proteins, water, chlorophyll, carotenoids, and fatty acid oxidation products that can be present in natural vegetable oils may contribute to changes in color, taste, and aroma, restrict their application, and reduce their shelf life time. Oils are refined to remove these substances. Refining generally includes degumming, neutralization, bleaching, and deodorization steps. In refining, vegetable oils are commonly heated between 60 °C and 100 °C before deodorization. Tasan and Demirci (2003) observed that the TFA 18:2 content increased 13.8-fold at the end of a refining process. Refined edible oils contain a small amount of *trans* fatty acids (0–2 %). Various foods like fast foods, packaged snacks, bakery products, and margarines contain *trans* fat. Fast food contains very high levels of *trans* fat, and it is possible to consume 10–25 g *trans* fat in 1 day. Customers who have a habit of consuming fast food every day have a daily intake of *trans* fat of about 5 g. This level of *trans* fat daily intake is associated with 25 % increase in the risk of ischemic heart disease (Stender et al. 2006).

TFAs are associated with cardiovascular disease, sudden death, and possibly diabetes mellitus. TFAs have hazardous effects on plasma lipoproteins that increase low-density lipoprotein (LDL) levels and decrease high-density lipoprotein (HDL) levels. This condition responsible to increase the LDL/HDL ratio is an important indicator of the risk of development of cardiovascular diseases. High consumption of TFA during pregnancy has been associated to effects on intrauterine development. A rise in allergic diseases has also been observed upon the high ingestion of these fatty acids. The association between TFAs in adipose tissue and the incidence of cancers of the breast, prostate, and colon is still equivocal.

Conclusions and Future Directions

Thermal processing of foods is necessary to make food digestible and palatable, to ensure microbial safety, and to produce a distinct taste, aroma, and texture. This may lead to extensive changes in the foods. The processes occurring during thermal treatment of foods are by far not completely understood and a multitude of compounds formed by thermal reactions not characterized. The nutritional effects of thermal treatment of foods are very diverse. The effects of thermal treatment can be nutritionally beneficial, for example, by increasing the bioavailability of bioactive components from an altered food matrix; however, also some of these bioactive components, particularly water-soluble vitamins and some heat-sensitive phytochemicals, will be lost during heat treatment of foods. The knowledge about the nutritional consequences of chemical and physical changes in food induced by thermal processing is scarce. It has been debated for many years whether thermal processing may create neoallergens. Even though sporadic reports on the formation of quite new allergens have occurred, there is still no good evidence that qualitatively new epitopes are formed during thermal processing of foods. For the influence of thermal processing on food allergenicity, an intelligent combination of biochemical, immunochemical, and clinical techniques must be applied for future research. There are number of toxic compounds formed during heating/cooking, but the question remains which toxicants are of greatest concern in foods from a dietary health perspective? Various compounds have been identified in foods having mutagenic, carcinogenic, or neurotoxic properties at high doses in animal studies. Such toxicants can be classified by structural characteristics or by the processing methods in which they occur. However, chemical changes during thermal processing are a very complex phenomenon. To understand the effect on various nutrients as well as formation of numerous chemical toxic compounds and their health effect requires interdisciplinary knowledge. The involvement of various disciplines and areas of expertise like agronomy, analytical chemistry, food chemistry, food technology, toxicology, epidemiology, nutrition, and consumer research is a very crucial step. Hence, there is need of a strong systematic interdisciplinary research project and effort to shed further light on these complex thermal processing issues.

Cross-Reference

- ▶ [Chemical Composition of Bakery Products](#)
- ▶ [Contamination from Industrial Toxicants](#)
- ▶ [General Properties of Major Food Components](#)

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Pressure Effects on the Rate of Chemical Reactions Under the High Pressure and High Temperature Conditions Used in Pressure-Assisted Thermal Processing

29

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Abstract

Thermal processing technologies are widely used to reduce microbial safety risks and extend the shelf life of foods. In spite of continuous improvements, the development of new technologies has become a necessary response to consumer

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demands for safer foods with closer-to-fresh quality, higher retention of nutrients, and higher bioavailability of phytochemicals promoting health. High-pressure processing (HPP) has become a well-established food pasteurization alternative with numerous and worldwide applications since the first products were first commercialized in Japan in the early 1990s. Efforts in the early 2000s to combine multiple pressure pulses and pressure shifting of pH with HPP treatments at moderate temperatures ($<100\text{ }^{\circ}\text{C}$) failed in achieving the inactivation of bacterial spores at acceptable levels. Pressure-assisted thermal processing (PATP), called pressure-assisted sterilization (PATS) when the process yields shelf-stable foods, is an emerging technology combining the application of high temperature ($>100\text{ }^{\circ}\text{C}$) and high pressure ($>600\text{ MPa}$). Under these conditions, bacterial spores can be inactivated, but the extent of chemical changes must be determined from a quality and safety point of view. However, adiabatic heating during pressurization increases temperature to lethal levels for microorganisms, which in combination with fast decompression cooling can lower the extent of chemical changes to levels below conventional thermal processing. Development of enzyme and microbial inactivation models and calculation methods to assist the design of PATP processes and equipment are advancing rapidly. However, elucidating reaction mechanisms in PATP-treated foods is challenging due to limitations when performing in situ measurements under high pressure. In this chapter, current knowledge of reaction kinetics at high pressure and elevated temperature and information on specific chemical reactions at the temperature and pressure levels required for the pasteurization and sterilization of foods, particularly those reactions known to yield toxic compounds, are presented. Predicting the direction of pressure effects on chemical reaction cannot be predicted unless its activation volume value (V_a) is experimentally determined. Reactions are accelerated or slowed by pressure if V_a is negative or positive, respectively. For example, acrylamide can be formed when foods are subjected to conventional thermal treatments above $100\text{ }^{\circ}\text{C}$. Under PATP treatment conditions, its formation in model systems was inhibited by pressure suggesting a positive V_a for this reaction, but this must be confirmed with experiments in foods.

Introduction

Conventional thermal processing, widely used to increase safety and extend the shelf life of foods, causes also extensive chemical changes (Eisenbrand et al. 2007). Consequently, there is an increasing demand to manufacture products with superior quality and long shelf life (Cruz et al. 2011). In spite of thermal processing technology improvements, new food conservation technologies have become necessary in response to consumer demands for higher safety standards and closer-to-fresh quality, including nutritional value and sensory properties. New products representing major potential opportunities have been engineered and manufactured using emerging technologies such as high-pressure processing (HPP) which can

yield safe food products with a high retention of nutritional compounds and sensorial properties (Spinner 2014; Cruz et al. 2011; Torres and Velázquez 2005). An emerging new food preservation method with high energy efficiency, and which can inhibit microbial growth with minor effects on food quality, is hyperbaric storage (HPS) using pressure levels in the 25–250 MPa range (Queirós et al. 2014; Fernandes et al. 2015; Duarte et al. 2014; Segovia-Bravo et al. 2012). Consumer acceptance of HPP food products keeps rising, and by 2018 they are expected to represent a \$12,000 million USD market, while sales of HPP equipment will reach \$600 million USD. Typically, HPP foods are treated at 500–600 MPa, initial temperature below 40 °C, and 3–5 min holding time. Commercial products include refrigerated products such as fruit juices, avocado products, deli meats, seafood, ready-to-eat meals, and salsas (Torres et al. 2009a; Torres and Velazquez 2008).

HPP treatments exert only a pasteurization effect since the inactivation of bacterial spores by pressure alone is not feasible (Torres and Velazquez 2008; Mújica-Paz et al. 2011; Serment-Moreno et al. 2014). Bacterial spores can survive up to 1000 MPa when treated by pressure alone (Patterson 2005). Although bacterial spores are highly resistant to harsh environments, they lose their resistance after germination. Thus, triggering germination as a preprocessing treatment would be an effective strategy to inactivate spores at low temperatures (<100 °C). It has been reported that pressure treatments in the range of 100–500 MPa can trigger the germination of *Bacillus* spores, suggesting that spores could be inactivated by pressure cycle treatments (Heinz and Knorr 2001; Meyer et al. 2000). However, this alternative is not effective on *Clostridium* spores characterized by a germination process that does not respond to high pressure (Paredes-Sabja et al. 2007). Although chemicals with GRAS-status has been identified as promoters of germination and demonstrated for the inactivation of *C. perfringens* in meats (Akhtar et al. 2009), the research is still fairly incomplete. Thus, it is not yet possible to use this alternative in the commercial production of HPP-treated foods. At present the only feasible sterilization alternative is to apply high pressure at elevated temperatures, and therefore, it is no longer possible to assume that the pressure treatment will not induce significant chemical changes in foods.

Pressure-assisted thermal processing (PATP), or pressure-assisted thermal sterilization (PATS) when achieving the required inactivation level of bacterial spores, has been reported to require pressure and temperature conditions higher than 600 MPa and 100 °C (Shao et al. 2010; Valdez-Fragoso et al. 2011; Wilson et al. 2008). *C. botulinum* and *B. amyloliquefaciens* are considered as the most high-pressure-resistant bacterial spores relevant to food intoxication and spoilage, but high pressure and temperature combination can inactivate these spores (Margosch et al. 2006). Wimalaratne and Farid (2008) reported that *Geobacillus stearothermophilus* and *Bacillus cereus* spores can be inactivated by PATS (~100 MPa and 80–125 °C) faster than using the same temperatures alone in both water and milk. PATP can yield shelf-stable foods of high quality while retaining constituents with desirable health benefits. However, PATP conditions can break covalent bonds, and thus losses of nutrients, color pigments, and flavor compounds should be expected (Ramirez et al. 2009). Furthermore, under PATP conditions the

formation of some toxic compounds may reach rates not observed in conventional thermal processes (Segovia Bravo et al. 2012).

Consumer perceptions that HPP products are closer to “natural” with high retention of nutrients including health-enhancing components with high market value have facilitated the acceptance of these products (Cruz et al. 2011). On the other hand, PATP is not yet a commercial technology and will require more complex safety validation procedures than HPP, particularly for the production of shelf-stable low-acid foods ($\text{pH} > 4.5$). In PATP, foods are preheated to 70–90 °C, and then the adiabatic compression heating of the food and pressurizing fluid increases the food temperature to levels lethal to bacterial spores. Moreover, subsequent decompression reduces food temperature to values below those causing significant thermal degradation. The rise and decrease in temperature due to adiabatic heating and cooling is an important advantage as it reduces the exposure of the food to high-temperature conditions. The analysis of chemical reactions in PATP-treated foods is necessary when marketing foods in countries regulated by novel food laws (Hepburn et al. 2008). In the EU and countries following similar regulations (e.g., Canada), this safety evaluation must follow the “substantial equivalence” comparative principle, i.e., the novel food must have similar nutrient content when compared with similar products obtained by conventional technologies. If toxic compounds are detected in the PATP products, a process of hazard identification, characterization, and assessment of the exposure risk to consumers must be carried out (Tritscher 2004). By contrast, in the USA, a PATP-sterilization process approved for mashed potatoes required no such characterization of toxicity risk nor of nutritional equivalency (The Weekly 2009).

Chemical Reactions Under High Pressure

Information on vitamin, pigment, and flavor compound retention under conditions ensuring spore inactivation requires substantially more research. Unfortunately, limitations of many high-pressure research publications include missing data such as sample temperature, pressurization rate, and incomplete sample characterization including dissolved oxygen concentration and at least initial and post-treatment pH; limitations of applying gas reaction chemistry principles to chemical reactions in foods under PATP conditions; or incorrect applications of physical principles. Although the maximum pressure covered in studies on chemical changes in foods and model systems under PATP conditions is ~850 MPa, temperatures above 100 °C required to achieve the sterilization of low-acid foods are less frequently included.

Effect of Dissolved Oxygen

Additional studies are needed to evaluate the effect of dissolved oxygen on the chemical degradation of flavor compounds and nutrients in PATP-treated foods since Oey et al. (2006) demonstrated that PATP can increase the degradation rates with the

dissolved oxygen concentration. Serment-Moreno et al. (2014, 2015) reviewed primary and secondary kinetic models that could be used to design PATP treatments.

Effect of Pressure-Induced pH Shifts

Water ionization changes with pressure resulting typically in a lower pH. These pressure-induced pH changes in foods have been poorly studied because in situ pH measurements were not possible until recently. Samaranayake and Sastry (2010, 2013) developed a pH sensor for pressures up to 825 MPa at room temperature, but the device needs to be validated for high-temperature use. It consists of a Nafion membrane placed between two reverse osmosis membranes that selectively isolate the migration of hydrogen ions from the test solution when a 10 V (DC) voltage is applied to the two chromel wires acting as electrodes (Fig. 1). The membrane assembly and the test solution are enclosed in silicon tubing sealed with luer fittings and allowing pressure transmission. Electrical connections are contained in a Teflon tube sealed with epoxy resin. A mathematical model was developed to relate

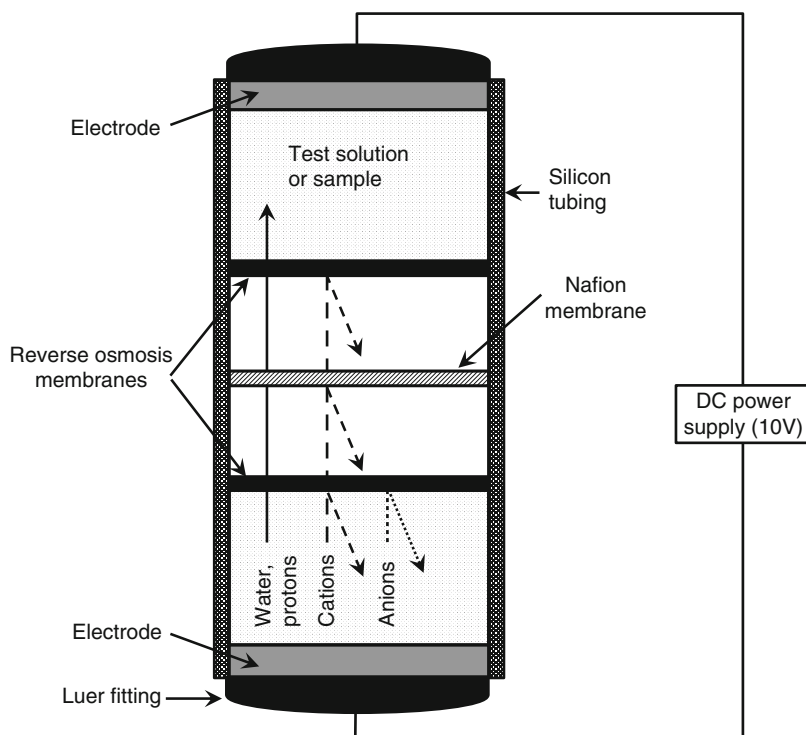


Fig. 1 Schematic construction of sensor capable of in situ pH measurements of semiliquid and liquid foods at high pressure (Modified from Samaranayake and Sastry 2013)

the pH shift to density (ρ) and proton conductivity (σ) ratios using atmospheric pressure as reference (subindex 0, Eq. 1):

$$\text{pH}_0 - \text{pH}_p = \log\left(\frac{\rho_p}{\rho_0}\right) + \log\left(\frac{\sigma_p}{\sigma_0}\right) \quad (1)$$

Water plays a critical role in the rate of reactions yielding charged particles (ions) from neutral species. These ions generate strong electric fields changing the orientation of surrounding water molecules. This electric field decays rapidly with the distance from the ions. Consequently, water molecules in the first hydration layer are situated in a field of considerably higher strength than those located outside of this shell (Danielewicz-Ferchmin and Ferchmin 1998). A strengthened electric field around ions attracts more solvent molecules, and therefore, the volume of the solvent in the hydration shell is smaller than that in the bulk. Molecular dynamic simulations of Li + F- ion pair in water showed that the first hydration layer contains between 1 and 2 more water molecules than the number observed for water in the absence of strong ions (Hamann 1981). This volume contraction due to a charged particle is known as electrostriction and depends on the charge and size of the ion and solvent properties.

According to Le Chatelier's equilibrium principle, the reaction molar volume change ΔV^0 can be used to predict the direction of the equilibrium displacement of chemical reactions. This value, defined as the difference between the partial molar volumes of products and reactants, is estimated from the pressure partial derivative of the reaction equilibrium constant K (Eq. 1, Torres et al. 2009b). Because of electrostriction effects, $\Delta V^0 < 0$, and thus pressure promotes the dissociation of ionizable substances, such as salts, acids, bases, and polyelectrolytes. The formation of ions results in a reversible and temporary change in pH that is reestablished upon pressure release (El'yanov and Hamann 1975; Hamann 1982). From a food processing perspective, the pressure-induced pH shift impacts protein denaturation (Smeller 2002), microbial growth (Mathys et al. 2009; Molina-Guitierrez et al. 2002), and the kinetics of chemical reactions (Jenner 2004; Hamann 1981). Although pH returns to its original value during depressurization, the pressure-induced pH shift will affect the rate of chemical reactions and the inactivation rate of enzymes and microorganisms while the food is under high pressure (Eq. 2, Paredes-Sabja et al. 2007):

$$\Delta V = -RT \left(\frac{\partial \ln K}{\partial p} \right)_T \quad (2)$$

$$(\text{p}K_a)_p = (\text{p}K_a)_0 + \frac{p(\Delta V^0)}{RT(1 + bp)} \quad (3)$$

where

$(\text{p}K_a)_p$ = pressure-shifted dissociation constant

$(\text{p}K_a)_0$ = dissociation constant at the reference pressure (0.1 MPa)

p = pressure (MPa)

ΔV^o = reaction molar volume change ΔV of the dissociating acid at 0.1 MPa
($\text{m}^3 \text{mol}^{-1}$)

R = universal gas constant, $8.31446 \text{ (Pa m}^3\text{)/(}^\circ\text{K mol)}$

T = absolute temperature ($^\circ\text{K}$)

$b = 9.2 \times 10^{-4} \text{ MPa}$ (assumed constant for all acids)

Samaranayake and Sastry (2010, 2013) determined pressure-induced pH shifts in buffer systems and semiliquid and liquid foods at 0.1–785 and 0.1–800 MPa, respectively. The pH of organic (acetate, biphthalate, phosphate, and sulfanilate) and biological (ACES, citrate, HEPES, MES, and TRIS) buffers decreased by 0.05–0.32 units at 785 MPa ($p < 0.05$) and returned to initial values upon depressurization. Differences in values predicted by Eqs. 1 and 2 for organic buffers were attributed to discrepancies in the optical and sensor approaches used for pH measurements. The effect of pressure up to 500 MPa on the pH of distilled water and commercial food products showed that the former experienced the highest pH decrease (-0.69 ± 0.07 units), while milk and fruit juices exhibited only a slight pH change (<0.3 units). The pH evolution of pressurized chicken broth and semiliquid foods was similar with a higher acidification at 100–300 MPa before stabilizing at 0.30 to 0.58 pH units below their initial value. The stability of milk and fruit juice pH might be due to the presence of weak organic acids acting as buffer agents in food systems. The high compressibility of fats and air bubbles in chicken broth and semiliquid foods may promote hydrogen bond formation causing a steep pH fall below 300 MPa, whereas the breaking of hydrogen bonds and exposure of hydrophobic moieties in denaturalized proteins would explain the subsequent pH stabilization at 300–800 MPa. The availability of a pH sensor is certainly promising, but further experimentation is needed, particularly in foods pressure treated at high temperature.

Rate of Chemical Reactions Under High Pressure

Kinetic studies on chemical reactions are fundamental to determine the final quality and safety of a food product. Chemical reactions can result in the formation of off-flavor volatiles or the degradation of desirable compounds and either can be the quality reason for product rejection by consumers. On the other hand, the formation of a toxic compound can compromise the product safety. Thus, understanding the mechanisms of chemical reactions and the factors impacting chemical reaction rates is important, enabling process designs and optimization of products with the desired quality (van Boekel 2008) and required safety (Segovia Bravo et al. 2012). For chemical reactions of order n , a primary model describing the effect of process time (t) on the level or concentration ($c(t)$) of a quality or safety factor as affected by pressure (p) and temperature (T) can be expressed as follows:

$$\frac{dc(t)}{dt} = k(p, T) c(t)^n \quad (4)$$

where $k(p, T)$ is the reaction rate constant at a given p and T level. Under isobaric and isothermal conditions, integration of Eq. 3 yields the following expressions:

$$\text{Zero order : } c(t) - c_0 = k(p, T) t \quad (5)$$

$$\text{First order : } \log c(t) - \log c_0 = k(p, T) t \quad (6)$$

$$\text{Second order : } \frac{1}{c(t)} - \frac{1}{c_0} = k(p, T) t \quad (7)$$

Zero-order reactions have been reported in the formation of volatile compounds and sugar dehydration in milk (Ramirez et al. 2009; Martínez-Monteaugudo and Saldaña 2014) and for the formation of free radicals in chicken meat (Medina-Meza et al. 2014), whereas thermolysin inactivation at 100 MPa has been reported to follow a second-order reaction (Fukuda and Kunijigi 1984). Deviations from the trends dictated by Eqs. 4, 5, and 6 are frequently observed (Serment-Moreno et al. 2014; Chakraborty et al. 2014). For example, Buckow et al. (2010) reported a kinetic expression with $n = 1.4$ for the anthocyanin degradation in blueberry juice treated at 100–700 MPa and 40–121 °C. Also, Buckow et al. (2009) used a 2.2 kinetic order to describe the polyphenoloxidase inactivation in cloudy apple juice at 0.1–700 MPa and 20–80 °C. Starch gelatinization at 0.1–600 MPa and 30–75 °C was described using a 1.65 reaction order expression (Buckow et al. 2007), while $n = 1.5$ resulted in the best fit for alkaline phosphatase at 400–500 MPa, 5–40 °C (Rademacher and Hinrichs 2006).

Nonlinearity of chemical reaction kinetics may reflect the involvement of a reaction mechanism with multiple steps. When measuring the greenness of broccoli juice treated at 0.1–850 MPa and 70–90 °C, Weemaes et al. (1999) modeled the decomposition of chlorophyll to pheophytin and its further degradation to pyropheophytin as two successive first-order reactions. Temperatures below 50 °C showed no chlorophyll loss in broccoli juice regardless of the pressure level, whereas the pyropheophytin concentration was negligible when the juice was pressure treated at 50–70 °C. Verlinde et al. (2009) developed a complex ordinary differential equation system based on the 5-methyltetrahydrofolic acid degradation pathway at ambient pressure. The concentration of the degradation products of 5-methyltetrahydrofolic acid solutions (0.4 μM) was quantitatively and qualitatively determined after treatments at 100–700 MPa and 35–60 °C. The formation of all intermediaries and final products considered in the differential equation system was assumed to follow first-order kinetics.

Experimental determinations of $c(t)$ conducted at “constant” p and T levels can be analyzed with conventional statistical or information theory criteria tools (Serment-Moreno et al. 2015) to select the order model with the best fit. A major challenge is to obtain experimental data at constant p and T levels starting from an unpressurized sample at refrigeration or room temperature, then preheated in the

vessel to reach lethal temperatures to the microorganism of concern, and finally subjected to compression heating. Differences in the thermophysical properties of food components and pressurizing fluid, combined with heat transferred between samples, pressurizing fluid and vessel, and of heat losses from the pressure vessel to the environment, result in sample temperature changing as a function of time and sample placement or position in the vessel. These temperature variations may affect the accuracy when determining the chemical reaction rates. This also means that the c_o value in Eq. 4, 5, and 6 is at best a “pseudo-initial concentration.” If c_o values at each test temperature do not change with pressure regardless of the treatment time, it can be assumed that the effects of experimental sample handling and heat losses have been minimized.

The Le Chatelier’s principle and the transition state theory can help in explaining the effects of pressure on chemical reactions. The Le Chatelier’s principle applies to a reaction system able to reach equilibrium in a short time such as the case of pressure-induced pH shifts. It states that if the intensity of a particular extensive variable changes, for example, pressure, the equilibrium shifts in the direction that reduces the change in the corresponding intensive variable, volume (Jenner 2004). The relative short processing times and the complexity of food matrices make it difficult to reach equilibrium for most chemical reactions. Food matrices are very complex systems with several components that simultaneously undergo chemical transformations at different rates. Consequently, the rate at which the reactions take place is often more important than the equilibrium point, and therefore, the transition state theory is used to determine whether a particular reaction is promoted or inhibited by pressure. The transition state theory based on the activated state concept provides a useful conceptual link between kinetic data and the molecular rearrangements of a chemical reaction.

Activation Energy (E_a) and Activation Volume (V_a)

The activated state is a most energetically favorable position (Laidler 2002) induced by the random collision of reagent molecules. It represents the maximum energy relative to the molecular motion and a minimum energy with respect to the reaction coordinate (El’yanov and Hamann 1975). Although the activated state is a very short-lived complex, it has its own thermodynamic properties. In the theoretical frame for the reaction pathway developed originally for ideal gas reactions (Serment-Moreno et al. 2014), it is assumed that a reactant fraction reaches first this activated state (Fig. 2a) requiring an energy level increase above that of the reactant characterized by the temperature-independent Arrhenius activation energy (E_a , Eq. 7). This unstable state is followed by the formation of reaction products characterized by negative or positive heat of formation defining the case of endothermic and exothermic reactions, respectively. If the molar volume of the intermediate state (activated complex) differs from that of its reacting components, the reaction velocity will increase or decrease with pressure, according to whether the intermediate state is less or more voluminous than the reactants

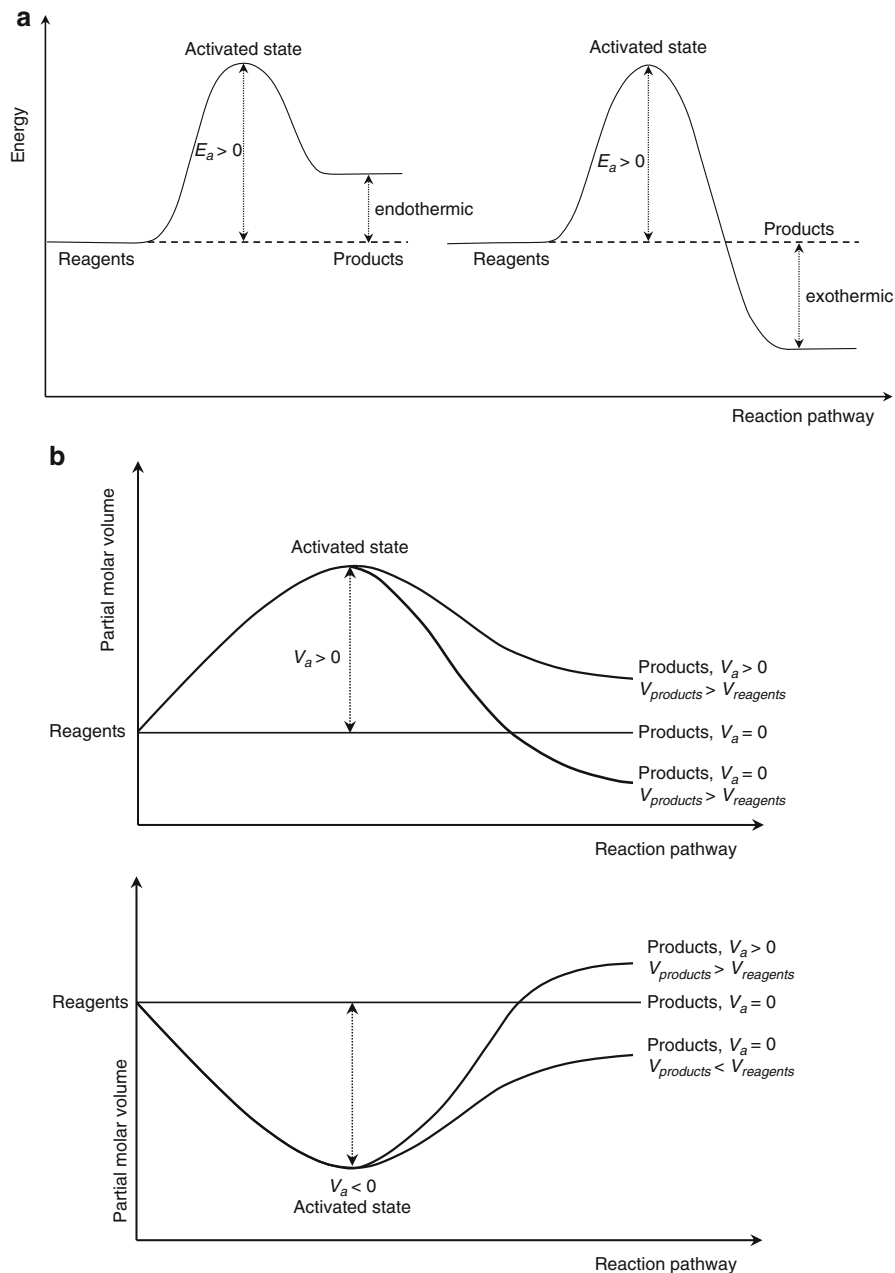


Fig. 2 Schematic definition of the activation energy (E_a) and of the activation volume (V_a) for a chemical reaction following the transition state theory. **(a)** Activation energy (E_a) value for endothermic and exothermic reactions. **(b)** Activation volume (V_a) for reactions inhibited ($V_a > 0$), inhibited ($V_a < 0$), or not affected by pressure ($V_a = 0$) with products having products with partial molar volume lower or higher than the reactants

(Wentorf and de Vries 2004). The pressure effect on E_a values can be analyzed utilizing the partial activation molar volume (V_a) concept (Fig. 2b), defined as the partial molar volume difference between the activated state and the reacting components at the same pressure and temperature (Eq. 8, McNaught and Wilkinson 1997). Equation 8 is integrated to obtain Eq. 9 where $\ln A$ is the integration constant. V_a values at constant temperature, obtained by linear regression of $\ln(k)$ versus pressure p , may be negative, positive, or zero (Torres et al. 2009b). Large magnitude V_a values (positive or negative) means that the reaction is highly sensitive to pressure, while a $V_a = 0$ indicates that it is pressure independent. Moreover, E_a value will decrease, remain unchanged, or increase with pressure if V_a is negative, zero, or positive, respectively. If V_a is negative, the reaction rate will increase with pressure, while the opposite effect will be observed if V_a is positive. The rate of chemical reactions with $V_a \gg 0$ will decrease with pressure to such an extent that no changes will be observed during the process time required for enzyme or microbial inactivation. On the other hand, quality losses (e.g., nutrient losses) and formation of undesirable compounds will increase significantly if the reactions yield a $V_a \ll 0$. Finally, the pressure and temperature effects on chemical reactions may be simultaneously modeled by combining Eqs. 7 and 9:

$$\ln(k) = \ln(k_0) - \frac{E_a}{RT}; \quad R = \text{universal gas constant}, \quad (8)$$

$$k_0 = \text{frequency constant}$$

$$V_a = -RT \left(\frac{\partial \ln k}{\partial p} \right)_T \quad (9)$$

$$\ln k = \ln A - \frac{(V_a) p}{R T} \quad (10)$$

Application Example of the Activation Volume Concept to Describe Pressure Effects on Chemical Reactions

The effect of pressure on the formation of 27 volatile compounds associated with cooked flavor in PATP-treated milk was analyzed by Vazquez et al. (2007) using the concepts described in the previous section. A previous principal component analysis (PCA) study had identified significant volatile profile differences among treated milk samples falling into one of three groups: (1) moderate PATP treatments and conventional pasteurization, (2) severe PATP treatments approaching conditions required for the inactivation of bacterial spores, and (3) conventional UHT sterilization (Vazquez et al. 2006). PATP milk treated at moderate temperature and pressure levels reached a refrigerated shelf life longer than 7 weeks and had a volatile profile similar to thermally pasteurized milk with a shelf life of about 2 weeks. The PCA for milk subjected to severe PATP processes, i.e., conditions

approaching those needed for commercial milk sterilization, showed a smaller volatile profile shift and in a different direction when compared to the one for PATP pasteurization treatments and commercial UHT milk.

The first-order kinetic model fitted well to the changes in the concentration of straight chain aldehydes yielding E_a values which decreased significantly with pressure suggesting that their formation reactions had $V_a < 0$. For example, E_a values for hexanal formation decreased nearly 40 times from 35.2 kJ mol^{-1} at 482 MPa to 0.9 kJ mol^{-1} at 655 MPa. In reactions following zero-order kinetics, E_a values increased with pressure for 2-methylpropanal and 2,3-butanedione suggesting formation reactions with $V_a > 0$ whereas they remained practically unchanged for the formation of hydrogen sulfide regardless of the pressure level suggesting that it originated from a reaction with $V_a = 0$. Most significantly, the concentration of the remaining 18 volatiles analyzed in PATP milk did not change under high pressure suggesting that their formation reactions had very large positive V_a values ($V_a \gg 0$). The observation that straight chain aldehydes were accelerated by pressure suggested that an antioxidant could be used to control this negative effect of pressure. L-Cystine, BHA, epicatechin, ascorbic acid, and β -carotene were all able to inhibit aldehyde formation, but the latter two were less effective (Vazquez and Qian 2007). Important conclusions from the PATP milk study are that moderate conditions ($\sim 500 \text{ MPa}$, $60 \text{ }^\circ\text{C}$ and up to 5 min) deliver milk with a much longer shelf life and a volatile profile comparable to that obtained by HTST pasteurization and that the increase, decrease, or lack of change caused by pressure on the formation of volatiles could be described with no need to assume alternative reaction pathways for PATP treatment conditions.

Effect of Pressure on Reactions Associated with the Loss of Food Quality

Pressure modifies interatomic distances, acting only on weak interactions where the bond energy is distance dependent, such as van der Waals forces, electrostatic forces, and hydrogen bonding (Saldaña and Martínez-Monteaudo 2014). There are three important consequences of these interatomic distance alterations: (1) changes in physical properties, such as melting point, solubility, density, and viscosity; (2) effects on equilibrium processes, such as dissociation of weak acids, acid–base equilibria, and ionization; and (3) effects on the rates of processes, such as delaying or accelerating the rate of specific reactions. In pressure-treated products, some desirable effects are the result of all three phenomena. For example, inactivation of microorganisms is a combination of changes in the physical properties of membrane lipids, in the chemical equilibrium controlling the intracellular pH, and in the rate of specific physiological functions that cause irreversible or lethal damage on bacteria cells (Molina-Gutierrez et al. 2002).

In tomato juice, the application of 500 MPa for 3 min increased the concentration of hexanal (Porretta et al. 1995), while in cherry tomato puree treatments of 800 MPa at $20 \text{ }^\circ\text{C}$ for 10 min significantly increased the concentration of straight

aldehydes, such as hexanal, heptanal, and octanal (Viljanen et al. 2011). According to these authors, the hexanal increase originates from the autoxidation of fatty acids. The stability of anthocyanins during PATP treatments has been studied in raspberries and strawberries (Verbeyst et al. 2010, 2011). The degradation kinetics was described by an Arrhenius–Eyring model showing a synergistic effect of pressure and temperature. A synergistic effect has been also suggested by Corrales et al. (2008) who reported that anthocyanin condensation reactions are promoted by combinations of pressure and temperature. Model solutions of cyanidin-3-*O*-glucoside (Cy3gl) and pyruvate treated at 0.1 MPa/70 °C showed around 5 % of condensation, while significant degradation of Cy3gl (around 25 %) was observed at 600 MPa and 70 °C. Apparently, the condensation reaction mechanism at 600 MPa is the same as the one reported for atmospheric pressure conditions. In the 0.1–600 MPa range, the reaction does not take place unless the processing temperature is 70 °C or higher. Condensation reactions of anthocyanins were also investigated by pressurizing wine at 600 MPa, 70 °C for 1 h. The concentration of malvidin-3-*O*-glucoside decreased and resulted in the formation of three unidentified chemical compounds with higher molecular weight (Corrales et al. 2008). Pressure-induced cyclizations also take place when peptide amides are treated at 800 MPa, 80 °C (Corrales et al. 2007). The proposed mechanism of H-Leu-Gly-NH₂ begins with the hydrolysis of the C-terminal amide, followed by a cyclization step yielding cyclo(Leu-Glu) and other unidentified organic compounds.

Lipid oxidation under high pressure is a topic of major research interest since it frequently results in formation of off-flavors perceived as rancidity and lower nutritional value due to the loss of polyunsaturated lipids (Ma and Ledward 2013). In general, lipid oxidation starts with the exposure of lipids to heat, light, or catalysts such as enzymes or metal ions resulting in the cleavage of a covalent bond and the loss of a proton. Furthermore, alkyl free radicals yield hydroperoxides by reacting with oxygen and other oxygen reactive species. Hydroperoxides react with other lipid molecules to yield more radicals or degrade into compounds with lower molecular weight such as aldehydes, ketones, alcohols, or epoxides (Medina-Meza et al. 2014; Martínez-Monteagudo and Saldaña 2014). HPP and PATP have been reported to promote lipid oxidation, although the mechanisms for free radical formation need to be further explored. Three considerations are used to explain the formation of free radicals at high pressure: (1) formation of pressure/temperature-induced radicals; (2) release of enzymes, metal ions, or pro-oxidant compounds from microstructure changes in membranes induced by pressure; and (3) the catalytic effect of metal ions from denaturalized proteins (Medina-Meza et al. 2014; Behnlian et al. 2014).

The pressure/temperature induction of prooxidant compounds has been widely studied in several food systems. Heat treatments at 60–120 °C for 14 min reduced conjugated linoleic acid (CLA) content in CLA-enriched milk by ~10–40 % (Martínez-Monteagudo et al. 2012). Pressure treatments at 100–350 MPa of enriched milk resulted in 60 % CLA retention, but more severe pressure–temperature conditions accelerated CLA degradation (~97 % loss at

600 MPa, 120 °C, 14 min). The potential oxidation mechanism was determined by quantifying the hydroperoxide concentration in CLA-enriched milk and controlled milk treated for 14 min at 600 MPa and 90–120 °C (Martínez-Montea-gudo et al. 2012). Higher hydroperoxide concentrations were found in non-enriched milk ($6.5 \pm 0.1 \text{ mmol ml}^{-1}$) when compared to CLA-enriched milk samples ($5.26 \pm 0.21 \text{ mmol ml}^{-1}$). The lower hydroperoxide concentration in CLA-enriched milk may reflect the antioxidant activity of CLA, which hindered the formation of free radicals responsible for lipid oxidation. A second explanation proposed by the authors is that CLA degrades into other primary oxidation products such as monomeric and cyclic peroxides rather than hydroperoxides. Additionally, the quantification of dissolved oxygen in milk indicated that oxygen might be capable of isomerizing CLA without altering the total CLA concentration (Martínez-Montea-gudo and Saldaña 2014). Conversely, Knockaert et al. (2011) reported no significant changes in β -carotene isomerization when carrots were treated under similar processing conditions (600 MPa, 121 °C, 5–10 min), stating that the cellular structure of carrot provided a protective effect. Further studies on CLA-enriched milk showed that the addition of nisin (16 mg g^{-1} of milk) reduced the population of *Bacillus amyloliquefaciens* spores in PATP-sterilized CLA-enriched milk (600 MPa, 120 °C, 5 min) and retained ~40 % of CLA initial concentration after 60 days of storage at room temperature (Martínez-Montea-gudo and Saldaña 2014).

Zhu et al. (2014) studied the effects of primary oxidation products (hydroperoxides) and secondary lipid oxidation products (thiobutyric acid reactive compounds) on the stability of fish oil emulsions containing eicopentaenoic acid (EPA; 67 mg g^{-1} oil) and docosahexaenoic acid (DHA; 226 mg g^{-1} oil). The formation of lipid oxidation products after 15 min treatments at 0.1–700 MPa at 20 and 50 °C and 600 MPa at 20–75 °C was used to evaluate pressure and temperature effects, respectively. The concentration of hydroperoxides and thiobutyric acid reactive compounds increased at pressures above 300 MPa. At 600 MPa, propanal levels remained similar to those measured for untreated samples unless the temperature was higher than 40 °C. Hydroperoxide values also showed a concentration drop at 400–500 MPa and temperatures higher than 30 °C, which was attributed to the formation of secondary oxidation products. The authors explained that the initiation and propagation steps of lipid oxidation were accelerated due to the presence of oxygen in the sample headspace and that high temperature increased oxygen availability in a closed system such as a PATP-treated emulsion (Zhu et al. 2014). Pressure treatments at 655 MPa, 75 °C, 3–10 min promote the formation of six- to ten-carbon alkyl aldehydes, 2-heptanone, and sulfur compounds in milk samples with a dissolved oxygen concentration of $9.33 \pm 0.06 \text{ mg kg}^{-1}$ of milk (Vazquez and Qian 2007). Oxygen removal with ultrasound and vacuum treatments yielded an initial oxygen concentration of $3.23 \pm 0.06 \text{ mg kg}^{-1}$ of milk, and the formation of most volatiles was significantly reduced with concentrations ranging from 15 to $139 \text{ } \mu\text{g kg}^{-1}$ of milk after 10 min treatments.

Moderate high-pressure treatments (150–450 MPa, 0–5 min) of mackerel at room temperature resulted in lower concentration of free fatty acids and tertiary lipid oxidation compounds during subsequent storage at $-10 \text{ } ^\circ\text{C}$ for 3 months, when

compared to untreated fish samples (Vázquez et al. 2013). Treatments of meat products in the 300–500 MPa range has been reported to induce lipid oxidation (Barbosa-Canovas et al. 2014). Bolumar et al. (2014) reported the formation of protein-derived radicals in chicken breast and minced beef loin after high-pressure treatments at 800 MPa and 20 °C for 10 min. The release of iron as a result of the heme–protein denaturation has been proposed to be a lipid oxidation, but the addition of EDTA (134 μM) and heptahydrated iron sulfate salt (50 μM) yielded similar radical concentration as in beef samples containing none of these chemicals, suggesting the participation of other mechanisms aside from heme-proteins. Bolumar et al. (2012) showed that pressure promoted the formation of free radicals in chicken breast. A critical pressure level (400–500 MPa, changing with temperature) was found above which the formation of free radicals was significantly enhanced. Interestingly, this critical pressure matched with the threshold pressure for protein denaturation and membrane damage, releasing metal ions and enzymes suggesting that the accelerated formation of free radicals may be associated to the release of metal ions or enzymes.

Volatile profiles of vegetable products including onion, potato, pumpkin, red beet (Kebede et al. 2014), broccoli, green pepper, spinach (Kebede et al. 2013), and carrot (Vervoort et al. 2013) after thermal (retort sterilization targeting $F_0 = 5$ min at 121 °C) and PATP treatments (600 MPa, 117 °C, 15 min) have been thoroughly characterized by coupling headspace solid-phase microextraction, gas chromatography, and mass spectrometry detection. Despite the wide variety of vegetables and volatile compounds studied, common trends in the volatile profiles after thermal and PATP treatments were observed. PATP-treated samples showed a higher content of alkyl aldehydes and ketones derived from the oxidation of lipids. In samples subjected to conventional thermal treatments, a higher concentration of Strecker degradation products, namely, furanic compounds and aldehydes, associated with the Maillard reaction was detected when compared to PATP samples (Kebede et al. 2013, 2014; Vervoort et al. 2013).

Effect of Chemical Reactions on Polymeric Structures at High Pressure

HPP treatments at 150–250 MPa and 50 °C for 10 min do not modify the composition and microstructure of lignocellulose in dry sugarcane bagasse or in sugarcane bagasse immersed in 44 % water. However, the HPP treatment of sugarcane bagasse immersed in mineral acids or sodium hydroxide under the same conditions resulted in the fragmentation of lignocellulose microstructure, particularly when the bagasse samples were pretreated in sulfuric acid at 121 °C. However, none of the HPP treatments significantly enhances the lignocellulose hydrolysis of sugarcane bagasse (Castañón-Rodríguez et al. 2013). Higher pressure levels (300–400 MPa) yielded a 1.2–1.9-fold increase of endocellulase hydrolysis of eucalypt pulp in 0.05 M sodium acetate buffer. Microscopic images, and a more detailed kinetic study at 400 MPa, revealed that high pressure promotes the swelling of the cellulose

structure which may facilitate the access to glycosidic bonds (Ferreira et al. 2011). Other studies have shown that the 1.5–4.0 % weight loss by water evaporation observed during the thermogravimetric analysis of eucalypt pulps is retarded when samples are first treated by HPP (Figueiredo et al. 2010). The same authors reported a 10 °C decrease in the glass transition temperature in HPP-treated eucalypt pulp, stating that the enthalpy difference of hydrogen bonding of water is lower than the enthalpy of hydrogen bonding of intermolecular cellulose polymers, which also resulted in the increased water retention capacity. The swelling of plant cell microstructure also took place in *Aloe vera* tissue treated at 350 MPa, 0.5 min, resulting in the increase of the mass water diffusion coefficient from 7.295×10^{-10} to $8.892 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ for a 70 °C drying process (Vega-Gálvez et al. 2011). Buckow et al. (2007) found that the rate constants for the gelatinization under isobaric conditions (0.1–650 MPa) of commercial maize starch suspended in water (5 % w/w) followed sigmoidal curves when plotted against temperature (30–75 °C). The inflection point of the sigmoidal kinetic curves represented the melting temperature of the starch granules (T_m), which remained constant at 67 °C for 0.1–300 MPa pressure treatments. Pressure levels higher than 300 MPa resulted in a sharp decrease of the melting temperature, reaching ambient temperature conditions ($T_m = 20$ °C) for 600 MPa treatments.

Effect of Pressure on Chemical Reactions Increasing Food Quality

Studies on the application of high-pressure processing (HPP) in several food systems have shown that pressure inactivates microorganisms in vegetative form without significant changes in sensory and nutritional properties (Mathys et al. 2009). Under the application of pressure at refrigeration or room temperature, covalent bonds can be assumed to remain intact (Martínez-Monteagudo et al. 2012). This assumption has been central in explaining the retention of ascorbic acid (Oey et al. 2006), folates (Butz et al. 2004), antioxidants (Matser et al. 2004), and anthocyanins (del Pozo Insfran et al. 2007) in HPP-treated samples. In the latter study, the anthocyanin content was evaluated in grape juice stabilized with ascorbic and water-soluble polyphenols extracted from rosemary and thyme before 15 min treatments at 400–500 MPa and room temperature. These extracts (copigmentation) were added to stabilize anthocyanins and ascorbic acid during pressurization and subsequent storage. In nature, intermolecular copigmentation is one of the mechanisms for the stabilization of anthocyanins, and it is also responsible for the characteristic color and stability of aged red wines. Anthocyanin monitoring for 21 days showed a higher content in samples stabilized prior to HPP treatments. More importantly, intermolecular copigmentation was not affected by pressure treatment, and therefore, it can be used prior to enhance anthocyanin retention.

On the other hand, covalent bonds cannot be assumed to remain unchanged under PATP conditions, and thus the effect of pressure on thermally driven reactions needs to be analyzed. For example, the glycosylation rate of bovine serum albumin under isothermal and isobaric conditions in the range of 60–132 °C and

0.1–600 MPa on the lysine availability of BSA investigated was significantly reduced by pressure (Buckow et al. 2011). Pressure inhibition was also reported by Schwarzenbolz et al. (2000) for the cross-linking and oligomer formation in β -casein and saccharides solutions at pH 7.4 subjected to 600 MPa and 60 °C for 2 h. These authors concluded that pressure accelerated sugar degradation which reduced protein cross-linking. Two Maillard reactions, the condensation reaction of amino compounds with carbonyl compounds and the melanoidin formation, studied at 50–500 MPa and 50 °C also showed a pressure inhibition (Tamaoka et al. 1991). An investigation on the browning reaction in a glucose–lysine model system at 40–60 °C under atmospheric pressure and 600 MPa showed that the rate under high pressure was influenced by the initial pH of the solution. In the pH 5.1–6.5, 7.0–7.5, or 8.0–10.5, the rate of browning was inhibited, unaffected, or accelerated by pressure, respectively (Hill et al. 1996). The effect of 400 MPa for 3 h at 60 °C on 10 % aqueous lactose isomerization in basic media and yielding lactulose was studied by Moreno et al. (2003b) who found also an inhibitory effect of pressure. CLA in milk suffers significant biological activity losses during processing at high temperatures such as those used in UHT treatments. CLA-enriched milk subjected to PATP treatments was studied as a means to deliver shelf-stable milk while retaining CLA activity. The effect of pressure (100–600 MPa), temperature (60–120 °C), and treatment time (0–14 min) on CLA content in milk and anhydrous milk fat (AMF) rich in CLA was evaluated by Martínez-Monteagudo et al. (2012). In milk treated up to 14 min at 100 MPa, CLA was stable (>80 % of retention), regardless of the temperature, while only 3.4 ± 2 % of CLA was retained at 600 MPa and 120 °C. In the case of AMF, CLA retention was considerably higher (40.2 ± 2 %) than in milk, suggesting that the presence of free metal ions in milk might catalyze CLA degradation. When the antioxidant catechin (1 g kg^{-1}) was added, CLA retention increased significantly (>90 %) in milk and AMF, regardless of the pressure and temperature conditions. Pressure and temperature effects on the stability of riboflavin, thiamine, and thiamine monophosphate in a model system and in minced pork were studied by Butz et al. (2007) finding that vitamins were more stable in pork than in model systems, where the reduction rate was up to 30 times faster. These results suggest that the outcomes from model systems should be used with caution when extrapolating them to foods. A treatment of 600 MPa and 100 °C for 15 min yielded a product with 10 % of vitamin loss, while the traditional thermal treatment (121 °C for 20 min) delivered a product with vitamin losses of up to 45 %. The stability of lycopene in tomato juice treated at 600 MPa and 100 °C for 10 min and at 700 MPa and 45 °C for 10 min was higher than in samples treated at 0.1 MPa and 100 °C for 35 min (Gupta et al. 2010). PATP treatments cannot only inhibit the degradation of bioactive compounds but also preserve the texture of shelf-stable fruits and vegetables. De Roeck et al. (2009) found that pressure inhibited cell wall depolymerization, which is the reaction responsible for tissue softening. Apple pectin solutions at pH 6.5 were subjected to different pressure and temperature combinations (0.1, 500, 600, and 700 MPa at 90, 110, and 115 °C). At all temperatures at 0.1 MPa, demethoxylation showed a higher rate constant than β -elimination. However, a temperature rise resulted in a

stronger acceleration of β -elimination when compared to demethoxylation. Under high pressure, β -elimination was retarded or even stopped, whereas demethoxylation was stimulated. These results are promising in terms of texture preservation by PATP treatments, as β -elimination has been reported as the main cause of thermal softening and low methoxylated pectin enhance tissue texture by forming cross-links with calcium ions.

Effect of Pressure on the Formation of Compounds Posing a Toxic Risk

The benefits of heat treatments as a preservation method are well established. However, formation of toxic compounds is known to take place also, and thus the chemical risk assessment of thermal processing has received considerable attention (Eisenbrand et al. 2007). Assessing the chemical toxic risks in PATP-treated foods is facilitated when the substrates and reaction mechanisms involved are known. Fortunately, chemical changes under high pressure and high temperature examined so far in foods and model systems have been shown to follow known reaction mechanisms making it unnecessary to postulate new ones. In the case of the acrylamide formation described below, this risk can be analyzed knowing the content of reducing sugars and free amino acids and that acrylamide formation occurs via the Maillard reaction. This reaction is not only responsible for the development of desirable color/flavor compounds in processed foods, but it is also responsible for the formation of anti-nutritive and off-flavor compounds. The Maillard reaction involves a complex sequence of reactions that can be markedly increased or decreased by pressure according to the mechanism for each reaction step. Different effects have been reported for the effect of high pressure on the Maillard reaction including an increased or decreased formation of intermediate and final products depending on the reaction stage evaluated (Moreno et al. 2003a; Isaacs and Coulson 1996; Schwarzenbolz et al. 2000, 2002). Therefore, evaluating the effect of pressure on the overall Maillard reaction can be misleading (Schwarzenbolz et al. 2000). Moreover, theoretical considerations reviewed in the previous section indicate that chemical reactions involving positive or negative activation volumes are inhibited or accelerated by high pressure, respectively. However, it is not yet possible to predict whether a reaction mechanism will involve a positive or negative activation volume. Therefore, demonstrating the decrease or increase in undesirable chemical changes in PATP-treated products requires experimental evidence.

At present, there is no experimental evidence that PATP and HPP of foods have created new chemical safety risks. However, the presence of health hazard compounds such as acrylamide and many others formed known to occur during thermal processing (e.g., heterocyclic aromatic amines, furans, etc.) should be evaluated in foods subjected to PATP treatments. The oxidation reactions of unsaturated fatty acids and the advanced glycation between amino acids and sugars should be investigated in high-fat products treated with PATP as these reactions have

undesirable nutritional and health effects (Kanekanian 2010). A more challenging situation is the potential formation of toxic compounds via reactions with $V_a < 0$ occurring at low rates in conventional processes. Such reactions will be accelerated by pressure and could generate new chemical risks never observed before. The solution to this challenge is the use of screening tests for toxic compounds.

The next sections will cover specific examples of chemical reactions responsible for toxic risks in thermally processed foods and the very few studies analyzing them under PATP conditions. Unfortunately, the kinetics of formation of acrylamide, polycyclic aromatic hydrocarbons, heterocyclic amines, N-nitroso compounds, and other chemicals known for their toxicological risks in thermal processing is mostly unknown under PATP conditions (Escobedo-Avellaneda et al. 2011; Segovia Bravo et al. 2012). Moreover, some studies have shown a toxicity reduction such as a decrease in the formation of biogenic amines in ripened meat products treated by HPP (Ruiz-Capillas et al. 2007; Ruiz-Capillas and Jiménez Colmenero 2004) and the inhibition of acrylamide formation in model systems subjected to PATP treatments (de Vleeschouwer et al. 2011).

Acrylamide. In 2002, findings of considerable acrylamide levels in starch-based foods were reported by the University of Stockholm and the Swedish National Food Authority (2002). Since acrylamide is neurotoxic, induces germ cell mutagenicity, and is classified as a potential human carcinogen (IARC 1994), finding it in foods generated a major international research effort including confirmations of initial findings (Matthäus 2004; Zyzak et al. 2003), mechanisms of formation (Zhang and Zhang 2007; Zyzak et al. 2003), improvements to analytical methods (Kim et al. 2007), and efforts to reduce its level in processed food (Haase 2004). The concerns about acrylamide presence in foods reflect the following safety risks. First, glycidamide, an acrylamide metabolite that binds to DNA causing genetic damage, has been found in humans exposed to acrylamide. In vitro and in vivo studies show the induction of gene mutations by acrylamide in cell cultures and animal studies (Gamboa da Costa et al. 2003). Neurological damage has been observed in workers exposed to high acrylamide doses (Fullerton and Barnes 1966), and lower fertility has been observed in rats fed 5–10 mg acrylamide/kg body weight per day (Swedish National Food Administration 2002). The Maillard reaction between amines and carbonyl moieties forming pleasant flavor and color compounds during the heating of foods is also the acrylamide formation mechanism in foods containing reducing sugars and high concentrations of the amino acid asparagine. Acrylamide formation investigated in food and model systems has shown that the limiting factor for the reaction is free asparagine (e.g., Weisshaar 2004). Only minor acrylamide amounts have been found in heated foods containing glutamine and methionine in addition to reducing sugars (Stadler et al. 2002). Under conventional thermal processing pressures, significant levels of acrylamide have been demonstrated to require temperatures higher than 120 °C (Pedrenski 2007). However, if the acrylamide formation reaction pathway were characterized by a large negative V_a value, pressure would accelerate it, and significant levels could be formed in PATP foods at lower temperatures. On the other hand, the pressure-induced pH shift during PATP treatments also could affect the amount of acrylamide formed.

Working with model systems, de Vleeschouwer et al. (2011) showed that acrylamide formation at 115 °C and 600 MPa was much lower than the concentration found in the same model subjected to conventional thermal treatments (1700 ppb vs. 6500 ppb). This pressure inhibition of acrylamide formation suggests that this reaction has $V_a > 0$, but this finding needs to be confirmed in food systems.

Polycyclic aromatic hydrocarbons, heterocyclic amines, N-nitroso compounds risk, and hormone-like peptides. The formation of polycyclic aromatic hydrocarbons (PAHs), a group of organic compounds with two or more fused aromatic rings, depends on the food temperature. Grilling meat, fish, or other foods with intense heat over direct flame leads to significant PAH formation. In the 400–1000 °C range, organic compounds fragment into smaller compounds yielding relatively stable PAHs. Benzo[α]pyrene, a five-ring PAH, has been extensively studied because it has been reported to be the most carcinogenic PAH (IARC 1983; Lee et al. 1981). Other PAHs including benzo[α]anthracene and dibenz[α,β]anthracene have been reported to be carcinogenic in animal model studies, and thus they are considered as probable human carcinogens. If their formation reaction has a $V_a > 0$, PAHs could be formed at the lower temperatures used in PATP-treated foods (typically under 120 °C).

Amino-carbolines, imidazoquinolines, imidazoquinoxalines, and imidazopyridines are also formed from heated foods. This toxic group of heterocyclic amines is characterized by two or three rings with an exocyclic amino group produced via the Maillard reaction from creatine or creatinine, some free amino acids, and sugars when heating meat or fish (Jägerstad and Skog 2005; Jagerstad et al. 1998). Cured meat and fish products treated by PATP containing nitrites used to control *Clostridium botulinum* should be tested for the presence of genotoxic N-nitroso compounds. There is no evidence on how pressure levels used in PATP treatments affect their formation rate, and as stated before, it is not possible to predict whether this toxic compound formation reaction has a positive or negative V_a value. Research on the formation under high pressure of other undesirable reactions such as the formation of peptides with hormone-like effects has been reported. Fernández García et al. (2003) reported that high pressure induces the formation of hormone-like substances by cyclization of glutamine at the N-terminus of certain peptides and accelerates the formation from aspartame of diketopiperazines, a peptide with biological activity.

Screening for mutagenic agents in PATP-treated foods. The identification of genotoxic substances is an important procedure in the assessment of processed food safety including novel foods such as PATP foods (Hepburn et al. 2008). Typically, the first recommended screening step is an Ames Salmonella/microsome mutagenicity assay. This rapid method test is designed to detect a wide range of chemical substances with the potential of causing gene mutations (Mortelmans and Errol 2000). The test is based on the use of histidine-dependent *Salmonella* strains differing in the mutations of the histidine operon genes. The test substances may appear to be noncarcinogenic unless they are previously metabolized into active forms by the cytochrome-based P450 oxidation system in the human liver. Since bacteria do not have this metabolic activity, the potential carcinogen in the

food extract needs to be activated using a rat liver extract (Ames et al. 1973). The histidine-dependent *Salmonella* strains are grown in media with very low levels of histidine, so only histidine revertants can grow and form colonies. The test is positive if the number of colonies growing in media without histidine is higher than the number of spontaneous revertants in media without the test substance. The test response is quantitative since the difference in the number of colonies is proportional to the dose effect of the test substance. Another test that should be used to screen PATP foods for the potential presence of toxic compounds is the single cell gel electrophoresis assay. This test, also known as the Comet assay, can detect DNA damage in eukaryotic cells (Östling and Johanson 1984; Singh et al. 1988). The standard alkaline Comet assay and its various modifications provide a relatively simple, sensitive, and rapid method of analyzing DNA damage and repair.

Conclusion

Although PATP products are not yet available to consumers, a near-future commercialization of this technology is expected. Commercial PATP prototypes have been available for more than a decade to evaluate the quality and safety of PATP foods at laboratory and pilot plant levels. However, the availability of models and data for the kinetics of chemical changes observed when treating foods by PATP is still limited, inconsistent, and lagging behind the standardized information available for conventional thermal treatments. Although the effects of temperature and pressure on the kinetics of chemical reactions can be analyzed utilizing the E_a and V_a concepts, there is insufficient experimental information to predict how pressure will affect the loss or increase the chemical safety and quality (including the concentration of nutrients and other desirable food composition factors) and when foods are treated under PATP conditions. Although the formation in foods of toxic compounds during PATP treatments, e.g., acrylamide and PAHs, can be studied knowing that the substrates and reaction mechanism are the same as those observed in foods treated by conventional thermal processing alternatives, the unpredictable effect of pressure on the kinetics of chemical reactions at the high temperatures required to produce shelf-stable PATP foods remains an unsolved challenge. Also necessary are further studies on bacterial spore inactivation, which is the key factor to determine PATP pressure and temperature levels necessary for microbial safety and stability. With further information on the inactivation of bacterial spores, e.g., an efficient method to trigger spore germination or decrease spore resistance, it may be possible to reduce the PATP conditions (below 100 °C and 600 MPa) to achieve commercial sterilization. Furthermore, lacking information on dissolved oxygen concentration under the PATP condition limits the understanding of oxidation reactions of nutrients and flavor compounds in PATP-treated food. Information on vitamin, pigment, and flavor compound retention under conditions ensuring spore inactivation is also fairly incomplete. This is important as the novel food regulations in Europe and many other countries require exhaustive nutritional

equivalence determinations in addition to chemical risk analysis before approving the marketing of PATP products. The determination of the potential presence of harmful compounds in PATP food products is challenging when the reaction mechanisms involved are known, but it is much harder if reactions forming them at negligible rates under the low-pressure conditions of conventional thermal treatments are accelerated significantly by the pressure levels used to achieve shelf stability.

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Cross-References

- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Drying](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Heating and Cooking](#)

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Part VI

**Chemistry of Bioactive Ingredients in
Functional Foods and Nutraceuticals**

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Abstract

Plant secondary metabolites are rich sources of bioactive compounds eliciting many beneficial health effects in man and animals. Plant-based foods, including vegetables, fruits, grains, seeds, nuts, and legumes, may contain hundreds of different phytochemicals. Recently, research on phytochemicals suggests their possibility as an important source of therapeutic and preventive agents against diseases. The types of foods containing these bioactive components are those

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functional foods that can provide desirable health benefits beyond their natural properties when consumed in a regular and consistent manner through diet. Alternatively, dietary supplements can be supplied to consumers in a concentrated form to deliver a specific bioactive phytochemical or a group of phytochemicals. Usually, these nutraceutical ingredients are administered with higher doses than in normal food or in a medicinal form with the purpose of improving human health. This chapter highlights the four most common groups of plant-derived bioactive components, polyphenols, alkaloids, terpenes, and saponins, mainly focusing on their chemistry, sources, and biological functions.

Introduction

Secondary metabolites are chemical compounds produced within the plants that are not directly involved in the normal growth, development, or reproduction of an organism. Some of them are found to act as defense compounds against diseases, predators, ultraviolet radiation, parasites, and oxidants, to facilitate the reproductive processes (e.g., serve as attractive smells and coloring agents), and for interspecies competition. Bioactive compounds of plant origin are those secondary metabolites possessing desired health/wellness benefit effects in man and animals (Kaur and Das 2011). Consistent evidence from epidemiological, *in vitro*, *in vivo*, and clinical studies has demonstrated that a diet rich in plant foods can reduce the risk of some degenerative diseases, such as diabetes, obesity, cardiovascular complications, and cancer. As an example, research studies have shown that about 20–50 % of all cases of cancer can be prevented by the plant-based diets (Glade 1999). Thus, dietary recommendations have always emphasized the consumption of various plant foods to reduce the risk of cancer and other chronic diseases.

With the development of analytical techniques such as gas chromatography (GC), high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance spectrometry (NMR), the isolation, purification, and structure determination of single compound from plant extracts have become available. These advanced techniques help us to identify a wide range of biologically active compounds, now known as “phytochemicals.” These phytochemicals including polyphenols, alkaloids, terpenes, saponins, etc. have been widely used in nutraceuticals as ingredients to provide a health benefit beyond basic nutrition. However, there are some studies suggesting that the potent biological functions could be assumed to be due to the synergistic and/or additive effects of phytochemicals and nutrients present in the plant foods rather than the purified chemicals themselves.

This chapter highlights the chemistry, sources, and biological functions of the four most common groups of phytochemicals including polyphenols, alkaloids, terpenes, and saponins. Also, the commercial nutraceuticals rich in the supplements are briefly introduced.

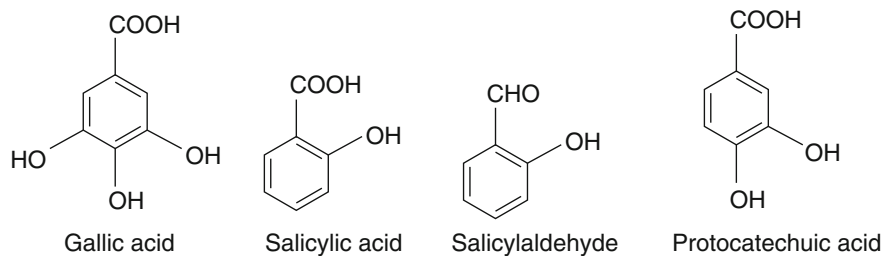


Fig. 1 Chemical structures of hydroxybenzoic acids

Polyphenols

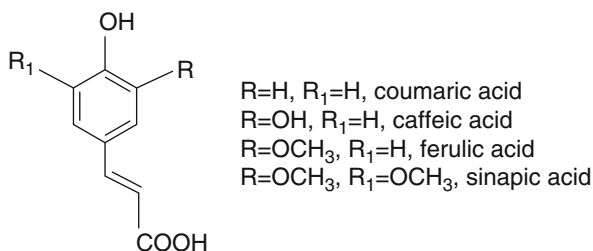
Polyphenols, a class of chemical compounds consisting of one or more hydroxyl groups ($-\text{OH}$) attached directly to an aromatic ring, are the most abundant secondary metabolites distributed in all vascular plants. They are important natural phytochemical compounds that play an important role in the taste, color, and nutritional properties of plant-based foods, such as vegetables, fruits, cereal, whole grains, coffee, tea, legumes, cocoa, and wine. Due to their abundance in the human diet, tremendous investigations have been conducted among nutritionists and food scientists to study the health effects of dietary polyphenols, including simple phenolics, coumarins, lignans, flavonoids, isoflavonoids, anthocyanins, tannins, quinines, and stilbenes in recent years. Increasing evidence indicates that polyphenols have several benefits on humans such as potent antioxidant properties, prevention of various diseases induced by oxidative stress, and prevention of particularly cardiovascular diseases, neurodegenerative diseases, and cancers (Bravo 1998). The health effects of polyphenols depend on the type of polyphenols, the amount consumed, and their bioavailability.

Simple Phenolics

Phenol, with one hydroxyl group ($-\text{OH}$) attached directly to an aromatic ring, is the simplest phenolic. Simple phenolics are substituted phenols (C_6C_n) possessing a singly substituted phenolic ring with carboxylic acid, alcoholic, or aldehydic groups. Phenolic acids, a class of chemical compounds possessing one carboxyl group attached to the benzene ring, have been studied extensively recently because of their potential protective role. Phenolic acids can be divided into two categories, benzoic acid derivatives (i.e., hydroxybenzoic acids) (Fig. 1) and cinnamic acid derivatives (i.e., hydroxycinnamic acids) (Fig. 2), based on the constitutive carbon frameworks.

The hydroxybenzoic acid ($\text{C}_6\text{-C}_1$ derivatives) (e.g., gallic acid, salicylic acid, salicylaldehyde, and protocatechuic acid) (Fig. 1) content is generally very low in edible plants, except for some red fruits. Hydroxybenzoic acids occur in high

Fig. 2 Chemical structures of hydroxycinnamic acids



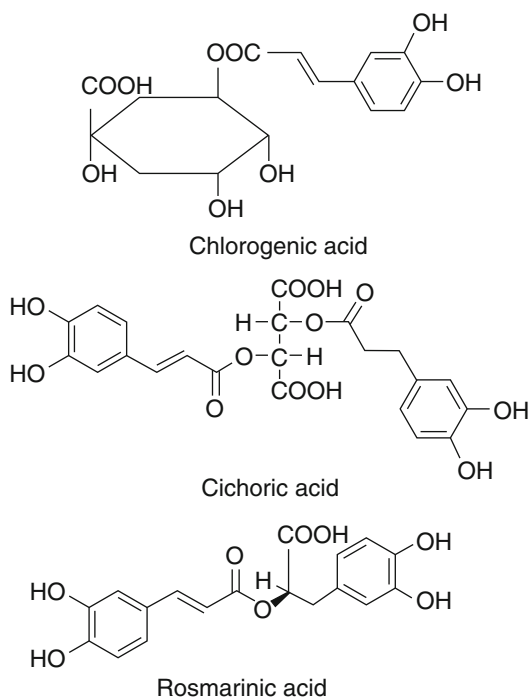
concentrations in blackberries (up to 270 mg/kg fresh weight). Protocatechuic acid is an important bioactive constituent of raspberry present up to 100 mg/kg fresh weight, while the concentration of protocatechuic acid is low in olive oil (about 0.22 mg/kg) (D'Archivio et al. 2007). Gallic acid occurs up to 4.5 g/kg fresh weight in tea leaves.

Hydroxybenzoic acids are potent antioxidants that may help protect the body from free radicals. Gallic acid has been used as an astringent and styptic, and it also possesses antineoplastic, bacteriostatic, and antimelanogenic activities. Salicylic acid exerts anti-inflammatory, keratolytic, analgesic, antipyretic, antifungal, and antiseptic properties for several skin conditions (e.g., dandruff and seborrheic dermatitis, acne, ichthyosis, and psoriasis). Protocatechuic acid has also been found to have several bioactivities such as anti-inflammatory, antifungal, free radical scavenging, antioxidant, apoptotic, cytotoxic, antihepatotoxic, chemopreventive, neuroprotective activity, and as platelet aggregation and LDL oxidation inhibitor (Khadem and Marles 2010).

Willow bark (*Salix* spp.), the bark from several varieties of the willow tree, including pussy willow or black willow, European willow or white willow, purple willow, crack willow, and others, is used as herbal medicine to treat low back pain owing to their anti-inflammatory activity. Salicin and related compounds such as salicortin and tremulacin are believed to be the active components inside. Pharmacopoeial-grade willow bark must consist of the dried bark from young branches of willow and must contain at least 1 % of salicin. The final active compound in willow bark is believed to be salicylic acid which is produced by the oxidation of salicyl alcohol, formed upon intestinal hydrolysis of salicin; however, other components such as flavonoids, tannins, and salicin esters from willow bark extracts may also contribute to its overall health effects (Setty and Sigal 2005).

The hydroxycinnamic acids (C₆-C₃ derivatives) are much more common than the hydroxybenzoic acids. The four most common hydroxycinnamic acids are coumaric acid, caffeic acid, ferulic acid, and sinapic acid (Fig. 2). These acids are usually found in plants in the combined forms as glycosylated derivatives or esters of shikimic acid, tartaric acid, and quinic acid rather than in the free form. As an example, chlorogenic acid (Fig. 3), an ester of caffeic acid and quinic acid, is present in high concentrations in many types of fruits. Coffee also contains high concentrations of chlorogenic acid that a single cup may contain between 70 and 350 mg of chlorogenic acid derivatives (Clifford 1999).

Fig. 3 Chemical structures of chlorogenic acid, cichoric acid, and rosmarinic acid



Although distributed throughout constitutive parts of the fruits, hydroxycinnamic acids occur at the highest levels in the outer parts of ripe fruits. Fruits such as blueberries, kiwis, plums, cherries, and apples have the highest hydroxycinnamic acid concentrations ranging from 0.5 to 2 g/kg fresh weight. Rice, oat, and wheat flours are also rich sources of phenolic acids with a concentration of 0.8–2 g/kg dry weight. Among the hydroxycinnamic acids, caffeic acid accounts for 75–100 % of the total contents in most fruits, and ferulic acid may represent up to 90 % of the total contents in cereal grains (Manach et al. 2004).

Hydroxycinnamic acids are known as potent antioxidants playing an important role in protecting the body from free radicals. Caffeic acid has been proposed to selectively block the biosynthesis of leukotrienes, components involved in allergic reactions, immune-regulation diseases, and asthma. Caffeic acid and some of its esters have also been suggested to possess anticarcinogenic bioactivity against colon carcinogenesis. Recent investigations have shown that a series of phenolic acids have the ability of inhibiting the transcriptional activity of AP-1 which is an activator protein implicated in the processes that control inflammation, cell differentiation, and proliferation. Next to its direct antioxidant effect, ferulic acid was found to induce antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase in rats. Animal studies also suggested anticarcinogenic activity of ferulic acid. Ferulic acid was found to have protective effects in the azoxymethane-induced colon carcinogenesis, and the induction of phase II

enzymes such as glutathione-*S*-transferase was suggested as a potential mechanism. Ferulic acid has also been shown to have anti-inflammatory effects (Gallaher and Bunzel 2012). However, further research is needed in order to confirm these benefits of phenolic acids.

Artichoke (*Cynara scolymus* L.) represents an important component of the Mediterranean diet. Artichoke leaf extracts are widely used alone or in association with other herbs in the production of alcoholic and soft drinks and preparation of herbal teas and herbal medicinal products. In various pharmacological test systems, artichoke leaf extracts have been reported to enhance detoxification reactions so as to protect the liver from damage. Artichoke leaf extracts can also be used as a kind of choleric, diuretic, and general stimulant. In addition, artichoke leaf extracts have been shown to lower cholesterol levels in the blood by inhibiting cholesterol synthesis in the liver, increasing cholesterol excretion in the bile, and mobilizing fat stores from the liver and other tissues. Findings from clinical studies have confirmed the therapeutic properties of artichoke leaf extracts to be effective in protecting patients from hyperlipoproteinemia and irritable bowel syndrome (IBS) without any adverse events. Moreover, artichoke leaf extracts have been observed to inhibit *N*-formyl-methionyl-leucyl-phenylalanine phorbol-12-myristate-13-acetate and hydrogen peroxide-stimulated reactive oxygen species (ROS) production to inhibit oxidative stress in human leukocytes in a concentration-dependent manner (Mulinacci et al. 2004).

The therapeutic properties of artichoke leaf extracts have often been ascribed to their high levels of bioactive phenolic compounds. Caffeic acid derivatives are the main phenolic constituents of artichoke head and leaf extracts. A wide range of caffeoylquinic acid derivatives are present in artichoke, of which cynarin (1, 3-dicaffeoylquinic acid) and chlorogenic acid (5-*O*-caffeoylquinic acid) (Fig. 3) are the most important ones. Flavonoids apigenin and luteolin, which present as glucosides and rutosides (e.g., apigenin-7-rutinoside, apigenin-7-*O*-beta-D-glucopyranoside, luteolin-7-*O*-glucoside, and luteolin-7-rutinoside), have also been identified in artichoke tissues. In addition, the bioavailability and metabolism of phenolic compounds from artichoke extract has been investigated in vivo by determining the urinary excretion of absorbed compounds, which demonstrated that the caffeic acid derivatives were metabolized to phenolic acids such as isoferulic, ferulic, vanillic, and dihydro ferulic (Lattanzio et al. 2009).

Echinacea (*Echinacea angustifolia*, *Echinacea purpurea*, *Echinacea pallida*) tissues such as roots, seeds, flower heads, and aerial parts can be made into extracts and tea as well as capsules and tinctures, and they are also widely used as herbal supplements in North America and Europe. Echinacea was initially used as an herbal remedy to treat headache, common cold, respiratory ailments, snakebites, and other infections by the American Indians and currently is used primarily to treat cold and influenza, build immune system, prevent upper respiratory tract infections, heal skin conditions, etc. (Tyler 1998).

Caffeic acid-derived compounds, unsaturated aliphatic compounds, and polysaccharides are believed to be the bioactive components of Echinacea. Echinacoside occurs at a high concentration of 0.3–1.7 % w/w in the roots of

E. angustifolia. Echinacoside has been shown to exhibit antioxidant and free radical scavenging activity which may account for its anti-inflammatory effects. Cichoric acid (2,3-*O*-dicaffeoyltartaric acid) (Fig. 3) is present in a relative high concentration of 1.2–3.1 % w/w in the flower heads and 0.6–2.1 % w/w in the roots. The concentrations vary generally depending on the species identity as well as the season harvest. Both in vitro and in vivo studies demonstrated the phagocytosis stimulatory activity of cichoric acid. Cichoric acid has also been shown to inhibit hyaluronidase, prevent free radical-induced type III collagen degradation, and inhibit the human immunodeficiency virus type 1 (HIV-I) integrase to prevent HIV from infecting host cells. In addition, other phenolics such as chlorogenic acid, tartaric acid, 2-*O*-caffeoyltartaric acid (caftaric acid), 1,3-*O*-dicaffeoylquinic acid (cynarin), lipophilic alkamides, and ketoalkynes identified in Echinacea may also contribute to different bioactivities for Echinacea (Mistikova and Vaverkova 2006).

Rosemary (*Rosmarinus officinalis* L.), widespread in Central America, Europe, and other regions, is widely used as flavoring component in foods, beverage drinks, and cosmetics. This plant has been used in folk medicine as an antispasmodic to treat renal colic and dysmenorrhea, for relief of symptoms caused by respiratory disorders, and for promoting growth of hair. Rosemary extracts have also been used as a diuretic, analgesic, choleric, expectorant, antirheumatic, antimutagenic, hepatoprotective agent, for human fertility, etc. Caffeic acid and its derivatives such as rosmarinic acid (Fig. 3) and chlorogenic acid have been thought to be the most important ones responsible for the therapeutic properties of rosemary extracts. Also, flavonoids, terpenoids (carnosol and carnosic acid), and essential oils are found in rosemary extracts. These compounds may work together to contribute to the bioactive function of rosemary (al-Sereiti et al. 1999).

Coumarins

Coumarins (Fig. 4) are a large class of C₆-C₃ derivatives belonging to the benzo- α -pyrone group, which are presenting in the free form or in combined form as heterosides and glycosides in certain foods or plants. The simple coumarins are the hydroxylated, alkoxyated, and alkylated derivatives of the benzene ring of a coumarin and the corresponding glycosides. Furanocoumarins are furano derivatives of coumarin having a five-membered furan ring fused with the benzene ring of a coumarin, and they can be divided into linear (psoralen) or angular (angelicin) types based on the skeleton structure. Pyrano coumarins are the third type of coumarins consisting of a six-membered pyran ring attached to the benzoid, divided into linear (xanthyletin) or angular (seselin) types (Gopi and Dhanaraju 2011).

Higher plants such as Rutaceae and Umbelliferae are the richest sources of coumarins. All the constitutive parts of the plant contain coumarins, while the highest concentrations are found in the fruits, followed by the roots, stems, and leaves. Coumarins are distributed at high levels in some essential oils. Cinnamon bark oil and cassia leaf oil are rich sources of coumarins with the concentration of

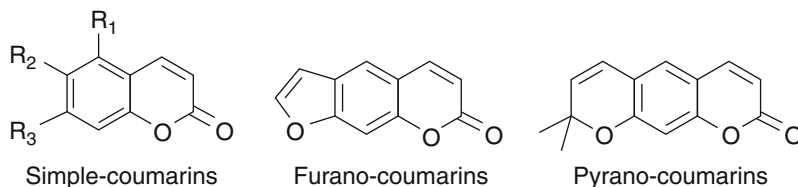


Fig. 4 Chemical structures of coumarins

7,000 and 87,300 ppm, respectively. Coumarins have also been found in lavender oil. In addition, fruits such as cloudberry and bilberry, chicory, and green tea and other foods have been reported to contain coumarins. The coumarin concentrations in diverse parts of the plant vary generally with the environmental conditions, species identity, as well as the seasonal changes (Jain and Joshi 2012).

Due to their diverse pharmacological properties, the coumarins have been studied extensively by food scientists, nutritionists, pharmacologists, and chemists. A growing body of studies demonstrated several bioactivities of coumarins and coumarin derivatives, including free radical scavenging activity, anticancer, anti-inflammatory, antimicrobial, anticoagulant, etc. These beneficial properties make them important starting products for developing novel derivatives as therapeutic agents. As an example, 7-hydroxycoumarin, a metabolite of coumarin, has been found to have antitumor effects against several human tumor cell lines such as breast cancer cell line MCF-7 and lung cancer cell line A549. In addition, coumarins and coumarin-related compounds such as esculetin have been reported to show inhibitory effects on cell growth of several carcinoma cell lines such as leukemia cells HL-60 and myeloid leukemia cells K562 (Lacy and O' Kennedy 2004).

Angelica archangelica L. (*A. archangelica*) is an aromatic, herbaceous plant commercially cultivated in Europe. Essential oils extracted from roots and seeds are widely used as flavoring ingredients in perfumery and cosmetics, in the production of absinthe and other alcoholic drinks, and in the preparation of tea and medicinal products (Bhat et al. 2011). This plant has been used in folk medicine as a remedy to treat disorders of the respiratory tract, nervous system, and gastrointestinal tract, as well as against cerebral diseases, nervous headaches, toothaches, fever, infections, skin rashes, and flu. *A. archangelica* extracts have also been used as a diuretic, antiseptic, and expectorant (Howes et al. 2003).

Essential oils and furanocoumarins are the chief constituents of *Angelica* fruits. Imperatorin and xanthotoxin are the major furanocoumarins in the tincture of the fruits with the concentrations of 0.90 mg/mL and 0.32 mg/mL, respectively. The essential oil and extracts containing furanocoumarins obtained from *Angelica archangelica* seeds showed significant efficacy against *Spodoptera littoralis* larvae in terms of acute toxicity and chronic toxicity, respectively, and both of them caused growth inhibition and showed antifeedant activity against *Spodoptera littoralis* larvae. *Angelica archangelica* seed extracts can serve as efficient substances for the development of botanical insecticides against phytophagous pest larvae (Pavela and Vrhotová 2013). Other secondary metabolites are also found in *Angelica*, including

a bitter principle, angelic acid, astringents, valeric acid, volatile oil, a peculiar resin known as angelicin, etc. These phytochemicals could work in coordination with each other to contribute to the biological function of *Angelica* (Kumar et al. 2013).

Lignans

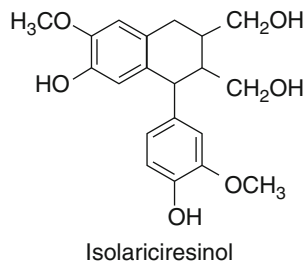
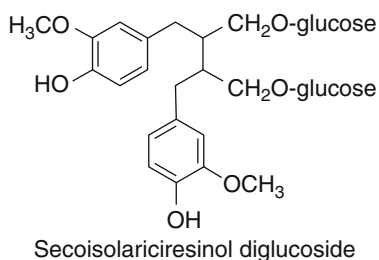
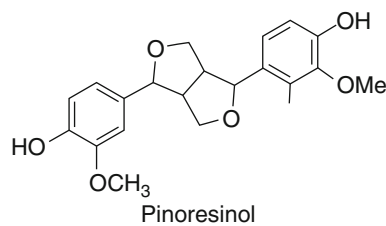
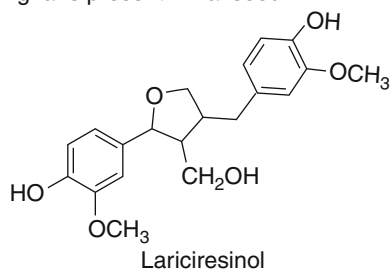
Lignans are a diverse group of bioactive phenolic compounds formed of two β - β -linked phenylpropane units; they are present in different parts of plant species in the free form or in combined form as glycoside derivatives. Flaxseed contains the most abundant lignans (Fig. 5). Secoisolariciresinol is present up to 3.7 g/kg dry weight, and matairesinol is found in a low quantity of about 10.9 mg/kg dry weight in flaxseed. Other foods such as cereals, fruits, legumes, vegetables, berries, and tea are also sources of lignans but in low amounts ranging from 0.1 to 81.9 mg/kg dry weight (Johnsson 2004).

After consumption, the plant lignans secoisolariciresinol and matairesinol have been described to be effectively metabolized to the mammalian lignans, enterodiol and enterolactone, by the intestinal facultative bacteria (Fig. 5). Thus, plant lignans are known as “phytoestrogens” and may exert the estrogen-like biological activities such as reducing the risk of hormone-dependent cancers (e.g., prostate, breast, and endometrial cancer) (Borriello et al. 1985). Also, plant lignans possess powerful antioxidant activity higher than that of vitamin E and may be effective in the treatment of several other diseases such as cardiovascular disease, coronary heart disease, and diabetes.

Flaxseed oil is also the richest source of the omega-3 fatty acid, a kind of polyunsaturated fatty acid, possessing several health benefits on humans. However, it is lignans in flaxseed that make it to be a hot research topic recently. The concentration of lignans in flaxseed is tens to hundreds of times as high as in most other plant-based foods. Flaxseed exhibits strong antitumor activities against colon, mammary gland, and lung cancer in vivo. Also, a diet rich in flaxseed has been shown to influence the hormone metabolism, thus decreasing the risk of prostate and breast cancer. Flaxseed has also been suggested to be a potential remedy for various diseases such as diabetes, cardiovascular diseases, hyperlipidemia, constipation, memory loss, and hypertension. However, more studies are needed to evaluate the biological activity of flaxseed on humans. The bioactive constituents that account for the function of flaxseed are thought to be the flaxseed lignans, especially secoisolariciresinol and matairesinol, unsaturated fatty acids, sterols, soluble flaxseed fiber mucilage, etc. (Mishra and Verma 2013).

Schisandra chinensis (Turcz.) Baill. (Schisandraceae) is a kind of medicinal plant widely distributed in the eastern parts of Russia, northeastern China, Japan, and Korea. The fruits and seeds of *Schisandra chinensis* (Turcz.) Baill. are widely used in traditional Chinese medicine for sedative, antiaging, and tonic purpose. They are also used in the production of nutraceuticals such as soft drinks and health foods and as an ingredient in some oral, skin, and hair health-care products. Current scientific findings suggest that lignans of *Schisandra chinensis* can stimulate liver regeneration, prevent liver injuries, and inhibit hepatocarcinogenesis as well as

Lignans present in flaxseed



Transformation of secoisolariciresinol and matairesinol to enterodiol and enterolactone

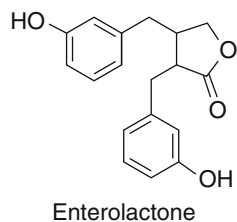
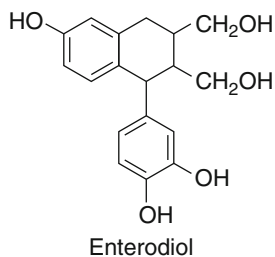
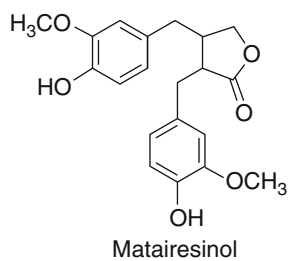
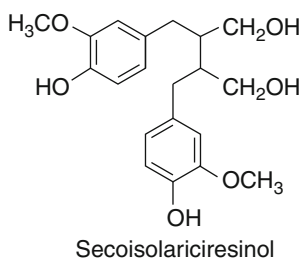


Fig. 5 Chemical structures of flaxseed lignans

lipid peroxidation in rats (Kubo et al. 1992). Also, other pharmacological effects of *Schisandra chinensis* including inhibiting platelet aggregation, lowering the serum glutamate-pyruvate transaminase level, and anti-oxidative, calcium antagonism,

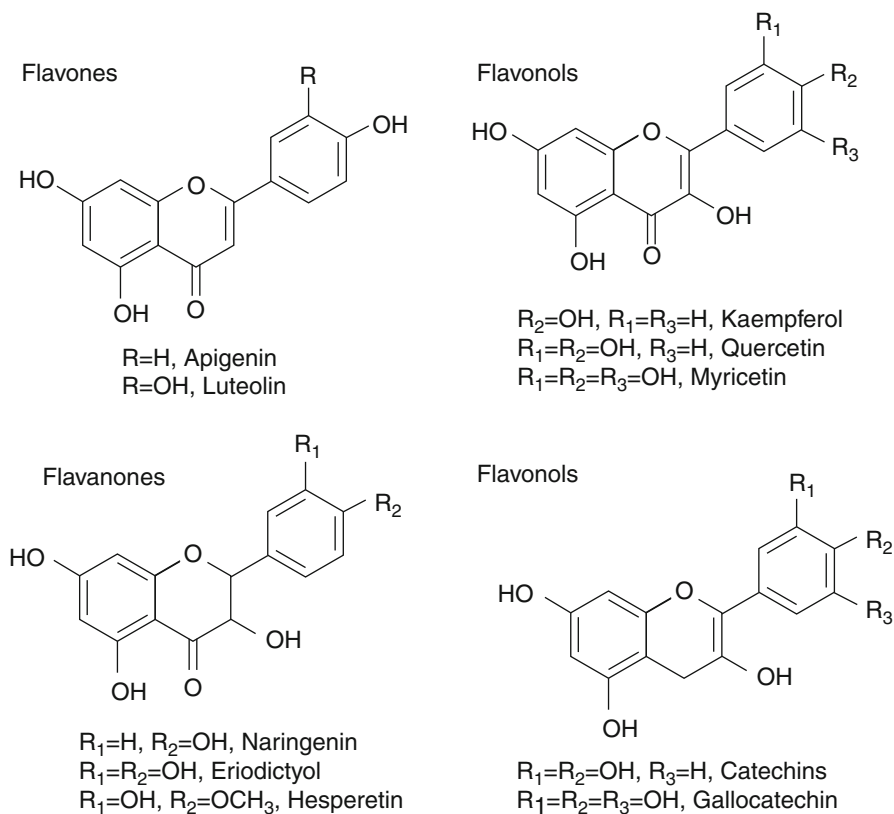


Fig. 6 Chemical structures of flavonoids

anti-HIV, and antitumor effects can also be attributed to its lignan constituents, particularly the dibenzocyclooctadiene-type lignans (Lu and Chen 2009).

Flavonoids

Flavonoids are one of the largest groups of phenolic compounds with more than 3,000 known structures. They are widely distributed throughout the plant kingdom. Flavonoids possess the general structural backbone of 15 carbon atoms (C₆–C₃–C₆) in which the two C₆ units are of phenolic nature and linked by a C₃ group. Flavonoids can be further divided into flavonols, flavones, flavanones, flavanols (catechins), etc. (Fig. 6) according to the oxidation state of the central pyran ring. Isoflavonoids and anthocyanins also belong to flavonoids.

Flavones and their 3-hydroxy derivatives flavonols are the most widespread flavonoids in plant foods. Flavones usually occur as glycosides of apigenin and

luteolin in plants. Apigenin has been shown to suppress 12-*O*-tetradecanoylphorbol-1,3-acetate (TPA)-mediated tumor promotion in mouse skin. Suppression of protein kinase C activity and nuclear oncogene expression is suggested to be the possible action mechanism. Apigenin has also been reported to exhibit anti-inflammatory, hypotensive, and antibacterial effects and diuretic activity and promote smooth muscle relaxation. Luteolin has also been reported to exhibit various biological activities such as antioxidant, antimutagenic, anti-inflammatory, and antibacterial.

Flavones are found in celery. Celery stalks and seeds are used to obtain extracts or essential oils, which are widely used as flavoring or spice and to prepare juice, tea, and medicinal products. Celery extracts alone or combined with other juices provide several health benefits such as lowering blood pressure, lowering total cholesterol and LDL cholesterol levels, and anti-inflammatory and anticancer activities. Apigenin and luteolin which are present in a relative high concentration in celery (22–108 mg/kg fresh weight) as well as coumarins, polyacetylenes, and phthalides identified in extracts of celery have been suggested to be responsible for the bioactive properties (Shahidi and Naczka 2003).

The flavonols, on the other hand, are widely distributed in plants. They are most often presenting in conjugated form as glycosides. To date, 200–300 flavonols have been identified from different kinds of plants with quercetin, myricetin, and kaempferol (Fig. 6) as the three most common ones. Onion is one of the richest sources of flavonoids that onion leaves contain 1,497.5 mg/kg of quercetin, 832.0 mg/kg of kaempferol, and 391.0 mg/kg of luteolin. Red wine is also a prominent source of flavonols (up to 30 mg/L). In addition, black tea contains up to 4.17 mg of quercetin/g in the human diet. Other fruits and vegetables such as berries, apples, broccolis, leeks, and curly kales are also sources of flavonols in our diet. Owing to the fact that the biosynthesis of flavonols is stimulated by light, the concentration of flavonol is generally higher in the outer parts (skins and leaves) of the plant foods than the other parts (Manach et al. 2004).

Flavonols have been reported to interfere with various biochemical signaling pathways, thus exerting several beneficial effects. Considered as the most abundant dietary flavonol, the possible health effects of quercetin have been investigated extensively. Quercetin is a powerful antioxidant playing an important role in protecting the body against reactive oxygen species. It also shows anti-atherosclerosis, anti-inflammatory, anticancer, and cholesterol-lowering properties and protects eye health (Erlund 2004).

Ginkgo (*Ginkgo biloba* L.) is a native Chinese plant now cultivated across the world. Both the leaves and the nuts of the tree have been used in traditional Chinese medicine in the past several centuries. Ginkgo nuts have been used extensively to treat pulmonary diseases (e.g., enuresis, cough, and asthma), bladder inflammation, and alcohol abuse. The leaf extracts are generally used to make extracts for medical purpose to alleviate asthma and cardiovascular disorders and treat skin infections, heart and lung dysfunctions, as well as dementia disorders such as memory impairment and concentration difficulties. In addition, the Ginkgo leaf extracts have the benefits of improving the mental capacities to delay the process of Alzheimer's

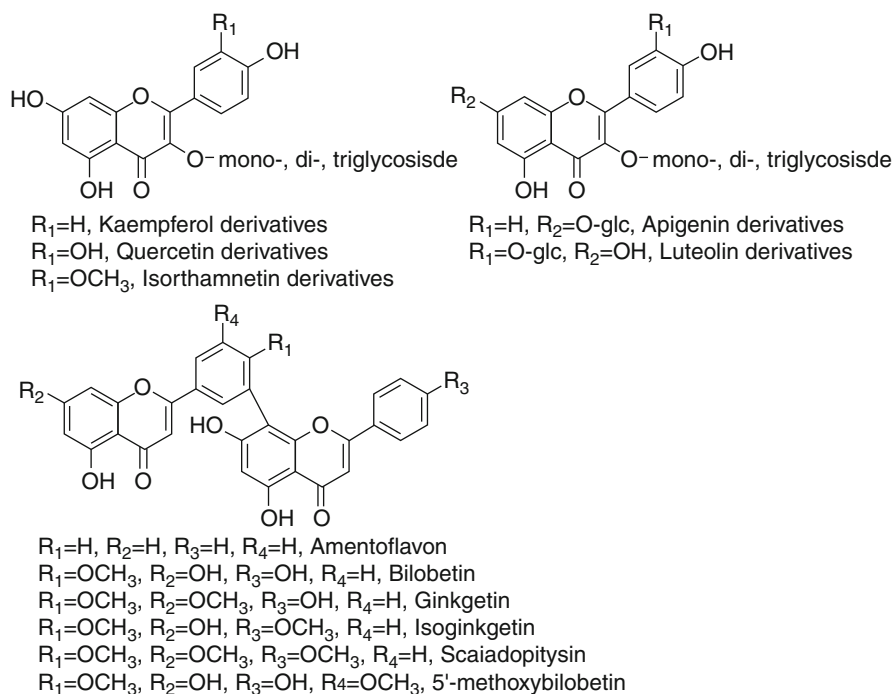


Fig. 7 Chemical structures of flavonoid constituents in Ginkgo

disease (Mahadevan and Park 2008). The physiological benefits of Ginkgo are mainly attributed to the active components, namely, flavonoids and terpenoids (such as ginkgolides and bilobalide), found in them.

The flavonoids (Fig. 7) present in the Ginkgo leaf extract are flavonols, flavonol glycosides, flavones, biflavones, proanthocyanidins, and associated glycosides of quercetin and kaempferol attached to *p*-coumaric esters 3-rutinosides or 3-rhamnosides, of which flavonols (quercetin and kaempferol coumaryl glycosides) are essential for the efficacy of Ginkgo leaf extracts. Rutin and quercetin and a mixture of flavonoids and terpenes from Ginkgo have been found to protect cerebellar granule cells from oxidative damage and apoptosis induced by hydroxyl radicals (Chen et al. 1999). Ginkgo leaves also contain toxic components, ginkgolic acids, the concentration of which must be less than 5 ppm in all commercial Ginkgo leaf products to minimize gastrointestinal and allergic reactions.

Flavanones (Fig. 6) are colorless compounds characterized by the absence of a double bond in the 2, 3-position of the pyrone ring and are isomeric with chalcones. The glycosylated flavanones are generally obtained by a disaccharide at position 7: either a bitter-tasting neohesperidose or a flavorless rutinose in human foods. High flavanone concentrations occur only in citrus fruit. Other plants such as tomatoes and mints contain low concentrations of flavanones. Hesperetin in oranges (*Citrus sinensis*), naringenin in grapefruits (*Citrus paradise*), and eriodictyol in lemons

(*Citrus limon*) are the major flavanone aglycones in the human diet. Hesperidin and narirutin occur in high concentrations at 200–600 and 15–85 mg/L, respectively, in orange juice. Forty to hundred and forty milligram of flavanone glycosides can be obtained from a single glass of orange juice. In addition, the whole orange fruit may contain five times more flavanones than the orange juice as the highest flavanones content is present in the solid tissues, especially the white spongy portion and the membranes separating the segments of the orange fruits (Kaur and Kaur 2014).

Hesperidin and naringenin themselves as well as the citrus fruit juice have been shown to possess anticarcinogenic and antitumor effects in animals studies. Satsuma mandarin juice which is rich in hesperidin has been reported to have possible chemopreventive effect against chemically induced colon carcinogenesis and lung tumorigenesis in rats. Naringenin and its analog liquiritigenin showed potent cancer chemoprevention activity against gastric carcinogenesis in both in vitro and rat studies. Hesperidin and naringenin may also prevent cardiovascular diseases by decreasing plasma low-density lipoprotein and hepatic cholesterol levels. Orange juice fed three times daily was found to decrease the ratio of low-density lipoprotein to high-density lipoprotein cholesterol in human studies. However, more human studies are needed to confirm these results. In addition, a mice model of acute colitis study revealed the potent anti-inflammatory function of naringenin and the aglycones. The anti-inflammatory activity may be attributed to their relative weak antioxidant activities. Naringenin was also shown to suppress intestine carbohydrate absorption and to promote extra-pancreatic action in a diabetic rat model, which contributes to its antidiabetic effects (Lim and Koffas 2010).

Catechins are the monomer form of proanthocyanidins. They are present in many kinds of fruits, among which apricots have the highest concentration at about 250 mg/kg fresh weight. Red wine also contains high amounts of catechins at the concentration of up to 300 mg/L. But the richest sources of catechins by far are known as green tea and chocolate. Up to 200 mg of catechins can be obtained from a cup of green tea (240 mL), while much less amount can be found in black tea because the monomer flavanols in tea leaves are oxidized to two types of more complex condensed polyphenols, theaflavins and thearubigins, during the “fermentation” procedure involved in producing black tea. Dark chocolate has about 54 mg of catechins per 100 g. Catechin and epicatechin are the most abundant flavanols found in fruits, while the main flavanols found in grapes, in certain seeds of leguminous plants, and more importantly in tea are known as gallicocatechin, epigallocatechin, and epigallocatechin gallate (D’Archivio et al. 2007).

The tea plant was first cultivated in China and has been consumed for almost 6,000 years. The first record of the health benefits of green tea appeared in a Chinese medical treatise in 2737 BC. The tea is mainly divided into three types, green, black, and oolong according to the way the leaves are processed. Green tea (unfermented) is produced by lightly steaming the leaves of the plant. Black tea (fully fermented) is produced by allowing the leaves to oxidize. Oolong tea (semi-fermented) is a traditional Chinese type of tea somewhere in between green and black in oxidation. Catechins including catechin (C), epicatechin (EC), gallicocatechin (GC), catechin gallate (CG), epicatechin gallate (ECG), epigallocatechin (EGC),

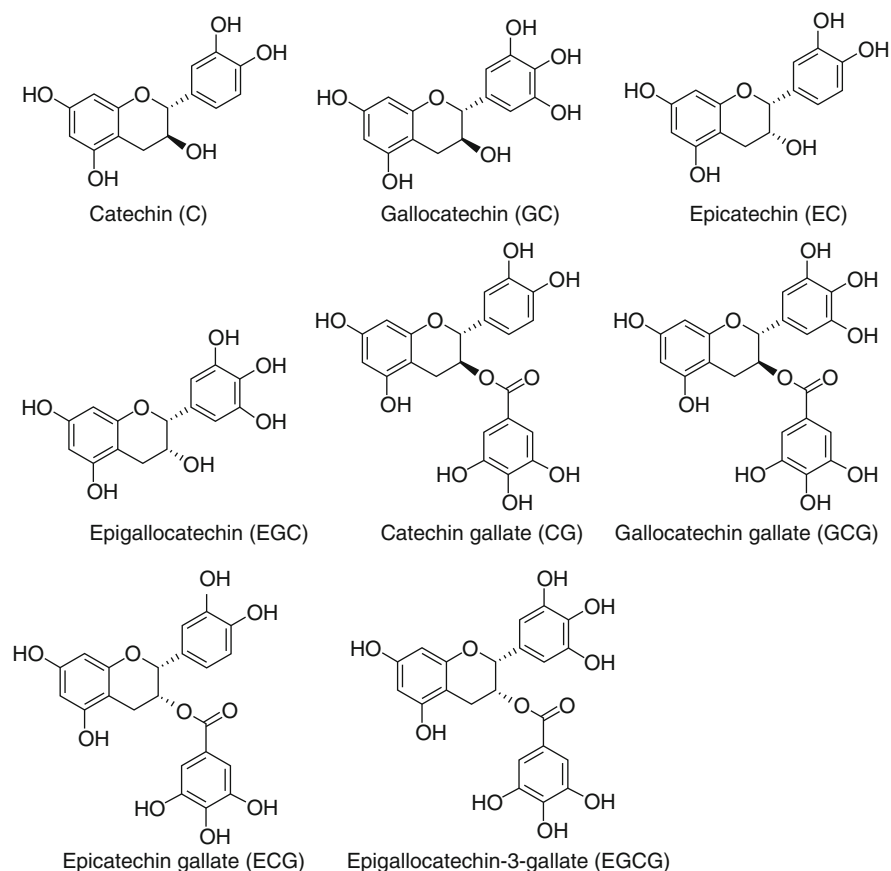


Fig. 8 Chemical structures of green tea catechins

epigallocatechin-3-gallate (EGCG), and gallocatechin gallate (GCG) (Fig. 8) are the main polyphenols present in green tea (Vidal et al. 2014). The total percentage of catechins in dried green tea leaves can reach approximately 30%. They are thought to be responsible for the majority of green tea's beneficial effects. Theaflavins and thearubigins (Fig. 9) are the major bioactive compounds in black tea. More than 20% thearubigins, 3–10% catechins, and 2–6% theaflavins are present in brewed black tea (Yang et al. 2008).

Mouthwashes of both green tea and black tea have shown to decrease dental plaque formation by inhibiting the growth of bacteria such as *Escherichia coli*, *Streptococcus salivarius*, and *Streptococcus mutans* that cause cavities in some studies. Green tea polyphenols also inhibited the growth of *Porphyromonas gingivalis* and its adherence to oral epithelial cells. Both green tea and black tea have been showing cardioprotective activity by lowering serum cholesterol, lipid peroxides, and triglycerides in animal and human studies. EGCG itself and the green tea have been shown to reduce the risk of Parkinson's disease in some

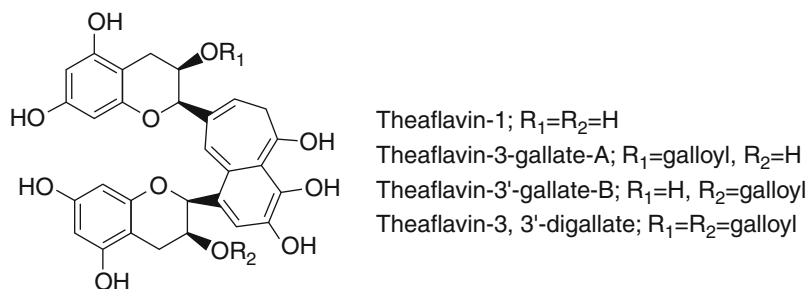


Fig. 9 Chemical structures of black tea theaflavins

experiments. Twenty-eight percent of Parkinson's disease was shown to be reduced by consuming three or more cups of tea every day in a case-control study in China. Green tea also has anticarcinogenic and antimutagenic properties. Green tea and/or green tea polyphenols have been shown to protect against carcinogenesis induced by ultraviolet light or chemicals in mice. The organ sites include the colon, breast, liver, pancreas, duodenum, esophagus, liver, and lung. EGCG is now believed to be the polyphenolic constituent that mainly contributes to the cancer chemopreventive effects of green tea. However, more laboratory, epidemiologic, and clinical studies should be conducted to further confirm the protective effect of tea against cancers and cardiovascular diseases (Rodriguez et al. 2006).

Isoflavonoids

Isoflavonoids, members of flavonoids that have their ring B fused with the C₃ position of ring C, are naturally occurring phenolics with phytoestrogenic activity (Fig. 10). Soybeans and soybean-derived products are the major sources of dietary isoflavones in the human diet. The concentrations of isoflavones in soybean products vary generally depending on the geographic zone, growing conditions, and processing. 580–3,800 mg of isoflavones is present in per kilogram of fresh soybeans, and 30–175 mg of isoflavones is found in per liter of soymilk (Kaur and Kaur 2014). Genistein, daidzein, glycitein, biochanin A, and formononetin are the main isoflavones found in soy. Each one of these compounds is present in four chemical forms, unconjugated (aglycone, IFA), sugar-conjugated (isoflavone glucoside, IFG), acetylglucosides, and malonylglucosides. Red clovers are also rich in isoflavones.

Since soybeans and soybean-derived products are a major part of human diet in many cultures, the role of isoflavones, thus, is of greatest interest. The soy isoflavones have structures similar to mammalian estrogens which confer them the capacity of working as phytoestrogens. These phytoestrogens can bind to the mammalian estrogen receptors and induce estrogenic and/or antiestrogenic effects both in vitro and in vivo. This might be the reason why the populations exposed to high dietary levels of soybeans generally have a reduced risk of estrogen-dependent cancers (e.g., prostate, breast, and perhaps colon cancer) (Kushwaha et al. 2014).

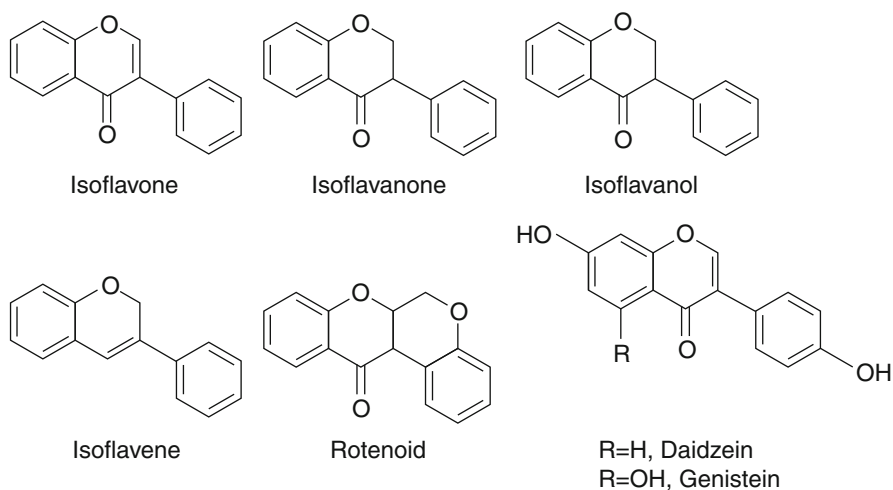


Fig. 10 Chemical structures of isoflavonoids

Though the cancer preventive role of soybeans and its components has been demonstrated by a number of experimental studies, the epidemiological data published so far have not drawn a consistent conclusion on soy intake and cancer risk. A small double-blinded, placebo-controlled study on highly purified genistein and daidzein indicated that they have negative effects on localized prostate cancer.

Epidemiological studies as well as clinical trials showed that Asian women who usually have diets rich in soy food (25–50 mg/day) have better bone health than western women who usually consume less soy food (less than 2 mg/day), characterized by higher bone mineral density and lower rate of bone fracture. In addition, Asian women have significantly lower incidence of hot flashes and other menopausal symptoms compared to western women, which is believed to be due to the effects of soy isoflavones. However, other studies showed that soy intake has no beneficial effect on menopausal symptoms (Rodriguez et al. 2006). High levels of serum cholesterol are associated with cardiovascular diseases. Soybean has been shown to exhibit cholesterol-lowering activity. Isoflavones were believed to be the specific components of soybean that offer protection against cardiovascular diseases by reducing plasma lipid and lipoprotein concentrations, which result in improved flow-mediated arterial dilation and systemic arterial compliance. However, isoflavone supplements were not shown to be effective in reducing cholesterol in two human studies (Nestel et al. 1997; Hodgson et al. 1998).

Anthocyanins

Anthocyanins are known as the largest and most important group of water-soluble vacuolar pigments that are responsible for the pink, red, blue, and purple color of the

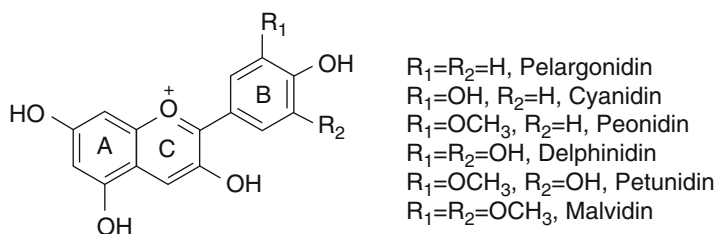


Fig. 11 Chemical structures of anthocyanidins

majority of fruits, flowers, vegetables, and grains (Shahidi and Naczek 2004). Anthocyanins are highly reactive species in plant tissues. Food processing can lead to the conversion of genuine anthocyanins to other molecules, resulting in either loss or destabilization of color and increasing of the range of available hues (Fig. 11).

The basic structures of anthocyanins are anthocyanidins, in which the two aromatic rings A and B are linked by a heterocyclic ring C that possesses oxygen. More than 23 different anthocyanidins have been found with pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin as the most common ones. Anthocyanins in plants mainly exist in the conjugated form as glycosides. More than 500 types of anthocyanins have been identified in plants. Individual anthocyanin varies in the hydroxylation and methoxylation patterns on the B ring; the nature, position, and number of conjugated sugar units; the nature and number of conjugated aliphatic or aromatic acids groups; and the presence or absence of an acyl aromatic group in the molecule. Cyanidins are the most common group of naturally occurring anthocyanins, followed by delphinidins. The substituent groups on the B ring of anthocyanidins and the pH conditions determine severely the color saturation and the stability of anthocyanins. Generally, higher degree of hydroxylation and lower degree of methoxylation of the hydroxyl groups of the aromatic rings result in higher saturated color. Anthocyanins are relatively stable in acidic solutions exhibiting a red color. However, they are unstable under neutral and weakly acidic conditions (Tsao 2010).

The fruit is the major source of dietary anthocyanins in the human diet. Usually, the amount of anthocyanins in the fruit is proportional to the intensity of the color. Strawberries contain about 0.15 mg of anthocyanins/g fresh weight, and cherries contain about 0.45 mg/g. Fruits with high color intensity such as blackcurrants and blackberries contain anthocyanins up to 2–4 mg/g fresh weight. Wine is also rich in anthocyanins that 200–350 mg of anthocyanins can be obtained from 1 l of wine. These anthocyanins are transformed into various complex structures during wine aging. Other food sources of anthocyanins include cereals and leafy and root vegetables (Yildiz 2010).

Berries contain a wide range of phytochemicals, such as flavonoids (e.g., anthocyanins, flavonols, catechins), phenolic acids (e.g., hydroxycinnamic acids), and tannins (e.g., proanthocyanidin, ellagitannins). Berry seed oils, berry oils, berry powders, and standardized berry extracts have been marketed as functional foods

and nutraceuticals. Anthocyanins and anthocyanin-rich berries or derived extracts have been suggested to possess a wide range of health benefits for humans and animals in a growing number of studies. Both *in vitro* and *in vivo* studies demonstrated the protective effects of anthocyanins, including antioxidant activity, anti-edema, decreasing capillary permeability, increasing the regeneration of visual purple, improving vision at dusk, anti-inflammatory activity, and anticarcinogenic activity (He and Giusti 2010). Berry extracts have also been shown to protect heart and brain health. However, it must be noted that not a single class of compounds may explain most of the health-promoting effects of consuming fruits and vegetables. Apparently, the phytochemicals contained in fruits and vegetables work collaboratively with other fruit ingredients to benefit our body.

Tannins

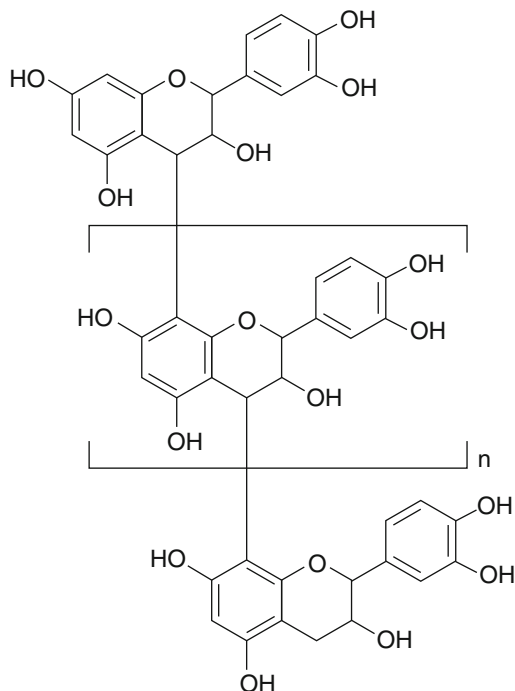
Tannins are polyphenols widely distributed with a wide diversity of structures capable of binding and precipitating proteins from aqueous solutions. Tannins are commonly classified into two categories, condensed tannins (also known as proanthocyanidins) and hydrolysable tannins, according to their structures.

Condensed tannins, also known as proanthocyanidins, chiefly comprise a flavan-3-ol unit to form dimers, oligomers, and polymers, up to 50 monomer units (Fig. 12). Proanthocyanidins have complex structures that individual proanthocyanidin varies in the number of flavan-3-ol unit, the location and type of interflavan linkage in the molecule, and the nature and position of substituents on the flavan-3-ol unit. Proanthocyanidins can be classified into several subgroups (e.g., procyanidins, propelargonidins, and prodelfinidins) based on their hydroxylation pattern of A and B rings (Shahidi and Naczk 2003).

Proanthocyanidins (condensed tannins) are responsible for the astringent character of fruits and beverages and for the bitterness of chocolate through the formation of complexes with salivary proteins. The astringency decreases over the course of maturation and often disappears when the fruit reaches ripeness. Proanthocyanidins occur in high levels in grapes, berries, apples, peanut inner skins, chocolates, pine bark, and cinnamon bark. Grape skins and seeds are particularly rich sources of proanthocyanidins that have received considerable attention from nutritionists, epidemiologists, and general consumers.

The abilities of grape seed proanthocyanidins to protect against lipid peroxidation and DNA fragmentation induced by 12-*o*-tetradecanoylphorbol-13-acetate (TPA) have been examined *in vivo*. It showed that grape seed proanthocyanidins have greater ability to decrease the production of reactive oxygen species in the liver and brain tissues of mice as compared to vitamin C and E. Moreover, proanthocyanidins showed a threefold to fourfold greater DNA fragmentation reduction ability than either vitamin C or beta-carotene in both hepatic and brain tissues. All these data support the powerful antioxidant activity of proanthocyanidins, which may be responsible for its cardioprotection, cancer chemoprevention, and lowering cholesterol level (Shi et al. 2003).

Fig. 12 Chemical structures of condensed tannins



Berries of *Vaccinium* sp. exhibit a wide range of biological activities with potential health benefits for humans and animals. Cranberry and wild blueberry and their derived materials have been shown to prevent bacterial motility-induced urinary tract infections. Proanthocyanidins have been suggested to be responsible for this protective effect due to their ability of preventing bacterial colonization. Proanthocyanidins fraction in wild blueberry has also been shown to prevent chemically induced carcinogenesis in vitro (Bomser et al. 1996).

Cinnamon bark has been widely used as a spice for centuries. In addition to its culinary uses, cinnamon bark has been employed in the form of a traditional herbal medicine for treating various health conditions. The available evidence from in vitro and animal studies suggested the protective effects of cinnamon as an antitumor, anti-inflammatory, antioxidant, antimicrobial, and cholesterol-lowering agent, as a treatment of infectious diseases, and for the prevention of cardiovascular diseases. The well-documented physiological effect of cinnamon is its activity for the treatment of type 2 diabetes (Gruenwald et al. 2010). However, further clinical studies are necessary to draw solid conclusions about these health benefits described above. A wide range of phenolic compounds, particularly, proanthocyanidins occurring in high amount in cinnamon bark, could in part account for the biological function of cinnamon (Mateos-Martín et al. 2012).

Hydrolysable tannins are molecules with a polyol (generally D-glucose) as a central core. Hydrolysable tannins have a more restricted occurrence than

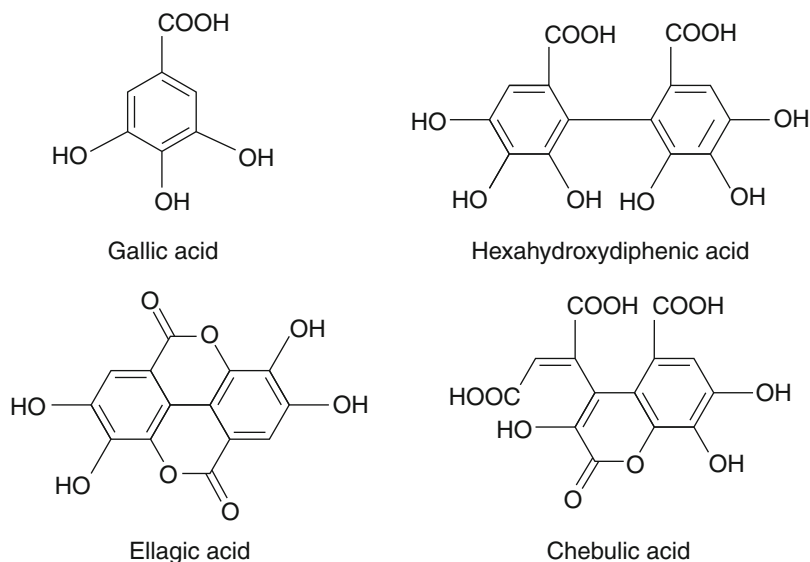


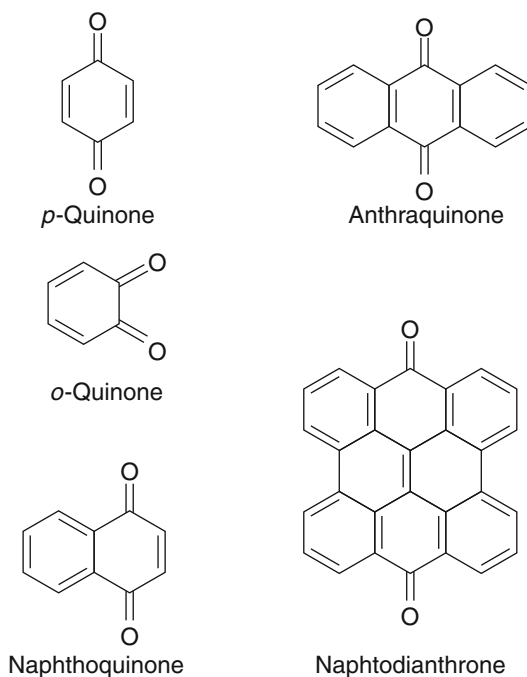
Fig. 13 Phenolic acid residues of hydrolysable tannins

condensed tannins in plants. They are further divided into two subclasses, gallotannins, which involve a glucose esterified to gallic acid, and ellagitannins, which are ellagic acid-derived hexahydroxydiphenic acid (Fig. 13). The distribution of gallotannins is rather limited in nature while ellagitannins are commonly found in many plant families. The profile of hydrolyzable tannins in a plant species is generally stable throughout the year (Amoo 2009).

Pomegranate (*Punica granatum* L.), a rich source of polyphenol compounds, particularly hydrolyzable tannins and anthocyanins, is widely used to process to juice and sauce. The total content of hydrolyzable tannins (e.g., gallagyl tannins, gallotannins, and ellagic acid tannins) and anthocyanins (e.g., pelargonidin, cyaniding, and delphinidin glycosides) in pomegranate juice ranges from 417.3 to 556.6 mg/L and from 161.9 to 387.4 mg/L, respectively. The hydrolyzable tannins have been reported to account for 92 % of the antioxidant activity of the whole fruit. The anthocyanins impart the red color to the fruit and juice. Other phenolic compounds such as catechin, gallic acid, and caffeic acid have also been detected in pomegranate.

Pomegranate fruit extracts/constituents exhibit several health benefits such as antioxidant, antiatherogenic, antitumor, antiviral, antifungal, antibacterial, free radical scavenging, prevention of heart diseases, and strengthening of the immune system in a growing body of in vitro and in vivo studies. All these protective effects could in part attribute to the diverse phenolic compounds in the pomegranate (Shahidi and Nacz 2003).

Fig. 14 Skeletal structures of quinones



Quinones

Quinones are kinds of phenolic compounds with a fully conjugated cyclic dione structure, such as that of benzoquinones, derived from aromatic compounds by the conversion of an even number of $-\text{CH}=\text{}$ groups into $-\text{C}(=\text{O})-$ groups with any necessary rearrangement of double bonds. Quinones show a wide range of pharmacological activities, which are the basis for their applications in the broad field of pharmacy and medicine. Plants which are rich in quinones are classically used in industry (dyestuffs) and pharmaceutical (laxatives) practice (Martínez and Bermejo Benito 2005). The most common skeletal structures of quinones found in plants are *p*-quinone, *o*-quinone, anthraquinone, naphthoquinone, and naphthodianthrone (Fig. 14).

Senna leaves are harvested from *Cassia senna* plants, and they are used to make herbal laxatives and tea to treat constipation. The active components in senna are anthraquinone derivatives, namely, rhein, aloe-emodin, chrysophanic acid, and physcion (Fig. 15), which are present both in free and glycoside forms. Sennosides A and B present in senna are the dianthrone derivatives of rhein with two glucose units. The leaves contain maximum sennosides at the time of flowering, and the handpicked pods are superior to black pods with regard to sennoside content. Owing to their chemical structures, the active components in glycoside forms are unabsorbed into the large intestine, where microbial metabolism takes place and the active aglycone is released. The intestinal bacterial flora also accounts for the

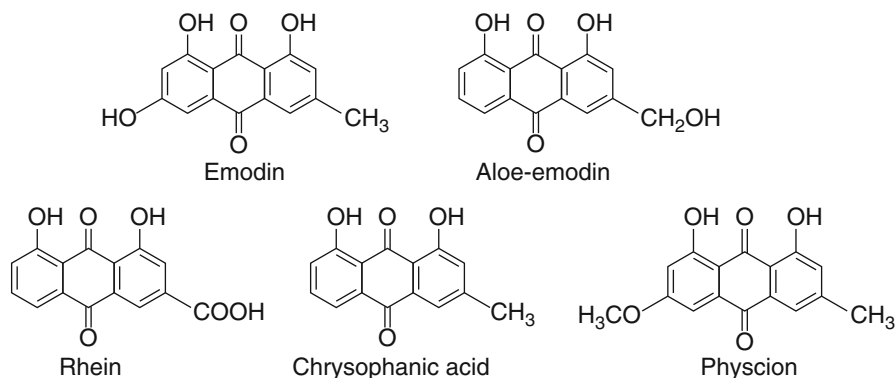


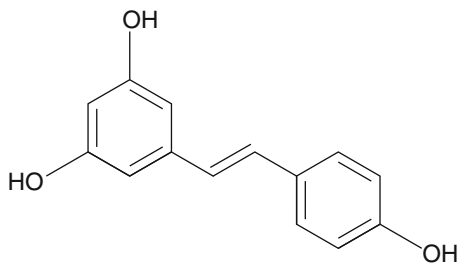
Fig. 15 Chemical structures of emodin, aloe-emodin, rhein, chrysophanic acid, and physcion

reduction of anthraquinone aglycones to the corresponding anthrones. These aglycones exert their laxative effect by damaging epithelial cells, which leads directly and indirectly to changes in absorption, secretion, and motility. The anthranoids are transformed after adsorption mainly into their corresponding glucuronide and sulfate derivatives, which appear in urine and bile (Selvaraj and Chander 2013).

Emodin, an active component present in the root and rhizome of *Rheum palmatum* L., has been shown to exhibit antitumor effects, but the mechanism is not fully understood. Several studies demonstrated that emodin induced cell apoptosis in human lung squamous carcinoma cell line CH27 and cells derived from human colon carcinoma. *Rheum officinale* H. Bn., a Chinese medicinal plant, also contains large amounts of anthraquinones as active compounds (e.g., emodin, chrysophanol, and rhein), which specifically inhibited one of the carcinogenesis-related enzymes (cytochrome P450). Additionally, chrysophanol, emodin, and rhein have been shown to inhibit benzo(α)pyrene-mediated DNA damage in human hepatoma cell line Hep62. In the same way, Kuo et al. (1997) tested four anthraquinones, emodin, emodin-1-glucoside, physcion, and physcion-1-glucoside, purified from another Chinese herb *Polygonum hypoleucum Ohwi* for their effects on antitumor activity in vitro. Emodin, on a percentage basis, was proven to be the most potent one against the various tumor cells proliferation, particularly K562 and Raji cell lines (Martínez and Bermejo Benito 2005).

Aloe-emodin is a natural anthraquinone from *Aloe vera* L. leaves that has been reported to exhibit anti-neuroectodermal tumor activity both in vitro and in vivo. Aloe-emodin has been demonstrated to be nontoxic for normal cells, but to possess potent neuroectodermal tumor cell selective cytotoxicity. Taking into account of its unique cytotoxicity profile and mode of action, aloe-emodin might represent a conceptually new lead antitumor drug. Aloe-emodin also significantly inhibited the growth of Merkel carcinoma cell, a free-floating cell line that was established from a metastasis of a Merkel cell carcinoma patient, and was characterized by

Fig. 16 Chemical structures of resveratrol



immunocytochemical methods using antibodies against the neuroendocrine and epithelial antigens. Aloe-emodin merits further investigation as a potential agent for treating these tumors (Wasserman et al. 2002).

Stilbenes

Stilbenes are a group of phenolic compounds that share a similar chemical structure to flavonoids, in which the two aromatic rings A and B are linked by a methylene bridge. Stilbenes are present in low quantities in the human diet. *Trans*-resveratrol (Fig. 16), mostly present in glycosylated forms, is one of the most recognized stilbenes. Cranberries and grapes contain the amounts of resveratrol between 0.16 and 3.54 mg/kg fresh weight, which contribute to the high concentration of resveratrol in red wine between 0.1 and 14.3 mg/L. Resveratrol is present in peanuts in a relative lower amount between 0.02 and 1.92 mg/kg (Lim and Koffas 2010).

Resveratrol is a potent antioxidant and thereby may play a role in the chemopreventive effects. Resveratrol has been shown to increase the plasma level of antioxidants and reduce the extent of lipid peroxidation in vivo, which may reduce the risk of coronary heart disease and myocardial infarction. Red wine has been reported to inhibit human LDL oxidation in vitro (Teissedre et al. 1996); this protective effect of red wine may be attributed to wine phenolics. In vitro and in vivo studies indicate that resveratrol can suppress platelet aggregation which is one of the major contributors to the atherosclerosis process. Resveratrol has also been shown to reduce the atherosclerotic plaques formation and restores flow-mediated dilation in vivo. These results may partly explain the mechanisms of resveratrol's cardioprotective effects.

Resveratrol has also been reported to inhibit carcinogenesis at the initiation, promotion, and progression stage in both in vitro and animal studies, such as prostate cancer, lung metastases, skin tumors, and colorectal cancer. Several mechanisms have been proposed on the cancer-prevention activity of resveratrol: resveratrol can inhibit the enzymatic activity of cyclooxygenase to inhibit the arachidonic pathway, in which tumor stimulator prostaglandins are produced; resveratrol can induce cell cycle arrest and apoptosis; and resveratrol can inhibit the preneoplastic lesion development to slow down the carcinogenic progression.

Caloric restriction has been thought to be the only way to extend life span since the 1930s. Resveratrol, a potent sirtuin activator, has antiaging and life-prolonging potentials. The biological effect is achieved by the activation of the NAD⁺-dependent deacetylase sirtuin, which contributes a lot to calorie restriction, thereby extending the life span. Other health benefits of resveratrol such as neuroprotective, antidiabetic, anti-inflammatory, and antiviral activity have also been described both *in vitro* and *in vivo* (Fernández-Mar et al. 2012).

Alkaloids

Alkaloids are a group of diverse low-molecular-weight compounds with nitrogenous bases (usually heterocyclic), which make them have potent bioactivity and bitter taste. Chemically, alkaloids are often constituted by one or more rings of carbon atoms with a nitrogen atom inside the ring, and most of them are derived from amino acids. However, its origin from different plant families or different groups of alkaloids makes the position of the nitrogen atom in the carbon ring vary, which leads to diverse structures and functions of the alkaloid family. Up to date, more than 10,000 naturally occurring alkaloids have been discovered from over 300 different plant species. Alkaloids are also isolated from animals, fungi, and bacteria. With the application of different purification and structural elucidation techniques, the structure and biological activity of natural alkaloids are becoming well known; some of them are utilized as pharmaceuticals, stimulants, narcotics, and poisons (Wink et al. 1998). Owing to the fact that alkaloids have a great structural diversity and without uniform classification, here we only highlight the six groups of most common alkaloids, including indole alkaloids, pyrrolidine alkaloids, tropane alkaloids, quinolone alkaloids, isoquinoline alkaloids, and izidine alkaloids.

The group of indole alkaloids is considered as the largest class of alkaloids, which contains more than 1,500 natural products that are traditionally used as medicinal agents. Chemically, indole alkaloids contain a structural moiety of indole (Fig. 17), and many of them also have the isoprene groups. The amino acid tryptophan is the biochemical precursor of all indole alkaloid syntheses (Lewis 2006).

Pyrrolidine alkaloid is a group of alkaloids consisting of 80 natural compounds, and most of them are derived from ornithine and lysine by adding acetate/malonate units. The basic unit is the five-membered carbon rings containing the nitrogen atom. The simple pyrrolidine alkaloid structures can be exemplified by hygrine and cuscohygrine (Fig. 18) isolated from plant Solanaceae. These simple pyrrolidine units can be further derived to other series of alkaloids by condensing with other compounds. Tropane alkaloid is a group of alkaloids that contains a tropane ring and is often esterified with a variety of acids, such as acetic acid, atropic acid, isobutyric acid, isovaleric acid, propanoic acid, tiglic acid, tropic acid, and 2-methylbutyric acid. Most of them occur naturally in species of Solanaceae, Convolvulaceae, and Erythroxylaceae.

Fig. 17 Chemical structural of indole

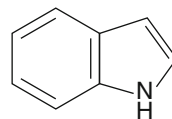


Fig. 18 Pyrrolidine alkaloids

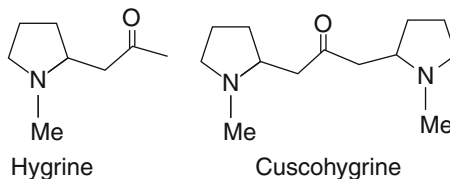


Fig. 19 Quinoline and isoquinoline

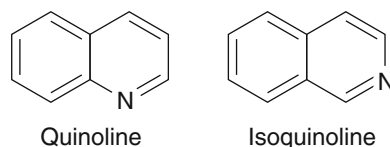
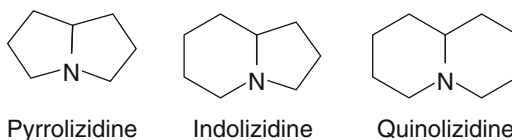


Fig. 20 Skeleton of izidine alkaloids



Quinoline and isoquinoline are the compounds with benzo-fused pyridines (Fig. 19). Quinoline forms part of the structure of quinine which has a 6-MeO substituent and a side chain attached to C₄ of it. Isoquinolines are the alkaloids that are mainly distributed in Papaveraceae (poppy family) and Berberidaceae (barberry family). Isoquinoline has a wide range of biochemical effects; they are usually considered as bioactive ingredients in herbal medicines. For example, they can be used as painkiller, inhibit the growth of cancer cells and bacteria, and stimulate bone marrow leucocytes as well as myocardial contractility.

Izidine alkaloids include some 500 compounds containing a pyrrolizidine, indolizidine, or quinolizidine skeleton (Fig. 20).

A necine base (the hydroxylated pyrrolizidine, Fig. 21) is the basic structure of the pyrrolizidine alkaloids, which could be derived to a series of alkaloids by esterifying with a carboxylic acid either as a monoester, a diester, or a cyclic diester with a dicarboxylic acid. The fourth class of these alkaloids is the *N*-oxides of the above structures. Most of these alkaloids show liver toxic effects.

In the nutraceutical industry, only a few of alkaloids are widely applied, such as the bioactive alkaloids that are isolated from yohimbe, bitter orange, golden seal, caffeine, and ephedra.

Yohimbine (Fig. 22) is an active indole alkaloid with sexual stimulant and aphrodisiac effects found naturally in *Pausinystalia yohimbe* (K. Schumann,

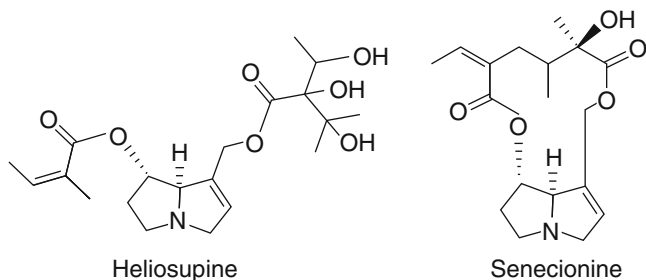
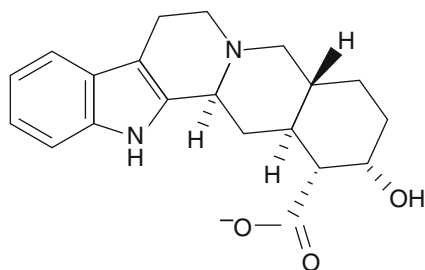


Fig. 21 Pyrrolizidine alkaloids

Fig. 22 Chemical structure of yohimbine



Yohimbe). It can increase blood flow to the penis by affecting the central nervous system, specifically stimulating the lower spinal cord region where the sexual responses are conveyed. In the United States, yohimbine in the hydrochloride form has been used to treat the erectile dysfunction as a prescription medicine. The commercial dietary supplements of yohimbe bark with different purity are also widely sold for the treatment of sexual dysfunction and enhancement of sexual satisfaction. However, there are lots of side effects related to the consumption of yohimbine as reported, such as elevated systolic blood pressure and heart rate as well as anxiety, headache, and increased urinary output. So, the patients who have high blood pressure or CVD should avoid the usage of yohimbine; ordinary people should also use it under the supervision of medical doctors (Drewes et al. 2003).

In traditional Chinese medicine, bitter orange can be used for treating a variety of clinical symptoms, including indigestion, diarrhea, dysentery, and constipation, and as an expectorant (Blumenthal 2004; Stohs and Shara 2007). At present, manufacturers like to advertise the extract of *Citrus aurantium* (bitter orange) for weight management and weight loss as well as enhancing sports performance. The main functional compound contained in the extract of bitter orange is *p*-synephrine, which is a protoalkaloidal constituent. Chemically, *p*-synephrine is derived from phenylethanolamine by attaching the hydroxyl group in the para position on the benzene ring of the molecule (Fig. 23). It has been suggested that the synephrine alkaloids extracted from *C. aurantium* are sympathomimetic agents containing both α - and β -adrenergic receptor agonists; these adrenergic agonists can lead to reducing gut motility. Through this signaling pathway, synephrine alkaloids could

Fig. 23 Chemical structure of *p*-synephrine

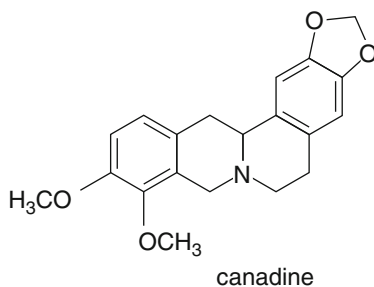
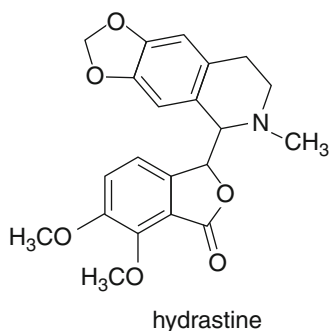
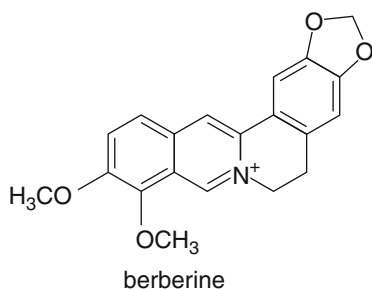
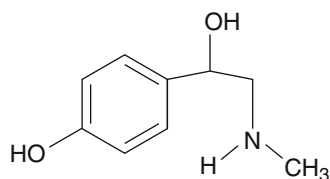
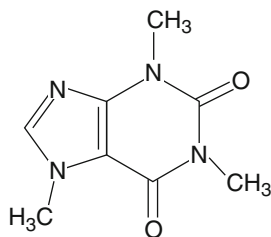


Fig. 24 Structures of three main goldenseal constituents

potentially decrease food intake by keeping the sensation of satiety and increase the burning of energy stored in our body (Haaz et al. 2006).

Goldenseal (*Hydrastis canadensis*) is a native North American plant, which contains a high amount of isoquinoline alkaloids and has been widely used to treat gastrointestinal disturbances, eye infections, and inflammation in a variety of regions for a long history (Upton 2001). The extracts of goldenseal root have been known to have antibacterial, immunostimulant, antimicrobial, and anticancer properties for a long time, all because of these three major alkaloids found in the extracts, berberine, hydrastine, and canadine (Fig. 24). In particular, berberine, the most active and abundant compound in this plant, exhibits multiple pharmacological activities (Brown et al. 2008). Nowadays, goldenseal is available in a wide array as herbal products and has been occupying great international market share. According to the American Herbal Products Association (AHPA), over 90 % of manufacturers sell goldenseal-containing products, and it can create more than \$50 million profit annually in the United States alone.

Fig. 25 Chemical structure of caffeine



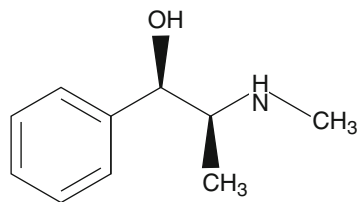
Caffeine (Fig. 25) is the most abundant purine alkaloid in the coffee plant, and it also belongs to a group of methylxanthines and methyl uric acids derived from purine nucleotides. Caffeine is well known as a stimulator of wakefulness and to enhance concentration and minimize the sensation of fatigue (Heckman et al. 2010). This kind of popularity was due to the active promotion of caffeine products by manufacturers. It gives people a wrong impression that caffeine only has benefit without any side effects. However, scientific studies suggested that high levels of caffeine consumption may have adverse effects on fertility and increase risk of restriction to fetal growth during pregnancy. The recommendation of caffeine consumption limit for women trying to become pregnant is <300 mg per day (Kuczkowski 2009). In addition, pregnant women are advised to drink no more than two cups of coffee or four cups of tea per day (Heckman et al. 2010). Regarding the health risks caused by caffeine, many countries should start to establish the regulatory boundaries around caffeine-containing products.

In the food market, caffeine is a common ingredient of lots of beverages (e.g., coffee, tea, cola); energy drinks are the typical commercial beverages that contain variable amounts of caffeine and other ingredients (e.g., guarana, taurine, sugar derivatives). The caffeine content inside those beverages could range between 50 and 505 mg per container (Reissig et al. 2009). In the pharmacy, caffeine can also be sold as over-the-counter cold preparations, stimulants, analgesics, and appetite suppressants (Barone and Roberts 1996).

Ephedra is the evergreen shrub-like plant that mainly grows in Central Asia and Mongolia. Ephedrine (Fig. 26) is the principal active compound isolated from various plants in the genus *Ephedra* (family Ephedraceae), which can powerfully stimulate the nervous system and heart by increasing the activity of adrenergic receptors. Chemically, ephedrine has similar molecular structure with phenylpropanolamine and methamphetamine as well as epinephrine (adrenaline), as it has a phenethylamine skeleton as its basic structure. In the market, it was used to sell in hydrochloride or sulfate salt form.

As a traditional medicine in China and India, ephedra has been used for a long time to treat colds, fever, flu, headaches, asthma, wheezing, and nasal congestion. In the recent time, manufacturers have applied the ephedra as an ingredient in dietary supplements for weight loss purpose. In the herbal market, consumers could find capsules, tablets, tinctures, and teas containing dried stems and leaves of the ephedra plant. It has also been suggested that using ephedrine or ephedrine plus

Fig. 26 Chemical structure of ephedrine



caffeine could significantly induce weight loss over a relatively short time (less than or equal to 6 months). However, there has no study that assessed their long-term effects (greater than 6 months). According to the US Food and Drug Administration (FDA), except for short-term weight loss, ephedra could greatly increase the risk of heart problems and stroke. The reasons are still unclear. In 2004, the FDA banned the US sale of dietary supplements containing ephedra. However, ephedra can still be used as traditional Chinese herbal remedies or sold as herbal teas in certain countries (Woolf et al. 2005).

Terpenes

The terpenes or isoprenoids isolated from plant source mostly are the secondary metabolites associated with plant growth and development. Terpenes are considered as the most diverse class of metabolites while there had been over 30,000 types of terpenes discovered. Chemically, isoprene which has the molecular formula C_5H_8 is the basic unit form for terpenes, so that the basic molecular formulas of terpenes could be showed as $(C_5H_8)_n$ where n is the number of linked isoprene units. This is called the isoprene rule or the *C5 rule*. Since the isoprene units may form linear chains by being linked together “head to tail” or form rings, terpenes contain varied compounds with diverse structure (Fig. 27). That is why people classify terpenes according to their molecular formula, such as monoterpenes, C10; sesquiterpenes, C15; diterpenes, C20; sesterterpenes, C25; triterpenes, C30; and so on (Dewick 2001).

In the nutraceutical industry, the most popular terpenes belong to the group of monoterpenes, sesquiterpenes, and tetraterpenes. For example, the monoterpene from thyme oil, parthenolide contained in feverfew, valerenic acid from valerian, lutein, and lycopene are considered as bioactive compounds in different products. Thymol (2-isopropyl-5-methylphenol, IPMP) belongs to the monoterpene group and is biosynthetically derived from cymene, isomeric with carvacrol. It can be extracted from *Thymus vulgaris* (common thyme) or various other kinds of plants as well as in oil of thyme. Purified thymol is a white crystalline substance with strong antiseptic properties and pleasant aromatic odor. In foods, thyme is used as a flavoring agent.

Thymol contains a phenolic structure (Fig. 28), which makes it to have the antimicrobial activity against bacterial strains including *Staphylococcus aureus* and *Aeromonas hydrophila*. The phenolic structure of thymol can inhibit the growth and

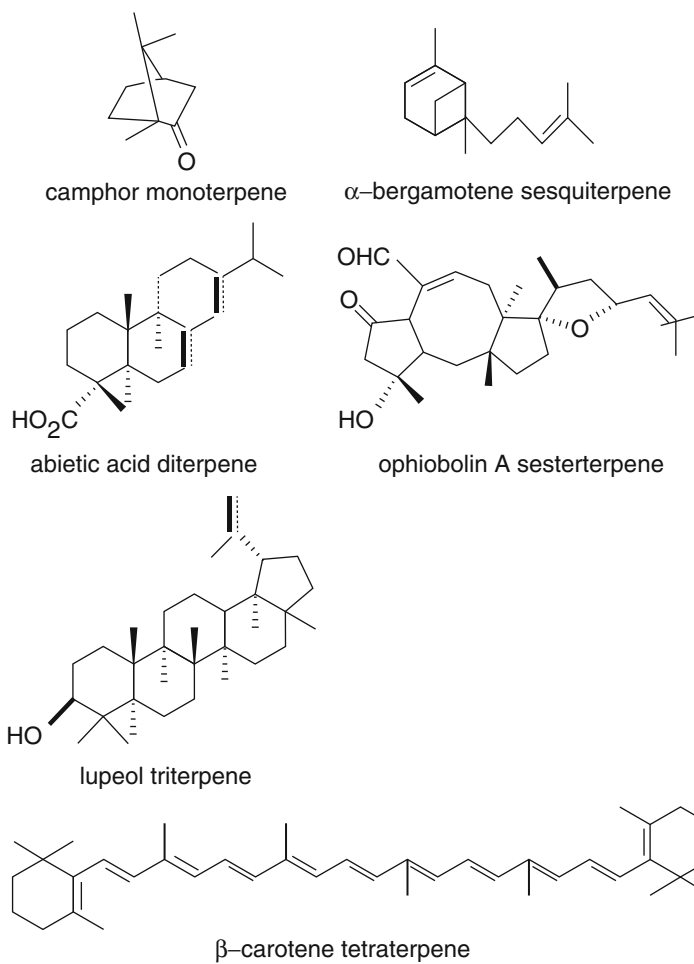


Fig. 27 Typical types of terpenes

Fig. 28 Chemical structure of thymol

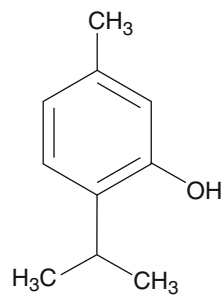
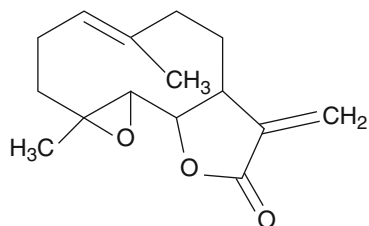


Fig. 29 Chemical structure of parthenolide

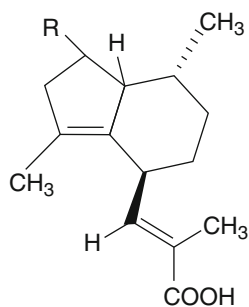


lactate production of bacteria as well as decrease their cellular glucose uptake. For medical use, thymol in alcohol solutions or in dusting powder could be used to treat ringworm infections, hookworm infections, and tinea. When combined with chlorhexidine, thymol could be more effective to reduce gingivitis and plaque. It also can be used as an anesthetic. In addition, thymol can be applied as an antiseptic in mouthwash, a preservative in halothane, an ingredient in perfumes as well as in cosmetics, soaps, and toothpastes (Dorman and Deans 2000).

Feverfew (*Tanacetum parthenium*) is a plant that originates from southeastern Europe, but is now widespread throughout Australia, Europe, and North America. In ancient times, feverfew was used to treat menstrual cramps or reduce inflammation; however, at present, it is widely used for preventing migraine headaches by relieving spasms in smooth muscle tissue, and the effectiveness and safety have been proven by several scientific studies. In the market, feverfew supplements are available as tablets, capsules, or liquid extracts, in which at least 0.2 % parthenolide should be contained. Parthenolide (Fig. 29) is a naturally occurring metabolite isolated from feverfew and belongs to the sesquiterpene lactone group. The purified parthenolide is a colorless, bitter-tasting lipophilic compound. Parthenolide was demonstrated to reduce migraine headaches in animal models by decreasing the initial excessive intracranial arterial constriction and directly inhibiting the contraction of vascular smooth muscle. It is also suggested that parthenolide can induce cancer cell apoptosis by inhibiting STAT- and NF- κ B-mediated anti-apoptotic gene transcription and directly bind with pattern recognition receptor NOD2. In addition, parthenolide is known to reduce inflammation through the inhibition of NF- κ B signaling pathway (Smolinski and Pestka 2005).

Valerian (*Valeriana officinalis*) is a perennial plant with a distinctive odor native to Asia, Europe, and North America. At present, valerian is widely applied as a mild sedative and sleep aid for insomnia and nervous tension. However, there is no conclusive evidence from clinical studies that could prove the efficacy of valerian in treating sleep disorders. In the market, valerian supplements are available as capsules or tablets which are usually made from its roots, stolons, and rhizomes. There are also herbal teas or tinctures available in the market made with dried roots of valerian. The active constituents of valerian remained unclear; it is suggested that the biochemical activity may result from interactions among multiple constituents instead of any single compound. Valerenic acids and its derivatives (Fig. 30) are terpenoids that are isolated from valerian, which belongs to the sesquiterpenoid group. In animal studies, the sedative properties of valerenic acids and its

Fig. 30 Chemical structure of valeric acid and their derivatives



Valeric acid (R=H)
 Hydroxyvaleric acid (R=OH)
 Acetoxyvaleric acid (R=OAc)

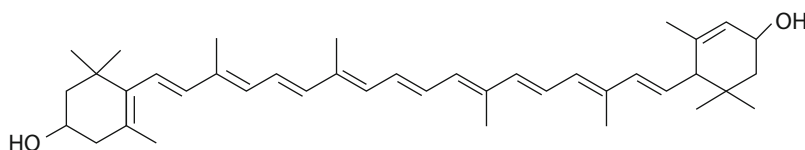


Fig. 31 Chemical structure of lutein

derivatives have been proven. The mechanism behind a valerian extract causing sedation is due to the increased amount of gamma-aminobutyric acid (GABA) in the synaptic cleft, which inhibits neurotransmission (Khom et al. 2007; Trauner et al. 2008).

Lutein (Fig. 31) is a xanthophyll belonging to the carotenoid group, which can protect our eyes from the acquired ocular diseases, such as age-related macular degeneration and cataracts. In the eye, lutein can accumulate in the retina and protect it from phototoxic damage by acting as a blue light filter and as an antioxidant. Nowadays, many manufacturers like to add the xanthophyll carotenoids to their multiple vitamin formulas and promote them as the essential nutrients which could greatly benefit your health and your lifestyle. Some companies even develop special eye vitamins containing large amount of lutein and other xanthophyll carotenoids. In manufacturing, marigold flowers are the dominant source of lutein for many lutein supplements. Also, dark green and leafy vegetables such as kale, spinach, and yellow carrots are the major source of lutein we can acquire from food. However, there is no scientific agreement as how much lutein should be taken daily for adequate eye and vision protection; some study shows that dietary intake levels of 6–10 mg/day result in positive effects, while over-consumption of lutein could lead to the bronzing of the skin (Schalch and Weber 1994).

Lycopene is a natural carotenoid predominantly synthesized by plants, which can protect the plants from photosensitization by absorbing visible lights during photosynthesis. Red fruits and vegetables such as apricots, tomato, pink guava, pink

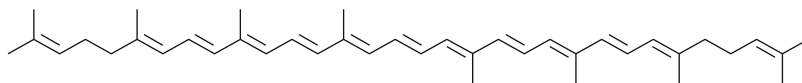


Fig. 32 Chemical structure of lycopene

grapefruit, watermelon, and papaya are the rich sources of lycopene. Especially, tomatoes and their based product are the most common sources of lycopene, which account for more than 85 % of consumption of lycopene in North America (Mangels et al. 1993). Chemically, lycopene is a linear hydrocarbon chain containing 11 conjugated and 2 nonconjugated double bonds (Fig. 32). Under the induction of thermo-energy or light or during chemical reactions, the bonds of lycopene can undergo isomerization from *trans*- to mono- or poly-*cis* isomers (Olson 1989).

As an efficient antioxidant, lycopene can neutralize oxygen-derived free radicals by breaking the double bonds between the carbon atoms. Since many degenerative diseases are caused by the oxidative damage from these free radicals, such as cancer, cardiovascular diseases, cataracts, and premature aging, many manufacturers like to develop dietary supplement products containing tomato lycopene and advertise them as “strong antioxidants that can maintain healthy status.”

In the market, lycopene is commonly available in oil-based softgel form, and it can be mixed with other formulations or just by itself. Tomato extracts are also a wide-selling and popular lycopene supplement due to its lycopene content. Also, there is no reported side effect for excessive intake of lycopene except it may cause the bronzing of the skin.

Saponins

Saponins are the bioactive compounds that predominately originate from plants and generally occur as polycyclic triterpenes or steroids with glycosides. Due to their chemical characteristics, saponins have lyobipolar properties, so that they can interact with cell membranes and have the ability to decrease the surface tension of aqueous solutions. Chemically, saponin can be divided into two groups, the triterpenoidal saponins and the steroidal saponins. Steroidal saponins contain 27 carbon atoms which form their core structures, such as spirostan (Fig. 33). However, in nature, (25*R*)-spirostan derivatives (“isosaponins”) and (25*S*)-spirostan derivatives (“real” saponins or neosaponins) are the most commonly occurring saponins, which have the A/B/C/D ring structures linked together in the order of *cis-trans-trans* (5 β derivatives) or *trans-trans-trans* (5 α derivatives). Triterpenoidal saponins or their nor-derivatives contain 30 carbon atoms which form their core structures, such as tetracyclic dammarans and pentacyclic oleanans (Fig. 34). There are also other triterpenoidal saponins, i.e., lupan, oleanan, ursan, and hopan, which have the A/B/C/D ring structures linked together in the order of *trans-trans-trans-cis* (lupan derivatives) or *trans-trans-trans-trans* (oleanan and

Fig. 33 Core structure of spirostan

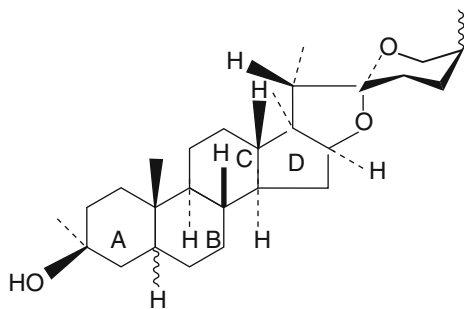
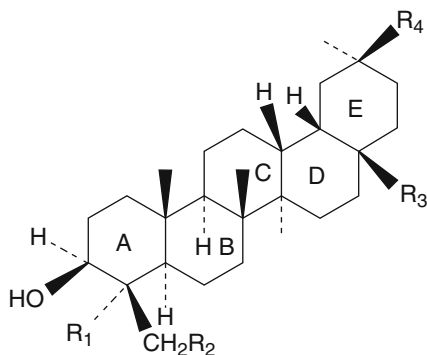


Fig. 34 Core structure of triterpenoid saponenin with arrangement of rings ABCDE



ursan derivatives), while in dammaran derivatives, the ring order is in *trans-trans-trans* (Hostettmann and Marston 2005).

Black cohosh (*Actaea racemosa* and *Cimicifuga racemosa*) is a perennial plant native to North America and is widely used for the treatment of night sweats, hot flashes, or other menopausal symptoms. The representative triterpene glycosides isolated from black cohosh are actein, cimigenol-3-*O*-arabinoside, cimigenol-3-*O*-xyloside, 23-*epi*-26-deoxyactein (*syn*-27-deoxyactein), and acetylshengmanol-3-*O*-xyloside (Fig. 35). However, the active compounds of black cohosh still remained unclear. In the market, black cohosh is usually sold as herbal supplement, which is made from roots and rhizomes. The commercial products of black cohosh supplements usually contain 1 mg of total triterpene saponins (using 26-deoxyactein as a standard) per 20-mg dose of extract (Jacobson et al. 2001).

Since the triterpene glycosides found in black cohosh have similar structure with estrogens, they may alter the effects of other chemicals with estrogen-like properties. Even though there is no scientific conclusion that black cohosh has significant effects on estrogen receptors in human, the combination of black cohosh with estrogen should be warned. Regarding the treatment of menopausal symptoms with black cohosh, there are no conclusive scientific results that can prove whether black cohosh is effective to menopausal symptoms since the results from different studies are mixed. Also, most of the studies related to black cohosh are in short

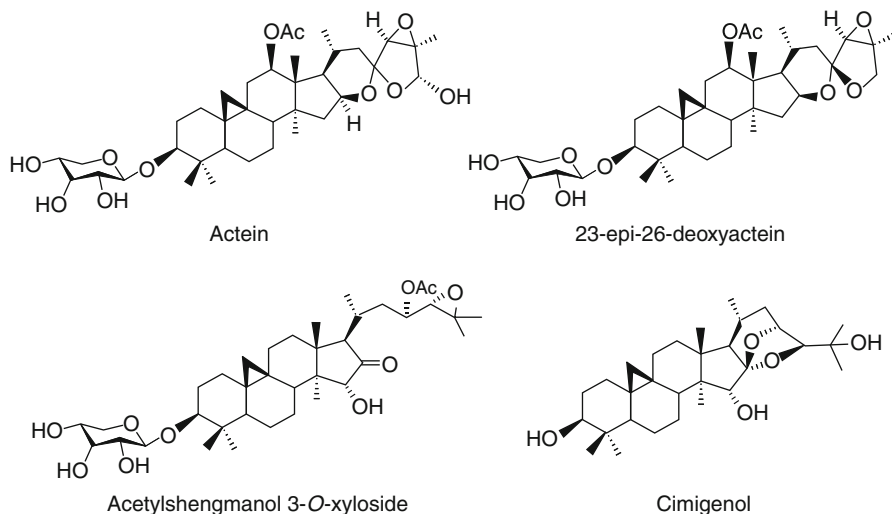
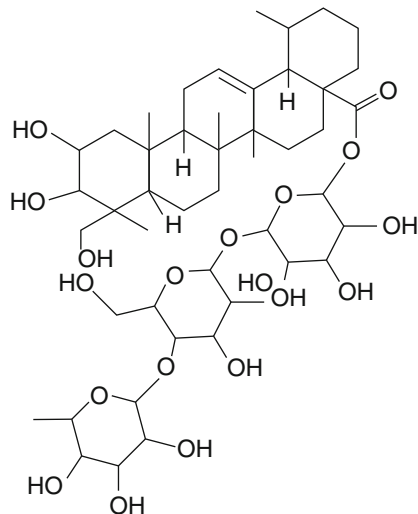


Fig. 35 Chemical structures of major triterpene glycosides found in black cohosh

term; there is lack of long-term study about the safety and effectiveness of the usage of black cohosh. The side effects of using black cohosh have been reported before, even though there are no conclusive scientific evidences that show that it may cause liver damage. However, a warning label should be added on black cohosh products and state that this product could induce harmful effects to your liver and should be used under the supervision of your doctor. So, further studies about the active compounds, mechanism, and potential effects of black cohosh on menopausal symptoms are still needed (Jacobson et al. 2001).

Centella asiatica (CA) is a medicinal herb containing high amounts of triterpenoid saponins which are the primary active constituents of CA. Taking *Centella asiatica* orally can treat the venous insufficiency and varicose veins since the CA can strengthen the walls of veins and stimulate the circulation. Asiaticosides are the major triterpene saponins responsible for the wound healing by increasing collagen formation and angiogenesis. Chemically, asiaticoside has a trisaccharide moiety which is linked with aglycone asiatic acid, madasiatic acid, and madecassoside (Fig. 36). Other than inducing the collagen synthesis, asiaticoside can further heal the wounds by increasing the tensile strength of the newly formed skin. However, there is also a study suggesting that asiaticoside could provoke hypertrophy in scars while inhibiting inflammation (Srivastava et al. 1997). Centelloside is a sugar-free triterpene saponin that is present in fresh plants, which can be used to treat venous hypertension (Heydari et al. 2007). In the market, *Centella asiatica* supplements are available as herbal teas or tablets, which are promoted as the reliever of anxiety, stress, and hypertension. Active constituents isolated from *Centella asiatica* are also widely applied in the area of skin care, which have been advertised as the healer of wounds, burns, and vein ulcers.

Fig. 36 Chemical structure of asiaticosides



However, the amount of active compounds of *Centella asiatica* is varied among the supplements, as well as in cosmetics.

American ginseng (*Panax quinquefolius*) and Asian ginseng (*Panax ginseng* C.A. Meyer) are the two major species of ginseng, which are well known for their preventive and therapeutic properties. Ginsenosides are the primary active ingredients of ginseng and belong to triterpene saponins. About 40 different types of naturally occurring ginsenosides have been found, and they were classified into two groups based on their chemical structure, the panaxatriol and the panaxadiol. In the panaxatriol group, there are ginsenosides Re, Rf, Rg₁, Rg₂, and Rh₁, while in the panaxadiol group, there are ginsenosides Rb₁, Rb₂, Rb₃, Rc, Rd, Rg₃, and Rh₂ (Fig. 37). Generally, Rg₁, Re, Rb₁, Rc, Rb₂, and Rd account for over 90 % of the total ginsenosides, and their ratio is varied among different species of ginseng. Studies have shown that pharmacological activities and therapeutic effects of ginseng could be affected by the ratio of different ginsenosides. For example, panaxatriol group can enhance memory whereas the panaxadiol group does not have this kind of function (Yun 2001). Through chemical analysis, the profiles of the American ginseng have only minor differences with Asian ginseng; only minor differences were found. For example, 24(R)-pseudoginsenoside F₁₁ but not ginsenosides Rf₁ and 2 and Rg₂ was obtained from North American ginseng. However, ginsenoside Rf and lower levels of ginsenoside Rg₂ have been isolated from Asian ginseng.

In China, ginseng roots are used in traditional medicinal therapies for over thousands of years and famous for their magical therapeutic effects. At present, manufacturers like to add the ginsenosides into supplements to generate the herbal supplement improving health status or to produce functional food for preventive benefits. Due to the different ginsenosides contained in ginseng, there are lots of biological activities that have been researched and reported, such as antioxidant

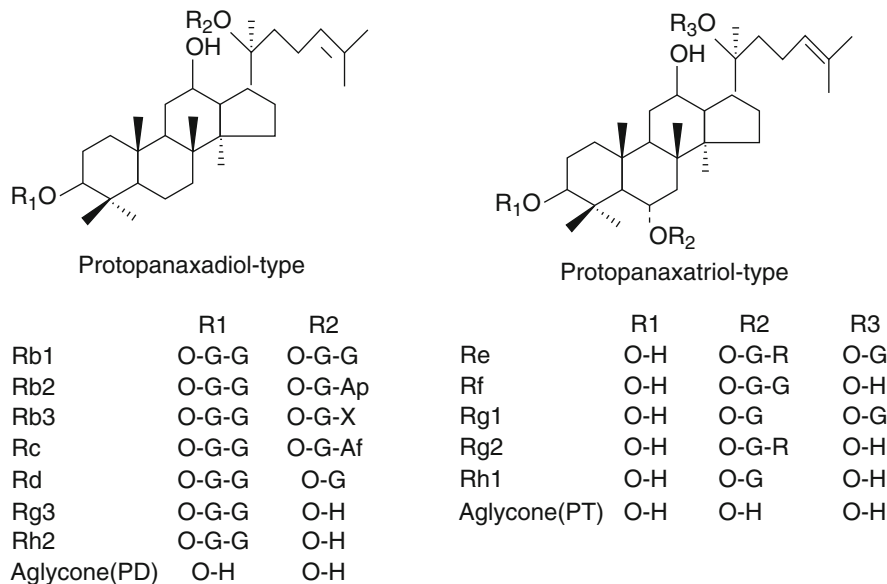


Fig. 37 Representative ginseng saponins

properties, anticancer effects, acceleration of metabolism, boosting immune system, and enhancing central nervous system. However, there are no conclusive doses of ginseng that should be taken daily to get the positive effects. Some studies suggested that for general preventive effects, 200–400 mg of *Panax* ginseng should be taken daily, whereas other studies show that 40 mg of *Panax* ginseng in a multivitamin could be enough (Jia and Zhao 2009).

Conclusion and Future Directions

Nowadays, people start to be concerned with their health status when they aged, so the function and safety of dietary supplements are getting more and more attention. There are more than 60 % of the American population and over 300 million people worldwide taking dietary supplements every day to maintain health. Especially, the botanical dietary supplements are isolated from natural plants holding more attention than others, and they have occupied an important and ever-growing portion of the market. In this chapter, we give the general and specialized reader a comprehensive insight into the most recent findings of some dietary supplement functional foods, which may contain simple phenolics, coumarins, lignans, flavonoids, isoflavonoids, anthocyanins, tannins, quinones, stilbenes, alkaloids, terpenes, and saponins that originated from plant sources.

Even though natural compounds can provide health-essential and health-improving nutrients, they can be toxic to our body or be transformed to toxic

compounds by our system. The safety, efficacy, or working mechanisms of large amount of botanical natural compounds remain unclear, but it does not stop the use and the sales of botanical supplements continue to expand rapidly in our market. That is why further research is needed to optimize the process of isolating these bioactive substances from their respective sources so as to improve the purity and quality of extracted bioactive compounds. Besides, clinical trials, which can generate reliable data on both the efficacy and the safety of these bioactive compounds on treating/preventing certain disease, are essential before widely applying them on the market.

Cross-References

- ▶ [Chemical Composition of Beverages and Drinks](#)
- ▶ [Chemical Composition of Cereals and Their Products](#)
- ▶ [Chemical Composition of Vegetables and Their Products](#)
- ▶ [Plant-Associated Natural Food Toxins](#)

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Abstract

Animals are rich sources of bioactive compounds that exhibit a variety of biological functions on human health. These bioactive molecules can be either essential to the animals' living or only produced integrally of more importance to other organisms. Over the past decades, an ever-growing number of natural compounds of animal origins have been extracted, identified, and applied as dietary or therapeutic supplements, which prevent, alleviate, or treat diverse

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diseases and associated symptoms. Some well-known examples are animal-derived polyunsaturated fatty acids and polysaccharides. Once consumed via animal food intake or supplementation, the ubiquitous chemical structures of these bioactives allow them to be either the substrates for important biomolecule synthesis or the modulators affecting the structure and function of organs and tissues. In addition to nutritional and medical potentials, some animal-derived bioactives have wide applications in the food, biotechnological, and agricultural fields. This chapter provides a comprehensive review of the ten types of animal-derived bioactive compounds, mainly focusing on their sources, chemistry, and biological functions.

Introduction

Bioactive substances are those chemical compounds possessing biological effects on living organisms. The bioactivities include preventive and therapeutic potential for human diseases, role as substrates for biomolecule and bio-structure synthesis, modulation of bio-system function, preservative effects against microbes, carrier for drugs, enzymes, and nutrients, and so on. The great biodiversity of animals makes them rich sources of bioactive compounds, and humans can directly acquire the health benefits by ingesting the animal-derived food products. In some cases, the bioactives are produced by the animals for maintaining their body functions and protecting themselves from external and internal stresses; in other cases, certain bioactive compounds integral to the existence of the animals may have much more significant functions in unrelated organisms (Colegate and Molyneux 2007).

The role of regular intake of foods containing bioactive substances in improving human health has been recognized for a long time, encouraging active search for novel bioactive compounds of animal origin and investigation of their applications in the fields of functional foods, pharmaceuticals, nutrition, biotechnology, and agriculture. These research efforts have generated fruitful results considering the number of valuable bioactive compounds identified, development of extraction and purification methodology, and elucidation of their structure-activity relationship. *In vitro* and *in vivo* studies are useful to provide some scientific evidence of possible bioactivities and targeting tissues, whereas clinical trials are necessary to evaluate the efficacy and safety of certain bioactive supplements for human consumption.

Omega-3 Fatty Acids

In chemistry, fatty acids (► Chaps. 13, “Chemical Composition of Fat and Oil Products”, and ► 14, “Chemical Composition of Fish and Fishery Products”) are defined as carboxylic acids with long aliphatic chains of usually an even number of carbons, which can be further categorized into either saturated or unsaturated on the basis of whether there are $C = C$ double bonds present in the chain. Omega-3 fatty

Table 1 Chemistry of ALA, EPA, DPA, and DHA

Name	Abbreviation	Chain length	No. of C = C bond	Position of C = C bond
α -Linolenic acid	ALA	18	3	9,12,15
Eicosapentaenoic acid	EPA	20	5	5,8,11,14,17
Docosapentaenoic acid	DPA	22	5	7,10,13,16,19
Docosahexaenoic acid	DHA	22	6	4,7,10,13,16,19

acids, as indicated by the name, are those polyunsaturated fatty acids (PUFAs) with two or more double bonds and one double bond is located at the third carbon atom counting from the methyl (CH_3) end of the carbon chain. The most important types of omega-3 fatty acids that are present in foods include α -linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), whose chemical identities are listed in Table 1.

On the basis of the percentage contribution toward omega-3 fatty acid (including ALA, EPA, DPA, and DHA) intake, the main food sources are cereals, cereal products, and cereal-based products and dishes (32 %), meat and poultry products and dishes (16 %), fats and oils (14 %), and fish and seafood products and dishes (13 %). More than 70 % dietary intake of EPA, DPA, and DHA is from fish and seafood and another 20 % from meat (Meyer et al. 2003). Herring, salmon, sardines, tuna, and halibut are among the types of fish which are most rich in EPA plus DHA content. The value can be as high as 1.7–1.8 g per 3-oz serving of fish (edible portion) (Covington 2004).

EPA and DHA are originally synthesized by marine plants including phytoplankton and algae, transferred through the food web and finally stored in the lipids of fish and other aquatic species. The stock of these long-chain omega-3 fatty acids tends to be particularly rich in fish living in the Atlantic region possibly because the low temperature of the region's cold water keeps the fatty acids' fluidity (Shahidi and Wanasundara 1998). Given the abundance in omega-3 fatty acids, fish oil is the ideal raw material source for fatty acid concentrate preparation. The methods suitable for large-scale production of omega-3 fatty acid concentrate include adsorption chromatography, fractional or molecular distillation, enzymatic splitting, low-temperature crystallization, supercritical fluid extraction, and urea complexation (Shahidi and Wanasundara 1998). The techniques differ from each other in the principle of separation efficiency, and the forms of fatty acids produced include free fatty acids, methyl and ethyl esters, or acylglycerols. It is a major challenge for food scientists to optimize current separation processes and develop new ones, which enable cost-effective production of fish oil concentrates with high percentages of EPA and DHA in their preferred forms.

The importance of omega-3 fatty acid supplementation relies on two aspects: first, omega-3 fatty acids cannot be synthesized by the human body and must be

obtained from diet; second, the health benefits of omega-3 fatty acid consumption have been well recognized since the early studies conducted by Burr and Burr (1929). Extensive evidence has been published regarding the biological effects of omega-3 fatty acids for the prevention and even treatment of a variety of diseases.

The positive role of omega-3 fatty acids in reducing the risk of cardiovascular disease has been suggested via epidemiological observations in Japan, South India, Quebec, and so on that regular fish eaters, especially those eating fatty fish (e.g., tuna, mackerel, trout, salmon) rich in DHA and EPA, suffer from lower death rate related to cardiovascular diseases. The mechanism for cardiovascular protection includes increasing the level of high-density lipoprotein (HDL) but adversely affecting low-density lipoprotein (LDL) and triglycerides, therefore improving the HDL:LDL ratio. Clinical trials are adopted to further explore the association between omega-3 fatty acid consumption and cardiovascular disease prevention (Ruxton et al. 2004). Although the reported outcomes depend on the dose, duration of intervention, and ratio of different types of omega-3 fatty acids, it is a general conclusion that omega-3 fatty acids can reduce cardiac events and mortality (Ruxton et al. 2004).

Another health benefit of omega-3 fatty acids is their capability to alleviate chronic diabetic complications, including hypercholesterolemia, hypertriglyceridemia, atherosclerosis, and hypertension. Intervention with omega-3 fatty acids has been recorded to reduce the mean transcapillary escape rate of albumin and blood pressure. There is increase in plasma HDL cholesterol and decrease in very low density lipoprotein (VLDL) cholesterol and triglyceride (Simopoulos 1991). The decrease in lipoprotein transfer across the vascular wall is postulated to result in change in vascular permeability. More biological impacts have been observed on diabetic patients after fish oil intake, including slight increase in plasma glucose, glycosylated hemoglobin, total cholesterol, and serum apoB (Simopoulos 1991).

Modulatory activity of omega-3 fatty acids on the immune system of animals and humans is useful to develop treatments for inflammatory diseases. For some inflammatory conditions such as chronic inflammatory bowel disease, asthma, and cystic fibrosis, either conflicting clinical data were generated or the size of population incorporated in the study was not large enough to reach a convincing conclusion (Ruxton et al. 2004). However, the potential of omega-3 fatty acids in treating rheumatoid arthritis is relatively clear. Meta-analysis of clinical trials data illustrated that 3-month fish oil supplementation significantly reduced the number of tender joints and duration of morning stiffness. Other improvements were reported later, such as reduced joint pain, reduced time to fatigue, increased grip strength, and decreased use of nonsteroidal anti-inflammatory drugs. But it must be pointed out that the practicality of omega-3 fatty acids as therapeutic agents for inflammatory diseases is limited by the high doses needed and hence high cost associated (Ruxton et al. 2004).

Perhaps the most well-known bioactivity of omega-3 fatty acids is their crucial role in brain development, function, and mental health. Omega-3 fatty acids, especially DHA, are essential in maintaining the fluidity of neuronal membranes,

and they assist neurotransmitter regulation (Ruxton et al. 2004). Deficiency in omega-3 fatty acids leads to impeded brain development and behavioral and learning disorders. Sufficient intake of DHA is recommended for women during pregnancy and lactation to ensure that their fetus and newborn babies acquire DHA delivered through placenta or breast milk for early brain development. DHA consumption is also a potential protective factor against cognitive impairment, cognitive decline, dementia, and Alzheimer's disease among elderly people. Finally, evidence is growing, which supports the correlation between poor omega-3 fatty acid status and increased risk of depression, and supplementation of EPA and DHA may alleviate the depressive symptoms (Ruxton et al. 2004).

Animal studies in latest decades have consistently suggested the anticarcinogenic activity of omega-3 fatty acids, characterized by delayed tumor appearance, decreased tumor growth rate, and reduced tumor size and number. Dietary intake of omega-3 fatty acids possibly modifies lipid metabolism. Mechanisms of protection against cancer are under investigation and may include modulation of prostaglandin production, immune function, free radical formation, membrane fluidity, intracellular transport systems, hormone secretion, calorie utilization, and gene expression (Simopoulos 1991).

Consumption of oily fish is a recommended approach to obtain the multiple health benefits of omega-3 fatty acids. Regional survey of consumers' dietary habit, however, suggests that an adequate daily EPA/DHA intake may not be achievable by eating oily fish or cod liver oil as neither of the products is popularly/frequently consumed. Therefore, other nonfish dietary options as omega-3 fatty acid sources could be helpful, which have been enabled by development of novel food manufacturing techniques. One example is to feed fish oils or their derivatives to poultry and livestock so as to increase the omega-3 fatty acid content of eggs and meat. Flavorless microencapsulated pure fish oil powder could be utilized to produce a variety of omega-3 fatty acid-fortified food products, without the concern of altering the original taste (Ruxton et al. 2004).

Conjugated Linoleic Acid

Conjugated linoleic acid (CLA) (► Chap. 16, "Chemical Composition of Meat and Meat Products") is the collective name for a group of geometric and positional isomers of linoleic acid (dienoic octadecadienoate, 18:2). All isomers contain conjugated double bonds, which are double bonds with a single carbon bond in between. The isomers differ from each other in the configuration of double bonds (either *trans* (*t*) or *cis* (*c*)), position (7, 9; 8, 10; 9, 11; 10, 12 or 11, 13), and geometry (*c/c*; *c/t*; *t/t*; *t/c*) (Schmid et al. 2006; Fig. 1).

The food categories that are rich in CLA consist of meat and dairy products derived from ruminants. As the *c*9, *t*11–18:2 isomer (also called rumenic acid) accounts for more than 80 % of the total CLA in foods, most studies measured the content of this isomer as an estimation of the total CLA (Schmid et al. 2006). Among meat types, lamb contains the highest CLA concentrations ranging from 4.3

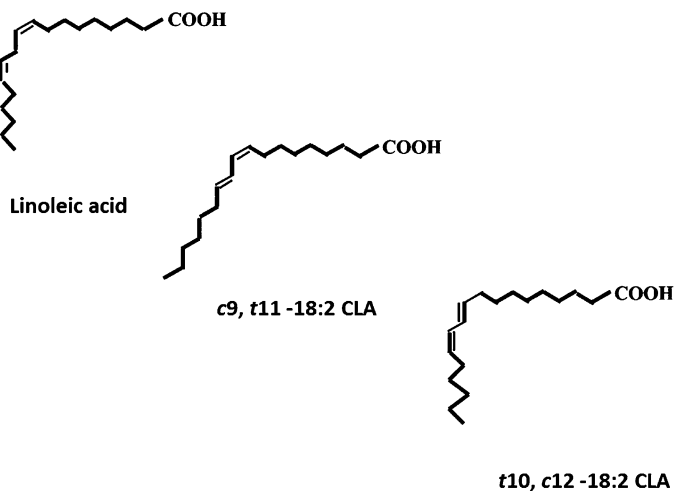


Fig. 1 Chemical structures of linoleic acid, *c9, t11* and *t10, c12* isomers of CLA (Belury 2002)

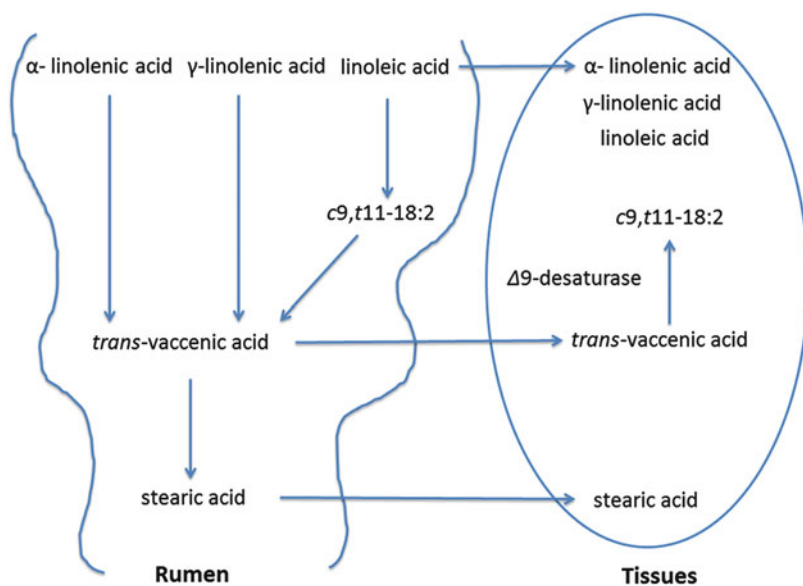


Fig. 2 Biosynthesis of *c9, t11*-18:2 CLA isomer in ruminants' rumen and tissue (Schmid et al. 2006)

to 19.0 mg/g lipid; the concentrations in beef are slightly lower at 1.2–10.0 mg/g lipid. There is also CLA present in fish and meat from monogastric animals such as turkey, pork, and chicken, but the concentrations are much lower (Schmid et al. 2006). This phenomenon is tightly related to the biosynthesis pathways of CLA as shown in Fig. 2.

The substrates for CLA synthesis are the PUFAs in the rumen of ruminants, whereas the microorganisms living in the rumen performs isomerization and/or hydrogenation during their lipid metabolism. Linoleic acid predominantly generates rumenic acid via isomerization and *trans*-vaccenic acid (*t*11–18:1) via further hydrogenation, which is an important intermediate precursor of CLA via desaturation occurring in the adipose tissue and mammary gland. α - or γ -linolenic acid, however, will only produce *trans*-vaccenic acid. The collective groups of microorganisms cooperate in the process of transforming PUFAs into stearic acid (18:0), the end product. Only the bacteria expressing the enzymes linoleate isomerase and CLA reductase are capable of isomerizing PUFA's *cis* double bonds into conjugated ones and further hydrogenating the formed conjugated fatty acids (Schmid et al. 2006).

The variations in the CLA content are not only observed between animal species but muscles of the same species. There are a couple of factors influencing the CLA content in meat, such as seasonal variations, animal genetics, and processing practices. The animal diet, which supplies the raw materials for CLA formation, should be the most important factor. Some effective approaches to increase CLA content in meats of ruminants are pasture feeding and adding oilseeds, vegetable oil, or fish oil to diet (Raes et al. 2004; Schmid et al. 2006). To enhance tissue CLA concentration of monogastric animals, CLA itself or its precursor such as *trans*-vaccenic acid needs to be included in the diet because fat modification is unfeasible before digestion and absorption (Schmid et al. 2006).

The bioactive properties of CLA are numerous including anti-adipogenic, antidiabetic, anti-atherosclerotic, and anticarcinogenic activities, which are discussed in details below.

On the basis of animal model experiments, CLA administration is effective in lowering body weight and modulating body composition by reducing adipose tissue mass (Belury 2002; Salas-Salvado et al. 2006). The modulation of body composition, however, may be gender specific that male responds better to dietary CLA. Long-term CLA administration is even reported to cause lipodystrophic consequences in female mice, characterized by complete ablation of brown adipose tissue, reduced leptin, increased fat accumulation in the liver, and development of insulin resistance (Belury 2002). Mechanism studies suggest the linkage between adipose tissue reduction and enhanced energy metabolism, as a result of stimulated sympathetic nervous activity by CLA. Meantime, CLA may inhibit triglyceride accumulation in adipocytes and induce adipocyte apoptosis or differentiation (Belury 2002). Only some of the studies performed on adult humans have verified the anti-adipogenic property of CLA, which varies with dose, duration, and isomeric composition of CLA administered (Belury 2002). More upcoming studies, hence, are necessary to elucidate the potential of dietary CLA to control body weight and fight against obesity.

Obesity is one of the key risk factors leading to development of type 2 diabetes, so it is reasonable to deduce that CLA may lead to favorable alterations in metabolic parameters of type 2 diabetic subjects due to its antidiabetic activity. In response to short-term CLA feeding, rats in experimental group are found to

show reduced fasting glucose, insulinemia, triglyceridemia, free fatty acid levels, and leptinemia than those in control group (Belury 2002). In some other studies, dietary CLA administration is accompanied by increased mRNA marker of adipose differentiation and glucose uptake into rat muscles. Similar to the cases happening in anti-adipogenicity, long-term feeding of CLA may induce insulin resistance and lipodystrophy (Belury 2002). The therapeutic potential of CLA against type 2 diabetes, hence, need further work to determine.

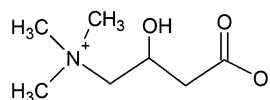
CLA, when supplemented to a hypercholesterolemic diet, can reduce serum triglycerides, LDL, total cholesterol, and atherosclerotic plaque formation in experimental rabbits and hamsters. The effects on experimental mice, however, are on the contrary that CLA stimulates formation of aortic fatty streaks (Belury 2002). Given the differential effects of CLA on the lipid profile and atherogenic markers in different animal models, it is still too early to conclude the application of CLA in preventing atherosclerosis and cardiovascular disease in humans.

The anticarcinogenic bioactivity of CLA is proposed based on the inhibition of numerous cancer models, particularly skin tumor, forestomach neoplasia, mammary, prostate, and colon tumorigenesis in experimental animals (Belury 2002). Although not all studies generate consistent results on its anticarcinogenic effect, CLA has not been reported to induce tumor. It is believed that dietary CLA mainly intervenes the initiation and promotion stages of carcinogenesis: in the initiation stage, CLA may modulate free radical-induced oxidation, carcinogen metabolism, and carcinogen-DNA adduct formation; in the promotion stage, CLA induces apoptosis and modulates cell proliferation via regulating cell cycle-related molecular signaling events (Belury 2002).

Chitin and Chitosan

The biopolymers known as chitin and chitosan are aminopolysaccharides, whose chemical structures are illustrated in Fig. 3. Chitin is a polymer of β -(1 \rightarrow 4)-*N*-acetyl-D-glucosamine, whereas chitosan is derived from chitin by various degrees of deacetylation at alkaline conditions, therefore as a copolymer consisting of β -(1 \rightarrow 4)-2-acetamido-D-glucose and β -(1 \rightarrow 4)-2-amino-D-glucose units with the latter usually exceeding 80 % (Jeon et al. 2000). The unique structures of chitin and chitosan make them to be highly insoluble materials with low chemical reactivity and allow them to provide supportive and protective functions in the exoskeleton and internal structure of invertebrates.

The main commercial sources of chitin are crab and shrimp shells, especially those obtained as food industrial waste for economic considerations. Industrial extraction of chitin from crustaceans is achieved by acid removal of calcium carbonate and then alkaline solubilization of proteins (Rinaudo 2006). Owing to the raw material sources and requirements on the purity and color for later utilization, the extracted chitin may be further decolorized and treated to remove pigments and impurities. Preparation methods of chitin and chitosan oligomers with about ten units or less include chemical (acidic) hydrolysis and enzymatic hydrolysis (Jeon

Fig. 3 Chemical structure of carnitine**Table 2** Applications of chitin and chitosan (Jeon et al. 2000)

Field	Application
Food	Antimicrobial and preservative agent; edible film
Nutritional	Dietary fiber; hypocholesterolemic or antihypertensive agent
Agricultural	Seed coating preparation; activator of plant cells
Pharmaceutical	Antibacterial, antitumor, or immunopotentiating agent; carrier for drug delivery
Medical	Accelerator for wound healing; artificial skin; fiber for absorbable structures
Biotechnological	Carrier for immobilized enzymes and cells; porous beads for bioreactors; resin for chromatography; membrane material

et al. 2000). Chitin and chitosan can also be chemically and mechanically modified to improve their strength, solubility, and tractability in purpose of generating novel properties, functions, and applications especially in biomedical area (Pillai et al. 2009).

The multidimensional properties of chitin and chitosan make them to be biomaterials of great functionality. Unlike most other natural polysaccharides that are neutral or acidic, chitin and chitosan are highly basic. Their ubiquitous properties include polyoxysalt formation, film forming ability, metal ion chelation, and optical structural characteristics. In addition, chitin and chitosan possess excellent biocompatibility, biodegradability, nontoxicity along with antimicrobial activity, and low immunogenicity. The wide applications of chitin and chitosan in the fields of food, pharmaceutical, medial, nutritional, biotechnological, and agricultural are listed in Table 2. Different physical and chemical properties are required in different applications.

Novel applications of chitin and chitosan are continuously revealed. For example, chitin and chitosan derivatives are utilized in water and wastewater treatment for the removal of metal ions, radionuclides, dyes, phenols, and other miscellaneous pollutants (Bhatnagar and Sillanpaa 2009). Applications in cosmetics recipe are also remarkable. Both chitin and chitosan have fungicidal and fungistatic properties. Chitosan is the only natural cationic gum becoming viscous when neutralized in organic acid solvents (Kumar 2000).

Milk Peptides

Milk proteins (► Chap. 17, “Chemical Composition of Milk and Milk Products”) are sources and precursors of a variety of bioactive peptides, most of which remain inactive until released from the protein sequence via enzymatic proteolysis.

Table 3 Bioactive peptides from milk proteins and their bioactivity (Meisel 2005)

Bioactivity	Bioactive peptide
Opioid agonist	Casomorphins, α -lactorphin, β -lactorphin
Opioid antagonist	Lactoferroxins, casoxins
ACE inhibitory	Casokinin, lactokinins
Immunomodulatory	Immuno peptides
Antimicrobial	Lactoferricin
Antithrombotic	Casoplatelins
Mineral binding, Anticariogenic	Phosphopeptides

The proteolysis can be facilitated by the cell wall bound proteinase and several intracellular peptidases of lactic acid bacteria during milk fermentation (Meisel and Bockelmann 1999). Digestive enzymes in the human gastrointestinal tract will also contribute to the breakdown of proteins and long polypeptides so as to activate the bioactive peptides.

The identification and characterization of bioactive peptides can be conducted following three different strategies: the peptides are either isolated from *in vitro* enzymatic and *in vivo* gastrointestinal digested proteins or synthesized chemically. Table 3 lists a total of eleven primary classes of bioactive milk protein derived peptides and their biochemical properties. Some of them share the same protein precursor, such as casein, the major protein in the milk. Moreover, certain peptides have been reported to be multifunctional, because the peptide sequences on the primary protein structure, which convey different bioactivities, sometimes overlap in regions called “strategic zones” and the zones are partially protected from proteolytic breakdown (Meisel 2005).

Casomorphins, α - and β -lactorphin, lactoferroxins, and casoxins are exogenous peptide ligands that can interact with opioid receptors. They are further divided into either opioid agonists or antagonists depending on whether they exert agonistic or antagonistic activity. The opioid peptides (except α -casein opioids) share the common structural motif of a tyrosine residue at the amino-terminal and another aromatic residue (e.g., phenylalanine or tyrosine) in the third or fourth position (Meisel 2005). The structure is not only crucial for fitting into the receptor’s binding site but expression of bioactivity. The opioid peptides possess naloxone inhibitable opioid activities in both receptor studies and bioassays. They can modulate social behavior and produce analgesia when administered to experimental animals (Meisel 2005). Other observed physiological effects include prolonged gastrointestinal transit time, antidiarrheal action, modulated intestinal transport of amino acids, stimulated secretion of insulin and somatostatin, and hence regulated post-prandial metabolism (Meisel 2005).

Angiotensin-I-converting enzyme (ACE) is a membrane-bound multifunctional carboxypeptidase in renin-angiotensin system. It is important for regulating peripheral blood pressure and local levels of certain ACE inhibitors and competitive substrates, which are endogenous bioactive peptides (Meisel 2005). Specifically, ACE converts angiotensin I to angiotensin II, which is an octapeptide with potent

vasoconstrictory ability (FitzGerald et al. 2004). Most of ACE inhibitory peptides are short-chain peptides with relatively small molecular size in order to fit into the active site of ACE. According to structure-activity studies, the C-terminal tripeptide sequence plays an important role that ACE prefers to bind to hydrophobic and positively charged amino acid residues (Meisel 2005). For peptides of longer chain, the conformation, instead of presence of certain amino acids, should contribute to ACE inhibitory potency. Through ACE inhibition, the bioactive peptides such as casokinin and lactokinins prevent the formation of the vasoconstrictory (hypertensive) angiotensin II but potentiate the vasodilatory (hypotensive) properties of bradykinin, therefore resulting in lowered blood pressure (Meisel 2005).

The immunopeptides can be derived from α - and β -caseins, and they are utilized to improve immune functions. The immunostimulating effects, as measured by immune cell proliferation and antibody synthesis, have been observed on the newborns, and the peptides also help to enhance adults' resistance to bacterial and viral infections (Meisel 2005). The molecular mechanism of these immunomodulatory effects is unclear, but the peptides likely bind to the receptors of lymphocytes and macrophages and stimulate their protective immune response.

Lactoferrin is a type of peptide from whey protein expressing antimicrobial activity. The antimicrobial mechanism of lactoferrin includes the actions of both iron-binding sites and a ubiquitous antimicrobial sequence near the N-terminus (Meisel 2005). Enzymatic cleavage of lactoferrin will generate a peptide fragment called lactoferricin, containing the 17–41 residues of lactoferrin. The smaller size of lactoferricin facilitates its access to the target sites on the microbial surface, and the net positive charge of the peptide will increase microbial membrane permeability, so lactoferricin has more potent antibacterial activity than the original undigested lactoferrin (Meisel 2005).

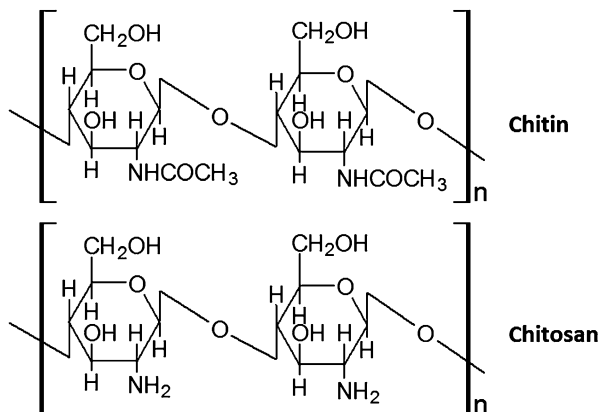
The antithrombotic property of casoplatelins is shown by its inhibition of platelet aggregation. The similarity in amino acid sequence allows casoplatelins to compete with the γ -chain of human fibrinogen on binding to the platelet surface receptor (Meisel 2005).

The last group of bioactive peptides derived from milk protein is called phosphopeptides, which function as carriers of different minerals, especially calcium. The mineral binding ability is mainly attributable to the negatively charged phosphate groups and meantime influenced by adjacent amino acids around the binding site (Meisel 2005). Besides, phosphopeptides also have anticariogenic effect, exhibited by their actions of recalcificating the tooth enamel and competing with dental plaque-forming bacteria for calcium, therefore inhibiting caries lesion formation (Aimutis 2004; Meisel 2005).

Increasing evidence shows that the milk protein may be a valuable source of peptides having cytomodulating activity. The antiproliferative and apoptosis-inducing peptides may be useful in cancer control, whereas the cell growth-promoting peptides have their applications in stimulating the digestive tract development of newborns.

Last but not the least, milk proteins have been recently found to be natural vehicles for delivering bioactives, due to their excellence in ions and small

Fig. 4 Chemical structures of chitin and chitosan



molecule binding, surface and self-assembly, gelation and pH-responsive gel swelling, complex formation with macromolecules, shielding capabilities, biocompatibility, and biodegradability (Livney 2010). The applications include but not limited to hydrophobic/heat-sensitive nutrient encapsulation and delivery as well as drug delivery and targeting.

L-Carnitine

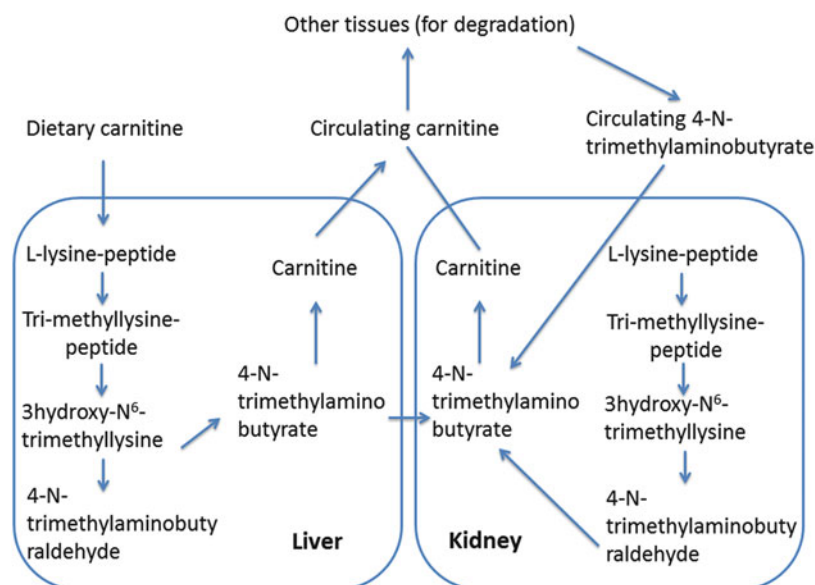
Free carnitine (3-hydroxy-4-*N*-trimethylaminobutyric acid, Fig. 4) was first isolated from bovine muscle by Russian scientists in 1905 (Zhou et al. 2007). This highly polar small amino acid derivative with a molecular weight of 162 D is widely distributed in food from animal sources such as milk and meat (Flanagan et al. 2010; Hurot et al. 2002). In Table 4, a list of animal and dairy products with corresponding carnitine content is provided.

Carnitine synthesis occurs mainly in the liver with the essential amino acids lysine and methionine as precursors and ascorbate, niacin, pyridoxine, and Fe²⁺ as cofactors (Flanagan et al. 2010). Carnitine can also be synthesized in kidney and brain. As shown in Fig. 5, the synthetic pathway in mammals starts with protein-bound lysine, which is modified to trimethyllysine by enzymatic methylation. The trimethyllysine undergoes four more enzymatic reactions to produce carnitine (Flanagan et al. 2010). The cardiac and skeletal muscles, where the highest concentrations of carnitine is stored, cannot synthesize carnitine but capture carnitine from plasma (Flanagan et al. 2010; Hurot et al. 2002). Only the L-isomer of carnitine is bioactive.

In humans, 25 % of L-carnitine is biosynthesized *de novo* while the other 75 % of exogenous origins including meat, poultry, and fish in diet (Zhou et al. 2007). As shown in Fig. 5, the dietary L-carnitine absorbed via both active and passive transport across enterocyte membranes, together with the free L-carnitine synthesized in the liver and kidney, constitutes the circulating L-carnitine which reaches

Table 4 Carnitine content in animal and dairy foods (Steiber et al. 2004)

Food	Carnitine ($\mu\text{mol}/100\text{ g}$)
Prepared steak	525
Raw beef (tenderloin, shoulder, rump)	3,691.4–4,160.5
Prepared ground beef	300
Raw beef liver	160.4
Prepared chicken	60
Prepared chicken egg	5
Cow milk	16.4

**Fig. 5** Biosynthesis and metabolism of carnitine (Flanagan et al. 2010)

cells of various tissues via the blood stream and the extracellular fluid and is metabolized for numerous physiological purposes.

In mammals, the metabolic significance of L-carnitine depends on the reversible esterification of the 3-hydroxyl group of carnitine by acyltransferases to form acylcarnitines. The bioactive functions include the mitochondrial oxidation of long-chain fatty acids, export of acetyl and chain-shortened acyl products from peroxisomes, preservation of the cellular CoA homeostasis, and so on (Steiber et al. 2004).

Carnitine is important to fatty acid oxidation because it is a component of a transport system consisting of three proteins: carnitine palmitoyltransferase I (CPT-I), acylcarnitine-carnitine translocase (CACT), and carnitine palmitoyltransferase II (CPT-II), which are localized at different submitochondrial sites

(Steiber et al. 2004). This transport system is essential for shuttling activated long-chain fatty acids across the mitochondrial inner membrane into the matrix, where β -oxidation takes place (Flanagan et al. 2010; Steiber et al. 2004). Therefore, active uptake of carnitine into the cytosol of cardiac and skeletal muscle cells is critical in muscle's energy metabolism from fatty acid oxidation.

Given the vital role of carnitine in fatty acid metabolism and energy production, deficiency in carnitine, from either genetic mutation of carnitine transporters or liver or kidney disorders, may adversely affect the function of cardiac/skeletal muscle and central nervous system (Flanagan et al. 2010). Considering its low toxicity and readily excreted property, L-carnitine supplementation is a relatively safe therapeutic approach to treat the complications induced by carnitine deficiency. Clinical application of L-carnitine potentially leads to improvements in painful neuropathies, Alzheimer's disease, cardiovascular disease, and immune function. Besides, there is increasing evidence on the benefits of carnitine in treating obesity, improving glucose intolerance, and total energy expenditure (Flanagan et al. 2010).

Choline

Choline (trimethyl- β -hydroxyethylammonium, Fig. 6) is a quaternary ammonium compound firstly discovered in pig bile isolate. Choline is a component of the phospholipid "lecithin" (phosphatidylcholine), in which form most choline is consumed through the human diet. The concentrations of choline in beef liver, beef steak, bovine milk, and eggs are 5831, 75, 150, and 42 $\mu\text{mol/kg}$, respectively (Zeisel and Blusztajn 1994). Although choline can be synthesized endogenously, dietary intake of extra choline is necessary to prevent liver and muscle dysfunction. The adequate intake values of choline are influenced by sex, pregnancy and lactation, clinical status, folate, vitamin B₁₂, and methionine nutriture as well as gene polymorphisms (Sanders and Zeisel 2007).

Free choline is directly absorbed mainly in the upper small intestine. About 50 % of choline is metabolized to betaine before it can be absorbed. Intestinal bacteria are responsible for metabolizing choline into trimethylamine, especially when large amounts of choline are ingested. Choline ingested in the form of lecithin is first hydrolyzed by the enzymes in pancreatic secretions and intestinal mucosal cells and then absorbed as lysolecithin, which reforms lecithin in the enterocyte. Free choline will be liberated at the tissues/organs by enzymatically cleaving the lecithin transported to the sites via systemic circulation. The other choline-containing compounds present in small quantities in the diet, including glycerylphosphorylcholine, phosphorylcholine, and sphingomyelin, are all cleavable either in the enterocyte or the tissues/organs to generate free choline (Zeisel 1981).

As an essential nutrient for human health, deficiency in choline will cause disorders in a variety of body systems and organs, and choline has a long history of being a therapeutic agent. Fig. 6 gives an overview of its biological functions.

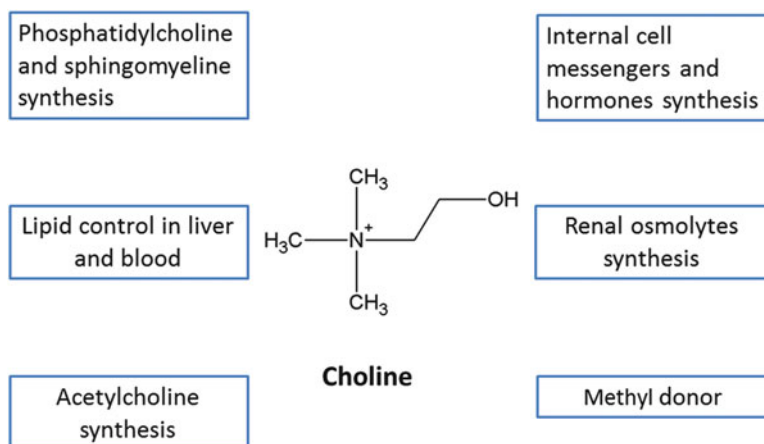


Fig. 6 Biological functions of choline (Gossell-Williams and Benjamin 2006)

Table 5 Physiological importance of choline metabolites and choline-containing compounds (Zeisel and Blusztajn 1994)

Compound	Biological functions
Phosphocholine	Intracellular storage of choline
Phosphatidylcholine	Building block of biomembranes; VLDL component
Sphingomyelin	Building block of biomembranes
Betaine	Methyl group donor; renal osmolyte
Acetylcholine	Neurotransmitter
Lysophosphatidylcholine	Second messenger modulating protein kinase C (PKC) activity
Lysosphingomyelin	Second messenger mediating growth factor actions, mitogen
Glycerophosphocholine	Renal osmolyte
Platelet-activating factor	Hormone

There are four major enzymatic reactions choline participates in mammalian tissues: phosphorylation, oxidation, acetylation, and base exchange (Zeisel 1981). Phosphate is transferred from adenosine triphosphate to the hydroxyl group of choline under the catalysis of choline phosphotransferase and the phosphorylated choline functions as the intracellular storage pool of choline. Choline phosphorylation is also the first step in the phosphatidylcholine synthesis. The oxidation product of choline is betaine aldehyde, which is then converted to betaine by the enzyme system choline oxidase. Choline acetyltransferase catalyzes the reaction of acetyl coenzyme A (acetyl-CoA) with choline to form acetylcholine. The base exchange reaction involves the reversible substitution of choline for serine, ethanolamine, or inositol in the presence of calcium ions within endogenous phospholipids. Table 5 summarizes the physiological importance of choline metabolites and choline-containing compounds.

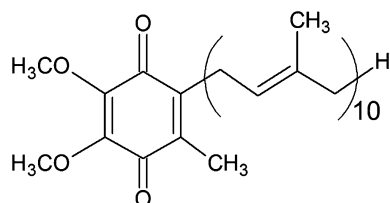
As discussed above, choline is a precursor for phosphatidylcholine synthesis via cytidine diphospho-choline pathway and phosphatidylcholine accounts for 50 % of phospholipids on cell membrane (Sanders and Zeisel 2007). Hence, sufficient choline is necessary to keep the structural integrity and transport functions of cell membrane. Besides, phosphatidylcholine is also an important component of VLDL, responsible for triglycerides excretion from the liver. Deficiency in choline, therefore, is associated with risk of fatty liver and hepatocarcinoma in prolonged deficiency. Several mechanisms have been proposed on the cancer-promoting effect of choline deficiency: lack of choline may lead to progressive increase in DNA synthesis and cell proliferation, so cells become more sensitive to chemical carcinogens; the oxidized choline metabolite “betaine” is a major methyl donor, so choline deficiency is linked with undermethylation of DNA, which results in chromosomal instabilities and abnormal expression of genetic information; increased lipid peroxidation in the liver observed during choline deficiency generates free radicals that could modify DNA and cause carcinogenesis (Zeisel and Blusztajn 1994).

Choline is the component of acetylcholine, the widespread neurotransmitter in the central nervous system. Choline and acetyl-CoA availability determines the rate of acetylcholine synthesis, so lack of choline will adversely affect the signaling function of cholinergic neurons. Choline enriched diet has the effects to promote acetylcholine release and has been applied to treat disorders caused by inadequacy in neurotransmitters (Zeisel 1981). Moreover, the choline’s stimulation of the cholinergic vagus nerve mediates its hypotensive effects (Zeisel 1981). Choline metabolism is closely related to folate metabolism, and both choline and folate are important for early brain development. It is noted in animal studies that maternal supplementation or deficiency in choline can cause permanent alterations in the offspring’s brain development (Sanders and Zeisel 2007). The hippocampus is the brain area most sensitive to choline availability, so choline supplementation may be used to improve elders’ memory (Zeisel 1981).

Coenzyme Q₁₀

Coenzyme Q is a group of naturally occurring lipophilic compounds sharing a common benzoquinone ring structure but differing in the length of the isoprenoid side chain. Coenzyme Q₁₀ (2, 3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone, Fig. 7), as the name indicates, is the group member comprising ten repeated isoprene units in the side chain (Bhagavan and Chopra 2006).

Q₁₀ is the most prevalent form of coenzymes in humans and most mammals. It can be biosynthesized *de novo* following three major steps (Overvad et al. 1999). The quinone ring is synthesized from the essential amino acid tyrosine or phenylalanine, whereas the isoprenoid side chain is formed from acetyl-CoA residues. In the last step, the ring and side chain structures are condensed together under the catalytic action of the enzyme polyprenyltransferase.

Fig. 7 Chemical structure of coenzyme Q₁₀**Table 6** The coenzyme Q₁₀ content in selected animal-origin food products (Overvad et al. 1999)

Food group	Food item	Cooking method	Q ₁₀ (µg/g food)
Meat and poultry	Pork heart	Fried	203 (151–282)
	Beef	Fried	31
	Chicken	Fried	17
	Pork chop	Fried	14 (9–17.8)
	Ham	Boiled	7.7 (5.4–9.4)
Fish	Herring	Marinated	27
	Rainbow trout	Steamed	11
	Salmon	Smoked	4.3
Dairy products	Yogurt	None	1.2
	Hard cheese	None	<0.2
	Cream cheese	None	<0.3
Egg	Hen's egg	None	1.5 (1–2.1)
	Hen's egg	Boiled	2.3 (1.7–2.9)

Coenzyme Q₁₀ is widely distributed in most tissues and exists in an oxidized form (ubiquinone) or a reduced form (ubiquinol) (Overvad et al. 1999). The total amount of coenzyme Q₁₀ in a normal adult human is around 0.5–1.5 g while the concentrations in different tissues vary. The highest concentrations of coenzyme Q₁₀ occur in tissues with high-energy requirements and lipid content, such as the heart, kidney, and liver at about 110, 70, and 60 µg/g tissue, respectively (Bhagavan and Chopra 2006; Overvad et al. 1999). The lowest concentration is found in lungs at 8 µg/g tissue. Except in brain and lungs, the major proportion of coenzyme Q₁₀ is in its reduced form (Overvad et al. 1999). Regarding the subcellular distribution, 40–50 % of coenzyme Q₁₀ is localized in the mitochondrial inner membrane (Bhagavan and Chopra 2006).

Tissue Q₁₀ of humans is either synthesized endogenously or obtained from food intake and oral supplements. A list of selected animal-origin food products and the amount of coenzyme Q₁₀ they contain are tabulated in Table 6. It is noted that meat, especially the heart muscle, is most rich in Q₁₀. There is also Q₁₀ present in vegetables, fruits, and cereals, but the values are low (<10 µg/g food). Variations in the values are caused by differences in animal species, seasonal variations, and analytical methods (Overvad et al. 1999).

Nowadays, coenzyme Q₁₀ is available over the counter as a dietary supplement and its popularity is increasing with the recognition by the public on its importance

to support human health. The fundamental roles of Q_{10} are in mitochondrial energetic production, antioxidation, as well as cell signaling and gene expression (Bhagavan and Chopra 2006). Q_{10} supplementation has been indicated to be potential treatment for heart disease, atherosclerosis, neurodegenerative disease, hypertension, male infertility, diabetes, and cancer (Bhagavan and Chopra 2006; Overvad et al. 1999).

As a cofactor in the mitochondrial electron transport chain, Q_{10} functions as a mobile redox agent shuttling electrons and protons in the respiratory chain and is therefore essential for adenosine triphosphate (ATP) production (Bhagavan and Chopra 2006). The rationale for the use of coenzyme Q_{10} as a therapeutic agent in cardiovascular disease is based on the important pathophysiological roles that mitochondrial dysfunction and energy starvation supposed to play in heart failure. It is observed that Q_{10} level in plasma and myocardial tissue is negatively correlated with the severity of cardiovascular symptoms and dysfunction. Q_{10} supplementation adjunct to standard medical therapy has been associated with signs of improvement in a couple of relevant clinical parameters in heart failure (Overvad et al. 1999; Sarter 2002; Singh et al. 2007).

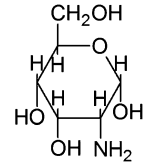
The reduced form of coenzyme Q_{10} , ubiquinol, acts to remove free radicals, therefore protecting membrane phospholipids, mitochondrial membrane proteins, and DNA from free radical-induced oxidative damage. The antioxidative capacity of ubiquinol is also facilitated by its ability to reduce tocopheryl radicals and semidehydroascorbate back to tocopherol and ascorbate, respectively (Singh et al. 2007). Ubiquinol and vitamin E are two endogenous antioxidants within LDL cholesterol to protect it against lipid peroxidation, so coenzyme Q_{10} is useful to prevent the pathogenesis of atherosclerosis (Littarru and Tiano 2007; Sarter 2002).

The level of Q_{10} declines with age (Overvad et al. 1999), so Q_{10} supplementation may be more important to elders, especially considering the potential efficacy of coenzyme Q_{10} in the treatment of degenerative neurodegenerative diseases such as Parkinson's and Alzheimer's diseases (Beal 2004; Shults et al. 2002). Substantial evidence suggests that mitochondrial dysfunction and oxidative damage may play a key role in the pathogenesis of neurodegenerative disease. Q_{10} , when administered with other types of antioxidants, may have additive or synergistic beneficial effects targeting mitochondrial uncoupling proteins and oxidative species reduction (Beal 2004).

Glucosamine

Glucosamine (2-amino-2-deoxy-D-glucose, Fig. 8) is an amino monosaccharide endogenously synthesized from glucose (Anderson et al. 2005) (James and Uhl 2001). Food sources of glucosamine include meat, poultry, and fish, whereas glucosamine sulfate, the salt of D-glucosamine with sulfuric acid, is one of the most common forms of dietary supplements (James and Uhl 2001). Glucosamine hydrochloride and *N*-acetyl-glucosamine are also available as glucosamine

Fig. 8 Chemical structure of “glucosamine”

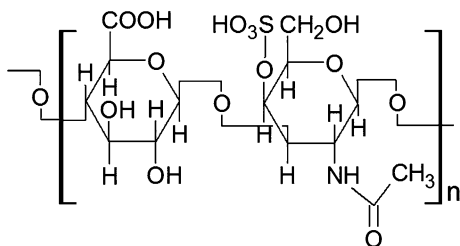


nutritional supplements, and all these glucosamine compounds are generally produced from chitin mentioned in “[Chitin and Chitosan](#)” (Anderson et al. 2005). Glucosamine supplementation methods include oral administration, intravenous injections, intramuscular injections, and intra-articular injections. By every route of administration, the absorption of dietary glucosamine sulfate into the bloodstream is fast. Near 90 % of orally administered glucosamine can be absorbed but only 26 % available for tissue use. Glucosamine is present in almost all human tissues, and the concentrations are high in liver, kidney, and cartilage. Liver is the site where glucosamine get combined with plasma proteins, reduced into smaller molecules, or used for other biological processes (James and Uhl 2001).

The phenomenon that glucosamine concentrates in cartilage suggests the important role of glucosamine in cartilage’s structure and function. Synthesized by the chondrocytes from glucose, glucosamine is used as a substrate for biosynthesis of proteoglycans and glycosaminoglycans, which are components of cartilage’s extracellular matrix. Lack of proteoglycans can lead to degeneration of articular cartilage, and glucosamine is essential to restore the proteoglycan-rich matrix, balance cartilage catabolism and anabolism, and protect damaged cartilage from metabolic impairment (James and Uhl 2001).

Given the importance in cartilage health, glucosamine has been used for medical treatment of osteoarthritis, which is a gradual disease characterized by changes in the subchondral bone as a result of continual wearing of the articular cartilage (James and Uhl 2001). Over the latest 40 years, a lot of studies have shown that glucosamine supplements likely have some efficacy in treating the symptoms of osteoarthritis. The improvements in outcomes are commonly measured by protection against joint space narrowing, improvement in mobility, drop in Lequesne’s severity index, and decrease in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain scores (Vangsness et al. 2009). The actions of glucosamine in treating osteoarthritis not only include stimulating proteoglycans and glycosaminoglycans synthesis but hindering the tissue-damaging enzyme “hyaluronidase,” repairing the damaged articular cartilage, and improving the lubricant properties of synovial fluid (James and Uhl 2001). Most clinical trials support the safety of glucosamine supplements to be equal to that of placebo (Vangsness et al. 2009). One of the most recognizable adverse effects of glucosamine supplementation is its interference of glucose metabolism. *In vitro* and animal studies have demonstrated that glucosamine leads to reduced pancreatic insulin secretion and insulin resistance in liver, muscle, and adipose tissue through activating the hexosamine biosynthetic pathway. However, these adverse effects are of low incidence under the glucosamine concentrations achieved by usual oral

Fig. 9 Chemical structure of chondroitin 4-sulfate



doses (Dostrovsky et al. 2011). As a conclusion, oral glucosamine administration appears to be moderately to highly effective in alleviating osteoarthritis symptoms and is well tolerated by humans with only infrequent side effects (Anderson et al. 2005).

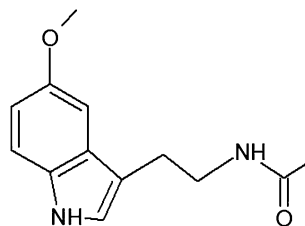
Chondroitin

Chondroitin is a linear polysaccharide composed of alternating disaccharide units of D-glucuronic acid and D-N-acetyl-galactosamine. Naturally isolated chondroitin contains small proportion of sulfate esters, while chondroitin sulfates possess same general structure but contain more sulfate ester groups mainly located at C-4 and/or C-6 of N-acetylgalactosamine and/or C-2 of glucuronic acid (Pigman 2012). Attached to the serine residues of the protein cores, chondroitin is a constituent of “proteoglycans,” the structural macromolecules of extracellular matrix organization (Asimakopoulou et al. 2008).

Chondroitin is present in up to 35–40 % in pig and ox cartilage. Isolation is also feasible from cartilage of shark, squid, and crab. Pure chondroitin 4-sulfate (Fig. 9) can be extracted from nasal septa or trachea of cattle using 1 % potassium carbonate with sodium chloride, and the protein impurities are removed by precipitation, adsorption, or proteolytic degradation (Pigman 2012). It is hard to separate chondroitin 4-sulfate from chondroitin 6-sulfate, though.

Similar to glucosamine, one key medical application of chondroitin sulfate is treatment of osteoarthritis. The mechanism may include stimulation of proteoglycan synthesis and inhibition of proteolytic enzyme synthesis so as to reduce the catabolic activity of chondrocytes. Meantime, chondroitin modulates the osteoprotegerin/receptor activator of NF- κ B ligand ratio to reduce bone resorption and exert anti-inflammatory activity (Martel-Pelletier et al. 2010). In all, chondroitin protects cartilage matrix and subchondral bone osteoblasts from cell damage and death. Sufficient quantity of chondroitin sulfate is essential to provide the cartilage with resistance and elasticity against tensile stresses (Martel-Pelletier et al. 2010). A set of clinical studies published lately have shown the pharmaceutical efficacy of oral chondroitin sulfate administration to improve the algo-functional symptoms and joint structure of knee, finger, and hip osteoarthritis (Bruyere and Reginster 2007; Uebelhart 2008). The well-documented good tolerability and safety of oral

Fig. 10 Chemical structure of melatonin



chondroitin sulfate administration further supported its great potential for long-term osteoarthritis treatment (Uebelhart 2008).

In addition to its role in cartilage function, chondroitin possesses many other biological functions as the compound interacts with a variety of molecules, such as growth factors, cytokines, chemokines, adhesion molecules, and lipoproteins (Asimakopoulou et al. 2008). Chondroitin sulfate that is chemically modified or with altered sulfation patterns has been developed in response to the need for new drugs or drug delivery systems targeting tumor cells and the interactions with effective molecules in extracellular matrix or on cell surface (Asimakopoulou et al. 2008). Therefore, chondroitin sulfate represents an important precursor molecule for synthesizing potent anticancer agent.

Melatonin

Melatonin (*N*-acetyl-5-methoxy-tryptamine, Fig. 10) is a hormone mainly synthesized and secreted by the pineal gland of mammals (Carrillo-Vico et al. 2005). The metabolic synthesis utilizes plasma tryptophan as precursor, which undergoes four intracellular enzymatic steps catalyzed by tryptophan hydroxylase, aromatic amino acid decarboxylase, arylalkylamine-*N*-acetyltransferase, and hydroxyindole-*O*-methyltransferase successively (Carrillo-Vico et al. 2005). Both melatonin synthesis and release are of rhythms under the control of a circadian clock in the hypothalamus (Pacchierotti et al. 2001). Specifically, the production of this pineal hormone is light-inhibited, so the level over nighttime far exceeds that of daytime (Bubenik 2002). The gastrointestinal tract of vertebrates is a rich source of extrapineal melatonin, and the tissue and plasma melatonin concentration is also associated with food intake (Bubenik 2002).

Melatonin can be bought as “food supplement” without prescription in some countries’ food stores. It is currently assumed that limited use of melatonin for its beneficial clinical impacts is safe (Bubenik 2002). The physiological roles of melatonin in regulating circadian and seasonal rhythms are the foundation for hypothesizing exogenous melatonin as a treatment for psychiatric and sleep disorders. In many psychiatric disorders, such as seasonal affective disorder, unipolar depression, and bipolar disorder, alteration or impairment of melatonin rhythm or pineal secretion has been reported, which may result in lower melatonin levels in patients’ serum than those in the control (Pacchierotti et al. 2001). Based on the

“melatonin replacement” hypothesis, replacing the age-related melatonin decline with physiological doses could improve the sleep quality of elderly insomniacs (Olde Rikkert and Rigaud 2001). Melatonin also appears to have efficacy in treating disturbed sleep in children with neurodevelopmental disabilities, especially on shortening the time to sleep onset (Phillips and Appleton 2004).

Another important action of melatonin is modulation of immune system. Exogenous melatonin, when binding onto its receptors on various immune tissues and cells, could stimulate immune responses by increasing lymphocyte growth, cell activity, and production of interleukin and interferon (Carrillo-Vico et al. 2005; Simonneaux and Ribelayga 2003). Besides, melatonin has been proposed as an antiaging and antioxidant agent, which could protect against free radical cytotoxicity. Not only can melatonin neutralize cytotoxic radicals (especially hydroxyl radical), melatonin would also stimulate the activity of antioxidant enzymes (e.g., superoxide dismutase, glutathione peroxidase) while inhibiting pro-oxidant enzymes (e.g., nitric oxide synthetase) (Simonneaux and Ribelayga 2003).

The effects of melatonin on immune responses and free radicals are believed to be associated with its anticarcinogenic potency. Mechanisms may include inhibition of cancer growth by antimetastasis or suppressing tumor linoleic acid uptake and metabolism as well (Mills et al. 2005). The importance of maintaining a sufficiently high plasma level of melatonin could be well described in slowing the progression of estrogen-responsive mammary tumors (Sanchez-Barcelo et al. 2003; Simonneaux and Ribelayga 2003). Specifically, the level of melatonin was found to be inversely correlated with the number of estrogen receptors in cancer patients. The receptor transcriptional activity could also be affected by interference of melatonin in signal transduction pathways. *In vitro* administration of melatonin induced uncoupled oxidative phosphorylation, morphological alteration, and autophagocytosis of human breast tumor cells (MCF-7) (Simonneaux and Ribelayga 2003). The findings encourage studies on the value of melatonin as therapeutic supplement in treating breast cancer (Sanchez-Barcelo et al. 2003; Simonneaux and Ribelayga 2003). Some other reports also indicate beneficial use of melatonin in prostate, endometrial, lung, gastric, and colorectal cancers, characterized by substantial reduction in death risk, low adverse effects, and low costs. Moreover, melatonin has also been claimed to protect against chemotherapy-induced blood cell damage, asthenia, stomatitis, cardiotoxicity, and neurotoxicity (Mills et al. 2005).

Conclusion and Future Directions

Animals produce a wide variety of natural compounds with beneficial biological functions in humans. The chemical species include fatty acids (omega-3 fatty acids, conjugated linoleic acid), polysaccharides and aminopolysaccharides (chitin, chitosan, chondroitin), peptides from milk protein, ammonium compounds (L-carnitine, choline, glucosamine), as well as quinones (coenzyme Q₁₀).

After being absorbed and metabolized in humans, these compounds exhibit diverse important biological functions which include but not limited to:

1. Preventive and therapeutic effects against cardiovascular diseases, diabetic complications, atherosclerosis, hypertension, neurodegenerative diseases, osteoarthritis, and so on
2. Antioxidant, anti-inflammatory, antithrombotic, anti-adipogenic, anticariogenic, and anticarcinogenic activities
3. Regulation of enzyme activity, energy production, and cell proliferation/apoptosis/homeostasis
4. Synthesis of neurotransmitters, osmolytes, molecules for biomembrane/extracellular matrix structure, hormones, and cell messengers
5. Antimicrobial/preservative agents
6. Carriers for drugs, minerals, enzymes, and other bioactives

Further research is needed to optimize the process of isolating these bioactive substances from their respective animal sources so as to improve the purity of extracted bioactives and lower the cost of dietary/therapeutic supplements production. Besides, clinical trials of high quality, which generate reliable data on both the efficacy and the safety of these bioactives on treating certain disease, are essential before widely applying them as therapeutic agents.

Cross-References

- ▶ [Chemical Composition of Eggs and Egg Products](#)
- ▶ [Chemical Composition of Fish and Fishery Products](#)
- ▶ [Chemical Composition of Meat and Meat Products](#)
- ▶ [Chemical Composition of Milk and Milk Products](#)

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Abstract

The use of foods that promote a state of well-being, better health, and reduction of the risk of diseases has become popular as the consumer is becoming more and more health conscious. This has resulted in the growth of functional foods in recent years, and there is an explosion of research being conducted on the area of bioactive substances from foods. An important constituent of them comes from microbial origin, taking into consideration of the vast variety of microorganisms including bacteria, mold, and yeast, which can cause bioconversion and biotransformation of food components. Foods that improve or change the intestinal microflora are of particular interest because of increasing knowledge of the role the intestinal microflora population plays in health and disease resistance. Recent advances on the research of prebiotics, probiotics, and synbiotics have provided valuable insights on how they impact intestinal flora and thus the health of the host, but it is very complicated and much more work still needs to be carried out. In an effort to review bioactive substances produced by microorganisms, this book chapter focuses on both active compounds produced in the fermentation process and in vivo by microbes in the intestine. Currently, there is a good selection of commercial functional products from microbial origin available in the market, and in this chapter, red yeast rice, *Cordyceps*, and fermented ginseng are given as examples.

Introduction

In the last decades, consumer demands for food have changed considerably. Food is no longer intended as to just satisfy hunger and provide necessary nutrients, but it is also believed to contribute directly to health. In this regard, functional foods, defined as ingredients with an additional health value, play an outstanding role. The increasing demand on such foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for improved quality of their later years (Roberfroid 2000). The world market for functional foods and drinks is expected to reach \$130 billion by 2015, according to Global Industry Analysts.

Among all known producers of small molecule natural products, microorganisms represent a rich source of biologically active metabolites that find wide-ranging applications as agrochemicals, antibiotics, immunosuppressants, antiparasitics, and anticancer agents (Gunatilaka 2006). Unlike other organisms, microbes occupy all living and nonliving niches on earth, and it has been estimated that less than 1 % of bacterial species and less than 5 % of fungal species are currently known, suggesting that millions of microbial species remain to be discovered (Ward et al. 1990).

Fermentation is a desirable process of biochemical modification of primary food products brought about by microorganisms and their enzymes. It is one of the most important forms of food preservation technologies in human diets,

because it is inexpensive and it preserves foods, improves its nutritional value, and enhances its sensory properties (Aloys and Angelina 2009). Fermentation may also lead to detoxification; to destruction of undesirable factors present in raw foods such as cyanides, phytates, tannins, and polyphenols; and to the removal of lactose (Schaafsma 2008). It is not surprising that their consumption has long been associated with good health. Indeed, as far back as 76 A.D., the Roman historian Plinio advocated the use of fermented milks for treating gastrointestinal infections. Nowadays, with greater emphasis on natural products and the role of food in health and well-being, food manufacturers are turning to fermentation not just for extending shelf life but also to create functional food products. The claimed health benefits of fermented functional foods are expressed either directly through the interaction of ingested live microorganisms, bacteria, or yeast with the host (probiotic effect) or indirectly as a result of ingestion of microbial metabolites produced during the fermentation process (biogenic effect).

The intestinal tract harbors a complex bacterial community, integrated by more than 800 different bacterial species, which have a marked effect on the nutritional and health status of the host (Nadal et al. 2007). The metabolic activity developed by the gut microflora contributes to the digestion of dietary compounds, salvage of energy, supply of (micro)nutrients, and transformation of xenobiotics (Santacruz et al. 2009). It also plays an enormous role in the bioavailability of phytochemicals, which are bioactive non-nutrient plant compounds. Dietary components with biological effects are susceptible to be metabolized by intestinal bacteria during the gastrointestinal passage prior to absorption. The colon has the highest bacterial load and constitutes an active site of metabolism. Phytochemicals and their metabolic products may also inhibit pathogenic bacteria while stimulating the growth of beneficial bacteria, exerting prebiotic-like effects (Gibson et al. 2005). Therefore, the intestinal microflora is both a target for nutritional intervention and a factor influencing the biological activity of other food compounds acquired orally. However, the metabolite-producing potential of intestinal bacteria differs among individuals, leading to individual differences in efficacy of phytochemicals.

This book chapter aims to give an overview of the current status of bioactive compounds from microbes, either those produced through fermentation with food-grade microorganisms or those that indirectly improve the health status of humans through stimulation of certain intestinal microorganisms. This chapter then gives some examples of fermented functional foods that are currently available in the market and focuses on those with clear chemistry and elucidated mechanisms. See section “[Examples of Fermented Functional Food Products and Their Bioactive Compounds](#)” for details on these examples including red yeast rice, *Cordyceps*, and fermented ginseng. Future prospects to exploit the potential health-promoting properties of such foods will continue to expand with a greater understanding of how microorganisms and their metabolites can directly interact in a positive way with the human host.

Probiotics and Prebiotics

More than a century ago when the Russian-born Nobel Prize recipient Eli Metchnikoff first observed the positive role played by some selected bacteria, people did not know the word “probiotics.” The term “probiotics” means “for life,” was first created by Lilly and Stillwell (1965) to contrast with “antibiotics,” and referred to growth-promoting factors produced by microbes that enhance the growth of other microorganisms. It was later redefined by Fuller (1989) as live microbial feed, which beneficially affects the host animal by improving its intestinal balance. After a few revisions, probiotics are currently defined by the Food and Agriculture Organization/World Health Organization (FAO/WHO) as “*live microorganisms which when administered in adequate amounts confer a health benefit on the host*” (World Health Organization 2001). Prebiotic, on the other hand, is “*a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal (GI) microflora that confers benefits upon host well-being and health*” (Roberfroid 2007). Based on the same idea, which is to improve host health via modulation of the intestinal flora, the synergistic combinations of pro- and prebiotics are called synbiotics. Essentially, both prebiotic and synbiotic are aiming to stimulate the growth and/or activity of probiotics in the GI tract. Thus, research on probiotics area has been put into the spotlight as it is the fundamental basis for prebiotic and synbiotic use. In the last 30 years, research in probiotics has progressed considerably, and significant advances have been made in the selection and characterization of specific probiotic cultures, especially in substantiation of the health claims relating to their consumption (World Health Organization 2001). Although many genera of microorganisms are considered to be probiotics, most of them are found in the genera *Bifidobacterium* and *Lactobacillus*. In addition to these genera, members from the genera *Streptococcus*, *Enterococcus*, *Bacillus*, and *Propionibacterium*, as well as strains in gram-negative bacterium *E. coli* and yeast *Saccharomyces*, can also be found in the probiotic market (Gerritsen et al. 2011).

Numerous health-beneficial effects are associated with usage of probiotics, prebiotics, or synbiotics such as effect on lactose intolerance, blood lipids, hypertension, constipation, irritable bowel syndrome (IBS), diarrhea, inflammatory bowel disease (IBD), cancer prevention, immune modulation, allergy and atopic disease in children, and mineral absorption (de Vrese and Schrezenmeir 2008). There are different degrees of evidence supporting the verification of such effects depending on the quality, scale, and specific settings of the experimental designs. It is of utmost importance to note that the health-beneficial properties of a given probiotic bacterium are specific to that strain and cannot be regarded as general for other strains of the same species or subspecies, not to mention other species of bacteria or yeast (Jungersen et al. 2014). Readers can refer to an excellent review written by deVrese and Schrezenmeir (2008) for the health effects of probiotics, prebiotics, and synbiotics. The present work mainly focuses on health benefits directly from interaction of ingested microorganisms with the host.

Health Relevant Effects of Probiotics

Although probiotics do not act exclusively in the large intestine via affecting the intestinal flora, most health effects attributed to probiotic microorganisms are related, directly or indirectly, i.e., mediated by the immune system, to the gastrointestinal tract (de Vrese and Schrezenmeir 2008). This is due to the fact that most probiotics are applied via oral ingestion and act in the GI tract, which contains at least tenfold more microbial cells (10^{14}) than human cells. The complex microbial community residing in or passing through the GI tract is collectively termed “gut microbiota,” composed of both autochthonous and allochthonous microorganisms, the relative proportions of which vary depending on a variety of intrinsic and extrinsic factors including changes to the diet. It is clear that gut microbiota can play a critical role in health and disease of the host (Eckburg et al. 2005). Based on research in recent years, it is suggested that changes in composition and diversity of the intestinal microbiota are related to diseases, including IBD, IBS, and obesity (Gerritsen et al. 2011; Kato et al. 2014).

Prevention of Diarrhea

Diarrhea is a major world health issue, responsible for several million deaths each year, majority of which occur among children in developing countries (World Health Organization 2001). According to National Digestive Diseases Information Clearinghouse (NDDIC), many factors can cause diarrhea such as bacterial or viral infection, parasites, functional bowel disorders, intestinal diseases, food intolerances and sensitivities, and reaction to medicines. Probiotics have the potential to reduce the rate of diarrhea, alleviate the symptoms, and shorten acute infections.

The best-documented health benefit of probiotics has been established using *Lactobacillus rhamnosus* GG (LGG), *L. reuteri*, *Bifidobacterium animalis* subsp. *lactis* BB-12 (BB-12), *B. bifidum*, and a few other probiotic strains for prevention and treatment of acute diarrhea mainly caused by rotaviruses in children (Sazawal et al. 2006). In a recent paper, Passaroello et al. (2014) reviewed studies on probiotics that were conducted using randomized controlled trials (RCTs) or their meta-analysis prior to January 2014 and concluded that probiotics may play a positive role for the treatment of acute gastroenteritis in children and for the prevention of antibiotic-associated diarrhea (AAD) both in children and adults. In Chatterjee et al. (2013) double-blinded, randomized, placebo-controlled, multicenter trial, a probiotic mixture of *L. acidophilus* LA-5 and BB-12 did not effectively reduce the incidence of AAD in adults; but the duration of diarrhea in the probiotic group was significantly shorter than that of placebo group. Moreover, in a similar study but on older inpatients (≥ 65 years) conducted by Allen et al. (2013), 2,981 patients were randomly assigned to either placebo group ($n = 1,493$) or probiotic group ($n = 1,488$). AAD including *Clostridium difficile* disease (CDD) occurred in 159 participants in the probiotic group and 153 patients in the placebo group during the 12-week study period. There was no difference between probiotic group and placebo group in preventing AAD or CDD in older inpatients.

Since the abovementioned studies showed inconsistent results, people probably would wonder: are probiotics useful for prevention of diarrhea or not? The answer is yes and no. Certain probiotic strains may benefit particular populations to some extent, whereas others do not show any effect depending on the circumstances. While the exact mechanisms involved in prevention and treatment of acute diarrhea remain unclear, several potential mechanisms have been proposed: (1) competitive exclusion that prevents the attachment of pathogens to the intestinal mucosal surface; (2) producing antimicrobial substances such as organic acids, hydrogen peroxide, diacetyl, short chain fatty acids (SCFAs), biosurfactants, and bacteriocins; and (3) restoring imbalanced gut microbiota (Kajander et al. 2007).

Alleviation of Inflammatory Disease and Bowel Syndromes

Inflammatory bowel diseases (mainly Crohn's disease and ulcerative colitis) and irritable bowel syndrome are two big health problems in adult population. Although causes to neither of them are well understood, researchers believe they may be caused or aggravated by alterations in the gut flora including infection (Shanahan 2000). Since mounting evidence has shown that intestinal microbiota may play a role in these diseases, many efforts have been undertaken to improve health and well-being of affected individuals by manipulation of gut flora using probiotics, prebiotics, and selected antibiotics. Numerous studies have demonstrated health-promotion effect of probiotics on IBD and IBS both *in vitro* and *in vivo*, whereas others reported no effect at all. As more and more positive outcomes have been documented in human studies and review papers concerning the potential mechanisms such as regulation of intestinal flora and immunological mechanisms, further investigation on the precise mechanisms of action of prebiotics and probiotics appears to be urgent. In such efforts, scientists in the pre- and probiotics field should aim to develop standardized protocols regarding experimental design, probiotic strains specific to certain health conditions, dosage and duration, outcome measures, and data analysis tools.

Cancer Prevention

According to National Cancer Institute's definition, cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Cancers as a group account for approximately 8.2 million deaths globally in 2012, within which liver and colorectal cancer caused ~745,000 and ~694,000 deaths, respectively (World Health Organization 2014). Due to their high lethality, cancers have always been fearful to human beings. U.S. President Richard Nixon even declared the "War on Cancer" in 1971, when cancer research has led to a number of new treatments for cancer based on improved understanding of molecular biology and cellular biology. Although treatments to many cancers are not yet definitive, cancers are considered largely preventable diseases through diet, medication, and vaccination. Numerous studies have shown that probiotics could potentially prevent or delay the onset of certain cancers based on *in vitro* and animal-based experiments. The cancer-related health-promoting property of probiotics has also been observed in clinical studies. EI-Nezami et al. (2006) reported that a probiotic preparation containing a mixture of the strains

L. rhamnosus LC705 and *Propionibacterium freudenreichii* subsp. *shermanii* (1:1, wt:wt) at a dose of $2-5 \times 10^{10}$ CFU/day for 5 weeks significantly ($p = 0.005$) reduced urinary excretion of AFB-N⁷-guanine (a marker for a biological effective dose of aflatoxin, which is a risk factor of liver cancer) compared to placebo group and thereby provide an effective dietary approach to decrease the risk of liver cancer. Liu et al. (2013) conducted a double-center, double-blind randomized clinical trial on the effect of probiotic treatment on serum zonulin concentration and subsequent postoperative infectious complications after colorectal cancer surgery and reported significant lower infection rate ($p < 0.05$), lower serum zonulin concentration ($p < 0.001$), shorter duration of postoperative pyrexia and antibiotic therapy, and lower rate of postoperative infectious complication ($p < 0.05$) in probiotic group compared to control group.

Based on in vitro and in vivo studies, Raman et al. (2013) suggested a few mechanisms of cancer prevention action of probiotics: (1) mutagen binding, degradation, and mutagenesis; (2) prevention of nontoxic procarcinogen conversion to harmful, toxic, and highly reactive format; (3) lowering intestinal pH by short chain fatty acid (SCFA) production during nondigestible carbohydrate degradation; and (4) modulation and enhancement of the host's innate immunity through the secretion of anti-inflammatory molecules. While probiotics sound promising in preventing cancers, researchers and physicians are rather skeptical about applying them on cancer patients due to indefinite strain, dosage, or means of administration.

Other Benefits and Use in Healthy People

Probiotic microorganisms are also claimed to have positive effects on lactose intolerance, *Helicobacter pylori* infection, constipation, immune response, allergy and atopic diseases in children, miscellaneous diseases due to microbial imbalance, common virus and respiratory tract infection, cardiovascular diseases, urogenital tract disorders, bacterial vaginosis, yeast vaginitis, and urinary tract infections (World Health Organization 2001; Eckburg et al. 2005). Unfortunately, none of the abovementioned claims has been clearly studied for mechanism of action. Investigations on the effect of probiotic bacteria on human health often showed inconsistent results, possibly due to differences in probiotic strains that are used, dosages applied, combinations of probiotic species and strains, population of interest, sample size, duration of intervention, format of delivery, diet control, lifestyle, or even host-dependent factor (host genotype). All of this, in combination with rather dynamic and maybe unique individual intestinal microbiota, makes it problematic to compare different studies on the effectiveness of probiotic interventions on IBD, IBS, obesity, lactose intolerance, cancer, or constipation.

However, the health benefits of probiotics are so promising that the global probiotic market potential is tremendous. The market revenue in North America is expected to reach up to 48.6 billion US dollars in 2018 (Transparency Market Research 2013). Various probiotic products such as yogurt, cheese, ice cream, chocolate, cookies, cereals, vegetable, meat, fruit juice, spray dried powder, and capsules are used by consumers who regard themselves as being otherwise healthy. They are more likely motivated to do so by the assumption that probiotics can retain

their health and well-being and potentially lower their risk of diseases related to imbalanced gut flora. There are a couple of points one needs to be aware of: (1) probiotics should not replace healthy lifestyle and well-balanced diet and (2) safety on long-term probiotic use in otherwise healthy population remains unclear.

Prebiotics and Synbiotics

According to the definition prebiotics selectively stimulate the proliferation of certain probiotic bacteria in the colon, especially those in *Bifidobacterium* and *Lactobacillus* genera. Prebiotic carbohydrates are dietary fibers that cannot be digested by human enzymes, but can be utilized by gut microorganisms via fermentation in the large intestine. In Roberfroid's (Roberfroid 2007) revisit to his prebiotic concept, only two types of food carbohydrates, namely, inulin and oligofructose, fulfilled the criteria for prebiotic classification. However, people argue that human milk should be considered the very first prebiotic, even synbiotic, most people have had as it contains substantial amount of oligosaccharides and may contain some naturally occurring probiotic bacteria.

In line with probiotics, many studies have demonstrated the health benefit of prebiotics or synbiotics on intestinal flora, cancer prevention, lipid metabolism, mineral adsorption and bone stability, and immunomodulation (Eckburg et al. 2005). It is generally believed that prebiotics promote/maintain human health via promoting healthy or balanced gut flora. Since no standard for healthy intestine flora has been identified yet, researchers consider it rather difficult to define such effect. Further advances in omics may provide approaches to uncover the gut molecular network and thus lead to a new vista for better understanding of the human gut ecosystem (Kato et al. 2014).

While there is no safety issue reported for probiotics use, ingestion of high quantities of prebiotics, on the other hand, may cause adverse conditions such as flatulence, abdominal disorders, and diarrhea due to fermentation in the large intestine (Roberfroid 2007). Despite all that, in the effort of conferring health benefit, prebiotics are often added to normal food, beverage, and probiotic foods as additive at low percentage to avoid the risk of gastrointestinal complaints in sensitive individuals. True synbiotic product should possess real synergistic effect from both probiotic and prebiotic component. Unfortunately, most food items containing both probiotic bacteria and prebiotic carbohydrates do not fulfill this criterion (Eckburg et al. 2005).

Microbial Metabolites from Fermentation Process

It is known that diet is one of the factors that have influence on health and certain diseases. Consumers are paying more attention to their health and expect the foods they eat can provide health benefits besides nutrients. For health-promoting effects

produced by animal or plants, please refer to ► [Chaps. 31, “Bioactive Substances of Animal Origin”](#) in this book. Metabolites generated by microorganisms during fermentation process, such as oligosaccharides, bioactive peptides, omega-3 fatty acids, and vitamins, are another good source of bioactive compounds. Recent research shows the above bioactive compounds have various health benefits and will be discussed in this chapter.

Oligosaccharides

Nondigestible carbohydrates such as oligosaccharides, dietary fibers, and resistant starches have various physiologic functions (Bird et al. 2006). Research suggested that the above carbohydrates can improve human health and reduce the risk of certain diseases (Zhang and Huang 2005). Among nondigestible carbohydrates, the oligosaccharides present important properties beneficial to the health of consumers so their application in food as food ingredients has been increasing. Fermentation is one of most common methods to produce oligosaccharides for food. Examples of most important oligosaccharides are fructooligosaccharides (FOS), glucooligosaccharides (GOS), isomaltooligosaccharides, soybean oligosaccharides, xylo-oligosaccharides, and maltitol. The health benefits of oligosaccharides may include: (1) providing low calories in body (0–3 kcal/g of sugar substitute); (2) improving the intestinal environment and changing the intestinal microflora (serve as pre-bio); (3) improving and suppressing diarrhea and symptoms of diarrhea; (4) stimulating intestinal absorption of minerals, such as calcium, magnesium, and iron (Manning and Gibson 2004); and (5) reducing the risk of cardiovascular disease, colon cancer, and obesity (Marionneau et al. 2001). In addition, the functional oligosaccharides are also used in feeds, pharmaceuticals, or cosmetics as stabilizers, bulking agents, immunostimulating agents, or prebiotic compounds (Simon 1996). Figure 1 shows the structures of the following oligosaccharides: fructooligosaccharides (FOS) (a) and galactooligosaccharides (b). Figure 2 shows a flowchart for the production of FOS. In this case, naturally occurring sugars (such as sucrose or molasses) can be used as raw materials. Commonly used microbes include *Aureobasidium pullulans*, *Aspergillus niger*, and *Lactobacillus bulgaricus*. The above microbes can produce the enzymes fructosyltransferase (EC 2.4.1.9) and fructofuranosidase (EC.3.2.1.26), which can synthesis fructooligosaccharides (GF, GFF, and GFFF; G, glucose; F, fructose). In order to achieve high yield of fructooligosaccharides, the optimum processing conditions should be achieve. The conditions include optimum pH (5.0–6.5), reaction temperature (50–60 °C), and high sugar concentration (70–80 %). Immobilized cells and enzymes are also used in order to improve the production efficacy and eliminate the process to remove cells. Depending on the application, the final product can be in liquid form after further concentration. The product can also be dried into solid (powder) form.

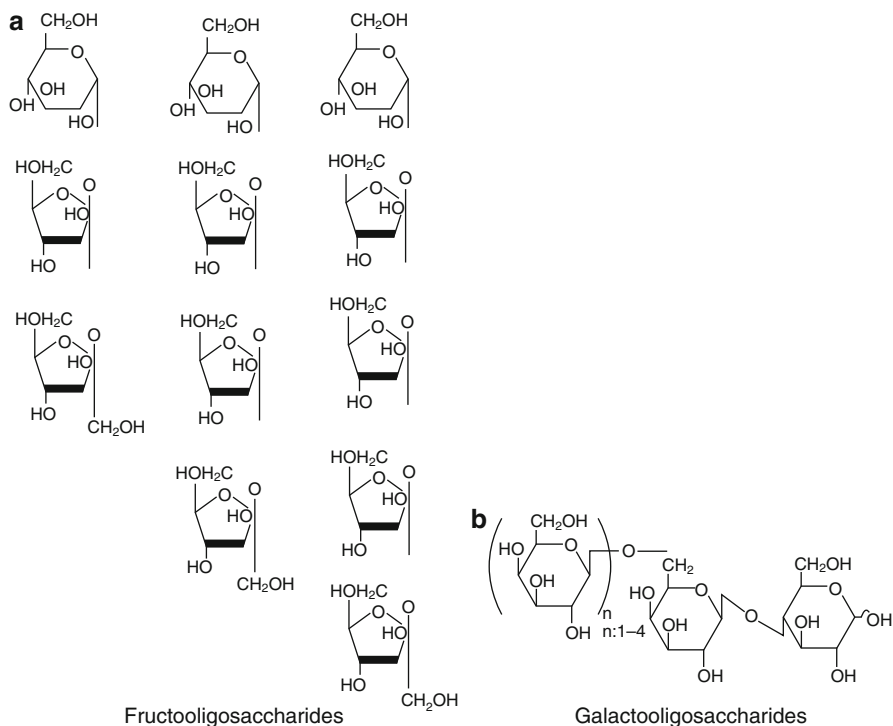


Fig. 1 Chemical structure of (a) fructooligosaccharides (FOS) and (b) galactooligosaccharides

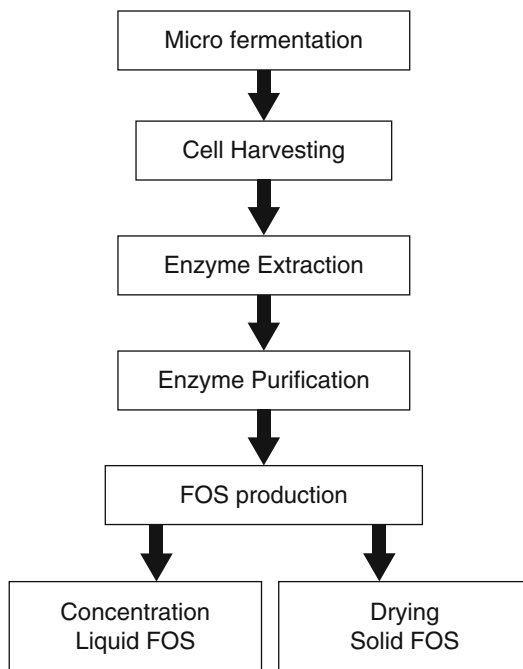
Bioactive Peptides

Protein constitutes a major nutrient of diet, which is hydrolyzed into a variety of peptides during gastrointestinal digestion. Some peptides may have structural characteristics similar to endogenous peptides that act in the organism as hormones and neurotransmitters. These exogenous peptides derived from food can interact with the same receptors in the organism and exert either agonistic or antagonistic activity. The food-derived bioactive peptides were first described in 1979 (Henschen et al. 1979). Since then, great progress has been made to the identification of peptides with different physiological effects. Many of the bioactive peptides can be found in fermented foods, particularly from fermented soy proteins and milk proteins, and are discussed in detail below.

Bioactive Peptides from Soy Proteins

Soybean is an important protein source and a good precursor for bioactive peptides. Interest in the composition of soy and its fermented products has grown since potential anticarcinogens and other bioactive agents have been identified. During fermentation, soy protein is hydrolyzed to produce many bioactive peptides with various specific biological activities. These include peptides with the following

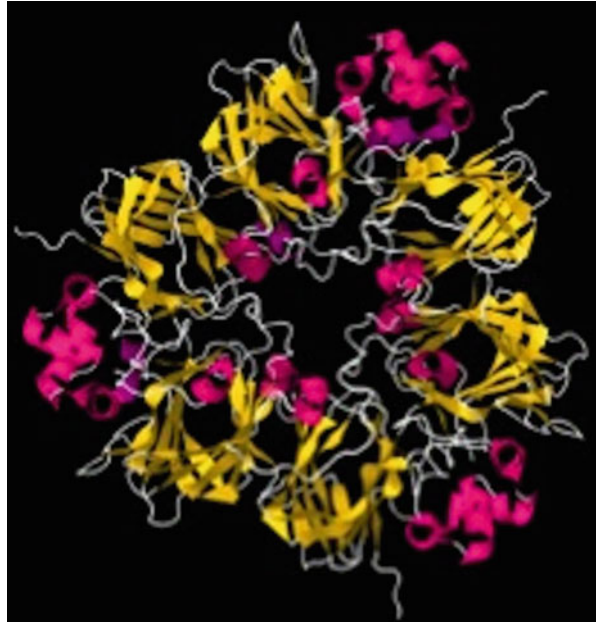
Fig. 2 Production flowchart for fructooligosaccharides (FOS)



properties: antihypertensive, hypocholesterolemic, antiobesity, opioid agonistic and antagonistic, antioxidant, anticancer, immunomodulatory, and antimicrobial (Gibbs et al. 2004), with antihypertensive and hypocholesterolemic activities most elucidated.

Antihypertensive peptides can inhibit the angiotensin-converting enzyme (ACE), which is a dipeptidyl carboxypeptidase associated with blood pressure regulation system. This enzyme increases blood pressure by converting the decapeptide angiotensin I into the potent vasoconstricting octapeptide angiotensin II, which leads to an increase in blood pressure. Therefore, inhibition of ACE will result in a hypotensive effect. ACE-inhibitory soy bioactive peptides have been found to lower systolic blood pressure and ACE activity in the aorta of spontaneously hypertensive rats (Li et al. 2002). Many literatures also indicate that soy proteins can reduce blood cholesterol concentrations in animals and humans (Potter 1995). Recently Leu-Pro-Tyr-Pro-Arg, a fragment peptide derived from soybean glycinin subunit, was found to lower serum cholesterol in mice. It was found in this experiment that Leu-Pro-Tyr-Pro-Arg also increased the excretion of bile acids in feces, suggesting that the peptides modulated cholesterol metabolism in the liver (Pais et al. 2006). Nattokinase is a bioactive peptide found in natto (fermented soy). Nattokinase was found to have beneficial effects against cardiovascular diseases (Pais et al. 2006). Figure 3 shows the structure of nattokinase from natto (fermented soy). It is necessary to know that nattokinase is not a kinase enzyme. It is actually a serine protease of the subtilisin family, and it exhibits a strong fibrinolytic activity.

Fig. 3 Chemical structure of nattokinase (Source: RCSB PDB protein data bank)



Bioactive Peptides from Dairy Products

Casein and whey proteins are the two main protein groups in bovine milk. Caseins comprise about 80 % of the total protein content in bovine milk and are divided into α -, β -, and κ -caseins. Whey protein is composed of β -lactoglobulin, α -lactalbumin, immunoglobulins (IgGs), glycomacropptides, bovine serum albumin, and minor proteins such as lactoperoxidase, lysozyme, and lactoferrin. The proteins (from casein or whey) have their own unique biological properties (Bennett et al. 2000). Furthermore, milk proteins can be degraded into numerous peptides by enzymatic proteolysis and may become bioactive peptides (Abubakar et al. 1998). Particularly, bioactive peptides can be generated during milk fermentation by the proteolytic activity of proteases. As a result, peptides with various bioactivities can be found in the end products, such as cheeses and fermented milks (yogurts and sour milk). These traditional dairy products may under certain conditions have specific health effects when ingested as part of the daily diet.

Many industrial dairy starter cultures are proteolytic to some extent. Therefore, bioactive peptides can be generated by the proteolytic activities of the strains of starter and nonstarter bacteria, such as *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactococcus lactis*, etc. (Rodríguez-Figueroa et al. 2012). The proteolytic system of lactic acid bacteria (LAB) is well studied, which consists of a cell wall-bound proteinase and a number of distinct intracellular peptidases, including endopeptidases, aminopeptidases, tripeptidases, and dipeptidases. Extracellular proteases cause degradation of casein into certain oligopeptides. The longer chain oligopeptides may be a source of

bioactive peptides when further degraded by intracellular peptidases released from lactic acid bacteria (Chen et al. 2007).

Bioactive peptides from fermented dairy products have some beneficial physiological effects, which include effects on cardiovascular system, effects on immune system, effects on nervous system, improved nutritional status and dental health, and antimicrobial properties (Contreras et al. 2009). Recent research has shown that enzymatic digestion of casein and whey proteins generates peptides that have the ability to inhibit ACE. For example, the ACE-inhibitory peptides, Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) with IC_{50} values (concentration of peptides mediating 50 % inhibition of ACE activity) of 9 μ M and 5 μ M, respectively, have been identified from a Japanese sour milk drink (Calpis[®]) fermented with *Lb. helveticus* and *Saccharomyces cerevisiae* strains (Nakamura et al. 1995). Furthermore, a dairy product, called Helveticus LBK-16H (Valio Ltd, Finland or Kaiku Vita brand[®], Spain), was found to exert significant antihypertensive effects in humans at daily doses of 150 ml. Two other commercial products, a casein hydrolysate containing the peptide FFVAPFPEVFGK (α 1-casein f23-34; Casein DP, Kanebo, Ltd, Japan, and C12 peptide, DMV, The Netherlands) and a whey protein hydrolysate (developed by BioZate, Davisco, USA) were also claimed to lower blood pressure in humans (FitzGerald et al. 2004). Recent studies have provided evidence that peptides found in some dairy products may play an active role in the nerve system, and these are known as opioid peptides. The first major opioid peptides discovered were β -casomorphins, which are the fragments of β -casein (Gilbert et al. 2011). Once absorbed into blood, these peptides can migrate into the brain and elicit pharmacological properties similar to opium or morphine, partly explaining drowsiness after drinking milk.

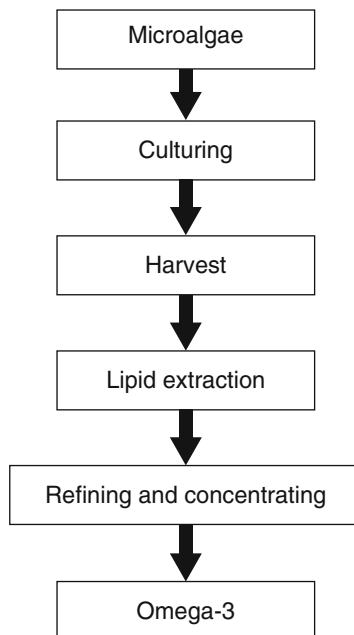
One of the most effective ways to increase the concentration of bioactive peptides in fermented dairy products is to ferment or coferment strains of LAB with highly proteolytic capacity. The LAB strains used for fermentation influence the release of effective bioactive peptides.

Fatty Acids

Fatty acids such as omega-3 fatty acids (also called n-3 fatty acids) have been found to be important to human health in many ways. Omega-3 fatty acids are polyunsaturated fatty acids (PUFAs) with a double bond (C = C) at the third carbon atom from the end of the carbon chain. The nomenclature of the fatty acid is based on the location of the first double bond, counted from the methyl end (the omega). The three types of omega-3 fatty acids important to human health are α -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). EPA and DHA are both commonly found in marine fish oils. The EPA and DHA are actually synthesized by algae and accumulate in fish while fish eat the algae.

The health benefits of omega-3 fatty acids have been extensively studied. Omega-3 PUFAs are known to have a variety of health benefits against cardiovascular diseases (CVDs), such as hypotriglyceridemic and anti-inflammatory effects.

Fig. 4 Production flowchart for omega-3 fatty acids



Also, many studies demonstrated promising antihypertensive, anticancer, antioxidant, antidepressant, antiaging, antiarthritic, and anti-inflammatory effects. DHA is helpful to support healthy brain development especially in young children along with supporting retinal development, and that is why DHA is widely used as a food supplement in infant formulas. As a matter of fact, the US Food and Drug Administration (FDA) approved the qualified health claims for DHA in 2004. Nowadays, DHA-fortified dairy items (milk, yogurt, cooking oil) can be found in the stores of many countries.

Omega-3 fatty acids can be found in fish oils, egg, and some plant oils. ALA can be found in walnut, chia seeds, berry oils, sage seed oil, algal oil, flaxseed oil, and etc. Commercially, omega-3 fatty acids are produced by fermentation process. For example, certain algae are used to ferment omega-3 fatty acids under controlled conditions. Then algae are harvested and further processed to extract and purify the omega-3 fatty acids, which can be packaged in capsules or spray dried into powders after mixing with carriers such as maltodextrin. Figure 4 shows the flowchart of producing DHA by algal fermentation. The above flowchart demonstrates an integrated system for the large-scale production of omega-3 fatty acids using microalgae. A microalgae strain is cultivated to increase cell density using photobioreactors. Then algal cells are separated from culture media by filtration and centrifugation. Lipid extraction from the harvested cells is commonly performed using organic solvent, for instance, a solvent mixture made of methanol/chloroform for the cell disruption and lipid extraction. Subsequently, fatty acids are separated from the total lipids by fractional (molecular) distillation or winterization, a process

where oil temperature is reduced to precipitate the more saturated lipids. If the omega-3 fatty acids are produced for the food and pharmaceutical industry, further extraction and purification processes are often necessary to improve the purity. Since omega-3 fatty acids are prone to lipid oxidation, controlling steps such as addition of antioxidants, removal of oxygen, and nitrogen flush are needed to prevent lipid oxidation and to achieve desired product shelf life. Recently, microencapsulation has become a useful technology to protect omega-3 fatty acids, and the product in powder form seems to be more convenient for food applications. For details on microencapsulation technology in food applications, see the ► [Chap. 34, “An Introduction to Food Nanotechnology”](#) by Chi-Fai Chau in this book.

Vitamins

B vitamins are a group of water-soluble vitamins that play important roles in cell metabolism. The B vitamins used to be a single vitamin, named simply as vitamin B. Later research shows that B vitamins are chemically distinct vitamins that often coexist in the same foods. In general, supplements containing all eight are referred to as a vitamin B complex. Individual B vitamin supplements are referred to by the specific name of each vitamin (e.g., B₁, B₂, B₃, etc.). It is well known that yeast can produce B vitamins during fermentation. Therefore, foods fermented by yeasts, such as bread and beer, are all good sources of B vitamins.

Examples of Fermented Functional Food Products and Their Bioactive Compounds

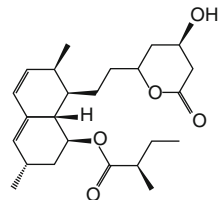
Traditional or indigenous fermented foods and beverages have been popular since the earliest recorded history. There is growing scientific evidence that many fermented foods are good for health or contain ingredients that are good for health. Meanwhile, new fermented foods with health benefits are emerging in the marketplace, as the concept of functional foods is gaining credibility. This section highlights some of the most representative examples of foods from microbial origin and the bioactive substances in them.

Red Yeast Rice

Overview

Red yeast rice (RYR), also known as Hongqu, red koji, or anka, is an Asian traditional fermentation food source. It is fermented by yeast *Monascus purpureus* that belongs to the family Aspergillaceae, and the use of RYR dates back to the Tang Dynasty, when it was used primarily as a preservative, coloring, and flavoring agent on fish and meat. RYR has long been recognized as a folk medicine for

Fig. 5 Chemical structure of monacolin K (lovastatin)



improving food digestion and blood circulation in China and has been consumed as a dietary supplement in the world. In the 1990s, many dietary supplement companies started to manufacture and advertise the use of RYR as a cholesterol-lowering agent due to its effect comparable to statins. However, it was not officially marketed until 2001 in the USA (Journoud and Jones 2004). Nowadays, many commercial RYR products are available around the world such as RYR supplement, Xuezhikang, *M. purpureus* rice, red mold rice, Cholestin, and HypoCol. Commercially RYR is produced by solid-state fermentation.

Monacolin K from RYR

RYR contains predominantly rice starches and sugars and also yeast polyketides, fatty acids, pigments, and condensed tannins. The classes of polyketide structures that arise from the fermentation process are called monacolins, and the major monacolin found in RYR is monacolin K (Fig. 5), which is identical in structure to lovastatin, Merck's prescription agent (Mevacor), and the first commercially available compound for the treatment of hypercholesterolemia.

Monacolin K is the major active compound in RYR responsible for its cholesterol-lowering effect. It decreases the amount of cholesterol by inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which is a regulatory and rate-limiting enzyme of cholesterol biosynthesis; therefore, it also helps to decrease blood pressure (Li et al. 2005). Although levels of monacolin K vary in the product, 2.4 g of RYR daily may contain about 4.8 mg of monacolin K, or 0.2 % of the total dose. RYR supplements may also contain isoflavonoids, monounsaturated fats, and sterols that help to reduce cholesterol levels even further.

The culture medium has a vital role in the production of monacolin K, and the fermentation process is investigated in the literature. One study utilized Plackett–Burman statistical analysis to screen 12 media components and to subsequently optimize significant parameters for the biotechnological production of monacolin K by *Monascus purpureus* MTCC 369. The production of monacolin K in the optimized medium was found to be four times higher than the basal medium in the submerged fermentation (Seraman et al. 2010).

Efficacy of RYR

Recently, many clinical trials have focused on the uses of RYR in disease state, and the strongest evidence for RYR use is in dyslipidemia versus other clinical conditions. RYR was shown to have an equal efficacy to statins when combined with or without other dietary supplements. One clinical trial found that all participants who

took RYR had significant decreases in low-density lipoprotein cholesterol (LDL-C), total cholesterol, triglycerides, and high-sensitivity C-reactive protein levels and an increase in high-density lipoprotein cholesterol level for 1 year when compared with baseline ($p < 0.001$), and the addition of phytosterol tablets to RYR did not result in further lowering of LDL-C levels (Becker et al. 2013). In another randomized, multicenter, and placebo-controlled clinical trial, beneficial effects of Xuezhikang, an RYR extract, in the treatment of hyperlipidemic patients were demonstrated (Li et al. 2010). Xuezhikang has been in clinical use as a Chinese proprietary medicine in China and is commonly prescribed to patients with ischemic cardiovascular disease as a supplementary therapy. A nutraceutical combination (NC), consisting of 500 mg berberine, 200 mg RYR, and 10 mg policosanols, was found to be effective in reducing cholesterol levels and improving endothelial function in patients with hypercholesterolemia, in a single-center, randomized, double-blind, placebo-controlled study (Affuso et al. 2010). On the other hand, information on diabetes, osteoporosis, cancer, nonalcoholic fatty liver disease, fatigue, and memory are currently limited although in vivo and in vitro studies have shown an effect (Yang and Mousa 2012).

Safety of RYR

Li and others evaluated safety and efficacy of RYR as an alternative therapy for hyperlipidemia and pointed out that lovastatin is associated with various adverse effects such as myopathy and abnormal liver function test results, which can lead to serious problems if patients are not monitored and treated (Lin et al. 2005). The natural inclusion of low-dose lovastatin in RYR and the lack of dietary supplement regulation by the FDA raise safety concerns for healthcare professionals as well as for patients.

The discovery of citrinin in RYR has also led to a controversy about the safety of RFR. Citrinin is a toxic fermentation by-product which can be found as a contaminant and can cause kidney failure in animals with a median lethal dose (LD50) of 35 mg/kg (Endo and Kuroda 1976). A total of 109 widely consumed RYR and related products were analyzed, and citrinin was detected in 31 samples (28 %) ranging from 16.6 to 52.5 lg/kg (Li et al. 2012). There have been efforts to develop an accurate, simple, and rapid micellar electrokinetic capillary chromatographic method to simultaneously detect lovastatin and citrinin in RYR to ensure efficiency and safety of these products. Efforts have also been dedicated to use mutant strains acquired by treatment with mutagenic agents to produce RFR with high concentration of monacolin K and low concentration of citrinin (Chen and Hu 2005).

In conclusion, when used as a dietary supplement to achieve and maintain healthy cholesterol levels, RYR has significant potential to reduce healthcare costs and contribute to public health by reducing heart disease risk in individuals with moderate elevations of circulating cholesterol levels. However, standardized manufacturing practices should be established for RYR sold as a dietary supplement in order to ensure equivalence of content of active ingredients in preparations being sold to the public and to limit the production of unwanted by-products of fermentation such as citrinin.

Cordyceps

Overview

Cordyceps species, including *C. sinensis*, *C. militaris*, *C. pruinosa*, and *C. ophioglossoides*, are prized traditional medicinal materials known since 2000 B.C. *C. sinensis* is the most explored species followed by *C. militaris*. It is a parasitic complex of fungus and caterpillar, which has been used for medicinal purposes for centuries particularly in China, Japan, and other Asian countries (Das et al. 2010). It is generally called “Dong Chong Xia Cao” in Chinese and “Tochukaso” in Japanese, meaning “winter-insect and summer-plant” because of the growing process: the fungus first parasitizes the larva of some species Hepialidae, forming a parasitic complex that comprises the remains of the caterpillar and the stroma of the fungus. Because the larvae are infected by fungi in the summer and autumn seasons, and consumed by mycelia and turned into “stiff worms” in winter, they are called “winter worms” (Dong Chong). In the spring and summer season of the following year, the stroma emerges from the ground, growing from the head of the larva, and is known as “summer grass” (Xia Cao) (Paterson 2008).

Cordyceps is commonly used to replenish the kidney and soothe the lung and for the treatment of fatigue. It also can be used to treat conditions such as night sweating, hyposexuality, hyperglycemia, hyperlipidemia, asthenia after severe illness, respiratory disease, renal dysfunction, renal failure, arrhythmias, and other heart diseases and liver diseases (Zhou et al. 2009).

During the last decades, as the findings of new uses for *Cordyceps* and development of *Cordyceps* preparations continue, some correlative and curative products have rapidly appeared one after another, such as *Cordyceps* capsule, *Cordyceps* oral liquid, *Cordyceps* drink, and so on, and become more and more favorable among the general public. Very few toxic side effects have been demonstrated with *Cordyceps* use, and no human or animal toxicity has been reported. In the USA, *Cordyceps* is considered a “food” by FDA and is classified as “generally recognized as safe” (GRAS). A growing number of researchers now consider it to be a “superfood” that can be included in almost every diet.

Fermentation of *Cordyceps*

Because of its highly specific growth environment and restricted geographical distribution (in Qinghai–Tibetan plateau), *C. sinensis* has a long reputation of being the single most expensive raw material used in Oriental Medicine. The extremely high price of *C. sinensis*, approximately USD \$20,000–40,000 per kg, has led to it being regarded as “soft gold” in China (Au et al. 2012). This has resulted in attempts to determine ways of cultivating it to make it a more affordable material for commercial trade. It is generally accepted that cultivated *C. sinensis* fungi possess the same functions as *C. sinensis* natural “herbs.” However, while cultivated products undoubtedly support ecologically sustainable use of *C. sinensis*, the actual similarities between the wild fungus and the cultures are not clear (Paterson 2008).

A number of culture techniques for *Cordyceps* have been noticed, for example, storage/stock culture, pre-culture, popular/indigenous culture (spawn culture, husked rice culture, and sawdust culture), and special/laboratory culture (shaking culture, submerged culture, surface liquid culture, and continuous/repeated batch culture) (Das et al. 2010). The optimal culture conditions such as initial pH value, various nitrogen sources, plant oils, and modes of propagation (shake-flask and static culture) are investigated based on the production of biomass, exopolysaccharide (EPS), adenosine, and, in particular, cordycepin production. For detailed discussion on cordycepin, see section “[Cordycepin](#)”.

A review on the bioactive ingredients and bioactivities of *C. sinensis* reveals that eight types of materials have been used, including (1) the crude powder, (2) the crude powder of the fruiting body, (3) the extracts from the crude powder, (4) the extracts from the crude powder of the fruiting body, (5) the crude powder of mycelia, (6) mycelial extracts, (7) the supernatants of submerged fermented cultures, and (8) the whole broth of submerged fermented cultures (Paterson 2008). The diversity of these materials brings up the major concern of whether the knowledge acquired regarding *C. sinensis* is applicable to wild “Dong Chong Xia Cao” or the fermented mycelia or mycelial fermentation products of *C. sinensis*.

Composition and Quality Control of *Cordyceps*

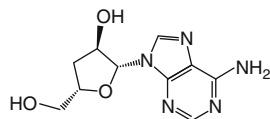
Besides cultivation of *C. sinensis* by fermentation technology, much effort has also been focused on discovering the alternative species. There are more than 400 types of the so-called *Cordyceps*; its substitutes have been found worldwide today, and *Cordyceps militaris* is the most commonly used substitute. In addition, there are counterfeits, and mimics such as *Stachys geobombycis*, *Stachys sieboldii*, *Lycopus lucidus*, etc., also emerge on the market (Hsu et al. 2002). Thus, it is a serious problem for authentication and quality control of *Cordyceps* on the market.

The methodology for quality control is crucial to ensure authenticity and quality of *Cordyceps* and *Cordyceps* products. The rational markers, which are related with the safety and efficacy of *Cordyceps*, are essential. At present, multiple markers such as nucleosides, ergosterol, mannitol, and polysaccharides are being used for quality control of *Cordyceps* and its products (Li et al. 2006). Another approach is using chemical profile instead of a single compound; therefore, distinct fingerprints could be revealed in water-soluble constituents derived from different sources of *Cordyceps*, instead of depending on the identities of any specific chemicals. The chemical constituents of natural *Cordyceps* include cordycepic acid, cordycepin, glutamic acid, amino acids, polyamines, cyclic dipeptides, saccharides and sugar derivatives, sterols, nucleotides and nucleosides, 28 saturated and unsaturated fatty acids, fatty acid derivatives and other organic acids, vitamins, and inorganic elements (Zhao et al. 2014).

Cordycepin

Cordycepin is the most important pharmacologically active compound in *Cordyceps*. It is an adenosine analogue (Fig. 6), 3'-deoxyadenosine (9-(3-deoxy- β -D-ribofuranosyl) adenine), with molecular weight of 251.24,

Fig. 6 Chemical structure of cordycepin



needlelike or flaky crystal appearance, melting point of 228–231 °C, and a maximum absorption wavelength of 259.0 nm (Tuli et al. 2013). Cordycepin is first extracted from *C. militaris* and is present also in other *Cordyceps* species.

Cordycepin is widely studied, and it performs a myriad of biological functions including antitumor, antibacterial, and antifungal functions, to name a few. Earlier reports suggested that cordycepin may be involved with a number of molecular processes, resulting in either inhibition or modulation of a number of genes related to inflammation or carcinogenesis, or directly influences protein synthesis (Ng and Wang 2005). Cordycepin regulates lipid concentrations in the plasma but has more favorable effects on the liver, implying its protective effect on the liver under fatty liver conditions (Sun et al. 2011). Upon effective treatment of obesity and obesity-related disorders, cordycepin may serve as a therapeutic agent by blocking both adipogenesis and lipid accumulation. Cordycepin also has a potent neuroprotective function by interfering with the synthesis of matrix metalloproteinase-3 (MMP-3) and by inhibiting free radical formation due to cerebral ischemia–reperfusion injury (Cheng et al. 2011). Besides, it is also found to have anti-inflammatory, antioxidant, anti-adipogenic, and antiaging activities.

Fermented Ginseng

Overview

Ginseng is one of the most popular and best-selling herbal medicines worldwide. Believed to be a tonic, prophylactic, and “restorative” agent, it has been used as a medicine and as a health food by healthy and ill individuals around the world, especially in Asian countries. It has a wide range of biological activities, such as anticancer, anti-inflammatory, hepato-protective, cardiovascular, and cognitive effects and so on (Ernst 2010). The term “ginseng” in the present paper is generally considered to mean the dried root of *Panax ginseng* C.A. Meyer, belonging to the *Panax* genus in the family of Araliaceae.

Global markets for ginseng and related products continue to expand with gradual elucidation of pharmacological mechanisms of ginseng. It is commercially consumed as ginseng teas, liquid extracts, capsules, soft and alcoholic drinks, and ginseng wines. Cultivated ginseng is classified into three types, depending on how it is processed: fresh ginseng (less than 4 years old), white ginseng (4–6 years old and dried after peeling), and red ginseng (harvested at 6 years old, steamed, and dried). However, due to the long duration of cultivation for marketable ginseng and plant diseases such as red skin and root rot, cultivation of ginseng is very difficult.

Therefore, researchers have studied the production of ginsenosides with fermentation by tissue and cell cultures (Wu and Zhong 1999), such as callus tissues, cell suspensions, normal roots, and hairy root cultures induced by *Agrobacterium rhizogenes*.

Recently, ginseng has been studied in a number of randomized controlled trials investigating its effect mainly on physical and psychomotor performance, cognitive function, immunomodulation, diabetes mellitus, cardiovascular risk factors, quality of life, as well as adverse effects (Ernst 2010). Currently, 15 systematic reviews of ginseng used to treat various conditions are available, mainly from Korean, Chinese, English, and Japanese databases. A journal in Korea named Journal of Ginseng Research is dedicated to publish in the diverse fields of ginseng research covering the disciplines of pharmacology, biology, chemistry, and veterinary science and cultivation, process, and production of ginseng.

Bioactivity and Bioavailability of Ginsenosides

Until now, ginseng has been reported to contain saponins, peptides, polysaccharide, fatty acids, vitamins, alkaloids, lignans, and flavonoids. The saponins, known as ginsenosides, are widely believed to be the major bioactive compounds of ginseng. Ginsenosides are attributed with cardioprotective, immunostimulatory, antifatigue, and hepato-protective physiological and pharmacological effects (Park et al. 2005). They are divided into three groups based on their structure, i.e., the Rb group (protopanaxadiol, including Ra₁, Ra₂, Rb₁, Rb₂, Rb₃, Rc, Rd, Rg, Rh, and others), the Rg group (protopanaxatriols, including Rg₁, Rg₂, Re, Rf, Rh, and others), and the Ro group (oleanolic acid) (Liang and Zhao 2008). The pharmacological actions of each of those classified compounds are different.

Ginsenosides themselves can exert various pharmacological activities, by directly being added to cell cultures in vitro or by being intraperitoneally (i.p.) or intravenously (i.v.) injected into experimental animals. These results have led to the misunderstanding that intact ginsenosides might be the real active principles in the body. Orally administered ginsenosides are very hard to break down by gastric juices or liver enzymes. Instead, they are metabolized by intestinal bacteria, and then these metabolites are absorbed from the intestine. Microbes cleave the oligosaccharide connected to the aglycone stepwise from the terminal sugar to generate major metabolites, namely, 20S-protopanaxadiol 20-O-β-D-glucopyranoside (M1) and 20S-protopanaxatriol (M4) (Hasegawa et al. 1996). These deglycosylated ginsenosides, including ginsenosides Rg3 and Rh2 and compound K, can be produced by the hydrolysis of sugar moieties from the major ginsenosides Rb1, Rb2, Rc, and Rd, accounting for more than 50 % of the total ginsenosides. Figure 7 outlines possible transformation pathways of Re, one of a major ginsenosides, by cell extracts from various food microorganisms to different deglycosylated metabolites (Bennett et al. 2000). These metabolites are known to be more readily absorbed into the bloodstream and expected to maximize efficiency of fermented ginseng in pharmacology. Therefore, a number of studies have investigated fermentation of ginseng or ginseng extracts for higher biopotency.

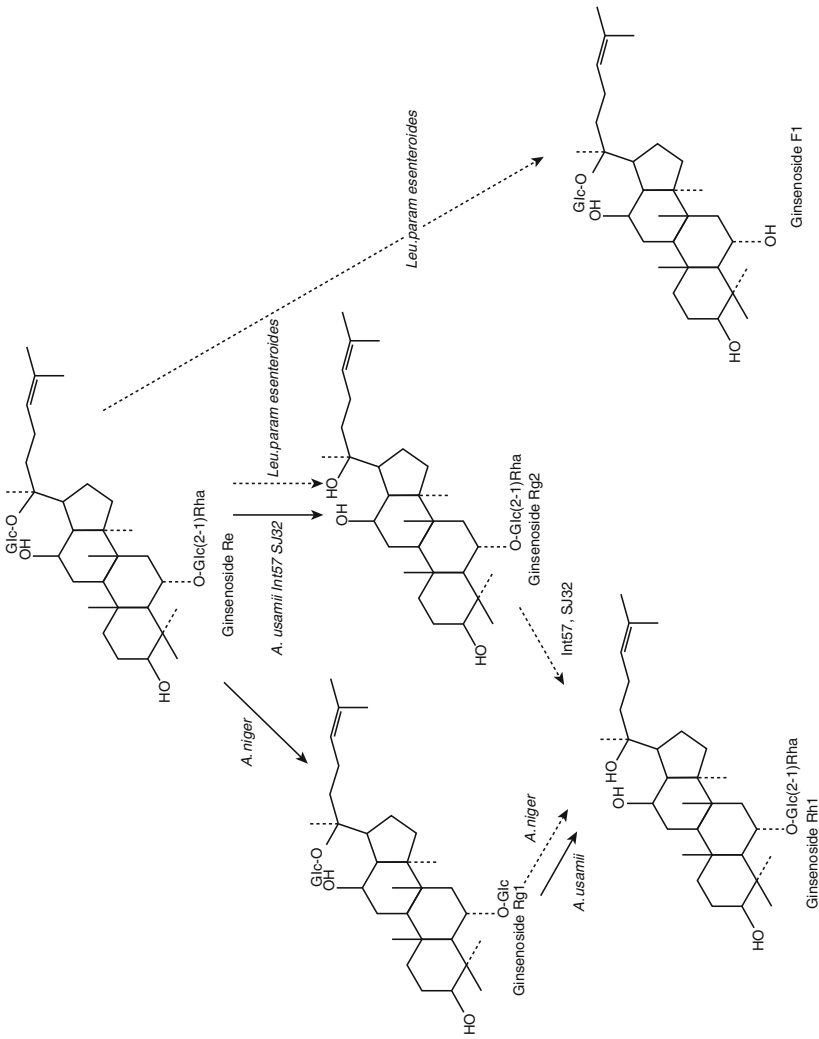


Fig. 7 Proposed transformation pathways of ginsenoside Re by cell extracts from various food microorganisms. → main pathway, - - -> minor pathway (With kind permission of Springer Science + Business Media)

In a previous study, edible *Lactobacillus* species were screened for their potential in metabolizing ginsenosides from red ginseng, and *Lactobacillus plantarum* M-2 that is food grade was selected for the microbial conversion of ginsenosides in red ginseng powder. This group further investigated changes in total sugars, uronic acid, polyphenols, and ginsenoside metabolites during fermentation by *L. plantarum* M-2 (Kim et al. 2010).

It was also found that the fermentation of ginseng increases its antitumor and immunostimulating activities of fermented ginseng extracts (FGE) when compared with those of non-fermented ginseng extracts (NFGE). FGE promotes antitumor activities to inhibit tumor metastasis, and its antitumor effects are associated with the enhancement of systemic as well as mucosal immune systems (Sato et al. 1994). FGE was also demonstrated to have improved antioxidant status than NFGE on lipid peroxidation and on major organs of aged rats. The results suggested that treatment of FGE could improve the antioxidant status during aging, thereby minimizing the oxidative stress and occurrence of age-related disorders associated with free radicals. In a transient focal ischemic rat model, orally administered red ginseng extract did not protect ischemia–reperfusion brain injury. However, fermented red ginseng with *Bifidobacterium* H-1 significantly protected ischemia–reperfusion brain injury (Bae et al. 2004). Another group also claimed that *Bifidus* fermentation of red ginseng increases its hypolipidemic and hypoglycemic effects (Trinh et al. 2007).

To conclude, fermentation of various ginsenosides is good for human body with enhanced bioavailability, and accumulating evidence strongly suggests that the metabolites of ginsenosides are the active molecules in the body. Fermented ginseng was developed to increase bioavailability of ginsenoside and standardize the effect of ginsenoside.

Others

There are a large variety of fermented foods and beverages with traditional and cultural values around the globe, such as fermented milk, fermented meat, fermented soy products, fermented fruits and vegetables, and fermented cereals and roots. The diversity of such fermented products derives from the heterogeneity of traditions found in the world, cultural preference, and different geographical areas where they are produced; examples are kimchi in Korea, natto in Japan, tempeh in Indonesia, and cheese in the West. Many of the fermented products consumed by different ethnic groups have therapeutic values, and in recent years more fermented functional food products are being marketed globally. Large portions of them are fermented dairy foods, including milk and yogurt, which are among the most accepted food carriers for delivery of viable probiotic cultures to the human GI tract. Active cultures may also be used to add function to foods that are not dairy-based, such as mayonnaise, fruit drinks, cereals, and meat.

Conclusion and Future Directions

To summarize, microbes are important sources for producing bioactive compounds that convey health benefits to humans. However, the mechanisms by which functional microbes and ingredients affect human gut health are still largely unknown. Recent advancement of knowledge in both microbiology in general, and the human intestinal microflora in particular, will drive the desire of consumers to buy and consume foods that impact favorably on the GI tract. The knowledge acquired by genomics on the genetics and physiology of a probiotic strain can be used for strain improvement. Other approaches such as microencapsulation have also been used to improve the resistance of sensitive probiotic bacteria against adverse conditions during storage, processing, and digestion. Besides, advances in “omics” (genomics, metagenomics, metabolomics, proteomics, transcriptomics) may help to understand the mechanism of action of each probiotic strain. In the future, fermented foods will become even more important in human diet and in maintaining health. On the other hand, more fermented foods with health-promoting properties will become available on the market, many directed toward consumers with very specific health and metabolism needs.

Cross-References

- ▶ [Chemical Composition of Milk and Milk Products](#)
- ▶ [Synthetic Bioactive Substances](#)

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Shiming Li

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Abstract

Three traditional macronutrients, proteins, carbohydrates, and fatty acids, are sufficient or even oversupplied for most of the current population globally, and consequently consumption of conventionally nonessential nutrients becomes vital. These nutrients include vitamins, minerals, coenzymes, some sulfur-containing compounds, and melatonin, as examples. The dietary source of these compounds usually includes vegetables, fruits, microorganisms, meats, and fish, and the content of these nutrients varies much. Some nutrients have sufficient supply from natural resource, but other nutrients have limited contents in the natural supply chain thus unable to meet the demand for health benefits. Fortunately, synthetic version of these nutrients has been available to use as

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early as the 1940s for human consumption but not until recent decades to reach a sufficient supply. This chapter is a summary of these nutrients that are synthesized either through a chemical process or microbial fermentation or both to supplement the essential nutrients with insufficient resources from natural food diets.

Introduction

Bioactive substances are nonessential traditional nutrients or extra-nutritional constituents naturally present in foods and food products. They typically occur in very small percentages in daily food ingestion and are expected to keep the body healthy and prevent various lifestyle-related diseases. They have been intensively evaluated for their effects on health-beneficial properties. The driving force behind this scientific inquiry was the result of many epidemiologic studies that have shown protective effects of these bioactive substances on many chronic diseases such as inflammation, metabolic syndrome, cardiovascular disease (CVD), and cancer. Many bioactive compounds have been discovered. These compounds vary widely in chemical structures and bio-functionalities and are grouped accordingly (Tringali 2001).

The sources of vitamins, as an example, come from meats, vegetables, fruits, and nuts (vitamins A, B, C, E, K). Phenolic compounds, including their subcategory, flavonoids, are present in various plants and have been studied extensively in cereals, legumes, nuts, olive oil, vegetables, fruits, tea, and red wine. Phenolic compounds by definition possess multiple hydroxyl groups on the benzene ring (s) and usually have strong antioxidant property. In addition, a myriad of studies have demonstrated favorable effects of polyphenolic compounds on health-beneficial property such as prevention of thrombosis and tumorigenesis and promotion of healthy cardiovascular system. Although some epidemiologic studies have reported protective associations between flavonoids or other phenolics and CVD and cancer, other studies have not found these associations. Examples of polyphenols and health-promoting effects are stated below. Hydroxytyrosol, one of the many phenolics in olives and olive oil, is a potent antioxidant. Resveratrol, found in peanut, black grape, red wine, mulberry, and *Polygonum cuspidatum*, has antioxidant, antithrombotic, and anti-inflammatory properties. Lycopene, a potent antioxidant carotenoid in tomatoes and other fruits, is thought to prevent cardiovascular diseases, to protect against prostate and other cancers, and to inhibit tumor cell growth in animals. Organosulfur compounds in garlic and onions, isothiocyanates in cruciferous vegetables, and flavonoids in citrus fruits, cherries, and herbs have antioxidant, anticarcinogenic actions and cardiovascular protective effects (Kris-Etherton et al. 2002). In summary, numerous bioactive compounds appear to have beneficial health effects.

Much scientific research of some bioactive substances has been convincingly conducted for daily dietary recommendations based on their efficacy and/or functionality, such as vitamins and minerals. However, some bioactive

compounds need more scientific evidence to determine the amount of recommended daily intake (RDI), even with the confirmative recommendation of a diet rich in a variety of fruits, vegetables, whole grains, legumes, natural unsaturated oils, and nuts, among others. It has to be pointed out that some bioactive substances have insufficient supply from natural source to provide consumers the recommended amount, such as vitamin B₁₂. Therefore, this chapter briefly reviewed some critical bioactive substances existing in foods and functional foods but usually being obtained from synthetic routes due to their limited supply from natural resource. Synthetic substances covered in this chapter are majorly vitamins, including vitamins B, C, and E, particularly B vitamins, thiamin, riboflavin, niacin, pantothenic acid, biotin, folic acid, cobalamin, pyridoxal, and its derivatives (B₆); sulfur-containing compounds, acetylcysteine, lipoic acid, methylsulfonylmethane, and adenosylmethionine; coenzyme Q10; melatonin; and raspberry ketone.

Synthetic Nutraceutical Substances

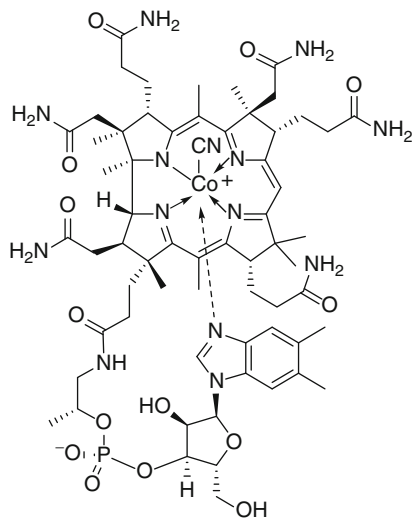
Synthetic Vitamins

Cobalamin

Vitamin B₁₂, or cobalamin in Fig. 1, is the most complex vitamin in chemical structure currently known to man. Vitamin B₁₂ is needed for building proteins and for forming nerve cells and red blood cells in the body. Symptoms of vitamin B₁₂ deficiency can lead to anemia, fatigue, mania, depression, weakness, constipation, loss of appetite, and weight loss, while a long-term deficiency of vitamin B₁₂ can cause permanent damage to the brain and central nervous system, such as numbness and tingling in the hands and feet. Additional signs of B₁₂ deficiency include difficulty in maintaining balance, confusion, dementia, poor memory, and soreness of the mouth or tongue. It may be a short time period to have slight signs of vitamin B₁₂ deficiency, but can take as long as two to several years for vitamin B₁₂ deficiency to become seriously symptomatic. The recommended daily allowance (RDA) of vitamin B₁₂ for adults is 2.4 micrograms, whereas the dosage of B₁₂ dietary supplement can be 100 times higher because of its bioavailability. Vitamin B₁₂ can only be synthesized by bacteria. Natural food sources of vitamin B₁₂ are found primarily in meat and other animal products, which means those who follow a plant-based diet have to find their source elsewhere. Neither plants nor animals are independently capable of constructing vitamin B₁₂. However, synthetic forms are readily available and added to many foods like cereals.

The chemical synthesis of B₁₂ was reported by many organic chemists. But the synthesis adapted by Robert Burns Woodward and Albert Eschenmoser in 1972 remains one of the classic feats of organic synthesis of B₁₂. Industrial production of B₁₂ is from fermentation of selected microorganisms. *Streptomyces griseus*, a bacterium once thought to be a yeast, was the commercial source of vitamin B₁₂

Fig. 1 Structure of vitamin B₁₂ (cyanocobalamin)



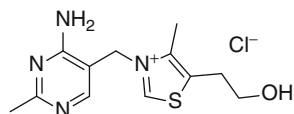
for many years. The species *Pseudomonas denitrificans* and *Propionibacterium shermanii* are more commonly used today. These are frequently grown under special conditions to enhance yield, and genetically engineered versions of one or both of these species are used. Since a number of species of *Propionibacterium* produce no exotoxins or endotoxins and are generally regarded as safe (GRAS) by the Food and Drug Administration of the United States, they are presently the FDA-preferred bacterial fermentation organisms for vitamin B₁₂ production (Riaz et al. 2007).

Cyanocobalamin is the most common and widely produced chemical compound that has vitamin B₁₂ activity. Although cyanocobalamin is a synthetic substitute of vitamin B₁₂, it can be transformed in the body to vitamin B₁₂ isomers that play the same role as vitamin B₁₂ inside the body. Cyanocobalamin is also produced by bacterial fermentation process, but the purification process is relatively easier than that of vitamin B₁₂. Fermentation by a variety of microorganisms yields a mixture of methyl-, hydroxy-, and adenosylcobalamin. These compounds are converted to cyanocobalamin by addition of potassium cyanide in the presence of sodium nitrite when heating (<http://lpi.oregonstate.edu/infocenter/vitamins/vitaminB12/>. Accessed 03 Mar 2015).

Thiamin

Vitamin B₁, thiamin, or thiamine in Fig. 2 is an essential nutrient required for maintaining body cellular function and organ functions. Thiamin deficiency leads to metabolic coma, particularly the nervous and circulatory systems, and even death in severe deficiency. Also, thiamin deficiency can lead to development of beriberi and/or Wernicke-Korsakoff syndrome. Symptoms of both diseases include severe fatigue and degeneration of cardiovascular, nervous, muscular, and gastrointestinal systems. Thiamin overdose is very rare and can cause weakness, headache,

Fig. 2 Chemical structure of thiamine



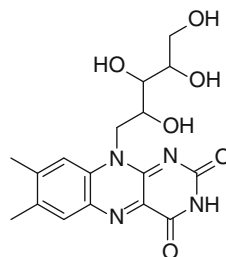
irregular heartbeat, and low blood pressure. Thiamin doses in the US recommended daily allowance (RDA) taken by mouth are 1.2 mg for male adults 19 and older and 1.1 mg for females.

Thiamin is a colorless compound with a chemical formula of $C_{12}H_{17}N_4OS$. Its structure consists of a diaminopyrimidine molecule and a thiazole ring linked by a methylene group. The thiazole is substituted with a methyl group and a hydroxyethyl side chain. Thiamin is soluble in water, methanol, and glycerol and insoluble in many organic solvents. It is stable at acidic pH and in cold storage condition, but unstable in alkaline, UV light, and gamma irradiation solutions. Thiamin is very reactive in Maillard-type reactions (Tanphaichitr 1999).

Diet sources of thiamin are widely spread, including liver, egg, fish, nuts, seeds, bread, green pea, squash, asparagus, beans, meat, vegetables, legumes, fruits, and grains. Complex thiamine biosynthesis occurs in bacteria, some plants, and fungi. The thiazole and pyrimidine moieties are biosynthesized separately and then combined to form thiamin monophosphate by the action of thiamine-phosphate synthase (EC 2.5.1.3). The biosynthetic pathways may differ among organisms. The enzymatic synthesis of thiamin monophosphate was developed, and its procedure can be briefly stated as hydroxymethylpyrimidine monophosphorylation and then diphosphorylation to obtain hydroxymethylpyrimidine diphosphate, which coupled with thiazole monophosphate under the enzyme catalysis in each step to generate thiamin monophosphate (Leger 1961). Thiamin cannot be synthesized within animal tissues and thus is a universally essential dietary component throughout the animal kingdom. All living organisms use thiamin in their biochemistry, but it is synthesized in bacteria, fungi, and plants. Animals must obtain it from their diet, and, thus, for them it is a vitamin. Supply from natural source of thiamin is not sufficient. Chemical synthesis of thiamine is very complicated, involving 15–17 different steps. Thiamine salts are stable and survive at temperature above 100 °C. The chemical characterization and synthesis of thiamin were carried out during the 1930s, with Roger Williams playing a major role. In the preferred synthesis pathway, the pyrimidine ring is built first, largely from organic nitrile compounds; then the thiazole ring is added by reaction with chloro-ketone, carbon disulfide, and ammonia; and finally an oxidation step with hydrogen peroxide removes a sulfur atom and introduces a ring and a double bond so as to complete the thiazole ring. Analysis of thiamin in food sources can be achieved by microbiological assay, fluorimetric assays, and high-performance liquid chromatography (Bates 2010).

Riboflavin

Riboflavin, or vitamin B₂ in Fig. 3, has a variety of functions in the body, including the metabolism of carbohydrates for the production of energy and the production of

Fig. 3 Riboflavin structure

red blood cells. The human body needs riboflavin to use oxygen efficiently in the metabolism of amino acids, fatty acids, and carbohydrates. Riboflavin is involved in the synthesis of niacin. It activates vitamin B₆ and helps the adrenal gland to produce hormones. It also helps the body make antibodies to fight disease and infection, regulates the thyroid gland, and is important in maintaining healthy hair, nails, and skin. Riboflavin is especially important in rapid growth period because it is involved in the formation and growth of cells, particularly red blood cells. People most likely to suffer from riboflavin deficiency problems are those with anorexia, older people with poor diets, alcoholics with impaired ability to absorb and use the vitamin, and newborn babies being treated for jaundice by exposure to ultraviolet light.

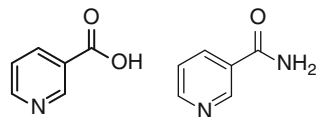
Riboflavin is an orange-yellow crystal with a bitter taste. It is relatively stable when exposed to heat, but tends to decompose in the presence of light for extended periods of time. It was not until the 1930s that the chemical structure of riboflavin was determined by Paul Karrer and Richard Kuhn independently and the compound was synthesized. Naturally, riboflavin can be synthesized in plants and microorganisms. Some foods rich in riboflavin are yeast, dark green vegetables, mushrooms, meat, eggs, legumes, nuts, milk and other dairy products, sweet potatoes, and pumpkins, among others. Bacteria in the human digestive tract are also able to synthesize some riboflavin, but not enough to meet the body's requirement.

Riboflavin is produced synthetically using either bacteria, yeast, or fungus. The bacteria or fungus is cultured in a large vat that has been seeded with small amounts of riboflavin. Over time, the organisms generate large quantities of riboflavin until some desired amount of the compound has been produced. The vat is then heated to a temperature sufficient to kill the bacteria or fungi, leaving crystalline riboflavin behind. The riboflavin is then separated and purified for human consumption (Fisher and Bacher 2008).

Niacin

Niacin and niacinamide (Fig. 4) are forms of vitamin B₃ and one of the eight B vitamins. All B vitamins help the body to convert carbohydrates into fuel glucose and to use fat and protein. These B vitamins, often referred to as B complex vitamins, are needed for healthy skin, hair, eyes, and liver. They also help the nervous system function properly. Niacin also helps the body to make various

Fig. 4 Structures of niacin and niacinamide



sex- and stress-related hormones in the adrenal glands and other parts of the body. Niacin helps improve circulation and has therapeutic effects. High dose of niacin is used for lowering hyperlipidemia (high cholesterol). Niacin is also used along with other treatments for circulation problems, migraine headache, and dizziness and to reduce the diarrhea associated with cholera. Niacinamide is used for treating diabetes and for preventing vitamin B₃ deficiency and related conditions such as pellagra. Niacin or niacinamide is also used for acne, leprosy, attention deficit hyperactivity disorder (ADHD), memory loss, and arthritis, preventing premenstrual headache, improving digestion, protecting against toxins and pollutants, reducing the effects of aging, lowering blood pressure, improving circulation, promoting relaxation, improving orgasm, and preventing cataracts. In addition, niacinamide is applied to the skin for treating a skin condition called inflammatory acne vulgaris (Gille et al. 2008).

Niacin can be found in many foods, including fungi (yeast, mushrooms), liver, meat, fish, milk, eggs, green vegetables, fruits, seeds (nuts, legumes, whole grain products), and cereal grains. Vitamin B₃ is often found in combination with other B vitamins, including thiamine, riboflavin, pantothenic acid, pyridoxine, cyanocobalamin, and folic acid. One recommended daily allowance of niacin is 2–12 mg/day for children, 14 mg/day for women, 16 mg/day for men, and 18 mg/day for pregnant or breast-feeding women. Symptoms of mild niacin deficiency include indigestion, fatigue, canker sores, vomiting, and depression. Severe deficiency can cause a condition known as pellagra. Pellagra is characterized by cracked, scaly skin, dementia, and diarrhea. It is generally treated with a nutritionally balanced diet and niacin supplements. Niacin deficiency also causes burning in the mouth and a swollen, bright-red tongue. Pharmacological doses of niacin (1.5–6 g per day) lead to side effects that can include dermatological conditions such as skin flushing and itching, dry skin, and skin rashes including eczema exacerbation and acanthosis nigricans.

Niacin, a water-soluble solid, is a derivative of pyridine with a carboxylic acid group (COOH) at the 3-position. Other forms of vitamin B₃ include the corresponding amide, nicotinamide (niacinamide), where at the 3-position is a carboxamide group (CONH₂). Nicotinic acid and niacinamide are interchangeable in the body. The synthesis of niacin starts from 3-picoline (3-methylpyridine). Nicotinic acid was first synthesized in 1867 by oxidative degradation of nicotine. Niacin is prepared by hydrolysis of nicotinonitrile, which, as described above, is generated by oxidation of 3-picoline. The catalysts used in the reaction above are derived from the oxides of antimony, vanadium, and titanium (Eggersdorfer and Adam 2011).

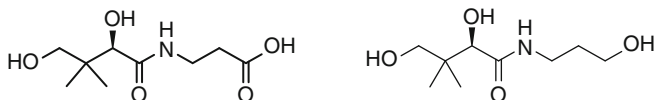


Fig. 5 Structure of (*R*)-pantothenic acid and panthenol

Pantothenic Acid

Pantothenic acid (Fig. 5) is vital for the synthesis and maintenance of coenzyme A (CoA) thus essential to almost all forms of life. Most tissues transport pantothenic acid into cells for the synthesis of CoA. Small quantities of pantothenic acid are found in most foods. The major food source of pantothenic acid is meat. Other good sources of pantothenic acid are grains and some vegetables and fruits. Specific foods contain pantothenic acids are beef, yeast, eggs, fresh vegetables, kidneys, legumes, liver, mushrooms, nuts, pork, royal jelly, saltwater fish, whole rye flour, and whole wheat.

Only (*R*)-pantothenic acid is biologically active and is extremely hygroscopic. Consequently, the major commercial form of pantothenic acid is calcium (*R*)-pantothenate. The key step in the present commercial chemical synthesis of (*R*)-pantothenic acid involves resolution of racemic pantolactone. Recent industrial synthetic efforts have been directed toward developing a method for enantioselective synthesis of (*R*)-pantolactone either by chemical or microbial reduction of ketopantolactone. Calcium (*R*)-pantothenate is used in food and feed enrichment. Panthenol and pantyl ether are industrially important derivatives of pantothenic acid and are used in hair care products (Rawalpally 2001).

Panthenol, called provitamin B₅, is the alcohol analog of pantothenic acid. In organisms it is quickly oxidized to pantothenate. When ingested, it is metabolized to pantothenic acid. Panthenol is a highly viscous transparent liquid at room temperature and soluble in water, alcohol, ether, and chloroform and slightly soluble in glycerin. Panthenol draws moisture from the atmosphere and readily binds to water molecules. When applied to the hair, panthenol will help to moisturize it and give it a shine and gloss. For this reason, it has become a very popular ingredient in shampoos and conditioners. In ointments, panthenol is an effective skin penetrator. It is sometimes mixed with allantoin, in concentrations of up to 2–5 %, and is used for the treatment of sunburns, mild burns, and minor skin disorders. It improves hydration, reduces itching and inflammation of the skin, and accelerates epidermal wounds' rate of healing. Panthenol is synthesized from a number of sources including honey, molasses, and rice.

Vitamin B₆

Vitamin B₆, referred to as pyridoxal, pyridoxamine, and pyridoxine (Fig. 6), is involved in the metabolism of amino acids, glucose, and lipids in the liver. It is also crucial in the synthesis of neurotransmitters, hemoglobin, and histamine, as well as proper gene expression. Vitamin B₆ plays a significant role in more than 100 metabolic reactions and is also involved in brain development during pregnancy and infancy as well as immune function. Hence, it is very important to consume enough of it on a

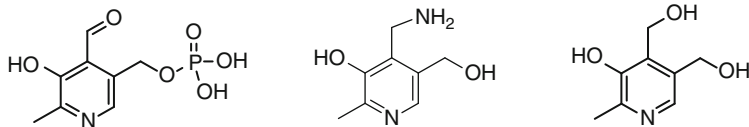


Fig. 6 Vitamin B₆ (pyridoxal phosphate, pyridoxamine, and pyridoxine)

daily basis. Vitamin B₆ is a water-soluble vitamin and naturally presents in many foods. Also it is added to many other foods. Usually, enough amounts of vitamin B₆ are ingested by eating a variety of foods, including poultry, fish, and organ meats, potatoes and other starchy vegetables, and fruits other than citrus. The US FDA (Food and Drug Administration) has established the following RDI (recommended daily intake) of vitamin B₆: 0.5 mg/day for children 1–3 years, 0.6 mg/day for children 4–8 years, 1 mg/day for children 9–13 years, 1.2 mg/day for females 14–18 years, 1.3 mg/day for males 14–18 years, 1.3 mg/day for adults 19–50 years, 1.5 mg/day for females 50 years and older, and 1.7 mg/day for men 50 years and older.

Although it is relatively rare, vitamin B₆ deficiency can have detrimental effects. Deficiency of vitamin B₆ can cause a range of symptoms, including anemia, itchy rashes, scaly skin on the lips, cracks at the corners of the mouth, and a swollen tongue. Other symptoms of very low vitamin B₆ levels include depression, confusion, and a weak immune system. Infants who do not get enough vitamin B₆ can become irritable or develop extremely sensitive hearing or seizures. Certain groups of people are at higher risk for vitamin B₆ deficiency, such as dialysis, arthritis, liver disease, ulcerative colitis, Crohn's disease, and HIV patients, as well as individuals coping with alcoholism and those who take certain medications like penicillamine and hydrocortisone. Oral contraceptives and other estrogens are found to interfere with vitamin B₆ metabolism, resulting in deficiency. People deficient in the vitamin should consider changing their diets to ensure that they are consuming enough of the nutrient from foods.

Many great sources of vitamin B₆ are in a wide range of foods. Vitamin B₆ is found in meat, poultry, fish, eggs, whole grains, legumes (bean and peas), potatoes, yeast, bananas, corn, cabbage, yams, prunes, watermelon, and avocado. One's daily quota of vitamin B₆ can be easily consumed through food, including these vitamin B₆-rich food sources.

Some scientists had thought that certain B vitamins (such as folic acid, vitamin B₁₂, and vitamin B₆) might reduce heart disease risk by lowering levels of homocysteine, an amino acid in the blood. Although vitamin B supplements do lower blood homocysteine, research shows that they do not actually reduce the risk or severity of heart disease or stroke. Vitamin B₆ can reduce nausea and vomiting for pregnant woman. The American Congress of Obstetricians and Gynecologists (ACOG) recommends taking vitamin B₆ supplements under a doctor's care for nausea and vomiting during pregnancy. Both chemical and microbial methods are available to prepare vitamin B₆ compounds, but vitamin B₆ is mainly produced by chemical synthesis (Mackey et al. 2005). For those who are interested in the

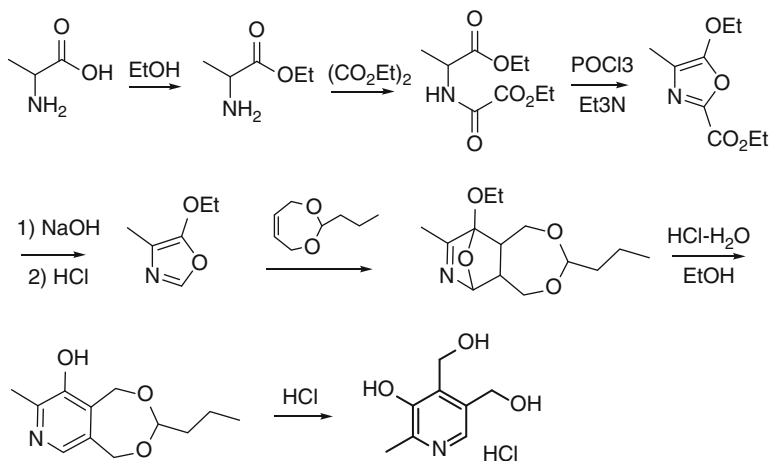


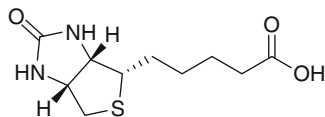
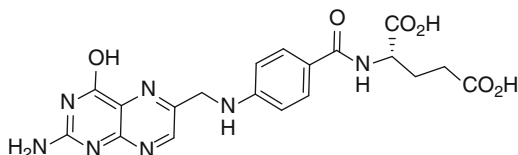
Fig. 7 Chemical synthetic route of pyridoxine

chemical structure transformation, Fig. 7 illustrates the most popular synthetic route of pyridoxine up to date to prepare pyridoxine from amino acid alanine (Firestone et al. 1967).

Biotin

Biotin, with other names of vitamin B₇, vitamin H, or vitamin B₈, is a colorless, water-soluble member of the B vitamin group. Figure 8 illustrates its chemical structure. There are eight different forms of biotin, but only one of them, D-biotin, occurs naturally and has full vitamin activity. Biotin can only be synthesized by bacteria, molds, yeasts, algae, and certain plants. Biotin is an essential growth factor found in all living cells. It functions as a cofactor for a group of coenzymes that catalyze transcarboxylation, decarboxylation, and carboxylation reactions related to biochemical processes such as gluconeogenesis and fatty acid synthesis. Sufficient intake of biotin is important as it has significant health functions. The European Food Safety Authority (EFSA) has confirmed the clear health benefits of the dietary intake of biotin in the contribution of normal macronutrient metabolism, normal energy-yielding metabolism, the maintenance of normal skin and mucous membranes, the normal function of the nervous system, the maintenance of normal hair, and normal psychological functions.

The food source of biotin includes raw egg yolk, animal liver, pork, salmon, sardine, nuts (almonds, peanuts, pecans, walnuts), soybean, other legumes (beans, black-eyed peas), whole grain, banana, mushroom, avocado, Swiss chard, raw cauliflower, and green leafy vegetables. Biotin is also available in supplement form and can be found in most pharmacies. Although rare, the symptoms of biotin deficiency include hair loss, dry scaly skin, cracking in the corners of the mouth (called cheilitis), swollen and painful tongue that is magenta in color (glossitis), dry eyes, loss of appetite, fatigue, insomnia, and even depression.

Fig. 8 Biotin structure**Fig. 9** Structure of folic acid

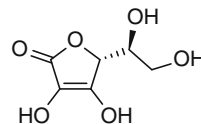
Biotin synthesis comes either from fermentation process or from organic synthesis. Early synthetic process developed in the 1940s uses fumaric acid as a starting material. The synthetic route of biotin has been modified or newly explored multiple times by different groups in which half comes from industry research groups to improve the efficiency of biotin synthesis. The detailed organic synthetic route has been reviewed by Seki et al. (2004; Zemleni and Mock 1999).

Folate

Folate is a naturally occurring B vitamin, and folic acid is the synthetic form of folate. Folate and folic acid (Fig. 9) are often interchanged. Naturally existing folate has many forms and is found in foods as well as in metabolically active forms in the human body. Folic acid is a stable form of folate. Folate helps the body make new cells. Folate is crucial for proper brain function and plays an important role in mental and emotional health. It assists the production of DNA and RNA, the body's genetic material, and is especially important when cells and tissues are growing rapidly, such as in infancy, adolescence, and pregnancy. Folic acid also works closely with vitamin B₁₂ to help make red blood cells and help iron work properly in the body. The function of folate coenzymes in the body is in mediating the transfer of one-carbon units. Folate coenzymes act as acceptors and donors of one-carbon units in a variety of reactions critical to the metabolism of nucleic acids and amino acids. There are two different pathways in folate coenzyme-assisted DNA metabolism: (1) the synthesis of DNA from its precursors (thymidine and purines) is dependent on folate coenzymes, and (2) a folate coenzyme is required for the synthesis of methionine, and methionine is required for the synthesis of S-adenosylmethionine (SAME). SAME is a methyl group donor used in many biological methylation reactions, including the methylation of a number of sites within DNA and RNA. Methylation of DNA may be important in cancer prevention (Bailey and Gregory 1999).

Food sources of folate include vegetables (romaine lettuce, spinach, asparagus, broccoli, cauliflower, beets, green beans, cabbage, bell peppers, leeks, tomatoes, celery, and green peas), legumes (black beans, navy beans, kidney beans, pinto beans, etc.), fruits (strawberry, papaya, orange, citrus, banana, melon, cantaloupe,

Fig. 10 Ascorbic acid (vitamin C)



pineapple), seeds (peanut, sunflower seed, dried peas, etc.), and animal products (eggs, kidney, liver, pork, poultry, shellfish, etc.). Due to its link with the nervous system, folate deficiency can be associated with irritability, mental fatigue, forgetfulness, confusion, depression, and insomnia. Folate deficiency can also result in intestinal tract symptoms (like diarrhea) or mouth-related symptoms like gingivitis or periodontal disease. The production of folic acid can be achieved either by chemical synthesis or by microorganisms (Lohner et al. 2012).

Vitamin C

Vitamin C (Fig. 10) is one of the many antioxidants, along with vitamin E, beta-carotene, and many other plant-based nutrients. Antioxidants block some of the damage caused by free radicals and other reactive oxygen species (ROS) that damage DNA. The buildup of free radicals and ROS over time may contribute to the aging process and the development of health conditions such as cancer, heart disease, and arthritis. Vitamin C is needed for the growth and repair of tissues in all parts of the human body. It is used to form an important protein used to make skin, tendons, ligaments, and blood vessels; to heal wounds and form scar tissue; and to repair and maintain cartilage, bones, and teeth. The body is not able to make vitamin C on its own, and it does not store vitamin C. It is therefore important to include plenty of vitamin C-containing foods in your daily diet.

Vitamin C is readily available from diet of fruits and vegetables. Dietary sources of vitamin C include oranges, green peppers, watermelon, papaya, grapefruit, cantaloupe, strawberries, kiwi, mango, broccoli, tomatoes, Brussels sprouts, cauliflower, cabbage, and citrus juices or juices fortified with vitamin C. Raw and cooked leafy greens (turnip greens, spinach), red and green peppers, canned and fresh tomatoes, potatoes, raspberries, blueberries, cranberries, and pineapple are also rich sources of vitamin C. Vitamin C is sensitive to light, air, and heat. Shortage of vitamin C is rare. Lack of vitamin C can cause muscle weakness, swollen and bleeding gums, loss of teeth, and bleeding under the skin, as well as tiredness and depression. Wounds would heal slowly. Vitamin C deficiency can lead to a disease called scurvy, and the healthcare professional may treat scurvy by prescribing vitamin C. Vitamin C is L-ascorbic acid, the naturally occurring enantiomer. Most of the ascorbic acid on the market is synthetic version, and the industrial synthesis of vitamin C mostly starts from glucose (Michels and Frei 2012; Tripathi et al. 2009).

Vitamin E

Vitamin E, composed of a collective name for a group of fat-soluble compounds, is biologically essential antioxidants derived from 6-chromanol. Naturally occurring

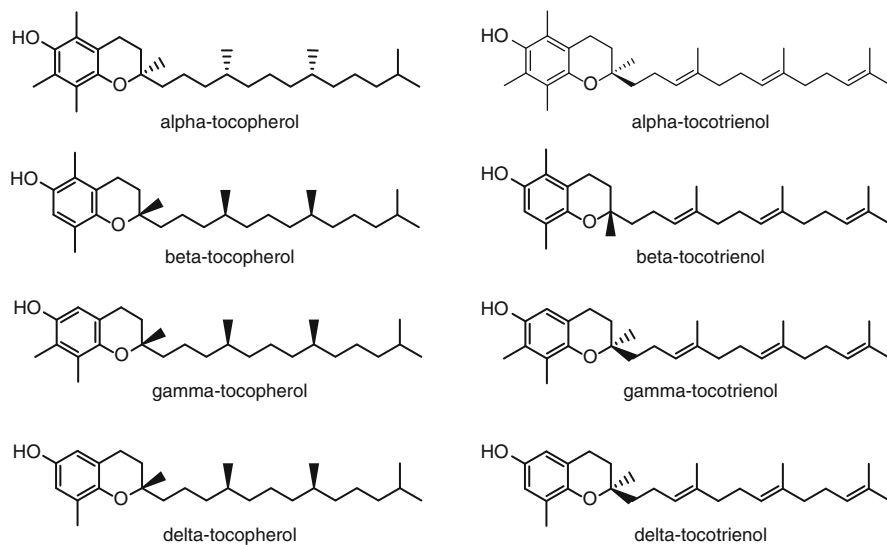


Fig. 11 Structure of alpha-tocopherol

vitamin E exists in eight chemical forms showed in Fig. 11 (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol) that have various levels of biological activity. Alpha-tocopherol is the only form that is recognized to meet human requirements. The concentration of alpha-tocopherol in blood depends on the liver, which takes up all forms of vitamin E after which are absorbed from the small intestine. The liver preferentially releases only alpha-tocopherol through hepatic alpha-tocopherol transfer protein and metabolizes and excretes the rest of other forms of vitamin E. As a result, blood and cellular concentrations of other forms of vitamin E are lower than those of alpha-tocopherol. Vitamin E is a fat-soluble distinctive antioxidant that reduces the concentration of ROS (reactive oxygen species) formed in the oxidation reaction inside the body. Scientists have been investigating whether, by controlling the concentration of free radical species, vitamin E helps prevent or delay the chronic diseases associated with free radicals. In addition to its antioxidant activity, vitamin E is involved in immune function and, as shown primarily by *in vitro* studies of cells, cell signaling, regulation of gene expression, and other metabolic processes. Alpha-tocopherol inhibits the activity of protein kinase C, an enzyme involved in cell proliferation and differentiation in smooth muscle cells, platelets, and monocytes. Vitamin E also increases the expression of two enzymes that suppress arachidonic acid metabolism, thereby increasing the release of prostacyclin from the endothelium, which, in turn, dilates blood vessels and inhibits platelet aggregation.

Vitamin E is found naturally in some foods, added to others, and available as a dietary supplement. Numerous foods provide vitamin E. Nuts, seeds, and vegetable

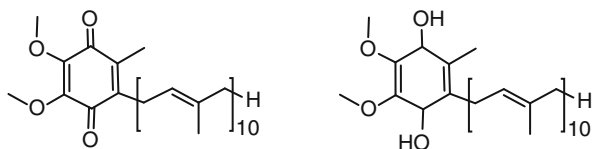
oils are among the best sources of alpha-tocopherol, and significant amounts are available in green leafy vegetables and fortified cereals. Most vitamin E in American diets is in the form of gamma-tocopherol from soybean, canola, corn, and other vegetable oils and food products. Vitamin E deficiency is rare, and overt deficiency symptoms have not been found in healthy people who obtain little vitamin E from their diets (Traber 2007).

The industrially most relevant alpha-tocopherol is generally built up by coupling of arenes with aliphatic precursors. For the synthesis of active vitamin E components, various strategies are compiled. In approaches to chiral chroman and side-chain building blocks, many asymmetric syntheses were employed, such as optical resolution and the use of chiral starting materials and chiral auxiliaries in stoichiometric or catalytic amounts, including catalysts from metal complexes, microorganisms, and enzymes. Most efforts were directed to alpha-tocopherol owing to its prominent biological activity (Netscher 2007).

Coenzyme Q10

Coenzyme Q10, abbreviated as CoQ10, is found in almost every cell throughout the body, especially in the heart, liver, kidney, and pancreas. It is present in most eukaryotic cells, primarily in mitochondria. It helps convert food into energy. A substance in the electron transport chain, it participates in aerobic cellular respiration, helping generate about 95 % of human body's energy in the form of ATP. Hence, the organs with the highest energy requirements, such as the heart, liver, and kidney, have the highest CoQ₁₀ concentrations. It is a powerful antioxidant and can neutralize free radicals. It is a fat-soluble substance primarily synthesized by the body and also consumed in the diet. CoQ10 is required for mitochondrial ATP synthesis and functions as an antioxidant in cell membranes and lipoproteins. Endogenous synthesis and dietary intake can provide sufficient CoQ10 in healthy people. But the CoQ10 level in tissue declines with age. Some scientists believe that CoQ10 may help with heart-related conditions, because it can improve energy production in cells, prevent blood clot formation, and act as an antioxidant. It is used as a medicine for treating heart and blood vessel conditions such as congestive heart failure, chest pain, high blood pressure, and heart problems linked to certain cancer drugs and also used for diabetes, gum disease, breast cancer, Huntington's disease, Parkinson's disease, muscular dystrophy, increasing exercise tolerance, chronic fatigue syndrome, and Lyme disease, among others. There are three redox states of CoQ₁₀: fully oxidized ubiquinone (Fig. 12), half-oxidized or half-reduced semiquinone (ubisemiquinone), and fully reduced ubiquinol (Fig. 12). The existence of this molecule in a completely oxidized form and a completely reduced form enables it to perform its functions in the electron transport chain and as an antioxidant, respectively. Also, it has been found that the bioavailability of the reduced form ubiquinol is higher than its oxidized form ubiquinone. These two forms are interchangeable in vivo and act as the function of CoQ10 (Monograph 2007).

Fig. 12 Structures of CoQ10 (ubiquinone) and ubiquinol



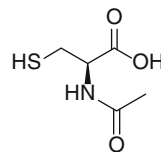
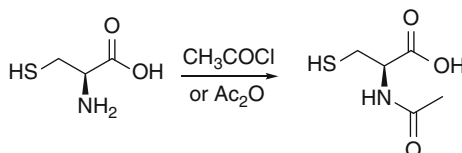
Most people get enough CoQ10 through a balanced diet. CoQ₁₀ is synthesized in most human tissues. Its biosynthesis involves three major steps: (1) synthesis of the benzoquinone skeleton from either tyrosine or phenylalanine, (2) synthesis of the isoprene side chain from CoA via the mevalonate pathway, and (3) the condensation of these two structural components. The enzyme hydroxymethylglutaryl (HMG)-CoA reductase plays a critical role in regulating the synthesis of CoQ10 and cholesterol synthesis.

Primary dietary sources of CoQ10 include oily fish, such as salmon and tuna, organs such as the liver, and whole grains. General rich sources of dietary CoQ10 include mainly meat, poultry, and fish. Other relatively rich sources include soybean and canola oils and nuts. Fruits, vegetables, eggs, and dairy products are moderate sources of CoQ10, which is unstable in heating conditions. Approximately 14–32 % of CoQ₁₀ was lost during frying of vegetables and eggs, but the CoQ10 content is unchanged when boiled. Some food examples with relatively rich CoQ₁₀ content are beef, herring, chicken, soybean, broccoli, cauliflower, orange, strawberry, egg, etc. CoQ10 can be prepared by chemical synthesis or fermentation process (Crane 2001; Lipshutz et al. 2002; Shinde et al. 2005).

Sulfur-Containing Compounds

N-Acetylcysteine

N-Acetylcysteine (NAC, Fig. 13) comes from the amino acid L-cysteine plus an acetyl (–COCH₃) group attached to the amino (NH₂) group. NAC is more water soluble, less toxic, and less susceptible to oxidation than cysteine. NAC is reported to be safe, even in large doses, and a better source of cysteine than cysteine itself. NAC is a precursor to glutathione (GSH). It is an antioxidant and used as a prescription drug and as a dietary supplement. As a drug, it is used as both an antidote for acetaminophen-induced hepatotoxicity and as a mucolytic agent for respiratory diseases. NAC is used to counteract acetaminophen and carbon monoxide poisoning. It is also used for chest pain, bile duct blockage in infants, amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease), Alzheimer's disease, allergic reactions to the antiseizure drug phenytoin (Dilantin), and an eye infection called keratoconjunctivitis. Clinical studies show NAC can treat drug-induced hepatotoxicity, prevent and treat conditions of oxidative stress and reduced GSH levels caused by diseases such as HIV/AIDS and cancer, and alleviate toxicity from chemo- and radiotherapy. Healthcare providers give *N*-acetylcysteine intravenously

Fig. 13 *N*-acetylcysteine**Fig. 14** One-step synthesis of *N*-acetylcysteine

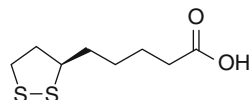
for acetaminophen overdose, acrylonitrile poisoning, amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease), kidney failure in the presence of liver disease (hepatorenal syndrome), chest pain in combination with nitroglycerin, and heart attack in combination with nitroglycerin and streptokinase and for helping to prevent multiorgan failure leading to death.

Oral supplementation with *N*-acetylcysteine (NAC) provides an alternate means of boosting intracellular glutathione via elevated intracellular cysteine. NAC is rapidly absorbed after oral administration and reaches a maximum plasma level in 2–3 h, with a half-life of about 6 h. NAC readily enters cells and is hydrolyzed to cysteine. Food is not a significant source of *N*-acetylcysteine. Synthesis of NAC is a one-step reaction (Fig. 14) from the acetylation of cysteine (Baniyasi et al. 2010; Grandjean et al. 2000; Grant et al. 2009; Tonbary et al. 2009; Yarema et al. 2009).

Lipoic Acid

Lipoic acid (1,2-dithione-3-pentanoic acid, LA, Fig. 15), an organosulfur compound derived from octanoic acid, has an antioxidant with metal-chelating and anti-glycation capabilities in both lipid and aqueous phases. It has been given for mushroom poisoning, heavy metal intoxication, and diabetic neuropathy. LA reduces glycation by inhibiting the lipid peroxidation. The combination of anti-glycation and hydrophobic binding capacity of lipoic acid enables lipoic acid to prevent glycosylation of albumin in the bloodstream (Abdul and Butterfield 2007). Lipoic acid is part of a redox pair, and the reduced form is dihydrolipoic acid (DHLA). LA is readily absorbed from diet and is rapidly converted to DHLA by NADH or NADPH in most tissues. Unlike glutathione, for which only the reduced form is an antioxidant, both the oxidized and reduced forms of lipoic acid are antioxidants. In general, DHLA has superior antioxidant activity to LA. DHLA can regenerate vitamins C and E from their oxidized forms. DHLA is also superior to GSH in regenerating vitamin C. DHLA can donate two hydrogens and neutralize free radicals without a free radical generation (Biewenga et al. 1997).

Fig. 15 Structure of lipoic acid

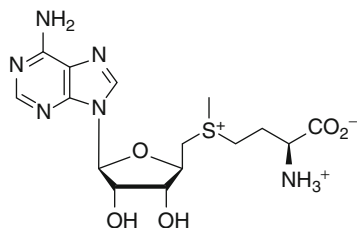


Chemically, lipoic acid exists in two enantiomers (exact same confirmation in mirror images): the R-enantiomer and the S-enantiomer. Naturally occurring LA is the R-form, but synthetic lipoic acid (known as alpha lipoic acid or α -lipoic acid, ALA) is a racemic mixture of R- and S-form. R-form is more biologically active than the S-enantiomer, but administration of alpha lipoic acid results in greater formation of DHLA due to a synergistic effect which each enantiomer exerts on the reduction of the other (Bast and Haenen 2003). Both LA and DHLA can chelate heavy metals, but the R-form is more effective. LA is most effective in chelating Cu^{2+} , Zn^{2+} , and Pb^{2+} , but cannot chelate Fe^{3+} . DHLA can form complexes with Cu^{2+} , Zn^{2+} , Pb^{2+} , Hg^{2+} , and Fe^{3+} , and the chelating compounds are poorly soluble in water. DHLA not only can chelate Fe^{3+} but also can reduce Fe^{3+} to Fe^{2+} – a prooxidant effect it shares with ascorbic acid. Small amounts of cadmium (Cd^{2+}) can cause significant lipid peroxidation in the brain, which can be prevented by lipoic acid (Packer et al. 1997). Lipoic acid is also beneficial in reducing ischemic-reperfusion injury by direct action as well as by glutathione protection and xanthine oxidase inhibition. Protection against peroxynitrite damage by lipoic acid is highly dependent upon the target molecule. Supplementation with both lipoic acid and acetyl-L-carnitine is an effective way of improving mitochondrial metabolic function without increasing oxidative stress (Hagen et al. 2002).

Lipoic acid is a cofactor for at least five enzyme systems. Two of these are in the citric acid cycle in which many organisms turn nutrients into energy. Lipoic acid is covalently attached to the enzymes, and the lipoyl group transfers acyl groups in 2-oxoacid dehydrogenase complexes and methylamine group in the glycine cleavage complex or glycine dehydrogenase. Lipoic acid serves as a cofactor to the acetoin dehydrogenase complex catalyzing the conversion of acetoin (3-hydroxy-2-butanone) to acetaldehyde and acetyl coenzyme A, in some bacteria, allowing acetoin to be used as the sole carbon source.

Lipoic acid is normally made in animals and is essential for aerobic metabolism. It is present in almost all foods, but slightly more so in kidney, heart, liver, spinach, broccoli, and yeast extract. It is readily digested, absorbed, and transported to tissues. LA induces cystine/cysteine uptake, thereby increasing synthesis of glutathione. Naturally occurring lipoic acid is always covalently bound and not readily available from dietary sources. In addition, the amount of lipoic acid present in dietary sources is very low. As a result, all lipoic acid available as a supplement is chemically synthesized. The first synthetic procedures appeared for both forms of LA in the mid-1950s. Advances in chiral chemistry led to more efficient technologies for manufacturing the single enantiomers by both

Fig. 16 Structure of S-adenosylmethionine



classical resolution and asymmetric synthesis. In the twenty-first century, LA racemic and R- and S-form of LA with high chemical and/or optical purities are available in industrial quantities. Although LA R-form is favored nutritionally due to its “vitamin-like” role in metabolism, both LA R-form and LA racemic are widely available as dietary supplements. Both stereospecific and non-stereospecific reactions are known to occur in vivo and contribute to the mechanisms of action, but evidence to date indicates LA R-form may be the major isomer (Shay et al. 2008, 2009; Durrani et al. 2010).

S-Adenosylmethionine (SAME)

S-Adenosylmethionine (Fig. 16, also known as SAM and SAME) is a molecule composed of adenosine (derived from ATP) and methionine. SAME is the principal biological methyl donor in metabolism, which donates a methyl group (methylation) to molecules such as DNA, proteins, phospholipids, or neurotransmitters. It is synthesized in all mammalian cells, but most abundantly in the liver. Biosynthesis of SAME requires enzyme methionine adenosyltransferase. DNA methylation declines with age, resulting in cellular dysregulation. Low DNA methylation can lead to mutations and chromosome instability. Excessive SAME methylation has shown to result in toxic methanol, formaldehyde, and formic acid in the brain (Lee et al. 2008). Although SAME is the principal methyl donor in all cells of the body, it is mainly synthesized, utilized, and degraded in the liver. Up to 80 % of methionine in the liver is converted to SAME, which is the source of glutathione, a principal antioxidant required for liver detoxification reactions. SAME has proven to be effective in the treatment of depression, with fewer side effects than is seen with conventional antidepressants (Papakostas et al. 2010). SAME can cause insomnia if not taken in the morning and is best taken on an empty stomach. The reactions that produce, consume, and regenerate SAME are called the SAME cycle.

Another major role of SAM is in polyamine biosynthesis. SAME is decarboxylated by adenosylmethionine decarboxylase (EC 4.1.1.50) and forms S-adenosylmethioninamine. This compound then donates its n-propylamine group in the biosynthesis of polyamines such as spermidine and spermine from putrescine. SAME is required for cellular growth and repair. It is also involved in the biosynthesis of several hormones and neurotransmitters that affect mood, such as

epinephrine. Methyltransferases are also responsible for the addition of methyl groups to the 2' hydroxyls of the first and second nucleotides next to the 5' cap in messenger RNA. SAmE has been studied in the treatment of osteoarthritis. It reduces the pain associated with osteoarthritis. Although an optimal dose has yet to be determined, SAmE appears as effective as the nonsteroidal anti-inflammatory drugs.

Several methods for the chemical and enzymatic synthesis of (–)-S-adenosyl-methionine are described and compared. Studies on the effects of solvents, pH, methylating reagents, and KI on the coupling of sodium homocysteine thiolate and 5'-chloro-5'-deoxyadenosine led to an improved procedure for the synthesis of (+/–)-SAmE (Matos et al. 1987).

Methylsulfonylmethane (MSM)

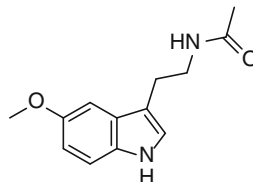
Methylsulfonylmethane (MSM, Fig. 17) is an organic form of naturally occurring sulfur, found in plants, animals, and humans. MSM has many functions in the body, and the most important is the formation of protein structures of connective tissue. The proteins that mostly required MSM include collagen and keratin. Proteins like collagen and keratin are part of structures like the skin, blood vessels, hair, nails, lymph tissue, and other connective structures. Thus, MSM consumption has multiple benefits: to boost the structure of the skin, the nails, and the hair, to increase hair and nail strength and appearance, to increase the mobility and flexibility of the joints, and to increase energy and better recovery from exercise. MSM is also used for chronic pain, osteoarthritis, joint inflammation, rheumatoid arthritis, osteoporosis, bursitis, tendonitis, tenosynovitis, musculoskeletal pain, muscle cramps, scleroderma, scar tissue, stretch marks, hair loss, wrinkles, protection against sun/wind burn, eye inflammation, oral hygiene, periodontal disease, wounds, cuts, and abrasions/accelerated wound healing. Other uses of MSM are for eye inflammation, mucous membrane inflammation, temporomandibular joint problems, leg cramps, migraine, headaches, hangover, parasitic infections of the intestinal and urogenital tracts including *Trichomonas vaginalis* and *Giardia*, yeast infections, insect bites, radiation poisoning, and boosting the immune system.

There is no recommended dietary allowance (RDA) for MSM or sulfur. Sulfur deficiency has not been described in the medical literature. MSM occurs naturally in many common foods such as tomatoes and milk. However, its concentrations are so small that it is not feasible to commercially “extract it from nature.” All MSM is manufactured by organic synthesis from two raw materials, dimethyl sulfoxide and hydrogen peroxide. This reaction forms MSM or dimethyl sulfone. After the reaction, a purification process removes the impurities from the MSM. Two commonly purification processes used in most chemical and food processing plants are distillation and crystallization. Distillation is the favored process for producing high-purity MSM (Brien et al. 2008, 2011; Parcell 2002; Tsui et al. 2012).

Fig. 17 Methylsulfonyl-methane



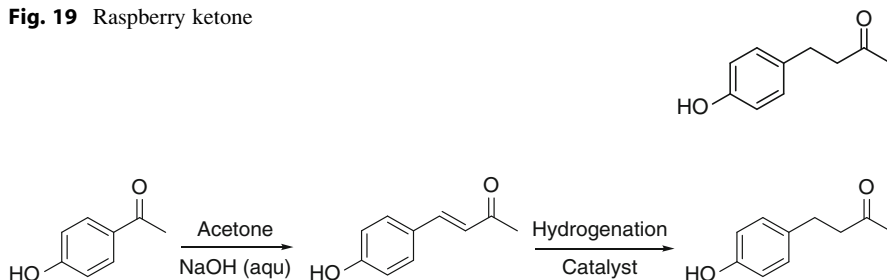
Fig. 18 Melatonin



Melatonin

Melatonin (Fig. 18) is a hormone secreted by the pineal gland in the brain. It helps regulate other hormones and maintains the body's circadian rhythm. The circadian rhythm is an internal 24-h "clock" that plays a critical role in our sleeping cycle. The level of melatonin starts to rise in the mid- to late evening, remains high for most of the night, and then drops in the early morning hours. Light affects the amount of melatonin the body produces. Melatonin also helps control the timing and release of female reproductive hormones. It is involved in the menstrual cycles and helps determine the timing a woman starts to menstruate, the frequency and duration of menstrual cycles, and when a woman stops menstruating (menopause). Natural melatonin levels slowly drop with age. Melatonin supplements are used to adjust the body's internal clock, such as jet lag and sleep-wake cycles in people whose daily work schedule changes, and for helping blind people establish a day and night cycle. Melatonin is also used for the inability to fall asleep (insomnia), delayed sleep phase syndrome (DSPS), insomnia associated with attention deficit hyperactivity disorder (ADHD), insomnia due to certain high blood pressure medications called beta-blockers, and sleep problems in children with developmental disorders including autism, cerebral palsy, and intellectual disabilities. It is also used as a sleep aid after discontinuing the use of benzodiazepine drugs and to reduce the side effects of stopping smoking. Scientists are also looking at other good uses for melatonin, such as treating seasonal affective disorder, controlling sleep patterns for people who work night shifts, preventing or reducing problems with sleeping and confusion after surgery, and reducing chronic cluster headaches.

Melatonin has strong antioxidant effects. Preliminary evidence suggests that it may help strengthen the immune system. Other uses include breast cancer, brain cancer, lung cancer, prostate cancer, head cancer, neck cancer, and gastrointestinal cancer. Melatonin is also used for some of the side effects of cancer treatment (chemotherapy) including weight loss, nerve pain, and weakness. Melatonin is synthesized chemically in three steps or less, which is suitable for massive production inexpensively (Buscemi et al. 2005, 2006).

Fig. 19 Raspberry ketone**Fig. 20** Synthesis of Raspberry ketone

Raspberry Ketone

Raspberry ketone (Fig. 19) is a chemical from red raspberries. It is reported to affect a body hormone called adiponectin. Hence, it is most commonly used for weight loss and obesity. It is also used to increase lean body mass. Raspberry ketone is also applied to the scalp to improve hair growth. However, there is no reliable scientific evidence for it improves weight loss when taken by people. Raspberry ketone is also used in foods, cosmetics, and other manufacturing as a fragrance or flavoring agent. The average daily intake of dietary raspberry ketone has been estimated as 0.42 mg/kg/day.

Raspberry ketone exists in a variety of fruits including raspberries, cranberries, and blackberries. The content of raspberry ketone in raspberry is very low in a range of ppm, and only 1–4 mg of pure raspberry ketone is extracted from about one kg of raspberries. Since the natural abundance of raspberry ketone in plants is very low, it is prepared chemically in industry by a variety of methods from various intermediates. One of the ways this can be done is through a crossed aldol-catalytic hydrogenation. Under the catalysis of sodium hydroxide, acetone and 4-hydroxybenzaldehyde can form the α,β -unsaturated ketone, which goes through catalytic hydrogenation to produce raspberry ketone (Fig. 20). This method produces a 99 % yield (Morimoto et al. 2005).

Conclusion and Future Directions

This chapter has introduced nutraceutical substances that are essential to human health but insufficient from the diet of daily intake, including vitamins, antioxidants, sulfur-containing nutraceuticals, and coenzyme Q10. The nutritional properties, natural resources, and synthesis of these nutraceuticals were discussed, and their nutritional value is scientifically proved to be essential. Hence, these nutraceuticals are readily available nowadays as forms of dietary supplements, food additives, and even medicines in some cases. Continued scientific research

concerning the biological activity, bioavailability, and also high dosage adverse effects of these substances is ongoing with the development of modern technology and up-to-date scientific results. Therefore, new findings of these nutraceuticals will be prevailed constantly in such aspects as health-promoting property from their interaction with gut microbiota, enhanced bioavailability from formulation or nano-emulsion, and more defined safety window of consumption from more animal and human trial studies.

Future research of nutraceuticals will be dedicated to more studies in biological activity, bioavailability, toxicity dosage, and also the resource with high quality and cost-effectiveness. Therefore, some nutraceuticals that cannot be obtained sufficiently from natural sources have to be made non-naturally to satisfy the need of human health, either by microbial fermentation or chemical synthesis or both. Challenges of synthesis exist for some nutraceuticals, such as coenzyme Q10. Hence, the near future goal will be to find efficient methods to make sufficient amount of each nutraceutical by adopting processes such as organic synthesis and microbial fermentation.

Cross-References

- ▶ [Bioactive Substances of Animal Origin](#)
- ▶ [Bioactive Substances of Microbial Origin](#)
- ▶ [Bioactive Substances of Plant Origin](#)
- ▶ [Chemical Composition of Beverages and Drinks](#)
- ▶ [Chemical Composition of Vegetables and Their Products](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Drying](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Freezing](#)
- ▶ [Nutritional and Toxicological Aspects of the Chemical Changes of Food Components and Nutrients During Heating and Cooking](#)
- ▶ [Overview of Food Chemistry](#)

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Part VII

Chemistry of Food Nanotechnology

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Abstract

Nanotechnology has been touted as the next revolution in many industries. It has offered enormous opportunities and provided new possibilities to many industries such as chemistry, materials science, medicine, and engineering. This emerging technology has also opened up a whole universe of new possibilities in the development and applications of nanotechnology in the food sectors. Considerable research efforts have highlighted the potential of nanotechnology in a wide range of food applications. While most nanotechnology-derived food products and applications are still at the early

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stage of research and development, food packaging and nanodelivery systems are relatively closer to be the promising sectors of the food industry. The diverse industrial applications in food and nutrition sciences may include the enhancement of uptake and bioavailability of nutrients and supplements, promotion of food safety and security, innovative tastes and textures, and preservation of quality and freshness. Future developments are limited only by the imagination. However, as novel properties and characteristics pose novel risks, uncertainty and risks of exposure for consumers to free engineered nanomaterials are emerging. Yet little is known about the impact of nanotechnology, especially engineered nanomaterials, on human health. From the food industry and public safety standpoints, it is anticipated that more scientific research in food nanotechnology will be conducted worldwide to fill the knowledge gaps and give more support to regulation and policy development for the safety and health of consumers and the environment.

Introduction

Nanoscience and nanotechnology are the engineering and manipulation of materials at the molecular scales, far smaller than a cell. They are so small that they cannot be seen under a regular microscope. In the 1980s, the word nanotechnology was initially referring to an ability to build machines from the bottom-up approach on the scale of molecules. The highly enlarged surface area per mass compared with bigger-sized particles of the same chemistry might render nanosized particles more biologically and chemically active (Oberdörster et al. 2005). Properties of the nanosized particles materials can be very different from those at the larger scale and potentially useful: for instance, gold nanoparticles are red and melt at a much lower temperature than bulk gold.

Since nanometer-scale technology has become an accepted concept, the word nanotechnology gained popularity across different science fields, such as chemistry, biology, physics, materials science, medicine, and engineering. Nanotechnology is likely to be under research in a number of areas, for example, bio-nanotechnology is a fusion of biology and nanotechnology and refers to the functional applications of biomolecules in nanotechnology. It encompasses the diverse applications in food and nutrition sciences, biomedicine, agriculture, and other fields. Scientists in the field of food and nutritional sciences have also tried to explore the possibility to apply the tools of nanotechnology in their research. From many futuristic ideas to more down-to-earth applications, there is much ongoing research about how nanotechnology could help enhance nutritional benefits and absorption of bioactive compounds. There is also much work being done to take advantage of nanotechnology to develop new delivery devices (i.e., liposomes), create new packaging materials, extend food storage, and improve food safety, taste, and texture.

An Overview of Food Nanotechnology

The National Nanotechnology Initiative (NNI) in the USA is a federal research and development program established to create a framework for understanding and controlling matter at the nanoscale. Nanotechnology leads to a revolution in technology and industry and is creating scientific advances. New products that are smaller, stronger, lighter in weight, and more reliable have been made. It is changing the world and the way we live.

An appropriate definition for nanotechnology should be broadly applicable to a wide range of products that include nanomaterials or otherwise involve nanotechnology. NNI describes nanotechnology as the understanding and control of matter at dimensions between approximately 1 and 100 nanometers (nm) with novel properties which are not feasible when working with bulk materials. It generally describes the fabrication of structures and devices where at least one dimension is less than 100 nm. Here are some examples of materials at the nanoscale in different dimensions: one dimension (thin coatings), two dimensions (nanowires and nanotubes), and three dimensions (nanoparticles or fine powder preparations).

Over the past few decades, several different definitions of nanotechnology have been discussed. These definitions have been generated for different purposes. In general, the term nanotechnology commonly refers to particles nanoscale in size or having at least one dimension falling within the size range of roughly 1–100 nm. Some other factors such as shape, charge, surface area to volume ratio, function, and other physical or chemical properties have also been considered as parameters to define nanoparticles. To consider whether a product contains nanomaterials or involves the application of nanotechnology, the ability of nanomaterials to exhibit novel properties or phenomena may be a consideration point even if their dimensions fall outside the nanoscale range (up to 1 μm) (FDA 2011). That means the size range (approximately 1–100 nm) may not be the only criteria in different cases. Therefore, the underlying scientific concepts of these technologies are more important than merely the semantics of a definition. It should be noted that one definition may offer meaningful guidance in one context, whereas it may be too broad or narrow to be of use in another. As different definitions and ongoing controversy on the implications of nanotechnology exist, it is understandable that an agency would choose not to arbitrarily offer a rigid and size-based regulatory definition to avoid any change or conflict as science evolves.

Regarding the size (between approximately 1 and 100 nm) as mentioned above, nanoparticles are also found in nature. Many natural food substances and ingredients have nanostructures in nature and have been eaten safely for generations. Broadly speaking, nanotechnology and nanomaterials are a natural part of food processing and conventional foods. Beta-lactoglobulin, α -lactalbumin, casein micelles, and recrystallized amylose are typical examples of food materials which may undergo structural changes at the nano- and micrometer scales during normal food processing (Sekhon 2010). Moreover, the characteristics and property of many foods may rely on their nanosized components such as foams and nanoemulsions.

Milk is an example of nanoemulsion in which incredibly small droplets of fat are suspended in water. In nature, protein, lipid, and starch molecules are extremely small down to a few nanometers in size. Scientists have tried to explore the possibility to apply the concept of nanotechnology in food and nutritional sciences. The application of nanotechnology or even microtechnology in food systems opens up many new possibilities for the food industry.

Nanotechnology, in its original sense, refers to the engineering and fabrication of functional systems at the molecular scale from the bottom up. As used today, this term also refers to a broader collection of mostly disconnected fields. Scientists in different fields have tried to explore the possibility to apply this emerging technology in their research. To date, much of the work being done under the name of nanotechnology is not nanotechnology in the original meaning of the word. As we have seen, the application of nanotechnology in food research is quite different from the approach and concept of molecular manufacturing in its original sense. However, it poses the question that the general definition of nanotechnology may not be completely applicable to food materials as well as the development of food nanotechnology, or in other word “nanofoods.”

Applications of Nanotechnology in Food Industry

After a couple decades of steady progress in nanotechnology research and development, scientists around the world have a much more clear view of how to create nanoscale materials with properties never envisioned before. Huge investment has been made in a global race to apply nanotechnologies in both academia and private industry. It is undeniable that this emerging technology has also introduced new chances for innovation in the food industry. There has been an increasing interest in the development and applications of nanotechnology in the food sectors. Considerable research efforts have highlighted the prospect that nanotechnology could be potentially used in a wide range of food applications, including food processing, water purification, improving supplements, delivery of bioactive compounds, food packaging and storage, food safety, deodorization, and increasing the variety of food textures, colors, and tastes (Chau et al. 2007; Cushen et al. 2012; Sanguansri and Augustin 2006; Sekhon 2010; FAO/WHO 2010).

Recent research has explored the possible applications of nanotechnology in food production and processing. That research and their applications in relation to the term “nanotechnology” can be summarized into two categories including (1) engineering materials and structures and (2) natural materials and structures. Different from the scenarios in the other nonfood sectors (e.g., electronic, lighting, robotics, atomic design. etc.), many applications in the food sectors are referring to the handling of natural nanostructures of some naturally occurring substances (e.g., proteins, starches, lipids, and sugars). The research of food nanotechnology might involve the changes in natural nanostructures at the nano- and micrometer scales during food processing, recrystallization of gelatinized amylose molecules into

nanoscale structures, enhanced efficiency of nanosized starch particles as an adhesive, and study of the nanostructure of homogenized milk droplets (Sekhon 2010).

The engineered nanomaterials used in different food applications may include both inorganic and organic substances. Different inorganic engineered nanomaterials such as nanosilver, nanosilica, nanozinc oxide, nanotitanium dioxide, nanocalcium, nanoiron, nanomagnesium, and nanoselenium have been widely used in the food industry. Common examples may include nanosilver and nanozinc oxide for antimicrobial action, nanosilica for food package and surface coating, nanotitanium dioxide for ultraviolet (UV) protection, and nanocalcium, nanoiron, nanomagnesium, and nanoselenium for the categories of health supplements. In addition to the uses of engineering nanoparticles or nanocomposites in food production and processing, many food nanotechnology applications in fact involve the development of nanostructures in foodstuffs. Nanostructured foods include many different forms such as nanoemulsions, liposomal nanovesicle, nanoencapsulation, emulsion bilayers, micelles, surfactant micelles, and reverse micelles (Weiss et al. 2006). Nanoemulsions have received much attention and have shown great promise in the nanotexturing of foodstuffs like beverage, mayonnaise, spreads, cream, and also a synthetic nanosized form of lycopene (FAO/WHO 2010; Hoppe et al. 2003). Commercially available nanostructured food products are, however, still limited in the marketplace.

There are two main ways of nanomaterial manufacture such as top-down and bottom-up approaches. Bottom-up manufacture usually includes crystallization, self-assembly, microbial synthesis, aggregation of substances, or biomass reactions (Cushen et al. 2012). In the food sectors, mechanical top-down approaches such as wet milling, dry milling, and homogenization have been commonly used to produce fine particles or suspensions. Currently, it should be noted that the number of food products using nanotechnology of any kind is still small.

Food Packaging and Storage

While most nanotechnology-derived food products and applications are still at the R&D and laboratory stage, applications for food packaging are relatively closer to being a promising sector of the food industry. This emerging packaging technology has the potential to change the atmosphere surrounding the food in the pack and delay oxidation. It can also help control microbial growth, respiration rates, and volatile flavors or aromas (Brody et al. 2008). The application of nanomaterials (e.g., metal and metal-oxide) in food packaging is a rapidly growing field and has led to the development of improved or novel food packaging materials. It forms the largest share of the current market for nano-enabled products in the food sector. Some example applications include the incorporation of nanoclay in plastic polymers as a gas barrier, nanosilver and nanozinc oxide for antimicrobial action, nanotitanium dioxide for ultraviolet (UV) protection, nanosilica for surface coating, and nanotitanium nitride for mechanical strength and as a processing aid. Furthermore, inorganic nanomaterials such as nanosilver, nanozinc oxide, or

nanomagnesium oxide may also be used as effective surface biocides or antimicrobial agents in food contact materials including plastics, rubber, and silicones (Bradley et al. 2011; Sekhon 2010).

Inclusion of nanocomposites (e.g., nanoclays, carbon nanoparticles, nanoscale metals and oxides, and polymer matrix) in packaging materials may improve their mechanical properties. This approach could be able to improve the barrier properties of packaging materials against oxygen, carbon dioxide, ultraviolet radiation, moisture, and volatiles, hence helping control moisture migration and reducing the possibility of food spoiling or drying out (Smolander and Chaudhry 2010). The use of nanocomposites can also provide some promising functionalities such as antimicrobial activity and antioxidant ability, thus promoting the prolongation of the shelf life of packaged food products. A known example is a transparent plastic film (called Durethan) embedded with an enormous number of silicate nanoparticles of clay. The use of functionalized nanocrystals in food packaging (e.g., beer bottles) has created an effective molecular barrier to gasses or moisture. The barrier can increase the shelf life effectively by reducing the entry of oxygen and minimizing the loss of carbon dioxide (Sorrentino et al. 2007). Nanosensors integrated in food packaging will be an innovative packaging technique to detect allergen proteins to avoid adverse reactions as well as to eventually improve food quality and safety (Brody et al. 2008). There is an expectation that nanosensors will be an important tool for food quality and safety in the future.

In many products and applications, such as plastic materials for food packaging, nanomaterials are claimed to be incorporated in a fixed, bound, or embedded form. It is possible that some novel applications may pose unknown or serious risks of exposure for consumers to free engineered nanomaterials. For example, carbon nanotubes have been reported to exhibit remarkable antimicrobial effects by puncturing the *Escherichia coli* cells and causing its cellular damage upon an immediate direct contact (Kang et al. 2007). It is not an easy task to make broad generalizations as to whether such a nanotechnology is good or bad in the food sector. An improved understanding of the benefits and the additional risks to consumer health or the environment may be needed.

Delivery of Bioactive Compounds

In addition to food packaging, nanodelivery systems based on encapsulation technology are perhaps another potential area of nanotechnology application in the food sectors. This technology has been used to develop nanostructured carriers for the controlled release of nutrients, additives, and supplements. These nanodelivery systems can be used for having a better dispersion of encapsulated ingredients, preserving core ingredients during processing and storage, masking unpleasant tastes and flavors, and controlling the release of core ingredients. While nanocarriers facilitate the delivery of a substance into the bloodstream, they could enhance the absorption and bioavailability of the encapsulated substance

(Chen et al. 2006; Shegokar and Müller 2010). The enormously increased biological activity, compared with conventional bulk equivalents, with the application of nanostructured carriers may require a risk assessment.

Nanoencapsulation evolved from the well-established microencapsulation process, but it involves much smaller particle sizes. It can be considered to be the miniaturization of microencapsulation. The nanoencapsulation technique is used to coat food ingredients or certain substances within another material at sizes on the nanoscale, in order to provide protective barriers, flavor and taste masking, increased bioavailability, controlled release, and better dispersion in aqueous systems (Chaudhry et al. 2008; Mozafari et al. 2006). For example, casein and hydrophobically modified starch can be used to produce nanostructured micelles as a natural nanovehicle for encapsulating hydrophobic active ingredients; α -lactalbumin nanotubes which are formed by the self-assembly of hydrolyzed milk protein α -lactalbumin possess a nanoscale cavity to encapsulate food components and nutrients (e.g., vitamins or enzymes) or to mask undesirable flavor or aroma compounds (Graveland-Bikker and de Kruif 2006; Srinivas et al. 2010).

Nanoemulsion and nanoliposome are the common examples of how nanoencapsulation techniques can be applied to an existing process in the food industry. Some low-fat products such as nanostructured spreads, mayonnaise, and ice creams are claimed to be as creamy as their full fat alternatives (Chaudhry and Castle 2011). The nanoemulsification technique may reduce the need for stabilizers in food products. Scientists believe that lipid-based nanoencapsulation systems (i.e., nanoemulsions, nanoliposomes) can be used as carrier vehicles of nutrients, nutraceuticals, enzymes, food additives, and antimicrobial agents (Mozafari et al. 2008). The applications of nanoemulsions could improve the stability and oral bioavailability of certain bioactive compounds such as polyphenols (Sekhon 2010). The formulation of nanoemulsions using some stabilizers can also allow natural fat-soluble colorants to be used in a novel way. Nanostructured lipid carriers may allow an oil-soluble pigment such as beta-carotene to be easily dispersed and stabilized in aqueous-based foods or beverages (Astete et al. 2009). Nanoemulsion techniques are expected to play a future role in revolutionizing the making of these products; however, they are still in developmental stages.

Food Safety

An overview of the existing research findings as well as the current and futuristic applications of nanotechnology for the food industry indicates that they can potentially offer a variety of benefits. The diverse industrial applications in food and nutrition sciences may include the following: enhanced uptake and bioavailability of nutrients and supplements, innovative tastes and textures, promotion of food safety and security, preservation of quality and freshness, and reduced dietary intake of fat, salt, and food additives. Novel techniques have paved the way for

detecting foodborne pathogens and developing various nano-based sensing approaches for pesticides, toxic anions, and ripening gas. However, the barrier of commercializing detectors and methods for rapid screening of analytes at low levels with accuracy and precision is still a challenge for real-world applications to the food industry (Nugen and Baeumner 2008; Valdés et al. 2009).

Taking food packaging, a promising sector, as an example, there are questions about the possible migration of nanomaterials from packaging into food. Knowledge gaps still remain as to whether nanoparticles could migrate from the packaging materials into food products. The highly increased surface area and chemistry of nanomaterials could possibly give rise to unwanted and also unexpected chemical reactions. It may pose unknown risks and uncertainties to consumers. Previous studies have shown that some nanomaterials could induce cell death in eukaryotic cells (Nel et al. 2006) and cytotoxicity in prokaryotic cells (Brayner et al. 2006; Thill et al. 2006). Universal analytical measuring tools, reliable technology, and protocols are also needed for arriving at solutions. Owing to the limited information of the potential toxicity of nanomaterials, risk assessment and management are therefore urgently required for existing products available on the market around the world. Some regulatory regimes including the European Union and the USA have required the pre-market approval with a safety assessment for the constituents of plastic food packaging materials.

Potential Risks of Nanotechnology

Although nanotechnology has introduced new opportunities for innovation in the food industry, it has also provoked public concern and debate. It is well known that nanomaterials are chemically and physically more reactive than larger particles of the same chemical composition. They may behave differently and interact with the living systems accordingly, causing unexpected toxicity (Das et al. 2009). The potential health risks of nanomaterials may depend on their properties including composition, particle size, shape, solubility, reactivity, and other physicochemical parameters (Chau et al. 2007).

The extent to which nanoparticles enter the body and their possible accumulation and translocation may determine their potential risks to the human health. It is generally believed that the scale (<100 nm) of nanoparticles could make it easier for nanomaterials to pass through biological barriers. Some studies have shown that particles less than 70 nm could even penetrate into nuclei and cause cellular damage (Geiser et al. 2005). However, particles up to 300 nm were also reported to be taken up by individual cells (Garnett and Kallinteri 2006). Little is yet known about the occurrence, bioaccumulation, fate, and toxicity of nanoparticles. As novel properties and characteristics pose novel risks, uncertainty and health concerns about the uses of nanomaterials are emerging. The safety concerns of different nanomaterials are different, and some nanomaterials may be more harmful than others.

Possible Routes of Exposure to Nanomaterials

In general, there are three main routes of exposure to nanomaterials, including dermal contact, inhalation, and ingestion.

The ability of nanomaterials to penetrate through the outer protective layers such as the epidermis or dermis determines their impact on health (Maynard 2006). The stratum corneum may act as a barrier layer (about 10 μm thick) to cutaneous absorption of most chemicals, ionic compounds, and water-soluble molecules (Hoet et al. 2004). It was reported that nanoparticles could penetrate into the dermis and translocate via the lymph to regional lymph nodes (Oberdörster et al. 2005). Titanium dioxide nanoparticles (~20 nm) were capable of passing through the skin and interacted with the immune system (Kreilgaard 2002). As yet, there is still limited information on the potential hazards of nanomaterials to the skin. Discussions about the health consequences of contacting nanomaterials and possible mechanisms of interaction are rather speculative, and further studies are needed.

In terms of aerodynamics, small particles less than 10 μm can pass through the nasal cavity into the lungs. With particles smaller than 4 μm , more than 50 % may penetrate more deeply into the alveolar region. The smaller the fine particles, the deeper they are drawn into the lungs (Hoet et al. 2004). While inhaled, titanium dioxide nanoparticles and carbon nanotube might accumulate in the lungs and induce chronic diseases such as pulmonary inflammation, pneumonia, pulmonary granuloma, and oxidative stress (Kim et al. 2003; Nel et al. 2006). Given our current state of knowledge, it is still not possible to reach generic conclusions on the toxicity of inhaled particles solely based on the consideration of size alone. As the overall surface of intestinal lumen available to nutrient absorption is more than 200 m^2 , the safety assessment of nanomaterials entering the body by ingestion becomes important. A particle translocation experiment has demonstrated that 34 % and 26 % of polystyrene nanoparticles (about 50 and 100 nm, respectively) could be absorbed, but polystyrene particles larger than 300 nm were absent from the blood, heart, or lung tissue (Jani et al. 1990). It has been reported that the smaller the particle, the faster it would penetrate across the intestinal mucus barrier. However, particles larger than 1 μm were unable to pass through the intestinal mucus barrier (Szentkuti 1997).

Safety Assessments

Nanomaterials such as titanium dioxide, aluminum oxide, zinc oxide, silica, and silver, which exhibit some novel properties not observed at the macroscale, may result in unpredictable safety problems and risks. For instance, aluminum oxide which is used in dentistry due to its inertness could spontaneously explode at nanoscale. Some *in vivo* experiments have shown that instillation of multiwalled and single-walled carbon nanotubes may agglomerate and cause pulmonary inflammation, interstitial fibrosis, granulomas, and death in rodents (Lam et al. 2004; Muller et al. 2005). Some other studies have observed that carbon nanoparticles diffused from the lungs to the surrounding blood vessel system and resulted in

further vascular diseases (Brown et al. 2000; Nemmar et al. 2002). The inhalation of titanium dioxide nanoparticles (~20 nm) also resulted in significant pulmonary inflammatory responses (Oberdörster et al. 1994). It was reported that titanium dioxide nanoparticles might penetrate through the skin barrier and cause intracellular damage (Oberdorster 2001). The genotoxicity of nanosilver particles themselves is found to be weak, but particles like silver nanopowder, silver/copper nanopowder, and colloidal silver might bind with DNA and affect the replication fidelity of genes (Yang et al. 2009).

The potential applications of engineered nanomaterials in food packaging have been and are being widely studied. For instance, engineered carbon nanotubes were added into food packaging with the purpose to improve its mechanical properties. Carbon nanotubes were also shown to be able to exhibit powerful antimicrobial effects on immediate direct contact with nanotube aggregates. The tiny nanotubes punctured *Escherichia coli* and caused cellular damage to bacterial cells (Kang et al. 2007). In such applications, nanomaterials may be incorporated in a fixed, bound, or embedded form in the packaging materials. The entry of these manufactured nanoparticles into food chain, however, may result in any unexpected bioaccumulation and harmful effects. Other applications, including certain foods and beverages containing free nanoparticles, may pose a greater risk of exposure for consumers to free engineered nanomaterials. Likewise, precautions are also required for the uses of nanostructured carriers to enhance the absorption and bioavailability of a given chemical substance. An enormous increase in bioavailability and/or tissue distribution of the substance (e.g., vitamin A), compared with conventional bulk equivalents, may not be desirable as it can exhibit a toxic effect given a large enough dose. It seems that even the use of nanostructured carriers to enhance the nutrient absorption may require a risk assessment.

Most of the existing toxicology research and safety assessments of nanotechnology are basically focusing on inorganic and nonfood materials or consumer products. Yet little is known about the impact of nanotechnology, especially engineered nanomaterials, on human health. Toxicological profiles of nanomaterials cannot be judged by extrapolation from data on their equivalent larger forms. It is probably wise to take a precautionary principle (e.g., a case-by-case approach) to deliberate the possible regulatory control as a proactive approach. It should be understood that engineered nanomaterials could be considered for use in food products or production only on the circumstances that they are deemed safe by intensive toxicology research which are practically limited, especially in the food sector.

Regulation of Nanotechnology

Like other new or modern technologies, there is a long-standing debate about the ethics and social issues involved in the development of nanotechnology. It has the potential to bring significant benefits but may also pose potential risks to human health and the environment. Broadly speaking, many of the issues are not particular to nanotechnology, but in fact are part of ongoing discussion about how different

technologies are used in our society. The risks arising from the exposure to various nanoparticles are not yet completely understood. It is important for governments and international agencies to collaborate to fill the knowledge gaps by conducting research related to the health and safety risks of nanomaterials, particularly the engineered nanomaterials that are deliberately introduced into the food chain (Reilly 2010).

As nanotechnology develops, countries and regions have begun to develop regulatory framework for foods. As a step toward developing the regulatory standards, it is necessary to envision that the emerging technology may offer diverse applications that will involve multiple regulatory agencies. It also requires government or regulatory agency to consider different criteria such as clear definitions, food safety assessments, public debate, and the need for regulation. Conflicting definitions of nanotechnology and blurry distinctions among different fields have complicated the development of a global harmonized regulatory framework. A precautionary principle should be taken to deliberate proper regulatory controls that address the definition and standard as well as regulate the labeling of food products.

In 2006, the US Food and Drug Administration (FDA) formed the Nanotechnology Task Force to address concerns and determine regulatory approaches that encourage the continued development of innovative, safe, and effective FDA-regulated products that use nanotechnology materials. Nanotechnology is an emerging technology that falls within the FDA's mission. It has the potential to be used in a broad array of FDA-regulated products. Nanotechnology products may be appropriately regulated by the product-focused, science-based regulatory policy (FDA 2013). The European Union (EU) policy on nanotechnologies is to take an "integrated, safe, and responsible approach" to their developments. The efforts include the review and adaptation of EU laws, monitoring of safety issues, and participation in dialogue with national authorities, stakeholders, and citizens. Since 2006, the European Food Safety Authority (EFSA) has been involved in the development of nanotechnology (EFSA 2013). EFSA has been asked by the European Commission to provide independent scientific opinion on the knowledge and potential risks of application of nanotechnology with regard to food and feed. The advice focuses on the use of nanotechnology, especially engineered nanomaterials, in the food and feed chain. The scientific advice then helps the European Commission to consider appropriate measures and assess existing legislation by considering EFSA's opinions. However, limitations in exposure data and a lack of validated methodologies make the risk assessments of nanomaterials difficult and subject to a high degree of uncertainty. EFSA's Scientific Committee has concluded that a case-by-case approach would be broadly applicable to protect the consumer.

Conclusion and Future Directions

From the food industry and public safety standpoints, this chapter is to give a general discussion on the development, applications, and risk of nanotechnology in relation to foods. Some food scientists would claim that the industry has already

embraced nanotechnology, but in fact, applications and available products in this area are still very scarce. Most developments of nano-derived food ingredients which are expected to be nearing the market are still in their formative stage in many regards. Unlike the development and applications of engineered nanomaterials, many claims of nanotechnology applications in the food industry, as a matter of fact, are basically a description of the characteristics and structure of naturally occurring food components at nanoscale level. Without a universal definition, some food components are regarded as nanomaterials simply because their particles or structures have at least one dimension falling within the nanoscale range from approximately 1–100 nm. Many food components such as protein, starch, phospholipids, lipid, and sugar possess nanostructures in nature. In fact, food proteins are true nanoparticles, and the molecules of starch and lipids may have one-dimensional nanostructures less than 1 nm in thickness. Casein micelles, emulsion in mayonnaise spreads, and foam in ice cream are also considered as nanostructured food components. The nanostructures of some nanomaterials in the food sectors existed naturally even before the word “nanotechnology” was used to describe the process for producing these structures. However, it should be understood that the uses of nanostructures of biological origin such as proteins, starch, phospholipids, lipids, etc. in food processing should not be considered as a manipulation of nanotechnology. The food sectors are only at an initial stage, and the future developments are limited only by the imagination.

It is worth noting that a number of nanomaterials found in literature are not likely to be used for any food-related applications. For example, there are some applications of carbon nanotubes in the areas of packaging or water treatment, but these engineered nanomaterials are basically manufactured for a diversity of nonfood applications (FAO/WHO 2010). Their functionalities in relation to the enhanced mechanical strength and electrical conductivity are of little relevance to the potential use in food products. To date, there are many different types of nanomaterials, whereas it should be understood that not all of them could be considered for uses in food products or production. From a food safety perspective, it therefore poses a question of whether these artificially engineered nanomaterials could be used in any food-related research and applications. It is anticipated that more scientific research in food nanotechnology will be conducted worldwide. This research will be used to fill knowledge gaps and give more support to regulation and policy development for the safety and health of consumers and the environment.

Cross-References

- ▶ [Applications of Nanotechnology in Developing Biosensors for Food Safety](#)
- ▶ [Advances of Nanomaterials for Food Processing](#)

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Abstract

Various biosensors have been developed to detect hazardous contaminations in foods, in order to get a fast and precise result. Typically, a biosensor is a device composed by a biorecognition element that specifically captures the target molecules from samples and a transducing element that transfers the interaction between the target molecule and the biorecognition element into a measurable signal which is proportional to the concentration of target molecules. Based on the types of transducers, biosensor can be grouped into optical, piezoelectric (mass-sensitive), thermal, and electrochemical sensors; based on the kinds of biorecognition elements, biosensors can be classified into nucleic acid/DNA sensors, immunosensors, enzyme-based sensors, and microbial sensors.

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Due to the recent improvements in nanotechnology, different nanoparticles (such as gold nanoparticles, liposomal nanovesicles, magnetic nanoparticles, and silica nanoparticles) have been applied in the development of biosensors, in order to enhance the assay sensitivity and working range. Choosing an optimal nanoparticle to enhance the performance of a sensor is a critical point in the development of biosensors. Therefore, this chapter firstly introduces various nanoparticles, including their properties, preparation methods, and different surface modification treatments and, furthermore, explains the applications of these nanoparticles on different types of biosensors with some examples specific to food safety.

Introduction

Owing to great progress in technologies that are utilized in bioassays, food safety has been attracting increasing attention globally. To protect consumers from foodborne diseases and to maintain the reputation of the food industry, rapid and sensitive detection systems have been developed. These systems are used to detect hazardous materials in food products, including foodborne pathogens, bacterial or fungal toxins, antibiotics, pesticides, herbicides, and heavy metals. Biosensors represent the new mainstream in the field of bioassays, because they integrate a biorecognition element that can specifically capture target molecules in samples and a transducer element that enables the specific interaction with the target analytes on the biorecognition element to be converted into a digital signal, using which the target concentration in the samples can be calculated (Luong et al. 2008). Therefore, a biosensor is a user-friendly device with the capacity to quickly provide reliable results. Since the end of twentieth century, nanotechnology has been utilized in the development of biosensors to enhance assay sensitivity, reduce assay time, and minimize the size of the devices to make them portable for point-of-care (POC) testing.

Among different applications of nanotechnology in the development of biosensors, the utilization of nanoparticles has become increasingly popular. The main advantage of using nanoparticles is their predictable physicochemical properties, which enhance the performance of the biosensors in which they are used. For instance, nanoparticles naturally possess a high surface-to-volume ratio, which enhances the immobilization efficiency of biomolecules on their surface and gives the immobilized biomolecules more orientational freedom, allowing them more quickly and effectively to react with the target molecules in samples than they could on a two-dimensional surface, such as that of an electrode of a quartz crystal microbalance (QCM) biosensor or the metal surface of a surface plasmon resonance (SPR) biosensor. Another benefit of the use of nanoparticles is their unique optical properties, which enable them efficiently to amplify the assay signal. For example, gold nanoparticles are frequently used as a labeling reagent in lateral flow assays that produces a red line on the test strip as an instant visual confirmation of the presence of target molecules without the need of any device.

Therefore, nanoparticles are used in biosensors because (1) they provide solid support for immobilizing the capture reagents and (2) they are labeling dyes for the detection reagents (Gilmartin and O’Kennedy 2012). Hence, the use of nanoparticles enables the assay signal to be amplified and the assay sensitivity to be consequently improved. Moreover, magnetic nanoparticles have been applied in pre-concentrating target analytes, based on the specific interaction between those target analytes and the capture reagents that are immobilized on the surface of these nanoparticles. This pre-concentration step can eliminate the need for a pre-enrichment step in the detection of low numbers of foodborne pathogens, and this improvement is very useful for on-site investigations. The nanoparticles that are most frequently utilized in biosensors can be categorized as gold nanoparticles, liposomal nanovesicles, magnetic nanoparticles, and silica nanoparticles, and the types of biosensors that are used to monitor food safety can be classified as optical biosensors, piezoelectric (mass-sensitive) biosensors, and electrochemical biosensors. The following sections will introduce the use of nanoparticles in biosensors and the performance of these nanoparticle-based biosensors.

Various Nanoparticles Used in Biosensors

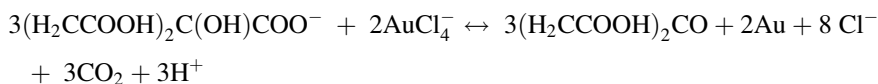
Nanoparticles are defined as particles that are intentionally produced in the nano-scale (1–100 nm). Since these particles are nanosized, their characteristics differ from those of microsized materials: they can exhibit enhanced surface activity, enhanced electric conductivity, enhanced magnetic properties, and quantum confinement effects. Owing to these characteristics, nanoparticles have been utilized in biosensors.

Gold Nanoparticles

Gold nanoparticles (AuNPs) are one of the most commonly used types of nanoparticle in biosensors, owing to their unique optical, thermal, and physical properties, a high surface area, low cost, and ease of preparation. They are used not only in biosensors but also as carriers in drug or gene delivery, as biocatalysts of enzymatic reactions, and as contrast agents in bio-imaging. Therefore, gold nanoparticles have been identified as future building blocks in nanotechnological applications. Various methods have been developed for generating gold nanoparticles with dimensions of a few nanometers to approximately 100 nm in relatively monodispersed forms; these include wet chemical synthesis and ultrasound-based methods. Most wet chemical methods are based on the reduction of a specific metal ion in the presence of a stabilizer, and they include the citrate reduction method and the Brust method. Brust et al. developed the Brust method in 1994. In this method, the gold nanoparticles are sterically stabilized by thiolated organic molecules since gold atoms automatically and specifically bind to the sulfur atoms of the thiol groups to form a sulfur–gold (Au–S) bond and the rest part of thiolated organic molecules

forms the steric hindrance to prevent the aggregation of gold nanoparticles. Typically, gold nanoparticles that are made by this method are stabilized in organic liquids, such as toluene (Brust et al. 1994). Since toluene is not miscible with water, this method is not commonly used to generate gold nanoparticles that are used for the conjugation with biomolecules.

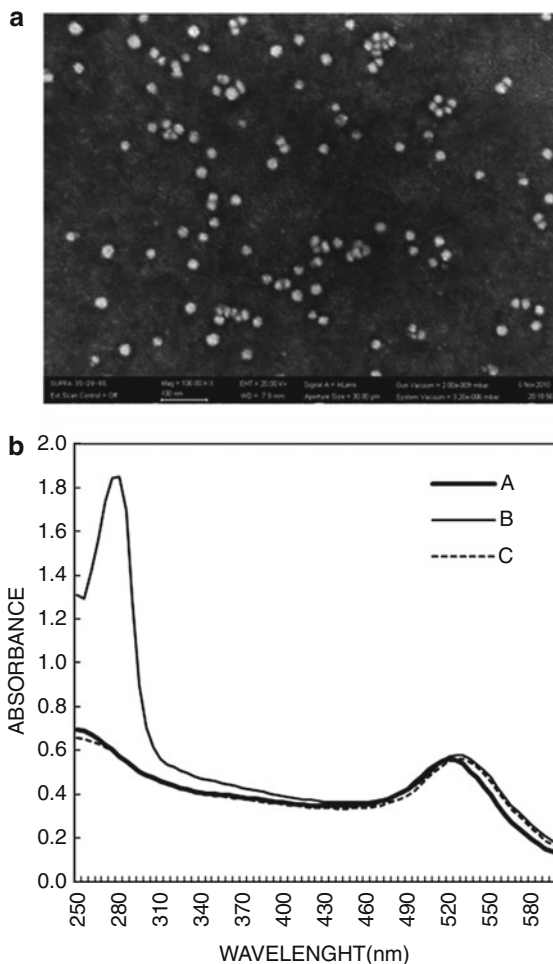
The well-known citrate reduction method is the simplest method for synthesizing gold nanoparticles and was developed by Turkevich in 1951. In this method, gold nanoparticles are synthesized in three stages – nucleation, growth, and coagulation. In the nucleation stage, hydrogen tetrachloroaurate (HAuCl_4) is mixed with a reducing reagent, sodium citrate, resulting in the reduction of a trivalent gold ion (Au^{3+}) to a zero-valent gold atom (Au^0), according to



Newly formed gold atoms rapidly collide with each other and create stable icosahedral nuclei with sizes of 1–2 nm. In the growth stage, Au ions are reduced on the surface of the nuclei until all of the Au ions have been consumed. In the final coagulation stage, stabilizers are added to prevent aggregation of the gold nanoparticles. Numerous factors influence the initial concentration of the nuclei, including the concentration of the reducing reagent, temperature, and reduction potential of the reaction. Increasing the concentration of sodium citrate increases the molar ratio of the reducing reagent to Au ions, increasing the number of nuclei. For a given amount of Au ions in a solution, as more nuclei are formed, smaller gold nanoparticles are obtained. Therefore, to synthesize smaller gold nanoparticles, a higher concentration of sodium citrate should be added. According to previous studies, gold nanoparticles with diameters in the range 9–120 nm can be obtained (Turkevich and Kim 1970). Citrate reduction rapidly turns yellow HAuCl_4 solution to a dark wine-red color as soon as sodium citrate is added, indicating the formation of gold nanoparticles. A scanning electron microscope (SEM) micrograph (Fig. 1a) reveals that the resulting gold nanoparticles have an almost spherical structure and are quasi-monodispersed. A UV–Vis spectrum includes an absorption peak around 520 nm as a result of the surface plasmon resonance of colloidal gold nanoparticles. After the conjugation with proteins, the absorption peak of protein-tagged gold nanoparticles moves toward a longer wavelength (from 520 to 525 nm), as shown in Fig. 1b. This phenomenon is called “red shifting.”

Typically, after the reduction reaction, citrate ions cover the surfaces of the gold nanoparticles, resulting in a net negative charge that provides electrostatic repulsion and thereby stabilizes these nanoparticles. However, these charged gold nanoparticles are very sensitive to changes in the dielectric properties of a solution, and irreversible particle aggregation can be induced by the addition of electrolytes (such as NaCl, KI, or KNO_3) to the solution. The presence of salts causes the attractive force to overcome the original repulsion among the surfaces of gold nanoparticles, resulting in the eventual aggregation of gold nanoparticles and a

Fig. 1 Gold nanoparticles (25 nm in diameter) produced by the citrate reduction method. **(a)** Phase contrast SEM image; **(b)** the UV–Vis spectrum of the AuNP modification procedure: *A*. AuNPs; *B*. AuNPs were modified with proteins before centrifugation; *C*. AuNPs were modified with proteins after centrifugation. Scan interval was set at 5 nm



change in the color of the solution from wine red ($\lambda_{\max} \approx 520$ nm) to blue ($\lambda_{\max} \approx 580$ nm). To avoid aggregation, the surfaces of gold nanoparticles can be further modified with biomolecules, such as DNA, antibodies, antigens, or protein-based reagents (such as bovine serum albumin, gelatin, or casein). These macromolecules electrostatically or sterically stabilize the gold nanoparticles, preventing any unexpected aggregation, and have applications in protein or DNA detection assays. Biomolecules have these effects because (1) they are charged when the pH of the solution in which they are present is below or above the pI of the biomolecules, (2) they are macromolecules with the ability to provide steric stabilization, and (3) they form hydrogen bonds with water molecules and so form a hydration shell around their surfaces (Molina-Bolivar et al. 1997). Biomolecules can be immobilized onto the surfaces of gold nanoparticles by covalent conjugation through their functional groups (-SH, -NH₂, -COOH, or -OH) using conjugation

reagents or by the nonspecific adsorption. Researchers favor the latter approach because it is easy to implement and provides an immobilization efficiency that depends on the pH of the reaction solution, the concentration of the biomolecules, and the reaction time. Through the optimization for these parameters, biomolecules can be easily tagged on the surface of gold nanoparticles.

Liposomal Nanovesicles

In 1965, Bangham became the first to use liposomal nanovesicles as a tool to study the properties of cell membranes, because they are similar to cell membranes. Since then, liposomal nanovesicles have been exploited in various fields such as drug delivery, gene therapy, cosmetics, food technology, and diagnostics. This broad range of applications is supported by their unique structure: each is a spherical vesicle that consists of an aqueous cavity that is surrounded by one or more phospholipid bilayers. Moreover, liposomal nanovesicles are effective carriers, owing to their large interior cavity, which can encapsulate large numbers of hydrophilic molecules, such as dye molecules, drugs, or DNA. Hydrophobic compounds can also be incorporated into their lipid bilayers. Additionally, various biomolecules such as antibodies, antigens, or nucleic acids can be covalently or non-covalently conjugated onto the liposomal surface, producing liposomal nanovesicles with a targeting function (Torchilin et al. 2003).

The bilayer membrane of liposomal nanovesicles is composed of self-assembled amphiphilic phospholipids that contain both hydrophilic and hydrophobic parts. The hydrophilic part is the polar head and includes a negatively charged phosphate, whereas the hydrophobic part is referred to the nonpolar tails and includes two fatty acid chains. In aqueous media, phospholipids automatically align with each other by means of lipid bilayers to form a sealed vesicle. In a lipid bilayer, two polar heads shield the nonpolar interior (fatty acid chains), minimizing the unfavorable interactions between the aqueous phase and the hydrophobic tails, resulting in an aqueous cavity within the liposomal nanovesicles, as shown in Fig. 2a. The composition of a liposomal nanovesicle can determine its surface charge and the rigidity of the membrane. All phospholipids are negatively charged, except phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which are neutral. PC, also known as lecithin, contains a choline head and is used as the main phospholipid in the preparation of liposomal nanovesicles, owing to its low price and chemical inertness. A PE molecule contains an unsubstituted quaternary ammonium group, which forms hydrogen bonds with its neighbors in the bilayer membrane or electrostatically interacts with neighboring phosphates, increasing the rigidity of the membrane. Additionally, the unsubstituted quaternary ammonium group of PE also provides an active amine group ($-NH_2$) on the liposomal surface for covalent conjugation with other biomolecules, such as antibodies or antigens. Moreover, to stabilize the liposomal nanovesicles by preventing their aggregation, charged lipids are added to the lipid mixture to provide repulsion among the liposomal surfaces. Negatively charged phosphoglycerol (PG) is the most

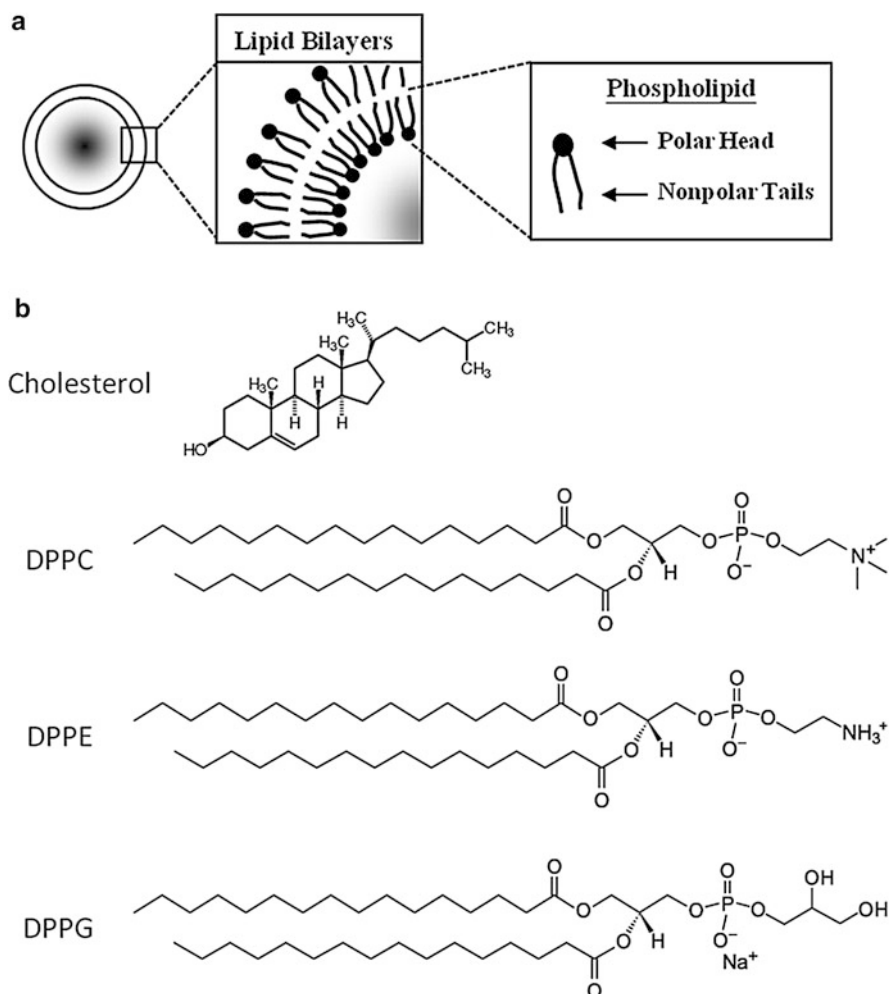


Fig. 2 The structure and components of liposomal nanovesicles. (a) The lipid bilayer structure of liposomal nanovesicles, which is composed by numerous phospholipids, and each phospholipid has a polar head and two nonpolar tails. (b) The common components of liposomal nanovesicles, including cholesterol, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (*DPPC*), 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (*DPPE*), and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (*DPPG*)

commonly selected charged lipid and is added to lipid mixtures to a concentration of around 5 mol% of the total lipids (Wen et al. 2005). The chemical structures of phospholipids influence the fluidity and stability of lipid bilayers of liposomal nanovesicles. Phospholipids can exist in different phases at different temperatures. The phase transition temperature (T_c) is the temperature at which the membrane is transformed from a tightly ordered “gel” phase to a “fluid” liquid-crystal phase in

which individual molecules can move with a high degree of freedom. Additionally, the T_c value of each phospholipid depends on its length and the degree of saturation of the hydrocarbon chains. In general, phospholipids with longer hydrocarbon chains or a higher degree of saturation have higher values of T_c , so the membrane that is formed from them is less fluid. Therefore, the chemical structures of phospholipids determine the fluidity of the bilayer membranes, which further affects the permeability, fusion, aggregation, protein binding, and stability of the liposomal nanovesicles (New 1990).

Like phospholipids, cholesterol is another principal compound that is used in the production of liposomal nanovesicles; it is also a constituent of the membranes in many eukaryotic cells. Cholesterol is an amphiphilic molecule that is composed of a 3-hydroxyl group (-OH), a planar steroid nucleus, and an aliphatic side chain. Even though cholesterol is an amphiphilic molecule, it cannot form a vesicle alone. Typically, it is inserted into a lipid membrane with its hydroxyl group toward the membrane surface, and its aliphatic side chain packed parallel to the fatty acyl chains of the phospholipids inside the bilayer membrane, such that it is located close to the first few approximately ten carbons of the phospholipid chain. The compact arrangement of the cholesterol and the phospholipid reduces the freedom of motion of the phospholipids, resulting in stabilizing the structure of the lipid bilayers. Cholesterol can regulate the stability of the lipid bilayer by decreasing the fluidity of the membrane when the temperature is higher than T_c and by increasing it when the temperature is lower than T_c . Cholesterol can also reduce the permeability of a bilayer membrane to water-soluble molecules, resulting in the maintenance of a constant concentration of the entrapped materials and a constant volume of the interior cavities of the liposomal nanovesicles (Liu et al. 2000). Therefore, the incorporation of cholesterol considerably alters the properties of liposomal nanovesicles. Figure 2b presents the common lipid components of liposomal nanovesicles. In bioassays, liposomal nanovesicles replace enzymes or fluorescent dyes as labels of the detection reagents. Liposomal nanovesicles can instantly provide a strong signal when a detergent is added, by releasing a large number of encapsulated markers, such as electroactive molecules, fluorophores, enzymes, and isotopes, which can be either encapsulated inside the cavities, tagged on the surfaces, or trapped in the lipid bilayers of the liposomal nanovesicles. This signal can be measured to determine the presence or concentration of the target analytes.

Various methods for liposome preparation have been published, but only a few methods can be applied for analytical applications since the requirements for this field include that the protocols are simple and the results are reproducible as well as the size of liposomal nanovesicles should be as homogeneous as possible in order to have a well-controlled amplification in the assay. Generally, liposomal nanovesicles are generated with the film method or reverse-phase evaporation method and then transformed to the desired diameter using the freeze-thaw, extrusion, or sonication method, followed by the elimination of non-encapsulated marker molecules (New 1990). The film method begins with a mixture of lipids in an organic solvent. After removing the organic solvent at reduced pressure on a rotary evaporator, the lipid film is hydrated by adding the hydrophilic markers. With

shaking, a heterogeneously sized population of liposomes is produced with the size over 1 μm . The reverse-phase evaporation method is based on the removal of organic solvent by evaporation from the emulsion of the aqueous solution of markers and the organic solvent with lipids. With continuous shaking and evaporation, the gel of lipids converts into a dispersion of liposomes with large size. On the next stage, these large-sized liposomes are transformed to nanosized vesicles by the freeze-thaw method, sonication method, or extrusion method. The freeze-thaw method uses several repeating cycles of quickly freezing (in dry ice/acetone bath or liquid nitrogen) and thawing (in warm water) of a liposome dispersion. The sonication method is processed on the ultrasonication of an aqueous dispersion of lipids with marker molecules, using a strong bath sonicator or a probe sonicator. The extrusion method uses a polycarbonate membrane with a defined pore size to filter the liposome dispersion. Finally, at the last stage, the non-entrapped markers are separated from liposomal nanovesicles by gel filtration chromatography, dialysis, or ultracentrifugation. The mechanical stress during centrifugation may cause the leakage of small molecules. If this occurs, it shall be replaced by other gentle methods such as dialysis or gel filtration chromatography.

Magnetic Nanoparticles

Magnetic particles have been increasingly utilized in the fields of bioanalysis and medicine since the mid-1970s. Owing to their nontoxicity, biocompatibility, injectability, and high degree of accumulation in target tissues or organs, magnetic nanoparticles have been extensively utilized in clinical examination and treatment, as, for example, a contrast agent for magnetic resonance imaging (MRI), or carriers of drugs to be released at a specific site, and the heating mediators of hyperthermia in treating cancer. Nowadays, magnetic nanoparticles are also used in biosensors, owing to their superparamagnetism, which refers to the fact that they become magnetized in an external magnetic field, and as soon as the external magnetic field is removed, they lose their magnetization as a result of thermal fluctuations, becoming suspended in solution. Hence, the conjugation of specific biomolecules on their surface makes magnetic nanoparticles suitable for use as capture reagents to isolate and concentrate target analytes from clinical extracts, food, or environmental samples and enables them to be used to identify target analytes with the use of a magnetic detector. The use of magnetic nanoparticles can effectively reduce the duration of absorption and separation steps for isolating the target analytes from samples, so they are utilized in rapid assays. Antibody-tagged magnetic nanoparticles are also called immunomagnetic nanoparticles (IMNPs) and they have been used as capture reagents in immunomagnetic separation (IMS) procedures for the isolation of pathogens or the identification of food allergens (Chu and Wen 2013). Magnetic nanoparticles with diameters of 25–100 nm are usually colloidal solutions that comprise an inorganic core of iron oxide (magnetite, Fe_3O_4 ; maghemite, $\gamma\text{-Fe}_2\text{O}_3$; or other insoluble ferrites), covered by water-stable polymers, such as dextran, synthetic polyacrylic acid, or silica. Owing to their extremely high

surface area-to-volume ratio, magnetic nanoparticles agglomerate easily to reduce their surface energy. Coating magnetic nanoparticles with a water-soluble polymer not only prevents their agglomeration but also provides additional functional groups (amino or carboxylic acid) on their surfaces, and these groups can be used in subsequent covalent conjugation with biomolecules, such as antibodies, peptides, proteins, and nucleic acids.

Numerous methods have been developed for synthesizing magnetic nanoparticles, such as hot-injection, heating-up, thermal decomposition, and metal reduction. In the hot-injection method, the excess precursor is rapidly injected into a reaction vessel that contains a hot surfactant solution, to produce a high degree of supersaturation, leading to burst nucleation by the release of the excess free energy of supersaturation (Murray et al. 1993). In the heat-up method, the precursors, surfactants, and solvent are mixed at a low temperature and heated to initiate the clustering and growth of metal particles (Park et al. 2007). Both methods are frequently used in the laboratory and can produce highly monodispersed magnetic nanoparticles. The heating-up method is particularly effective for large-scale production, owing to the simplicity of its implementation. The thermal decomposition reaction provides one of the simplest and most important means of producing magnetic nanoparticles, which are formed by the decomposition of metal precursors, such as organometallic complexes. These complexes are easily decomposed by heat, light, or sound, because they are metastable. Metal carbonyls and their derivatives are frequently used to synthesize metallic nanoparticles, because reactive carbonyls are easily dissociated when heated, causing the zero-valent metal atoms to nucleate and grow to form nanoparticles. For instance, iron pentacarbonyl, $\text{Fe}(\text{CO})_5$, has been used to make monodispersed magnetic iron and iron oxide nanoparticles by thermal decomposition in the presence of surfactants (such as oleic acid and oleylamine). These methods produce iron nanoparticles with different diameters from 5 to 19 nm, and nanoparticles of high quality can be achieved by carefully optimizing the synthetic conditions, such as surfactant concentration, heating rate, and the type of solvent (Huber 2005). The last method for synthesizing magnetic nanoparticles involves the reduction of metal salts using reducing reagents in the presence of surfactants. In this method, stable metal precursors (oxides, nitrates, chlorides, acetates, and acetylacetonates) are reduced using conventional hydride-based reducing agents (sodium borohydride and lithium superhydride) or organic reducing agents (polyols, hydrazine, and dihydrogen gas). For instance, FeCo nanoparticles have been synthesized by the co-reduction of ferrous sulfate (FeSO_4) and cobalt chloride (CoCl_2) using sodium borohydride (NaBH_4), and Fe_3O_4 nanoparticles have been formed by the reduction of iron acetylacetonate, $\text{Fe}(\text{C}_5\text{H}_7\text{O}_2)_3$, with 1,2-hexadecanediol in benzyl ether in the presence of oleic acid and oleylamine (Chaubey et al. 2007).

When hydrophobic surfactants are used to synthesize and stabilize magnetic nanoparticles, these nanoparticles remain under hydrophobic conditions and so are not suitable for bioassays. Two methods have been utilized to make the surface of magnetic nanoparticles hydrophilic – surfactant addition and surfactant exchange. In the surfactant addition method, amphiphilic molecules are added to magnetic

nanoparticles and their hydrophobic parts (hydrocarbon chains) form a double layer with the original hydrophobic chains on the surface of the nanoparticles while the hydrophilic polar functional groups are exposed, making the entire nanoparticle water soluble. For example, polyethylene glycol (PEG)-modified phospholipid has been used to functionalize FeCo nanoparticles: the hydrocarbon chains of phospholipids bind to the surfaces of the nanoparticles by hydrophobic interactions, forming stable double layers, while the hydrophilic PEG chains were exposed to the aqueous medium (Seo et al. 2006). In the second method, surfactant exchange, the original surfactant on the nanoparticles is directly exchanged with a new bifunctional surfactant, which uses one functional group to bind chemically and strongly to the nanoparticle surface, with the second polar functional extended out of the nanoparticle. Accordingly, functionalized nanoparticles can be dispersed in water and further covalently conjugated with biomolecules. As an example, dopamine has been used to replace the originally coated surfactants and then coated on the surface of an iron oxide nanoparticle with a five-membered ring, yielding magnetic nanoparticles that are highly stable under physiological conditions (Xu et al. 2004). Dopamine-PEG (thiol-terminated PEG) has been used to modify the surfaces of magnetic nanoparticles. The use of dopamine-PEG not only enhances the stability of FePt nanoparticles in aqueous solution but also provides a convenient means of immobilizing biomolecules on the surface of nanoparticles because of the terminal thiol groups of PEG (Hong et al. 2005). Moreover, apart from polymer-based surfactants, various small bifunctional molecules, such as cystamine and 2,3-dimercaptosuccinic acid (DMSA), have been used to replace the originally coated hydrophobic layer on magnetic nanoparticles. Cystamine forms metal-S bonds with magnetic nanoparticles and its amine group conjugates with biomolecules (Xu et al. 2010). DMSA forms carboxylic chelate, which bonds to the magnetic nanoparticles and subsequently covalently conjugates with ligands through intermolecular disulfide cross-linkages (Jun et al. 2005). Like PEGylated or other polymer-based magnetic nanoparticles, magnetic nanoparticles that are coated with small molecules on their surfaces sufficiently remain their small diameters without aggregation.

Silica Nanoparticles

Silica nanoparticles are spherical vesicles which consist of silica shell and cannular core where a large number of molecules, such as iron oxide, organic and inorganic dye molecules, or even drugs, can be entrapped. Hence, compared with a single dye molecule, a silica nanoparticle contains tens of thousands of dye molecules, producing a significantly stronger signal in bioassays when dye-doped silica nanoparticles are used as labeling reagents for the detection of biomolecules. Moreover, silica nanoparticle is a good carrier since silica is not subjected to the degradation caused by microbes, its porosity does not change with different pH values of solutions, and silica is chemically inert and optically transparent. The shell of silica nanoparticles acts as an isolator to protect the encapsulated molecules

from some harsh conditions, such as high temperature, extremes of pH (high acidity or alkalinity), or high salinity (Drake et al. 2004). Therefore, compared with other fluorescent labeling reagents, organic dye-doped silica nanoparticles get an excellent photostability. In addition, iron oxide can also be enraptured in silica nanoparticles for the purpose of isolating and concentrating the target molecules from samples. In order to generate a targeting ability on silica nanoparticles, biomolecules, such as aptamers, protein A/G, enzymes, antibodies, antigens, and nucleic acids, are conjugated to the surface of nanoparticle with the use of various conjugation methods, such as amino group cross-linkage, disulfide-coupling chemical binding, cyanogen bromide modification, and avidin–biotin linking bridge. According to their different surface modifications and loading molecular, silica nanoparticles have presented a wide range of bioanalytical and biotechnological applications in the detection of various organic and inorganic molecules, drug delivery for cancer therapy, and molecular imaging for signal amplification (Biju 2014).

There are two methods to prepare silica nanoparticles: the Stöber method and the reverse microemulsion method. The former method is mainly used to prepare pure and organic dye-doped nanoparticles, while the latter method can be widely applied to prepare pure, inorganic and organic dye-doped and magnetic nanoparticles (Drake et al. 2004). In a Stöber method, alkyl silicates are hydrolyzed and subsequently silicic acids are condensed in alcoholic solution using ammonia as catalyst, to form Si–O–Si bridges (Stöber et al. 1968). During the process, absolute ethanol and tetraethyl orthosilicate (TEOS) are firstly mixed in a conical flask placed in an ultrasonication bath. After the addition of ammonia as a catalyst, silica nanoparticles are obtained. Thereafter, silica nanoparticles are separated from the colloidal solution by high-speed centrifugation and then washed with absolute ethanol for two to three times to eliminate undesirable particles. Finally, silica nanoparticles are dried in an oven at 100 °C for a few hours to stop continuous reaction. The size of silica nanoparticles becomes larger with the increase of TEOS or catalyst concentrations, since the increase of either reagent can speed up the rate of hydrolysis and condensation, leading to shortened nucleation period, and then the total number of nuclei formed will be fewer in number, leading to a larger diameter of silica nanoparticles in the end (Ibrahim et al. 2010). The second method for generating silica nanoparticles is the reverse microemulsion method, based on the polycondensation of TEOS in a reverse microemulsion system (water in oil; W/O), where a homogeneous mixture of water, oil, and a surfactant is maintained. This method can produce monodispersed particles in the nanometer-size range and has been frequently used in producing organic dye-doped silica nanoparticles. In this method, water and dye are firstly dispersed and become numerous water nanodrops in the bulk oil phase (organic phase) and stabilized by the surfactant. These water nanodrops are used as nanoreactors for the synthesis of silica nanoparticles. As soon as silica matrix is loaded into the water droplets, the hydrolysis and condensation reactions of alkoxy silanes begin, and the size of silica nanoparticles is affected by the molar ratio of water to surfactant, the type of organic solvent and surfactant, and the amount of TEOS (Santra et al. 2001).

In addition, low dye encapsulation efficiency and the leakage of encapsulated dye molecules are main issues in the production of dye-doped silica nanoparticles. The internal environment of silica nanoparticles is hydrophilic and negatively charged, so organic dye molecules tend to be in the bulk oil phase, due to their hydrophobic properties. Therefore, the addition of a hydrophilic portion (such as dextran) to organic dye molecules makes organic dyes water soluble and subsequently increases their amounts in water nanodrops, resulting in a higher encapsulation efficiency. Moreover, the protonation of dye–dextran complexes with acidic solutions (such as HCl, H₂SO₄, and acetic acid) provides a stronger retaining force between the dye complex and the negatively charged silica matrix, which can obviously reduce the leakage of encapsulated dye molecules (Zhao et al. 2004). Hence, with optimal modifications for organic dye molecules, it is useful to manufacture photostable dye-doped silica nanoparticles as labeling reagents to amplify the assay signals of biosensors.

Biosensors that Use Nanoparticles

A biosensor is a device that can rapidly generate a measurable signal when target analytes interact with the complementary biorecognition layer, which is either integrated in or intimately associated with a physicochemical transducer. Thus, biosensors are integrated devices that comprise two units – a biorecognition element and a transducer (Luong et al. 2008). Based on the analysis mechanism of the transducer, a biosensor can be classified as optical, electrochemical, or piezoelectric (mass-sensitive). To detect specifically the target analytes in samples, capture reagents are immobilized on the biorecognition element, which may be an antibody, antigen, receptor, aptamer, nucleic acid, molecularly imprinted polymer (MIP), cells, or tissues. The specific interaction between the immobilized capture reagents and the target analytes causes a physicochemical change in the reaction cell that can be measured and output as a digital reading through the transducer. Hence, biosensors provide a convenient means of detecting various target molecules and are suitable for the rapid monitoring of food safety.

Optical Biosensors

Optical biosensors have been most extensively used in immunosensors. Optical biosensors, which are based on different labeling techniques, can be used to measure the absorbance of light, fluorescence, phosphorescence, electrochemiluminescence, and polarization. The surface plasmon resonance (SPR) spectroscopic sensor is the most widely used optical sensor. The extensive use of SPR spectroscopy may follow from its provision of label-free, rapid detection of various antigens with high sensitivity and specificity. SPR spectroscopy can also be utilized to investigate the affinity and kinetics of interactions between two molecules, such as antibody–antigen, receptor–ligand, and protein–nucleic acid interactions,

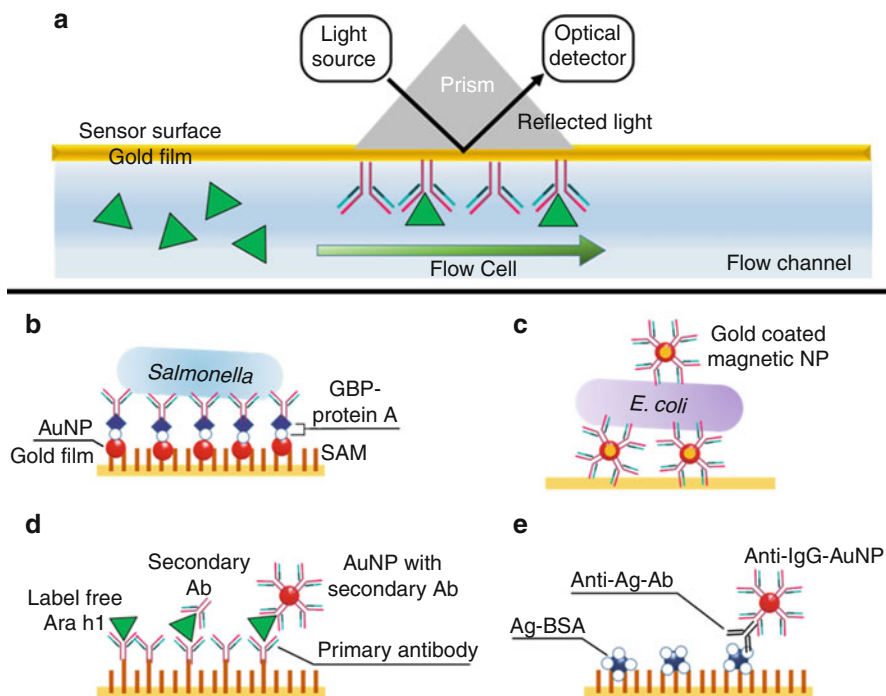


Fig. 3 Schematic representation of the nanoparticle-based applications in SPR biosensors. (a) The illustration of an SPR device. (b) Detection of *Salmonella* using GBP-protein A modified AuNPs. (c) Isolation and detection of *E. coli* by gold-coated magnetic nanoparticles. (d) Detection of Ara h1 through label-free, secondary antibody (Ab) or AuNPs modified with secondary Ab. (e) Detection of antigen (Ag) using anti-Ag Ab and the AuNPs modified with anti-IgG Ab

as well as DNA/RNA hybridization. A typical SPR spectroscope consists of a light source, a glass prism, a transducer that is covered by a gold film, a flow system, and a detector (Fig. 3a); it is based on the excitation of a charge-density oscillation at the interface between a metal and a dielectric (sample solution) (Homola et al. 1999). Incident plane-polarized light, with a range of incident angles, is directed through the prism to the gold-sample solution interface. The intensity of the reflected light is measured using a detector. At the prism-gold interface, total internal reflection (TIR) occurs when the light passes from which is the material with a high refractive index across the interface to which is the material with the lower refractive index at an angle that is larger than the critical angle. The TIR of an incident light can generate a propagating plasma wave with the release of an evanescent field wave. At particular wavelengths and angles, the photons of incident light resonate with the free electrons on the surface of the gold film, reducing the intensity of the reflected light. The angle at which the decrease in the intensity of the reflected light happens is greatest is referred to as the SPR angle. The SPR angle is sensitive to the refractive index in the evanescent field at the gold surface that is in contact with

the samples as they migrate through the flow system. Therefore, the refractive index is strongly affected by the total amount of biomolecules that are immobilized on the gold electrode. Since the interaction between the target analytes and the immobilized capture reagents at the surface of the gold film alters the refractive index, the SPR angle is shifted. This angular shift is expressed in resonance units (RU) and is directly proportional to the increase in the mass (concentration) of target analyte. Accordingly, SPR can provide real-time analysis of specific interactions between molecules without the need for prior dye or enzymatic labeling. SPR has been applied in various areas, such as clinical diagnosis, the detection of environmental pollutions, and food safety.

Since target analytes may present at a concentration lower than nanomolar levels, recent developments in SPR have focused on the application of nanoparticles in a sandwich format to strengthen the SPR response. Most of the nanoparticles that are used in SPR for the analysis of target DNA fragments are small (10–15 nm in diameter), and they can largely improve assay sensitivity, as they conjugate with DNA probes, which are used to hybridize with the target DNA fragments that are captured by the immobilized DNA probes on an SPR gold chip (Yao et al. 2006). Theoretically, increasing the size of nanoparticles enhances the SPR response, because larger nanoparticles cause greater changes in the refractive index. However, larger nanoparticles may be less able to maintain colloidal stability following the conjugation of biomolecules on their surface. Additionally, in a study by Mirkin, DNA probes that were labeled with variously sized gold nanoparticles (DNA-AuNP) varied significantly in the equilibrium binding constant (K_{eq}) in aggregate formation. The K_{eq} increased from 2×10^0 for 15 nm DNA-AuNPs to 2×10^6 and 9×10^8 for 60 and 150 nm DNA-AuNPs, respectively (Hurst et al. 2008). Hence, the size of gold nanoparticles significantly affects the kinetics of DNA hybridization for gold nanoparticle-labeled DNA probes. In a study by Kwon, large gold nanoparticles (40–50 nm in diameter) were used to detect of thrombin (Kwon et al. 2012). The large gold nanoparticles provided a significantly better ($>10^3$ times) detection limit than that obtained in a previous study in which smaller gold nanoparticles (~15 nm) were used. Kwon's study also focused on finding the optimal shape of gold nanoparticles for an SPR sensor. Thus, gold nanocages, gold nanorods, and gold quasi-spherical nanoparticles were applied conjugated with antithrombin antibody for the detection of thrombin in combination with SPR measurements of an aptamer-modified gold chip surface. All of these selected particles had at least one dimension in the 40–50 nm range to make the size effects negligible. The results thus obtained reveal that gold quasi-spherical nanoparticles provided the best detection limit of 1 aM, followed by nanorods (10 aM) and nanocages (1 fM). Therefore, the conjugation of gold quasi-spherical nanoparticles with antibodies provides a good way to amplify the signals in SPR immunosensors, and this enhancement can be maximized by optimizing the size and shape of these nanoparticles.

Nanoparticle-based SPR immunosensors have been developed to detect *Salmonella* spp., *Escherichia coli*, Ara h1 (a major peanut allergen), ochratoxin A (a fungal toxin), and the residues of antibiotics. To detect large molecules, such

as pathogens or food allergens, antibodies can be immobilized on the gold chip of SPR by physical adsorption, covalent bonding with the self-assembled monolayer (SAM) of alkanethiols, or specific interactions between streptavidin and biotin. However, such processes involve multiple steps and take a long time, and they may sometimes cause protein denaturation or reduction of antibody immunoactivity because of the inaccessibility of the antigen-binding sites that is caused by the chemical modification by the conjugation reagents or the random orientation of the immobilized antibodies (Zhu and Snyder 2003). Numerous studies have demonstrated that antibody immobilization using protein A or protein G can improve both the orientation of antibodies and assay sensitivity. Protein A and protein G are antibody-binding proteins that can specifically bind to the Fc fragment of antibodies, while protein G can bind to antibodies from a wider range of mammalian species than protein A, and with a stronger affinity (Bjorck and Kronvall 1984).

To optimize the antibody immobilization method to maximize assay sensitivity in the detection of *Salmonella enterica* serovar Typhimurium, Ko et al. used a gold-binding polypeptide (GBP)–protein A (GBP–ProA) fusion protein as a linker to coat anti-*Salmonella* antibodies on the gold chip of an SPR immunosensor to retain the freedom of orientation of the immobilized antibodies, enabling them effectively to capture bacterial cells from samples, as displayed in Fig. 3b. GBP can strongly bind to the surface of gold, owing to its three repeated anchoring components with the sequence MHGKTQATSGTIOS (Braun et al. 2002). Additionally, to strengthen SPR signals without loss in a label-free detection system, rather than labeling them with antibodies, gold nanoparticles were directly immobilized on gold chips using a SAM of aminoethanethiol (AET). These coated gold nanoparticles were then used to interact with the gold-binding domains of GBP in GBP–ProA complexes, and the protein A portion of the GBP–ProA complexes was subsequently used to capture the anti-*Salmonella* antibodies for the detection of *Salmonella* Typhimurium. The results thus obtained demonstrate that the modified chips provided a tenfold signal enhancement in the detection of *Salmonella* Typhimurium over the bare chip, and the detection limit of the SPR with the modified chips was 10^5 cells/mL. The enhancement of assay sensitivity may have arisen from the fact that the gold nanoparticle-assembled chips had a larger surface area, which could immobilize more GBP–ProA complexes, resulting in the immobilization of more antibodies than on the bare chips. Additionally, this work demonstrates that the use of a GBP–ProA linker is an easy way to immobilize antibodies on a gold surface and this linker has the potential to be used on other devices with coated gold surfaces, such as a quartz crystal microbalance (QCM) (Ko et al. 2009). Like gold nanoparticles, magnetic particles also have been utilized in SPR biosensors for the detection of pathogens. In 2012, Ozlem et al. used three antibody immobilization methods to detect *Escherichia coli* using an SPR sensor, which were nonspecific adsorption, specific adsorption by the avidin–biotin interaction, and SAM coating with 11-mercaptoundecanoic acid (11-MUA). Among these three methods, the SAM method yielded the highest sensitivity, followed by the specific adsorption and then the nonspecific adsorption, with corresponding limits of detection (LOD) of 35, 6.2×10^3 , and 4.8×10^5 CFU/mL, respectively.

The nonspecific adsorption method provided the lowest sensitivity, perhaps because of the random orientation, conformational changes, and steric hindrance of the antibodies as well as the inducible denaturation of proteins as they come into contact with a metal surface. Moreover, even though SAM coating significantly increased the detection limit, the assay with this surface modification still exhibited some limitations, perhaps owing to the limited penetration of bacteria into the electromagnetic field, the low refractive index between the bacteria cytoplasm and the aqueous reaction environment inside the SPR channel, and the accessibility of bacterial epitopes to the antibodies. To improve further the assay sensitivity, gold-coated magnetic nanoparticles (Fe_3O_4) have been applied in SPR analysis: the nanoparticles were tagged with 11-MUA molecules to conjugate covalently with avidin, and avidin-tagged nanoparticles were mixed with biotin-labeled anti-*E. coli* polyclonal antibodies to form antibody-tagged nanoparticles (Fig. 3c). Owing to their superparamagnetic property and metallic character, the antibody-tagged and gold-coated magnetic nanoparticles have been used not only to capture *E. coli* cells from samples but also to enhance SPR signals. These nanoparticles can reduce the LOD for *E. coli* to 3 CFU/mL and enable the entire assay, including capturing, washing, and measurement, to be conducted in 70 min (Torun et al. 2012).

Most commercial SPR sensors are expensive and bulky but provide good results with high sensitivity and specificity, because they use an integrated prism. Optical fibers are compact and affordable and can couple light to surface plasmons, but they are not as sensitive as an SPR sensor with a prism, due to the inherent characters of fiber optics. However, a fiber optical system is suitable for use with nanoparticles because it does not incorporate a microfluidic device, which could easily become clogged with nanoparticles. Hence, Pollet utilized antibody-linked magnetite nanobeads as signal enhancers in an optical fiber SPR biosensor for detecting peanut allergen Ara h1 (Pollet et al. 2011). Magnetic nanoparticles were prepared by the hydrolysis method on anhydrous FeCl_3 and further silanized to produce the carboxyl surface groups. Anti-Ara h1 antibodies were covalently conjugated on these carboxyl groups using a carbodiimide reaction. The LOD of the label-free assay was 9 $\mu\text{g/mL}$, that of the assay using secondary antibodies as the signal enhancer was 0.21 $\mu\text{g/mL}$, and that of the assay using secondary antibody-tagged nanoparticles was 0.09 $\mu\text{g/mL}$ (Fig. 3d). Therefore, the use of magnetic nanoparticles (~19 nm) significantly enhanced the assay sensitivity to approximately 100 times that achieved using the label-free format. Additionally, this optical fiber SPR sensor could be easily regenerated by a treatment with acid for 2 min with a glycine buffer at pH 1.7, and the duration of this procedure was half that of the ELISA protocol. This fiber device can be used for making a portable SPR sensor, as it does not incorporate a prism and so can be miniaturized.

Nanoparticle-based competitive SPR sensors have been developed to detect low concentrations of small molecules, such as toxins or antibiotics. For instance, an SPR biosensor that is based on signal enhancement by gold nanoparticles has been used to detect ochratoxin A (OTA) (Yuan et al. 2009). In this assay, OTA was covalently tagged on the surface of carrier protein ovalbumin (OVA) using PEG as a linker, because the insertion of a water-soluble linker between the small molecule

and the protein can significantly increase the immobilization of the small molecules on the surface and thereby improve assay sensitivity (Yuan et al. 2008). Additionally, long-chained PEG molecules can effectively prevent the nonspecific binding of biomolecules onto the surfaces of gold chips and efficiently project the tagged small molecules out from the sensor surface, maximizing their interaction with antibodies in the solution and increasing the efficiency of regeneration for surface modification. OTA-PEG-OVA complexes were immobilized on the mixed SAM (mSAM) that was formed by the deposition of a mixture of 11-mercaptoundecanol (11-MUOH) and 16-mercaptohexadecanoic acid (16-MHA) on a bare gold chip, since the mSAM enabled the immobilized biomolecules to be better orientated. In this competitive assay, free OTA in samples competes with the immobilized OTA-PEG-OVA to bind to antibody-tagged gold nanoparticles that have a mean diameter of 40 nm. Large gold nanoparticles were used in this work because, in a previous study of the detection of chloramphenicol using an SPR biosensor, the LOD (0.74 pg/mL) with large gold nanoparticles (40 nm) was significantly lower than that (4.9 fg/mL) with small gold nanoparticles (10 nm) (Yuan et al. 2008). The use of antibody-tagged gold nanoparticles to enhance the signal reduced the detection limit from 1.5 ng/mL for the directly detected OTA on the surface of the modified SPR chips to 0.042 ng/mL. Moreover, with a simple extraction using 50 % methanol and further treatment with poly(vinylpyrrolidone) (PVP), the LODs for OTA in oats and corn were 0.3 and 0.5 ng/g, respectively, whereas the LODs for OTA in wine and other beverages ranged from 0.058 to 0.4 ng/mL (Yuan et al. 2009).

The size of the gold nanoparticles in SPR is also a critical factor in determining the performance of complete SPR biosensors in the detection of small molecules. To investigate the effect of the size of the gold nanoparticles and the assay format on the detection limit of an SPR biosensor in the detection of fluoroquinolone (FQ) antibiotic residues, four assay formats were tested (Fernandez et al. 2012). All of these formats exploited the same mSAM method to immobilize the FQ-tagged bovine serum albumin (FQ-BSA) on the surfaces of gold chips. To stabilize gold nanoparticles, which had been produced by the citrate reduction method, the surfaces of gold nanoparticles were firstly functionalized with the hydrophilic O-(2-carboxyethyl)-O'-(2-mercaptoethyl)-heptaethylene glycol (acid-PEG-thiol) and O-(methyl)-O'-(2-mercaptoethyl)-hexaethylene glycol (m-PEG-thiol) and covalently conjugated with antibodies through the carboxyl groups of the acid-PEG-thiol molecules. Their four formats were (1) a single antibody step with anti-FQ antibodies (format A), (2) two antibody steps with anti-FQ antibody and anti-IgG-coupled 10 nm gold nanoparticles (format B₁), (3) two antibody steps with anti-FQ antibody and anti-IgG-coupled 19 nm gold nanoparticles (format B₂), and (4) a single antibody step with anti-FQ antibody coupled with 19 nm gold nanoparticles (format C) (Fig. 3e). The use of IgG-labeled or anti-IgG-labeled gold nanoparticles significantly improved the assay sensitivity over that of format A. Anti-IgG gold nanoparticles improved the LOD by a factor 14, reducing it from $0.98 \pm 0.38 \mu\text{g/L}$ to $0.07 \pm 0.01 \mu\text{g/L}$. Anti-FQ antibody-labeled gold nanoparticles reduced the detection limit to $0.11 \pm 0.01 \mu\text{g/L}$ and reduced the

number of steps required. Hence, the application of antibody-tagged gold nanoparticles is a favorable strategy to improve the sensitivity of SPR biosensors and can be optimized by using variously sized nanoparticles or various surface modification methods to functionalize these nanoparticles.

Typically SPR sensors have low resolutions while analyzing low-molecular-weight samples and low-concentration samples. In order to solve these issues, the techniques of surface plasmon resonance-phase imaging (SPR-PI) have been developed. SPR-PI measures the phase shift of the p-polarized reflected light happening as surface plasmon polaritons are injected onto the surface of the gold thin film in the Kretschmann coupling configuration. The absorption onto the interface can be measured by detecting changes in the phase shift from light incident at a fixed angle (Nikitin et al. 2000). Generally, the device using SPR-PI provides a lower detection limit than traditional intensity-based SPR imaging measurements (Halpern et al. 2011). Recently, an SPR-PI device was established with the use of 120 nm DNA-modified silica nanoparticles (SiNPs) to amplify the SPR-PI signal. SiNPs were selected in this study because their optical effect on the measurements of the phase grating is straightforward and easier to model than gold or silver nanoparticles. The detection limit of this “nanoparticle-enhanced SPR-PI” was 25 f. as analyzing 38 mer ssDNA using a three-sequence hybridization format. The enhancement of using silica nanoparticles on the detection limit might be due to the absorbance of nanoparticles that largely increased the interfacial refractive index, leading to a great phase shift. In addition, the detection limit of 25 f. for SiNP-enhanced SPR-PI is about 20 times more sensitive than that with nanoparticle-enhanced SPRI. The authors of this study suggested that not only DNA-modified SiNPs but also protein-modified or carbohydrate-modified SiNPs are capable to be applied in this SPR-PI system for various ultrasensitive biosensing applications (Zhou et al. 2012).

Piezoelectric (Mass-Sensitive) Biosensors

The word “piezoelectricity” originates from the Greek word *piezein* and means “electricity under pressure,” since piezo means pressure. French physicists Jacques and Pierre Curie discovered this phenomenon in 1880 (Vaughan et al. 2001). When a force is applied to a piezoelectric material, electric charges are generated in the material, producing an electric field, so mechanical energy is converted into electric energy. Such a phenomenon is called the direct piezoelectric effect. In 1881, Lippmann predicted the existence of a reverse piezoelectric effect, in which the application of an electric field causes the deformation of the piezoelectric material. The piezoelectric quartz crystal in QCM biosensors is such a material. The induced vibration of a quartz crystal depends on the angle at which the mother quartz crystal is cut. The AT-cut quartz crystal (cut at $35^{\circ}15'$ to the optic z-axis) is the one typically used for QCM biosensors, because it has a very stable frequency and high thermostability with a relatively low $\Delta f/f$ ($\sim 10^{-8}$) and a near-zero temperature coefficient of resonant frequency between 0°C and 50°C , respectively.

In 1959, Sauerbrey established the relationship between the change in resonant frequency of the quartz in a QCM and the change in mass of molecules attached to the surface of a gold electrode in the QCM, leading to the development of a QCM as a commonly used biosensor that relies on the increase in mass that is caused by the absorption of target molecules (Lu et al. 2004). In this device, quartz is a precise and stable oscillator, and two gold electrodes are attached to both of its surfaces, in order to generate an electric field to deform the quartz and then induce the quartz to oscillate at a specific resonant frequency. In QCM immunosensors, antibodies or antigens are immobilized on the surface of a gold electrode by chemical modification techniques with the use of cross-linking reagents. The consequent increase of the mass of the gold electrode reduces the resonant frequency of the quartz. Hence, the change in mass at the solid/liquid interface can be calculated from the measured frequency change, using the Sauerbrey equation,

$$\Delta F = \frac{-2\Delta m f^2}{A\sqrt{\mu\rho_q}} = -C_f \Delta m$$

where ΔF = change in resonant frequency of crystal (Hz), Δm = change in mass of the molecules bound on the gold electrode (ng), f = intrinsic frequency of crystal, A = piezo-electrically active area, ρ_q = density of quartz, μ = shear modulus of quartz, and C_f = integrated QCM sensitivity. Therefore, the thickness, chemical structure, shape, and mass of the wafer determine the fundamental frequency of the quartz crystal (O'sullivan and Guilbault 1999). Typically, a thinner quartz disk resonates at higher frequencies, leading to a higher sensitivity. As a directly responsive microsensor, the QCM is extensively applied in the detection of target molecules in liquid samples because it supports rapid analysis and free labeling, is relatively easy to use, and exhibits both high selectivity and sensitivity. Therefore, the QCM has been used in clinical diagnosis, the analysis of pesticide residues, the detection of pollution by microorganisms, environmental monitoring, and the detection of (fungal) toxin. To enhance the assay sensitivity of QCM biosensors, nanoparticles can be either applied on the gold electrode for the immobilization of capture reagents, such as antibodies, for detecting the target antigens, or they can be labeled with detection reagents, such as reporter probes, as "mass enhancers" for measuring the DNA targets (Fig. 4a).

To improve the detection limit of the QCM, nanoparticles have been used as "mass enhancers" because they have a larger mass than the DNA targets. In 2006, Mao et al. developed a nanoparticle-based QCM DNA sensor for the detection of *eaeA* gene fragments (151 bases) of *E. coli* O157:H7, amplified using asymmetric PCR with biotin-labeled primers (Mao et al. 2006). In this study, streptavidin-coated ferrofluid nanoparticles are generated by coating BSA over the surface of an Fe_3O_4 core (120 nm in diameter) and covalently conjugating streptavidin with BSA, forming streptavidin-functionalized nanoparticles with an average diameter of 145 nm. Mao et al. achieved a detection limit in detecting *E. coli* O157:H7 that was four orders of magnitude lower (10^{-12} M DNA) than that, 2×10^{-8} M DNA,

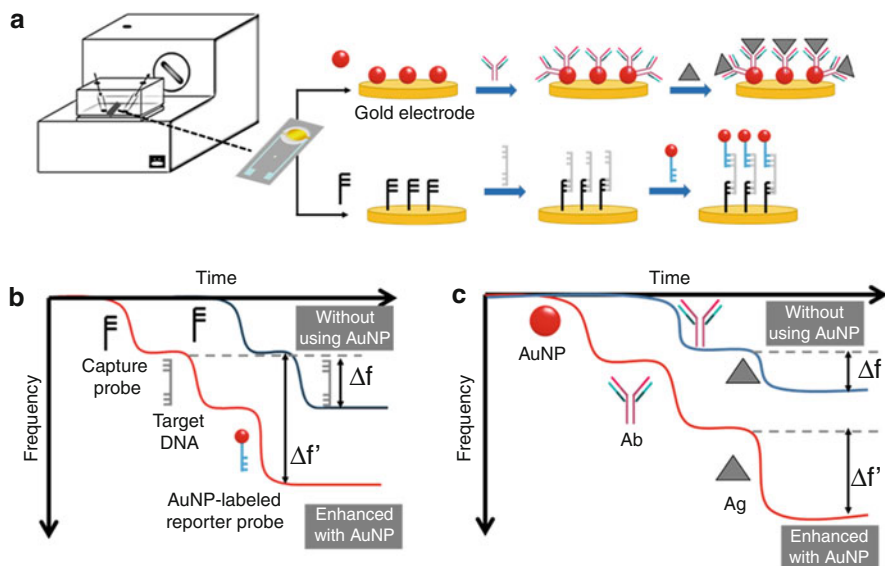


Fig. 4 Schematic representation of the nanoparticle-based applications in QCM biosensors. (a) The illustration of the sensor fabrication and detection procedure for a QCM immunosensor and a QCM genosensor. (b) Comparison of the frequency changes with or without the AuNP-labeled reporter probe in a QCM genosensor. (c) Comparison of the frequency changes with or without the AuNP modification on a gold electrode in a QCM immunosensor

obtained previously using a non-amplification QCM DNA sensor (Deisingh and Thompson 2001). Moreover, this QCM DNA sensor was used to enumerate the target bacteria in a range of 2.67×10^2 to 2.67×10^6 CFU/mL, with a complete assay time less than 30 min. The detection limit was 2.67×10^2 CFU/mL in the detection of PCR products without any culture enrichment, and this value was about one order of magnitude lower than the lowest detection limit that has been achieved for the PCR gel-based detection method. Therefore, this QCM DNA sensor provides a more sensitive and quicker method than fluorescent measurement after gel electrophoresis analysis of the PCR product, for detecting and quantifying pathogenic bacteria by identifying a specific DNA fragment. However, it was found that streptavidin-coated nanoparticles strongly adsorbed onto the gold surface of the electrode resulted in false-positive results. In order to eliminate this nonspecific interaction, several frequently used blocking reagents for streptavidin or streptavidin-coated nanoparticles were tried, including 11-hydroxy-1-undecanethiol, thiolated PEG, and BSA. The change of frequency was significantly reduced from ~ 75 Hz (without blocking) to < 3 Hz after the electrode was blocked with BSA. Thus, using biomolecule-tagged nanoparticles as the “mass enhancer” can significantly improve the sensitivity of a QCM biosensor, but it is essential to avoid any nonspecific interaction between the nanoparticle and the electrode, by treating the electrode with a suitable blocking reagent.

In 2008, besides ferrofluid nanoparticles, gold nanoparticles (AuNPs) were applied to detect *eaeA* gene fragments (104 bp) of *E. coli* O157:H7 (Chen et al. 2008). In this work, thiolated DNA probes, which were complementary to one end of the target DNA, were tagged onto AuNPs with an average diameter of 20 nm (Fig. 4b). The addition of sequence-specific oligonucleotide-functionalized AuNPs as “mass enhancer” reduced the detection limit of this AuNP-based QCM sensor to 1.2×10^2 CFU/mL of *E. coli* O157:H7 with a working range of 1.2×10^2 to 1.2×10^6 CFU/mL. These results are comparable to those of Mao et al. (2006). These AuNPs acted not only as “mass enhancers”; they were also “sequence verifiers” in the detection of *E. coli* O157:H7. In addition, the effect of various modifications for the capture probes on the assay sensitivity was studied. The biosensors using the capture probes with additional 12 dT (P1-30/12 T) and 24 dT (P1-30/24 T) gave significantly greater frequency change than that using the capture probe without additional dT (P1-30), while detecting the target DNA. The result indicates that the capture probes with space segments (additional 12 dT or 24 dT) can improve the hybridization efficiency with the target DNA, leading to an improvement in assay sensitivity.

Two QCM immunosensors in a sandwich format were developed for detecting *Salmonella enterica* serovar Typhimurium. One of these immunosensors, developed by Su et al., used magnetic beads with a diameter of 2.8 μm as mass enhancers (Su and Li 2005); the other, developed by Salam et al., utilized 40 nm AuNPs as mass enhancers (Salam et al. 2013). In Su’s work, protein A was firstly immobilized on the Au electrode to capture anti-*Salmonella* antibodies. Protein A is a cell wall component of *Staphylococcus aureus* and specifically binds to the fragment crystallizable region (Fc region) of antibodies (Björk et al. 1972). Therefore, protein A was applied as the linker to immobilize antibodies on the surface of Au electrode with a correct orientation, in order to improve the capture efficiency for target bacterial cells. In the direct detection of *Salmonella* Typhimurium, both the changes in resonant frequency and motional resistance (ΔF and ΔR) were measured and were found to be proportional to the bacterial concentration in the ranges 10^5 – 10^8 and 10^6 – 10^8 cells/mL, respectively. When antibody-tagged magnetic beads were used as the “separators/concentrators” for sample pretreatment and as the “mass enhancers” in QCM analysis, the detection limit reached as low as 10^2 cells/mL, based on the ΔR measurement. However, magnetic beads could not be used to measure ΔF , because ΔF was not related to the bacterial concentration, as it could be either positive or negative at the same concentration of bacterial cells. This fluctuation of ΔF may have been caused by the variation in the sizes of the *Salmonella*–bead complexes. In 2003, Olsen et al. utilized the somatic O antibody and the flagellar H7 antibody to form a rigid bond and a flexible connection between *Salmonella* Typhimurium and the surface of the electrode of a QCM sensor, respectively. When the attachment of bacteria was rigid and strong, the response of the QCM sensor was correlated with the concentration of bacteria at the liquid/solid interface. However, when the attachment of bacteria was flexible, the intensity of the signals was inversely proportional to the bacterial

concentration (Olsen et al. 2003). When magnetic beads were used with a QCM sensor, bacteria–bead complexes with various sizes (from several microns to tens of microns) were formed. Small complexes might have bound tightly to the surface of the electrode, resulting in a positive response of signal that was directly correlated to the change in mass. In contrast, large complexes might be attached flexibly to the electrode, causing a negative response of signal change that was inversely proportional to the increase in mass (Su and Li 2005). In this study, ΔF measurement was more sensitive than the ΔR measurement in the direct detection (without using magnetic beads); however, with the use of magnetic beads, the ΔR measurement is more sensitive and reliable than ΔF measurement as well as provides a lower detection limit than the most reported QCM immunosensors for bacterial detection. Therefore, it is very important to use the proper parameter to evaluate the performance of a QCM immunosensor while using micrometer-sized magnetic beads as mass enhancers to detect bacterial cells. Recently, Tothill's group used AuNPs as mass enhancers in the detection of *Salmonella* Typhimurium using an automated QCM instrument. In their work, the assay sensitivity was improved by optimizing the method of immobilization for capturing antibody and using anti-*Salmonella* polyclonal antibody-tagged AuNPs as mass enhancers. The SAM technique was utilized because it can generate a reproducible, ultrathin, and well-ordered layer for immobilizing capture antibodies. Hence, various thiol compounds were applied, and short-chain thiols covered less of the electrode surface. Therefore, 11-mercaptoundecanoic acid (11-MUDA) was selected for the formation of SAM on the electrode for the further immobilization of anti-*Salmonella* monoclonal antibody by the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/*N*-hydroxysuccinimide (EDC/NHS) cross-linking method (Park et al. 2004). Moreover, when the antibody-tagged AuNPs were used, the QCM immunosensors had a lower limit of detection (LOD) ~ 10 – 20 CFU/mL than those of the direct and sandwich assays without AuNPs (1.83×10^2 and 1.01×10^2 CFU/mL, respectively) (Salam et al. 2013). Hence, AuNPs have substantial potential to reduce the LOD of a QCM sensor in the detection of pathogenic bacteria.

Nanoparticle-based QCM immunosensors have been used to detect not only pathogens but also the major wheat allergen gliadin (Chu et al. 2012). In the development of a sensitive QCM for detecting gliadin, Wen's group utilized AuNPs to modify the surface of the gold electrode in the QCM to increase the number of antigliadin antibody-binding sites and, consequently, the mass of capture antibodies and target molecules on a QCM chip. Hence, in this work, AuNPs are used to immobilize capture antibodies, unlike in other studies, in which AuNPs have been used as mass enhancers, as displayed in Fig 4c. In order to immobilize AuNPs on the NH_2 -modified gold electrode, glutaraldehyde was firstly added to create carbonyl groups on the surface of electrode that were further covalently linked with cysteamine to provide free thiol groups for conjugating with AuNPs. After the immobilization of AuNPs, cysteamine was added again to form NH_2 groups on the AuNP surface. These NH_2 groups were further used for the covalent

conjugation with anti gliadin antibodies with the use of glutaraldehyde. Three sizes of AuNPs were used and their efficiency of immobilizing anti gliadin antibodies evaluated. The results thus obtained demonstrate that coating antibodies on the 17, 25, and 40 nm AuNP chips changed the frequency by 120 ± 2 , 170 ± 6 , and 109 ± 3 Hz, respectively. The decrease in the frequency of the 40 nm AuNP-modified chip might have been caused by the aggregation of 40 nm AuNPs on the chip, resulting in a less surface area for coating antibodies. Hence, AuNPs with a diameter of 25 nm were utilized in this study. After optimization, the linear dynamic range in 60 % ethanol was from 1×10^1 to 2×10^5 ppb gliadin, and the calculated LOD was 8 ppb. However, the detection limit of this developed was reduced to 1 ppm of gliadin, while analyzing spiked food samples. This reduction of detection limit might be due to interference of the food matrix by fat, carbohydrate, and other components, which are able to block the antigen-binding sites of antibodies and then reduce the antibody–antigen interaction. In addition, the results of the detection of gliadin in ten commercial food products by the AuNP-modified QCM system were consistent with those obtained using an AOAC-approved gliadin kit. Therefore, the AuNP-based QCM platform potentially provides an alternative means of detecting the residues of food allergens to help people with food allergies avoid allergen-containing food.

Electrochemical Biosensors

Electrochemical biosensors are alternative devices to optical and mass-sensitive biosensors for use in the identification and quantification of hazardous materials, such as pathogens, bacterial toxins, viruses, and pesticides, in food. The wide range of applications of electrochemical sensors follows from their simplicity, moderate cost, and portability. The electrochemical assay is based on the induction of oxidation–reduction reactions to generate electrons or ions, which change the electric properties of sample solutions. These reactions are induced by the interaction of targets with the biorecognition elements of the biosensors. The electric signal is produced by either the release of ferrocene, In^{+2} salts, and redox mediators or by the enzymatic reactions of the enzymes that are tagged on the detection reagents, which may include peroxidase, glucose oxidase, alkaline phosphatase, and catalase (Sadik et al. 2009). Typically, electrochemical sensors have poor sensitivity and easily give false negative, owing to interference from the nontarget components of the samples. Additionally, the materials that are used to label detection reagents, such as antibodies, may reduce the ability of the detection reagents to bind to the target molecules or reduce their stability. Fortunately, these limitations of electrochemical biosensors have been overcome by the use of nanoparticle-based transducers. Modification of the surface of an electrode with nanoparticles can significantly increase the surface area on which the biorecognition elements can become attached to the transducer, increasing the assay sensitivity. Gold nanoparticles are one of the most commonly used

nanomaterials because their redox properties cause their use markedly to increase the sensitivity of electrodes. Based on measurable parameters, such as current, potential, and impedance, electrochemical biosensors can be classified into voltammetric, amperometric, potentiometric, conductometric, or impedancemetric devices.

The Czech scientist Jaroslav Heyrovsky discovered voltammetry in the 1920s. In a voltammetric sensor, samples are analyzed by measuring current as a function of the electric potential of an electrochemical cell. This method exhibits high selectivity because the analyte can be identified by its voltammetric peak potential, based on its inherent chemical properties. If the potential is maintained at a certain value, then a faradic process occurs, which involves the electrode and a solution species; this process leads to the flow of a current, which is the rate at which charge passes through the interface between the electrode and solution. Therefore, the current can be measured to evaluate the rate of the faradic process and to estimate the concentration of the solute species. The development of the voltammetric method improves its ease of operation, reduces its cost, and reduces the assay time. The cyclic voltammetric method is frequently selected as the first experiment in an electrochemical study or in the development of an electrochemical biosensor. Cyclic voltammetry (CV) enables the redox characteristics of a target analyte to be easily and rapidly observed over a wide range of electric potentials. In England in the 1950s, Geoffrey Barker proposed square-wave voltammetry (SWV), which is another frequently used method for trace analysis (Barker and Gardner 1992). In this method, a discontinuous potential change, as in square-wave polarography, is used to reduce the effect of the double-layer charging current on the working electrode, improving the measurement of the faradic current in the sample analysis.

Voltammetric biosensors have been successfully applied to detect food pathogens with the use of nanoparticles to enhance assay sensitivities. For example, iron oxide/gold core/shell nanoparticles (Fe@Au) and biolabeled CdS nanocrystals have been used for the detection of *Salmonella enterica* serovar Typhimurium (Freitas et al. 2014). In this work, magnetic nanoparticles were generated by a thermal decomposition method and then coated with gold, using gold (III) chloride, to become Fe@Au nanoparticles. To develop hydrophilic surfaces of Fe@Au nanoparticles to enable them to conjugate with antibodies, they were modified with SAM, and three materials were tested to identify the one that provided the highest peak current and a stable baseline. The three SAM materials were cystamine, mercaptopropionic acid (MPA), and a mixture of 2-mercaptoethanol and 12-mercaptododecanoic acid (ME-MDDA). The results thus obtained reveal that cystamine yielded the highest peak current (25 μA), followed by ME-MDDA (23 μA) and then MPA (8 μA). However, the mixed ME-MDDA monolayer provided the most stable baseline, so ME-MDDA mixtures were used to generate the covalent conjugation with anti-*Salmonella* antibodies through the carboxyl end groups of MDDA using the EDC/NHS method. Magnetic nanoparticles were first used to isolate bacterial cells from

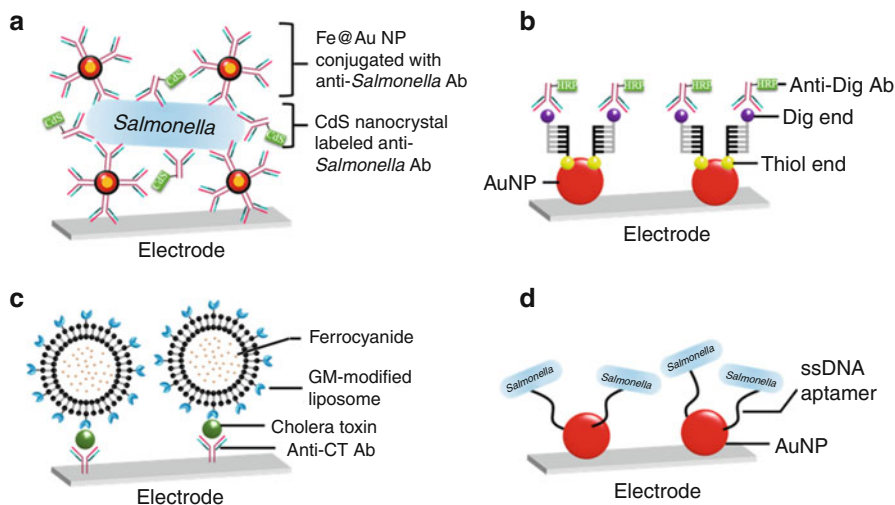


Fig. 5 Schematic representation of the nanoparticle-based applications in electrochemical biosensors. (a) Separation and detection of *Salmonella* with the iron/gold core/shell nanoparticle (Fe@Au NP) and CdS nanocrystal. (b) Detection of Dig- and Thiol-labeled PCR amplicons with the AuNP-modified electrode. (c) Detection of cholera toxin using GM-modified liposome-encapsulating ferrocyanide. (d) Detection of *Salmonella* using ssDNA aptamer-modified AuNPs

samples. Many studies have proved that the use of magnetic nanoparticles or beads to isolate and concentrate target molecules is easy and fast and can minimize damage to the target molecules and improve the sensitivity and specificity of the assay. Nanoparticles are easily suspended in samples to form a colloidal solution, and their nanosize provides a high surface area/volume ratio, which favors the conjugation of a large number of biomolecules; these inherent properties improve their accessibility and efficiency of capturing target molecules. Therefore, the Fe@Au nanoparticles were used herein to isolate *Salmonella* Typhimurium from samples, and then with these captured bacteria cells attached to an electrode using magnet that is placed below the electrode. Moreover, for intensifying the signal, the antibody-tagged CdS nanocrystals were added, and the peak currents were measured using various standard *Salmonella* solutions and a square-wave voltammetric device, as shown in Fig. 5a. Based on the data thus obtained, a calibration curve was established from 1×10^1 to 1×10^6 cells/mL, with a detection limit of 13 cells/mL. The entire assay was completed in 1 h, so this method quickly and sensitively detects the contamination of *Salmonella* Typhimurium and improves the control of the quality of food samples.

Besides being applied in immune-based assay, voltammetric biosensors have been used to detect DNA molecules. An electrochemical biosensor that is based on gold nanoparticles has been utilized to detect the PCR amplicons of the *tnpA* gene that is encoded in the *Salmonella*-specific IS200 mobile element and thereby to

identify the presence of *Salmonella enterica* serovar Typhimurium (Marques et al. 2009). To enlarge the surface area on which PCR amplicons are immobilized, gold nanoparticles are added to a mixture of graphite–epoxy composite (nanoAu-GEC), which is then coated on the electrode. The results thus obtained indicate that an electrode that is coated with 7.5 % gold nanoparticles (nanoAu (7.5 %)-GEC electrode) yields the highest peak current because gold nanoparticles in such a composite aggregate less than others with higher percentages of gold nanoparticles and so are more available for the electrochemical oxidation at the electrodes. Moreover, a primer set of the *tnpA* gene that is labeled with thiol and digoxigenin (Dig) on each 5' end of primers was utilized to produce a double-tagging PCR amplicon, which was immobilized on the nanoAu-GEC using its thiol end and could interact with the HRP-labeled anti-Dig antibody through its Dig end, as displayed in Fig. 5b. After the substrate (hydrogen peroxide) and redox marker (hydroquinone) were added, the current was measured until a steady-state current was reached. The linear range of this electrochemical genosensor was from 10 to 500 fmol and the detection limit was 9 fmol (60 pM). Therefore, the use of gold nanoparticles can effectively improve assay sensitivity by ensuring a favorable orientation for the immobilized biomolecules and excellent transducing properties. Moreover, such voltammetric biosensors can be applied to analyze any PCR amplicon with a double labeling using a PCR reaction, to measure the amount of a specific DNA fragment.

An amperometric biosensor detects by the application of a constant potential and measuring the current, which positively corresponds to the redox reaction involved in the interactions of the target analytes with the biorecognition element. Thus, the working electrode is maintained at a constant potential with respect to the reference electrode. The working electrode is commonly made from noble metals, graphite, or conduction polymers, whereas the reference electrode is normally Ag/AgCl. The current is directly proportional to the concentration of the target molecules in the samples. Amperometric biosensors have high sensitivity with a wide linear range, but they have limited specificity, which is shortcoming that can generally be overcome by using mediators or selective membranes. For example, ganglioside (GM1)-functionalized liposomal nanovesicles (238 nm) that encapsulate potassium ferrocyanide have been used in electrochemical immunosensors for detecting cholera toxin (CT), as displayed in Fig. 5c (Viswanathan et al. 2006). CT is a bacteria toxin that is secreted by *Vibrio cholerae* and can cause human diarrhea. To improve the electronic properties and the strength of the glassy carbon electrode, Nafion-supported multiwalled carbon nanotubes (MWCNT) that are embedded in poly (3,4-ethylenedioxythiophene) (Nafion-MWCNT-PEDOT) are coated on the surface of the electrode. CT in the samples is captured by anti-CT monoclonal antibodies that are immobilized on the caste film and detected by GM1-liposomal nanoparticles as a “sandwich-type” assay. The encapsulated electroactive redox markers (potassium ferrocyanide) are released from the bound GM1-liposomal nanovesicles using a methanolic solution of Triton X-100

and then adsorbed onto the Nadion-MWCNT-PEDOT-coated electrode for analysis using a square-wave stripping voltammetric assay. The linear range of this assay is 10^{-14} to 10^{-7} g/mL with a detection limit of 10^{-16} g of CT. Hence, using potassium ferrocyanide-encapsulated liposomal nanovesicles provides a very effective means of amplifying the signal in the detection of ultra-trace levels of CT.

The impedancemetric sensor is another often used electrochemical biosensor, which has been successfully utilized to detect *E. coli* and *Salmonella enterica* serovar Typhimurium and to detect simultaneously both *E. coli* O157:H7 and *Staphylococcus aureus*. An impedancemetric sensor is used to measure changes in ion concentration that are caused by a biorecognition event, and it normally includes the two metal electrodes. An AC voltage is applied between the two electrodes to cause the flow of a current. A change of the electric impedance between the two electrodes, such as occurs at an interface between the working electrode and the sample solution or in a bulk region of the sample solution, can be measured, and the measurement used to identify bimolecular reactions in the electrochemical cells, such as DNA hybridization, ligand–receptor interaction, antibody–antigen interaction, or the excretion of cellular metabolic products (Shinde et al. 2012). For instance, a label-free electrochemical impedance immunosensor was developed using an AuNPs/PAMAM-MWCNT-Chi nanocomposite-modified glassy carbon electrode to detect *Salmonella* Typhimurium in milk (Dong et al. 2013). In that work, a solution of PAMAM-MWCNT-Chi was firstly dropped onto the electrode to form a membrane, and then gold nanoparticles were absorbed on the surface of the membrane through an interaction with PAMAM dendrimers. In the PAMAM-MWCNT-Chi mixture, a fourth-generation (G4) poly(amidoamine) (PAMAM) dendrimer had 64 primary amine groups on its surface, which absorbed gold nanoparticles because of amido-Au affinity. The anti-*Salmonella* antibodies were covalently conjugated onto the surface of the gold nanoparticles for capturing *Salmonella* cells. The use of PAMAM dendrimers enabled more gold nanoparticles to be attached to the electrodes, increasing the amount of immobilized antibodies and improving the sensitivity of the assay. The generated electrochemical signal was determined by measuring the change in the electron transfer resistance (ΔR_{ct}), which was increased by the capture of *Salmonella* Typhimurium on the gold nanoparticles. The linear range of detection of *Salmonella* Typhimurium was from 1.0×10^3 to 1.0×10^7 CFU/mL, and the detection limit was 5.0×10^2 CFU/mL. This sensor also had high selectivity toward *Salmonella* Typhimurium, since ΔR_{ct} increased to 806 Ω when *Salmonella* Typhimurium was detected, but it increased to less than 100 Ω when *E. coli* and *Staphylococcus aureus* were detected. The use of an AuNPs/PAMAM-MWCNT-Chi nanocomposite film to modify the glassy carbon electrode of an impedancemetric sensor provided high sensitivity and specificity, enabling the detection of pathogens without labeling and supporting the potential use of this nanocomposite film-based impedancemetric sensor in detection of pathogens for public and environmental protection. Moreover, the antibodies

that are coated onto gold nanoparticle-modified glassy carbon electrodes can be replaced by a *Salmonella*-specific recognition aptamer for the detection of *Salmonella*, as recently reported and shown in Fig. 5d (Ma et al. 2014). Aptamers are single-stranded DNA or RNA molecules and can provide a sequence-dined structure with a binding affinity that enables them to identify a specific target molecule. Owing to their small size, ease of synthesis and labeling, low cost, and high affinity and specificity toward targets, aptamers have been extensively applied in bioassays. Hence, in such bioassays, as more bacteria cells are added to the electrochemical cell, the current falls further because more cells are captured by the aptamers that are immobilized on the electrode, increasing the impedance of the electrode to a greater extent. The detection limit of this aptamer-based biosensor is 3 CFU/mL. This new method can be used to detect pathogens rapidly and sensitively with a high specificity. Therefore, in a label-free impedancemetric sensor for the detection of food pathogens, gold nanoparticles can be applied on the electrode to increase the amount of immobilized biomolecules, such as antibodies or aptamers, and to improve conductivity to improve the sensitivity of the assay.

Conclusion and Future Directions

To improve the assay sensitivity, various nanoparticles have been used in biosensors for the detection of hazardous contaminations in food, such as food pathogens, food allergens, bacterial toxins, fungal toxins, and pesticides. The benefits of using nanoparticles in biosensors include increasing the surface area for the immobilization of capture reagents on the transducer element of a biosensor, as well as providing the orientational freedom for capture reagents to interact with the target molecules in samples. Moreover, nanoparticles can be labeled on the detection reagents to be used as signal enhancers. Conclusively, gold nanoparticles, magnetic nanoparticles, gold-coated magnetic nanoparticle, and silica nanoparticles have been applied in SPR biosensors, for either improving the immobilization of antibodies or for being the signal enhancer. Regarding QCM biosensors, gold nanoparticles are the most often used nanoparticles for being the “mass enhancers” or for improving the efficiency of immobilization for capture antibodies on the gold chip, while magnetic nanoparticles are mainly used for mass enhancement. Finally, for electrochemical biosensors, gold nanoparticles are mostly used for improving the efficiency of immobilization for DNA probes or antibodies, while liposomal nanovesicles and CdS nanocrystals are used for signal enhancement. These applications of nanoparticles in biosensors are organized in Table 1. With a proper design and a thorough optimization for the size and shape of nanoparticles, the usages of nanoparticles have a great potential to enhance a performance of a biosensor, and this is the reason why the application of nanoparticles in biosensors has become increasingly popular.

Table 1 Nanoparticle-based SPR biosensors in food safety

Target	Assay format	Type of NPs	Function of NPs	Sample	Working range	Limit of detection	Refs
SPR biosensors							
<i>Escherichia coli</i>	Immune direct	Gold-coated magnetic NP	Isolation and signal enhancer	PBS	$30-3 \times 10^4$ CFU mL ⁻¹	3 CFU mL ⁻¹	Torun et al. 2012
<i>Salmonella</i> Typhimurium	Immune sandwich	Gold NP	Immobilization	PBS	10^4-10^6 cells mL ⁻¹	10^4 cells mL ⁻¹	Ko et al. 2009
Ara h1	Immune sandwich	Magnetic NP	Signal enhancer	Chocolate	0-1.8 ug mL ⁻¹	0.09 ug mL ⁻¹	Pollet et al. 2011
Ochratoxin A	Immune competitive	Gold NP	Signal enhancer	HBS-EP buffer	0-1 ug mL ⁻¹	0.042 ng mL ⁻¹	Yuan et al. 2009
Fluoroquinolone	Immune competitive	Gold NP	Signal enhancer	PBS	Not available	0.07 ug L ⁻¹	Fernandez et al. 2012
OCM biosensors							
<i>Escherichia coli</i> O157: H7	DNA sandwich	Magnetic NP	Signal enhancer	PBS	$2.67 \times 10^2-2.67 \times 10^6$ CFU mL ⁻¹	2.6×10^2 CFU mL ⁻¹	Mao et al. 2006
<i>Escherichia coli</i> O157: H7	DNA sandwich	Gold NP	Signal enhancer	PBS	$1.2 \times 10^2-1.2 \times 10^6$ CFU mL ⁻¹	1.2×10^2 CFU mL ⁻¹	Chen et al. 2008

<i>Salmonella</i> Typhimurium	Immune direct	Magnetic NP	Separation+	PBS	10^5-10^8 cells mL ⁻¹ (ΔF)	10^2 cells mL ⁻¹ (ΔR)	Su & Li 2005
			Concentration+	Chicken meat	10^6-10^8 cells mL ⁻¹ (ΔR)		
			Signal enhancer				
<i>Salmonella</i> Typhimurium	Immune sandwich	Gold NP	Signal enhancer	PBS	$10-10^5$ CFU mL ⁻¹	$10-20$ CFU mL ⁻¹	Salam et al. 2013
	Immune direct	Gold NP	Immobilization	60 % ethanol	$1 \times 10^1-2 \times 10^5$ ppb	8 ppb	Chu et al. 2012
Electrochemical							
<i>Salmonella</i> Typhimurium	DNA sandwich	Gold NP	Immobilization	PBS	10-500 fmol	9 fmol	Marques et al. 2009
	Immune sandwich	Liposomal nanovesicles	Signal enhancer	PBS	$10^{-14}-10^{-7}$ g mL ⁻¹	10^{-16} g	Viswanathan et al. 2006
<i>Salmonella</i> Typhimurium	Immune direct	Gold NP	Immobilization	PBS	10^3-10^7 CFU mL ⁻¹	5×10^2 CFU mL ⁻¹	Dong et al. 2013
	Immune direct	Fe@Au NP	Immobilization	PBS	10^1-10^6 cells mL ⁻¹	13 cells mL ⁻¹	Freitas et al. 2014
<i>Salmonella</i>		CdS NP	Signal enhancer				
	Immune direct	Gold NP	Immobilization	PBS	$2.4-2.4 \times 10^3$ CFU mL ⁻¹	3 CFU mL ⁻¹	Ma et al. 2014

Cross-References

- ▶ [Advances of Nanomaterials for Food Processing](#)
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Abstract

Modern food for human consumption has transformed significantly from simple raw foods to post-processed foods. Among the various technologies to manipulate the raw materials of food, nanotechnology attracts a lot of attention from food manufactures. The obvious benefits of nanoscale food and associated nanomaterials for food processing have been highlighted, such as higher bioavailability in the gastrointestinal tract of humans and a superior gas barrier for the packaging of oxygen-sensitive foods. The methods to encapsulate food

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components using nanoscale vesicles consist of emulsion, liposomes, solid lipid nanoparticles, and various novel vesicles. Multiple functions of higher bioavailability and reduced oxidation are therefore added to the raw foods. Food packaging utilizing nanotechnology include active packaging and intelligent packaging (or so-called 'smart packaging'). The discovery of improved properties of the gas barrier by the introduction of nanoscale clays (and organic modified ones) into the packaging polymers emerged two decades ago. As security and safety issues are frequently raised, packages embedded with nanosensors to communicate with consumers were introduced later. This chapter addresses the nanomaterials for food packaging and encapsulation, and food safety.

Introduction

The idea of nanotechnology has been widespread in various disciplines since the lecture of Nobel laureate Richard P. Feynman in 1959. The synthesis of nanomaterials can be achieved through either top-down methods or bottom-up approaches, and many significant advances in nanomaterials have been documented. For instance, carbon nanotube, discovered by Sumio Iijima, has a hollow structure that is known to show unusual properties of heat and electrical conductance as compared with traditional graphite. In nano-electronics, the giant magnetoresistance (GMR) effect of ferromagnetic materials and conductive materials layered in a few atomic spaces was utilized in hard disk drives with huge capacity and various electronic devices. In food chemistry and processing, the development of nano-sized nutritional food was found to increase the gastrointestinal absorption in the human body. The addition of nanoscale gold in wine showed accelerated conversion of aldehydes in wine. The gas barrier of food packages was also advanced with the application of nano-clay exfoliated in traditional packaging polymers such as PET (polyethylene terephthalate) and nylon. These novel packaging materials benefit the packaging of beverages greatly, allowing for an extended shelf-life.

It is expected that more and more applications of food nanotechnology will emerge. The contamination of food during processing and storage can often threaten the health of consumers; however, the expiration of food cannot rely solely on the package labels. Therefore, nanoscale biosensors for the surveillance of the shelf-life of food emerged to meet those demands. As the demand for food safety from consumers has increased tremendously, guidance for the manufacturing and storage of food has been established in the USA, European countries, and other developed countries. The spirit of the guidance is to ask food manufacturers to undertake scientific evaluation of safety for food manipulated with nanotechnology. Based on this background knowledge, this chapter focuses on the nanotechnology-driven materials for food processing, particularly packaging materials.

Nanotechnology in Food Packaging

Nanotechnology is known as one of the novel techniques that influence the world economy and society in the early twenty-first century. Broadly speaking, nanotechnology is “a technique at the nanoscale.” The nanoscale is determined on a range from 1 to 100 nanometers. The US National Nanotechnology Initiative declared that nanotechnology is concerned with materials whose structures and components express significantly improved physical, chemical, and biological properties due to their nanoscale size. Another definition of nanotechnology is the design, manipulation, building, production, characterization and application, by controlling the shape and size, properties-responses and functionality of structures, devices and systems of the order or less than 100 nm (Sattler 2011; Bhushan 2004). In 2008, more than US\$15 billion was spent on the research and development of nanotechnology worldwide. Nanotechnologies are projected to impact at least US\$3 trillion across the global economy by 2020 (Roco et al. 2011). In recent years, nanotechnology has become an emerging area with more than 50,000 technology articles published globally (Huang et al. 2011).

The emergence of nanomaterials has benefited the food industry, allowing new processing methods and advanced functions of foods; the food components are manipulated at a micro-/nano-length scale, leading to the desired structures of food. The interaction of nano-sized food components has been discovered to improve food functionality, such as nutrient accessibility, microbiological stability, gas transport properties, and food texture (Aguilera 2005; Sanguansri and Augustin 2006). Additionally, different nanomaterials exist for packaging food components. For instance, the encapsulation of food ingredients to give nanoemulsion could increase nutrient accessibility (Brusewitz et al. 2007). Hybrid films of PET and clay have exhibited better gas barrier properties, resulting in an extended shelf-life of food while retaining the transparency of the PET films (Wu et al. 2002).

Active Packaging Polymers

Food packaging was originally employed as a barrier to preserve food during the shelf-life. In the US Federal Food, Drug and Cosmetics Act, the purpose of a package is defined as to prevent the manufacture, sale, or transportation of adulterated, misbranded, or poisonous deleterious foods, drugs, medicines, and liquors. Physically, it prevents the scattering of foods and the mixing of components in the package and containers. In addition, the packaging is used to maintain the quality of the food at the final stage of processing. The reasons for the quality of food being lost are rooted in the interaction of food with substances permeated from the environment, e.g., the loss (or gain) of water, ethylene, or oxygen. Substances building up in the package can also lead to the deterioration of food quality, such as cooking odors in oxidized fats and oils. The science of food packaging is based deeply in the polymer processing in which various forms of barriers (packages) are produced. The complex structure of the barrier is divided into several types, such as the monolithic polymer

film, lamination of two or more polymers, reactive laminates, coated laminates, and membranes filled with impermeable inorganic particles. The packaging materials are intended to interact with food components in either a passive or active manner. The packaged foods can remain in a fresh status because the properties of food, including water activity, pH, the presence of bacteria, oxygen, light, and carbon dioxide (CO₂), are maintained by packages. The micro-climate within the package, in particular moisture and gas, is controlled for specific foods. The permeability of packages of vegetables should be higher to prevent the condensation of vapor inside the package. Moisture uptake should be inhibited for dried foods.

The limitations of food processing, packaging, and marketing collectively led to the successful development of novel packaging materials and structures in the last two decades. The packaging constraints include occluded gases and odors built into the package and the permeation of gases. The oxygen dissolved in food can be removed in the vacuum or through nitrogen flushing. However, such processing methods are limited for beverages because of frothing (the formation of bubbles). The oxidation of fats and oils can increase development of aldehyde in the food in a time-dependent manner. Similarly, a simple package cannot remove CO₂ released from roasted coffee beans. Therefore, functional packaging (or so-called 'active packaging') is considered instead of simply providing a barrier for food. Moreover, the 'green' lifestyle and consumer awareness of environmental protection also drive the selection of polymers for the packaging of food, e.g., the use of recyclable or degradable polymers. The cost reduction in food processing also leads to high economic demand to use functional packaging materials by food manufacturers. All of these constraints led to the development of active packaging (Labuza and Breene 1989).

In recent years, packaging polymers for food have advanced significantly in both food quality and convenience. They not only solve existing drawbacks of traditional food packaging, such as nutrient loss, odor formation, microbial spoilage, and premature ripening, but also enable more convenient food use, such as easy-to-use packages and self-heating/-cooling cans. Their functions can vary from passive protection of food to intelligent sensors embedded in the packaging structure, such as to release flavor into food when there is a demand by the consumers.

In response to consumer demands, active packaging and intelligent packaging have been introduced in recent years. These two types of packaging materials are differently defined: the former can detect and respond to changes in and out of the food package, while the latter can sense and display quality changes in the food to consumers. Therefore, intelligent packaging indicates food availability for consumers during its shelf-life. Some examples of innovations in active packaging are as follows:

- For meat, bread, fruits, and vegetables, antimicrobial release of surface amines from nylon films can retard the spoilage of foods.
- The release of antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) from the package can be used to prevent fat oxidation.

- Based on a similar concept, CO₂ absorbers for coffee, release of CO₂ from a sachet for the inhibition of Gram-negative bacteria, ethylene scavenging for climacteric fruits, and moisture control on the surface of packaging materials for meat, bakery items, and cut fruit and vegetables have been practiced in the food industry. Currently, scavengers of oxygen, CO₂, ethylene, and moisture are the most commonly used commercially.

In contrast to active packaging, intelligent packaging provides indicators for communicating with consumers (Schilthuisen 1999). For instance, radio frequency identification (RFID) tags and magnetic strips are currently used for the surveillance of food. Food in modified atmosphere packages (MAPs) is monitored with gas concentration indicators such as redox dyes, pH dyes, and enzymatic labels. Temperature indicators and microbial indicators are also embedded in the packaging of food for the surveillance of freshness.

Polymers are macromolecules consisting of repeated monomers. Depending on the molecular weights and the intra-/inter-molecular interactions, the physical status of the polymers can vary from liquid, gel, elastomer, semi-rigid to very hard solid. The wide range of mechanical properties of the polymer can be best fitted into the various needs of the food packaging. Polyolefins is the collective description for a type of polymer made from hydrocarbon monomers. Polyethylene (PE) and polypropylene (PP) are the most common polyolefins for packaging purposes. PEs are classified by their density into different PEs, such as low-density PE (LDPE), high-density PE (HDPE), and other PEs. The density of PEs is affected by the degree of chain branching. LDPE is usually used for packaging of bread and baked goods. HDPE has been developed for dairy products and bottled water. Polybutene-1 shows flexibility and creep resistance. It has been used to mix with PE for peelable packaging. 4-Methylpentene-1-based polyolefin is a transparent polymer used in the gas-permeable packaging of fruit and vegetables. Polyester is a large class of packaging polymers, among which PET is the best known for beverage bottles. These polymers are transparent and semi-crystalline. Polycarbonate (PC) is a polymer prepared by the condensation polymerization of diacid and diol. Its clarity is close to that of glass bottles and it also has high heat resistance. Polystyrene (PS) has three different forms, including general PS, oriented PS, and high-impact PS. General PS is a widely employed polymer for packaging of yoghurt and meat, egg, and fruit and vegetable trays. Oriented PS is usually laminated for food plates and trays. High-impact PS is produced by mixing it with the impact modifier polybutadiene. Therefore, it is suitable for shipping packaging for foods. Polyvinyl chloride (PVC) is a rigid polymer, making it suitable for boxes of food. However, the plasticizer used in the production of PVC boxes or other packaging structures raises high attention due to the toxicity of the plasticizer in humans. Polyvinylidene chloride (PVDC) is structurally similar to PVC, but an additional chloride atom is present in the repeat unit. Its trade name is Saran™ PVDC wrap; either single-layer or coextruded films are used to prevent gas transmission for food packaging. Nylon is also a large class of polyamide produced by the condensation of diacid and diamine. Among various nylons,

Grilamid[®] L 25 shows good flexibility and is a food contact-acceptable polymer. It is polymerized from a single monomer, aminolauric acid. Introduction of a bulky tertiary group in the monomer of nylon results in the amorphous polymer, Nylon 6-3-T. This type of polymer is crystal-clear and exhibits high impact strength for food packaging. Ethylene-vinyl copolymers exhibit an antistatic property, clarity, and printability for food packaging. It can be fabricated into rigid or flexible packaging.

The use of renewable raw materials for the production of polymers is an emerging area. Biodegradable or compostable polymers are also considered in food packaging because of the high demand of environmental protection and a green lifestyle. However, there are limited numbers of renewable/degradable polymers available on the market. Polylactic acid (PLA) is a biodegradable polymer derived from cornstarch. Polyglycolic acid (PGA) is structurally similar to PLA. Cellophane[™] is a kind of cellulose ester obtained from wood, cotton, or other sources. Polycaprolactone (PCL) is biodegradable polyester made from cyclic ϵ -caprolactone. Polyhydroxyalkanoates (PHAs) are naturally derived polyesters.

Nanocomposite Packaging Materials

There are many potential applications of nanomaterials in food packaging, including nanocomposites, nano-coating, surface biocides, active packaging, and intelligent packaging. Nanocomposites could be added into the packaging materials to improve their physical performance, durability, barrier properties, and biodegradation. Nano-coating techniques could improve the barrier properties by the incorporation of coating nanomaterials onto the inside or outside of the packaging surface, or by sandwiching some nanomaterials in a laminated layer. For surface biocides, nano-scale biocidal agents would be incorporated onto the packaging surface to maintain the hygienic condition of the food contact surface, such as food-processing or food-handling equipment. The principle of active packaging is intended to change the character of the composition of food and the atmosphere surrounding the food in the pack. Thus, active packaging techniques could be applied to incorporate nanomaterials with specific properties, such as antioxidant or antimicrobial agents, and consequently affect the packaged food by releasing the nanomaterials internally into it. Furthermore, intelligent packaging could be designed to monitor and report the conditions, such as gas release and temperature changes, of the food by using appropriate nanosensors (Bradley et al. 2011).

In the late 1990s, the nanocomposites emerged in response to the demand for high barriers for food packages. The nanocomposites are a class of nanomaterials that have polymers that are intimately contacted with nanoscale particles. For instance, nylon-6 incorporated with ultra-thin nanoscale silicate platelets could reduce the oxygen transmission rates to four times lower than original nylon-6 (Akkapeddi et al. 2003). The silicates, known as montmorillonite (MMT; $[\text{Na,Ca}]_{0.33}(\text{Al,Mg})_2(\text{Si}_4\text{O}_{10})(\text{OH})_2 \cdot n\text{H}_2\text{O}$), are a class of layered clay which can be broken down into nano-sized platelets. The strength of the oxygen barrier was

further increased by thermal blending of polymeric oxygen scavengers. For instance, commercially available Amosorb[®] is fabricated by incorporating poly (1,2-butadiene) segments into the backbone of PET at percentage of 0.5–12 wt% (Cahill and Chen 2000). The improved barrier properties of this type of packaging material enhance the storability of oxygen-sensitive drinks such as fruit juices and beer. Similarly, lamination of an ethylene-vinyl alcohol (EVOH) polymer between two layers of PET, which is defined as a passive barrier, has also been reported (Mahajan et al. 2013). The commercially available nanocomposites include nylon6-MMT (Nanacor, Inc.), nylon6-MXD6 (Mitsubishi Gas Chemical Co., Inc.), polyamide 6-silicate (Byaler Polymers), nylon co-injection stretch blow molding with PET (Aegis[™] OXCE, Honeywell Polymers), etc.

An interesting application of nanocomposites as a food packaging material for soldiers has been developed by the US Army Natick Soldier Research, Development and Engineering Center (NSRDEC). Meal, Ready to Eat[™] (MRE[™]) is designed to withstand rough conditions as it is carried by soldiers, and everything can be eaten without cooking. Civilian MRE[™] foods are also available for the emergency use in natural disasters.

The reduced gas permeability of nanocomposites has been modeled as the tortuous pathway where moisture, oxygen, and other gases require a longer diffusion length to transport across the packaging membrane. Looking into the interfacial region of polymer and nano-clay, the free volume of the nanocomposites is attenuated as indicated by positron annihilation lifetime spectroscopy (Choudalakis and Gotsis 2009). As a result, the hopping rate of migrant gases between free volumes is decreased. The first successful nanocomposite was developed by Toyota Corporation in 1986 as nylon-6 was blended with nanoscale MMT for better heat resistance and mechanical strength (Kawasumi 2004; Krishnamoorti and Vaia 2002). To date, various polymers have been studied to incorporate nanoscale clays such as MMT, kaolinite, mica, graphite, silicon dioxide (SiO₂), and calcium carbonate (CaCO₃) (Duncan 2011). The organic modified MMTs have been reported to decrease oxygen permeability because the *d*-spacing between the clay platelets increased as the number of long alkyl chains on the octadecyl ammonium modifier increased (Osman et al. 2005). Interestingly, a layer-by-layer (LbL) method has been developed to modulate the gas permeability. The MMT clay solution bearing a negative charge to the solution of positive-charged poly(ethylene imine) is coated alternately along the polymer solution. It was reported that if the polymer-clay nanocomposite could efficiently be oriented perpendicular to the diffusion direction of gases, the permeability can be reduced to an almost undetectable level (Priolo et al. 2010).

Because of the awareness of a sustainable lifestyle by customers, bio-based polymers are considered potential sources of polymers for the production of nanocomposites. These polymers are biodegradable, but they are generally hydrophilic. Therefore, the polymers are engineered with nanomaterials to improve the mechanical and gas barrier properties (Uskokovic 2007). The source of biopolymers can be plant-derived such as starch, cellulose, or protein, animal-derived such as protein and polysaccharides, microbial-derived such as polyhydroxybutyrate

(PHB), or synthetic polymers derived from a natural monomer such as PLA (Arora and Padua 2010). Among these bio-based polymers, starch is cost effective compared to petroleum raw materials. Currently, corn is the major source of starch for application of biodegradable polymers, while other sources such as potatoes, wheat, rice, barley and oats are also being considered to replace starch. With a similar mechanism to the synthetic nanocomposites mentioned earlier, the addition of MMT clay in biodegradable starch resulted in reduced water uptake due to the tortuous diffusion pathway in the starch-clay films (Cyras et al. 2008).

The material that enrobes food to extend its shelf-life and which can be eaten together with the food is called edible film, and provides functions such as a barrier to oxygen, moisture, solute movement, and aromas (Weiss et al. 2006). It is time-consuming for a food product to reach the consumer's table; typically, the food production steps include handling, storage, and transportation. During these processes, food may start to dehydrate, deteriorate, and lose its appearance, flavor, and nutritional value (Pavlath and Orts 2009). In order to maintain the freshness and safety of food products, edible film can be applied to the food surface to provide protection. Nano-lamination, a kind of nano-coating, is currently used for the production of edible film, based on two or more nano-layers (1–100 nm/layer) bound together by chemical or physical interactions. For instance, the LbL deposition technique is suitable for construction of edible film, with precise control on the thickness and properties of nano-lamination. Based on electrostatic forces, each nanolayer deposited in the LbL technique can be self-assembled on the food surface (Decher and Schlenoff 2003). Polysaccharides, proteins, and lipids are film-forming materials that are electrostatically charged and are suitable to incorporate enzymes, flavors, colors, or specific materials with functionalities such as antimicrobial, anti-browning, and antioxidant functions. Normally, the nanolaminate film can be coated on the food surface by immersing the food into a series of coating solutions or by directly spraying the coating solution onto the food surface (Weiss et al. 2006). Medeiros et al. (2014) applied the alginate/lysozyme nanolaminate to extend the shelf-life of 'Coalho' cheese, a semi-hard cheese of economic and social importance in Northwest Brazil which has been confirmed to have the presence of spoilage microorganisms (*Staphylococcus aureus* or *Salmonella* spp.) (Borges et al. 2003; Perry 2004; Santana et al. 2008). Alginate is a natural anionic polysaccharide and lysozyme is a protein with good antibacterial properties (Saether et al. 2008; Ibrahim et al. 2001). Addition of a nanolaminate coating of alginate/lysozyme on 'Coalho' cheese was capable of ensuring its quality and safety. The cheese was repeatedly immersed into the alginate solution and lysozyme solution for the formation of "Algi-Lyso-Algi-Lyso-Algi" five-layer nanolamination. Compared with uncoated cheese, the coated cheese gave lower values of mass loss, pH, lipidic peroxidation, and proliferation of microorganisms after 20 days. Thus, the alginate/lysozyme nanocoating could be used as a gas barrier and antibacterial coating to extend the shelf-life of 'Coalho' cheese (Medeiros et al. 2014). Regarding to the applications of nanocoating for fruits, 'pectin and chitosan' and 'κ-carrageenan and lysozyme' coating layers have been developed for protecting the surface of Tommy Atkins mangoes and Rocha pears, respectively

(Medeiros et al. 2012a, b). Pectin is an anionic biopolymer with a low permeability for oxygen, while chitosan is a natural cationic polysaccharide with good gas barrier properties. In addition, κ -carrageenan is a sulfated polyanionic polysaccharide with good gas barrier and optical properties. The results of these studies showed that nanolaminate films possibly reduced gas flow and microbial proliferation on the fruit surface and also maintained the quality (color and texture), subsequently extending the shelf-life of mangoes and pears.

In addition to acting as a gas barrier, nano-composition may exhibit antimicrobial or antioxidant properties by incorporating specific nanoparticles into polymeric materials. This system will be more effective than the traditional systems because nanoparticles and nano-scaled films have high surface-to-volume ratios (Damm et al. 2008). Therefore, the efficiency of this nano-composition system is associated with particle size, size distribution, the degree of nanoparticle agglomeration, nanoparticle content, and interactions between the nanoparticle surface and base polymer (Kim et al. 2007). Silver nanoparticles are the most widely used for the development of nanocomposite antimicrobial systems due to their wide-ranging antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi (Cushen et al. 2012). The general explanation for the antimicrobial activity of silver include (a) structural changes and deformation due to binding of silver to negatively charged biomacromolecular components (disulfide or sulfhydryl groups of enzymes) and nucleic acids to alter their properties (Butkus et al. 2003; Feng et al. 2000); (b) membrane damage caused by the release of silver ion (Ag^+) on the cell membrane surface catalyzing the formation of reactive oxygen species (ROS) (Kim et al. 2007); and (c) a high permeability of the cell membrane due to the accumulation of silver on the cell membrane surface resulting in cell death by osmotic pressure (Sondi and Salopek-Sondi 2004). Recently, Lin et al. (2015) developed a silver/titanium dioxide/chitosan adipate ($\text{Ag}/\text{TiO}_2/\text{CS}$) nanocomposite for fruit storage. The minimum inhibitory concentration of $\text{Ag}/\text{TiO}_2/\text{CS}$ nanocomposite was $0.38 \mu\text{g Ag/mL}$ and reduced 6 logs of *Escherichia coli* after 24 h of incubation. The $\text{Ag}/\text{TiO}_2/\text{CS}$ nanocomposite could exhibit a higher antimicrobial ability than silver nitrate (AgNO_3) or nano-Ag particles at $100 \mu\text{g/mL}$. Hence, this nanocomposite could be applied as an antibacterial protective coating for fruit storage (Lin et al. 2015). In order to study the size effect, Moura et al. (2012) incorporated two batches of silver nanoparticles (41 and 100 nm) into a hydroxypropyl methylcellulose (HPMC) matrix as a nanocomposite coating, and the smaller silver nanoparticle showed a better antibacterial ability against *E. coli* and *S. aureus*. A technique of combining antimicrobial silver with LDPE nanocomposite films for the modified atmosphere packaging could significantly extend the shelf-life of chicken breast fillets (Azlin-Hasim et al. 2015).

Intelligent Packaging Technology

The intelligent functions of food packaging can be divided into the following: communication with consumers (or retailers), indicators for gas inside the package,

records of temperature history, physical shock indicators, etc. Of course, combinations of the above sensors and newly developed sensors to meet specific demands will emerge in the near future. The value propositions for the development of intelligent packaging are consumer engagement, quality assurance, authentication, and efficient tracking of the products, respectively.

Intelligent packaging technology could be developed to detect the gas composition changing in the package headspace as a seal and leak indicator. Gases such as oxygen and CO₂ are used to monitor food quality because changes in the concentrations of these gases are mostly caused by chemical or enzymatic reactions in foods while the package is not sealed appropriately. Thus, by detecting the atmospheric changes in the package headspace with nanomaterials, the quality of food products can possibly be evaluated. For example, detection of CO₂ being formed from a calcium hydroxide-containing nanosensor, or detection of ethanol or oxygen by a redox indicator incorporated into PP and carbon nanotubes (Pereira De Abreu et al. 2012).

Regarding food safety, the expiration date is usually the only information to determine whether the food is expired or not. With nanotechnology, nanosensors embedded in the package can indicate when food is spoiled or unpalatable. For example, gold nanoparticles (AuNPs) can respond to the melamine content in milk by aggregation in which the color changes from red to blue in a dose-dependent manner (Ai et al. 2009). It has been proven that melamine and cyanuric acid (CA) form a specific triple hydrogen-bonding structure (Lehn et al. 1990), and, therefore, this recognition between melamine and CA was used for detecting the presence of melamine in milk. Firstly, a kind of thiol-functionalized CA derivative was tagged on AuNPs to form well-dispersed colloids in distilled water with a wine red color. After exposure to melamine, the hydrogen-bonding recognition between melamine and CA tagged on the AuNP surface resulted in the aggregation of AuNPs, leading to the color change of AuNPs from red to blue. The result of this assay could be observed with the naked eye within 1 min and the detection limit was 2.5 ppb melamine in milk. This rapid assay has great potential for being applied as an on-site and real-time detection method, and could be integrated into food packages to detect the presence of melamine in milk products. Other than optical methods, electrochemical sensing of toxins in the food has been devised. Microcystin-LR (MC-LR) is known as a toxin secreted by cyanobacteria, which causes acute liver failure and promotes the primary liver cancer after a long exposure to the contaminated drinking water (Zhou et al. 2002). In 2009, a sensor was developed to detect MC-LR in water with the use of an anti-MC-LR antibody tagged single-wall carbon nanotube (SWNT-Ab) immobilized on paper to prepare a smart electronic textile using electrochemical current-time transients (Wang et al. 2009). A detection limit of 0.6 nmol/L of toxin was achieved to satisfy the World Health Organization guideline for MC-LR in drinking water (1 ng/mL). The sensitivity of this electrochemical sensor was comparable with that of the traditional enzyme-linked immunosorbent assay (ELISA). As the entire assay time including sample preparation only required 30 min, there was a greater improvement than the general operation time for ELISA (>14 h).

The detection of microorganisms is an urgent issue since food-borne pathogens are highly infectious to people consuming contaminated food. The most convenient approach to detect microorganisms is the immunologic assay (Heo and Hua 2009). To that end, the surface of nanomaterials is functionalized with an antibody for either optical detection or electrochemical sensing. Although there have been numerous nanosensing probes devised for biomedical applications, real foods contain a complex matrix that can lead to a poor signal-to-noise ratio. For the detection of *E. coli* in food, antibody-tagged magnetic nanoparticles were developed to selectively capture *E. coli* for subsequent quantification (Yang et al. 2007). Such a detection method is efficient as the isolation of the target analyte prior to detection is not required. Concerning personalized food, additives-encapsulated nano-formulation has been developed. Users can heat up food with a microwave to trigger the release of the colorant and flavor according to their choice. Additionally, different activation methods for the release of flavor, color, and nutrient contents from nano-emulsions have been developed such as ultrasound and pH.

Nanotechnology in Encapsulation

Currently, the definition of nanotechnology is large and includes research, technology development, and structure from 1 to 100 nm (Uskokovic 2007). Nanoencapsulation, a branch of nanotechnology, is a remarkable technology in food processing that entraps active agents within a carrier material and it is an effective tool to enhance delivery of bioactive molecules (e.g., antioxidants, minerals, vitamins, phytosterols, lutein, fatty acids, lycopene) and living cells such as probiotics into food (Zuidam and Nedović 2009; De Vos et al. 2010). The encapsulated substance is called the core, fill, active, internal, or payload phase. Otherwise, the substance used to entrap payload phases can be named the membrane, shell, capsule, and external phase (Zuidam and Nedović 2009; Fang and Bhandari 2010). Nowadays, nanoencapsulation remains one of the most promising technologies, having the feasibility to pack bioactive compounds. Nanoencapsulation of bioactive compounds has many advantages for targeted site-specific delivery and efficient absorption through cells. Nanocapsules are vesicular systems in which the bioactive compound is covered by a cavity surrounded by a polymer membrane, whereas nanospheres are matrix systems in which the bioactive compound is uniformly dispersed, as shown in Fig. 1 (Anandharamkrishnan 2014).

Materials for Nanoencapsulation

Many materials could be used for nanoencapsulation, with various types and properties. However, regulations for food additives are more rigid than other industry standards, e.g., for pharmaceuticals. In fact, the whole food process should

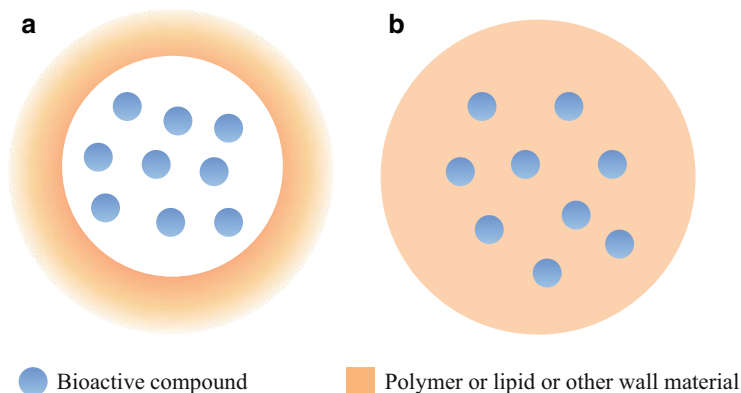


Fig. 1 Structural scheme of (a) nanocapsules and (b) nanospheres

be designed in order to meet the safety requirements of governmental agencies such as the European Food Safety Authority (EFSA) or US Food and Drug Administration (FDA). Most materials used for nanoencapsulation are biomolecules. Suitable materials for nanoencapsulation are selected depending on the different purposes of the food process. However, materials used for the design of the external phase of encapsulates must be food-grade and biodegradable. The most widely used materials in food applications are polysaccharides, and starch and cellulose and its derivatives are also commonly used. Plant exudates and extracts – gum arabic, gum tragacanth, gum karaya, mesquite gum, galactomannans, pectins, and soluble soybean polysaccharides – are also employed. Recently, marine extracts such as carrageenans and alginate are also materials for encapsulation. Microbial and animal polysaccharides such as dextran, chitosan, proteins, and lipids are also used in food applications. Furthermore, other materials are employed such as PVP (polyvinylpyrrolidone), paraffin, shellac, and inorganic materials (Zuidam and Nedović 2009). Bioactive compounds can be classified as lipophilic and hydrophilic on the basis of their solubility in water. Hydrophilic compounds are soluble in water but insoluble in lipids and organic solvents. Lipophilic compounds are insoluble in water but soluble in lipids and organic solvents (Anandharamkrishnan 2014).

Types of Food Encapsulation

Nanoencapsulation allows protection of the sensitive bioactive food ingredients from unfavorable environmental conditions, eradication of incompatibilities, solubilization, or masking of an unpleasant taste or odor. In this section, the present status of the art of applications of nanoencapsulation, including nanoemulsions, nanoliposomes, and solid lipid nanoparticles (SLNs), are discussed.

Nanoemulsions

Nanoemulsions are nanoscale droplets formed by dispersing one liquid in another immiscible liquid by physical shear-induced rupturing. Nanoemulsions have some excellent physical properties as compared to microemulsions. The droplet sizes in nanoemulsions are much smaller than visible wavelengths; therefore, most nanoemulsions appear optically transparent (McClements and Li 2010). This very special nature of nanoemulsions allows them to be applied as the nutrient carriers in beverages. An interesting property of nanoemulsions is that they are metastable and can be diluted with water without change in the droplet size distribution. Nanoemulsions can deliver poorly water-soluble food ingredients, such as fish oil and lipophilic vitamins, owing to their ability to raise bioactive solubilization and their potential for enhancing absorption in the gastrointestinal tract (Talegaonkar et al. 2010). Chitosan-coated lipid droplets can potentially be applied as useful carriers for the oral delivery of lipophilic compounds.

In order to have stable nanoemulsions, some parameters such as homogenization pressure or the homogenization cycle need to be studied. In 2015, Ha et al. tried to enhance the bioaccessibility of lycopene through nanoemulsion (Ha et al. 2015). Lycopene is a carotenoid in tomato, which has antioxidant, anti-inflammatory, anticancer, and anti-cardiovascular properties, but its bioactivity would be easily diminished in food processing. The size reduction of emulsions from 925 to 184–308 nm by an increase of homogenization pressures and cycles could enhance the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging ability and bioaccessibility of lycopene nanoemulsions.

Liposomal Nanoparticles

Liposomes are vesicular structures of colloidal dimensions, consisting of amphiphilic lipid-forming bilayers enclosing part of the aqueous phase in which they are dispersed. The lipids used are predominantly glycerophospholipids and sphingophospholipids; liposomes therefore have many of the properties of natural membrane-bounded structures. According to liposome's bilayer structure, vesicles can be classified as unilamellar vesicles (ULVs) and multilamellar vesicles (MLVs) (Nagle and Tristram-Nagle 2000). Liposomes trapped in water-soluble compounds have been widely applied in pharmacology and cosmetology. Antimicrobials being encapsulated in liposomes helps in preserving their effectiveness and stability in various applications (Were et al. 2003). Moreover, Colas et al. (2007) showed that nanoliposomes were prepared from different lipids to trap nisin and target bacteria. The stability of nanoliposome-encapsulated nisin was different with various nanoliposomes, such as at least 14 months at 4 °C for DPPC (dipalmitoylphosphatidylcholine):DCP (dicetylphosphate):CHOL (cholesterol) vesicles and 12 months at 25 °C for DPPC:SA (stearylamine):CHOL vesicles. Xia et al. have demonstrated that coenzyme Q10 (CoQ10) entrapped into nanoliposomes could inhibit the increase in the z-average diameter of nanoliposomes during storage for 8 months at 4 °C (Xia et al. 2007). Additionally, nisin-loaded ProLipo[®] H liposomes were transferred into cheese milk. After 6 months, 90 % of the initial nisin activity was recovered in nisin-loaded liposomes (Benech et al. 2002).

The encapsulation of pediocin AcH within PC liposomes enhances the activity of pediocin in slurries of heated beef muscle and tallow (Degnan and Luchansky 1992). In addition, Degnan et al. assessed that some nanoliposomes as pediocin AcH vectors in slurries of non-fat dry milk, butterfat, beef muscle tissue, or beef tallow were higher than free pediocin AcH (Degnan et al. 1993). Therefore, from the results, it proved the efficiency of liposomes in increasing bacteriocin activity and minimizing the negative effects observed for non-encapsulated pediocin (Colas et al. 2007). Moreover, liposome has been applied to enhance the antimicrobial activity of *Clove* oil. As *Clove* oil is unstable and easily loses its biological activity when exposed to air, light, moisture, and high temperatures, encapsulation of *Clove* oil by liposome could also be a useful tool to protect the sensitive ingredients from destruction. Liposome-encapsulated *Clove* oil (5 mg/mL), which had its highest entrapment efficiency at about 20.41 %, was used in tofu as an antimicrobial reagent, with a 3- to 4-log reduction of *S. aureus* after 24 h of incubation, and even in 120 h of incubation (Cui et al. 2015). The size and zeta potential of *Clove* oil were 149.2 nm and -24.5 , respectively. This result demonstrated that the use of liposome as a nanocarrier could enhance the antimicrobial activity of *Clove* oil against *S. aureus*, hence extending the shelf-life of tofu.

Solid Lipid Nanoparticles

SLNs have attracted the interest of scientists in pharmaceutical and food applied research in recent years (Awad et al. 2008). SLNs are particles consisting of a solid lipid shell matrix (Müller et al. 2000). SLNs have some distinct advantages for food applications, such as having high encapsulation efficiency, they avoid the use of organic solvents in their preparation, and the possibility of large-scale production and sterilization. SLNs have been used as a delivery system for unstable and lowly soluble food compounds. For instance, resveratrol presence in foods such as grapes, berries, and red wine is beneficial to human health, but has some limitations such as a short elimination half-life ($t_{1/2}$) in the human body, low oral bioavailability, and rapid metabolism. To overcome these limits, Pandita et al. applied SLNs to increase its bioactivity, photostability, and $t_{1/2}$ (Pandita et al. 2014). The resveratrol SLNs (RLNs) were composed of stearic acid, lecithin, PHOSPHOLIPON[®] 90G/poloxamer 188, and resveratrol. The particle size and entrapment efficiency were 134 nm and 88.9, respectively. The RLNs were capable of releasing 85 % of the preloaded resveratrol in 120 h. The RLN and drug suspension $t_{1/2}$ were found to be 11.51 and 2.37 h, respectively, in in vivo models. Therefore, using SLNs as a carrier for resveratrol could significantly prolong the $t_{1/2}$ of resveratrol, subsequently resulting in a better chance for resveratrol to reach the target sites as well as an improvement in its bioactivity.

Nanotechnology in Quality Monitoring

Food quality is the point of most concern for consumers as they purchase food products. The quality of a food product needs to be of benefit to both the safety and health of users. In order to monitor the quality of food products, various sensors

have been developed for detecting food pathogens, toxins, pesticides, herbicides, heavy metals, artificial food additives, or volatile organic compounds (VOCs). Due to the advanced developments of nanotechnologies, various nanomaterials have been applied in the establishment of sensors for monitoring food quality, by enhancing either the sensitivity or specificity of sensors.

Nanotechnology Applications in Food Storage

As storage time elongates, the quality of food products reduces by either the degradation of food components through oxidation or hydrolysis reactions or the growth of spoilage microorganisms. Therefore, nanosensors can be applied for the real-time monitoring of food quality in food storage. The applications of nanotechnologies, such as constructing nanostructures on the surface of the sensor, can either improve the selectivity or enhance the assay sensitivity.

Regarding the wine industry or the process of food fermentation, the measurement of VOCs, such as ethanol, is essential. Normally, gas chromatography (GC) and mass spectroscopy (GC-MS) are widely used for detecting VOCs (Kim et al. 2012). However, the GC-MS device occupies a large space and requires a well-trained technician to operate it. Therefore, it is not suitable for in-site detection in the field or in farm areas. Surface-enhanced Raman scattering (SERS) utilizes metallic nanostructures to enhance the electromagnetic field at localized surface Plasmon hot-spots to analyze vibrational fingerprints of molecular structures (Fan et al. 2011) and signal molecule detection has been proved by SERS (Kneipp et al. 1997). In 2014, Wong et al. developed a multiplex SERS for detection of the mixture of acetone and ethanol vapor using leaning nano-pillar substrate (Wong et al. 2014). As the VOC molecule was absorbed at the tip of leaning nano-pillar substrate, SERS signals were produced due to the occurrence of field enhancement at the localized surface plasmon hot spots between adjacent leaning nanopillars. Without any labeling method for enrichment of gas molecules on the surface of sensor, the limits of detection (LODs) for acetone vapor and ethanol vapor by this multiplex SERS are 0.0037 and 0.0017 ng, respectively.

Electrochemical gas sensors are also a promising method for detecting VOCs. Nanopowders of metal oxides, such as tin dioxide (SnO_2), tungsten trioxide (WO_3), zinc oxide (ZnO), and maghemite ($\gamma\text{-Fe}_2\text{O}_3$), have widely been used in developing gas sensors (Zhang et al. 2006; Baruwati et al. 2006; Wang et al. 2006), and SnO_2 is usually applied in detecting ethanol due to its advantages of easy production, low cost, and rapid response and recovery time (Comini et al. 2002). However, the sensors using SnO_2 normally need a very high working temperature above 300 °C. In addition, several organic polymer semiconductors also have been used in developing gas sensors, but they cannot provide good selectivity. Therefore, to overcome these disadvantages of metal oxides and organic polymers, hybrid composite sensors are being intensively investigated. Recently, a novel flexible ethanol gas sensor was developed by depositing a mixture of SnO_2 nanopowder, and poly-diallyldimethylammonium chloride (PDDAC) on as-patterned interdigitated

electrodes (Zhan et al. 2013). Compared with the sensor with SnO₂ alone, the SnO₂-PDDAC sensor provided significantly higher signals. In this hybrid sensor, SnO₂ provided a good selectivity to ethanol, while PDDAC acted as the binder and the dopant. The LOD of this sensor is 10 ppm of ethanol at room temperature and displays a good selectivity to ethanol.

Nanotechnology Applications in Food Safety

Safety is always the first priority for consumers when they buy any food products. Usually, foods are rich in nutrients for microorganisms, such as bacteria, molds, yeasts, or viruses, to grow. The presence of microorganisms or their toxins may cause foodborne diseases or outbreaks. Therefore, the development of rapid assays for detecting microorganisms or their toxins is essential for food safety. Commonly, foodborne diseases can be classified into two categories: infection and intoxication (Jay et al. 2005). Infection refers to the diseases caused by ingestion of the food contaminated by a large number of microbial cells. As these vital cells grow and invade the gastrointestinal system of human bodies, they may cause diarrhea, vomiting, or fever. For example, *Salmonella* is the common foodborne pathogen that causes infection in poultry products (Finstad et al. 2012), while *E. coli* causes infection in meat products, such as pork or beef (Ju et al. 2012). On the other hand, intoxication refers to the ingestion of food contaminated by toxins formed prior to consumption. The ingestion of microorganisms may be harmless. Thus, regarding detecting the source of intoxication, the target should be the toxins, instead of microorganisms. Staphylococcal toxins of *S. aureus* and aflatoxins of *Aspergillus flavus* are the classical bacterial toxin and mycotoxin representatives (Grumann et al. 2014; Richard 2007). In order to enhance the assay sensitivity and shorten the entire assay time for detection, various nanotechnologies have been applied in developing methods to detect foodborne pathogens or their toxins.

A biosensor integrates a biorecognition element that can specifically capture target analytes in samples, and a transducer element that enables the specific interaction with the target analytes on the biorecognition element to be converted into a digital signal using which the target concentration in the samples can be calculated (Luong et al. 2008). Biomolecules such as antibodies, enzymes, nucleic acid, lectin, or even cells are applied on the surface of the biorecognition element to specifically interact with the targets in samples and this interaction can be subsequently measured through an optical, electrochemical, or piezoelectric (mass-sensitive) mechanism of the transducing system. Currently, various types of biosensors have been developed with the applications of nanotechnology to detect the contamination of specific pathogens, based on analyzing its surface protein, toxin, or a specific DNA fragment of this microorganism. Commonly, nanoparticles are applied in biosensors to either enhance the signal intensity for a detection reagent or to improve the binding efficiency for a capture reagent.

E. coli O157:H7 is enterohemorrhagic and the characteristic symptoms of its infection are diarrhea, severe abdominal cramps, and hemolytic uremic syndrome, which may lead to acute renal failure in children. In order to get rapid detection and quantification of *E. coli* O157:H7 in meat and water samples, an electrochemical biosensor was constructed, based on the electrocatalytic properties of AuNPs for hydrogen evolution reaction (HER) and superparamagnetic microbeads (MBs) as pre-concentration or purification platforms (Hassan et al. 2015). In this study, the MBs were tagged with anti-*E. coli* O157 antibodies as a capture platform to specifically isolate and concentrate *E. coli* O157:H7 from samples, and then sandwiched with AuNPs modified with secondary antibodies also against *E. coli* O157:H7. Finally, 'AuNPs-*E. coli* O157:H7-MB' complexes were detected on screen-printed carbon electrodes with the use of chronoamperometric measurement. The LODs of this developed assay were 148, 457, and 309 CFU/mL in buffer solution, minced beef, and tap water samples, respectively. The electrocatalytic properties of AuNPs on hydrogen formation from hydrogen ions (HER) were utilized to enhance the assay signal. This strategy of labeling with gold nanoparticle conjugates to improve the assay signals has also been applied in the detection of anti-hepatitis B virus antibodies in human serum (De la Escosura-Muñiz et al. 2010) and cancer circulating cells (Maltez-da Costa et al. 2012) of clinical samples.

Optical immunoassay is also a common method for the detection of foodborne pathogens. With the use of antibody-tagged fluorescent nanoparticles, the target microbial cells can either be detected by fluorescent microscopy or flow cytometry. Recently, Chen et al. (2015) utilized fluorescent silica nanoparticles (SiNPs) for indirect immunofluorescence detection of *E. coli* O157:H7. The dye-doped SiNPs were synthesized with the w/o microemulsion method using 3-aminopropyltriethoxysilane (APTES) and fluorescein isothiocyanate (FITC), and subsequently polymerized with carboxyethylsilanetriol sodium salt (CEOS). In order to tag anti-*E. coli* O157:H7 antibody on the surface of SiNPs, firstly the surfaces of SiNPs were covalently conjugated with protein A using the carboxyl linker and then the Fc fragments of antibodies specifically bind to protein A. As compared with the conventional FITC dye molecules, the FITC-doped nanoparticles maintained a high fluorescence intensity even after 300 s of continuous excitation, indicating their good photostability. In addition, the result of detecting *E. coli* O157:H7 in beef samples using FITC-doped nanoparticles by flow cytometry was matched with that determined with the plate-counting method on Luria-Bertani agar. Compared with the conventional fluorescent dyes, FITC-doped nanoparticles provided a higher luminescence signal intensity and better photostability (Shen et al. 2012). Therefore, they have a high potential to be a signal amplifier in bioassays for detecting pathogens.

The presence of mycotoxins in foods is another big issue for food safety. Among the various mycotoxins, aflatoxin B₁ (AFB₁) is one of the important contaminants to monitor for in agricultural and food products due to its multiple toxic effects, especially carcinogenesis (Heidtmann-Bemvenuti et al. 2011). ELISA is the typical immunoassay technique for measuring AFB₁ and several commercial kits for

detecting AFB1 are on the market. However, this method needs to be performed for a couple of hours to form the detectable immune complexes through the diffusion-dependent heterogeneous reactions (Pound 1998). In addition, AFB1 is a non-polar molecule so it requires organic solvents such as methanol to effectively extract AFB1 from samples. However, the presence of methanol induces the denaturation of antibodies, resulting in the decreased assay sensitivity of an ELISA (Russell et al. 1989). In order to overcome these issues, the Dzantiev group utilized magnetic nanoparticles as carrier for immobilization of antibodies to generate a pseudo-homogeneous mode for an antibody–antigen immune interaction in samples, and resulted in a reduction of ELISA duration (Urusov et al. 2014). This developed assay gave an LOD of 20 pg/mL AFB1 while testing barley and maize extracts with an entire assay duration of 20 min. This low detection limit was below the maximum officially permissible level in different agricultural and food products. Thus, this mode using magnetic nanoparticles to immobilize antibodies was a potential alternative method for ELISA. In particular, it provided an important advantage of shortening the entire reaction time for those targets that require an organic solvent for their extraction.

Conclusion and Future Directions

Nanotechnology is currently a general term for both the public and scientific communities, and new findings in nanomaterials can spread to different industries. For instance, the improved heat resistance of nanoscale clay-exfoliated polymers was introduced first by an automobile producer. Improved gas permeation was later discovered for the packaging of food using a similar concept of clay-exfoliated polymers. The scientific community and industry also fully utilize nanotechnology based on its advantages. For example, the nanosensors fabricated as a type of microfluidic devices meet the industrial testing standards for food contamination. These testing devices provide multiples advantages compared to GC and tandem mass spectroscopy and other chromatographic instruments, including that they are portable to the site of food specimens, ultrasensitive to food-borne pathogens, their speed of testing, etc. In conclusion, added value of nanotechnology in food processing is synergistic, and food safety can also be improved with applied nanotechnology. It is expected that fundamental studies of nanotechnology will bring new insights to food science. The nanomaterials used in the food industry will definitely benefit consumers in the near future.

Cross-References

- ▶ [An Introduction to Food Nanotechnology](#)
- ▶ [Applications of Nanotechnology in Developing Biosensors for Food Safety](#)

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