Integrating Food Science and Engineering Knowledge
Into the Food Chain

Kristberg Kristbergsson
Semih Ötles *Editors*

Functional Properties of Traditional Foods

Springer

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Functional Properties of Traditional Foods

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Series Preface

The ISEKI-Food series was originally planned to consist of six volumes of texts suitable for food science students and professionals interested in food safety and environmental issues related to sustainability of the food chain and the well-being of the consumer. As the work progressed, it soon became apparent that the interest and need for texts of this type exceeded the topics covered by the first six volumes published by Springer in 2006–2009. The series originate in work conducted by the European thematic network ISEKI-Food an acronym for "Integrating Safety and Environmental Knowledge In to Food Studies." Participants in the ISEKI-Food network come from most countries in Europe and most of the institutes, and universities involved with food science education at the university level are represented. The network was expanded in 2008 with the ISEKI Mundus program with 37 partners from 23 countries outside of Europe joining the consortium, and it continues to grow with approximately 200 partner institutions from 60 countries from all over the world in 2011. Some international companies and nonteaching institutions have also participated in the program. The network was funded by the ERASMUS program of the EU from 1998 to 2014 first as FoodNet coordinated by Professor Elisabeth Dumoulin at AgroParisTech site de MASSY in France. The net then became known as ISEKI-Food and was coordinated by Professor Cristina Silva at the Catholic University of Portugal, College of Biotechnology (Escola) in Porto, from 2002 to 2011 when Professor Paola Pittia at the University of Teramo in Italy became coordinator of ISEKI-Food 4.

The main objectives of ISEKI-Food have been to improve the harmonization of studies in food science and engineering in Europe and to develop and adapt food science curricula emphasizing the inclusion of safety and environmental topics. The program has been further expanded into the ISEKI-Food Association (https://www.iseki-food.net/), an independent organization devoted to the objectives of the ISEKI consortium to further the safety and sustainability of the food chain through education. The motto of the association is "Integrating Food Science and Engineering Knowledge into the Food Chain." The association will continue working on the

ISEKI-Food series with several new volumes to be published in the near future. The series continued with the publication in 2012 of *Novel Technologies in Food Science: Their Impact on Products, Consumer Trends and The Environment*, edited by Anna McElhatton and Paolo J. Sobral. The book is intended for food scientists, engineers, and readers interested in the new and emerging food processing technologies developed to produce safe foods that maintain most of their original freshness. All 13 chapters are written from a safety and environmental stand point with respect to the emerging technologies.

We now see the publication of the *Trilogy of Traditional Foods* written for food science professionals as well as for the interested general public. The trilogy is in line with the internationalization of the ISEKI consortium and will offer 74 chapters dedicated to different traditional foods from all over the world. The trilogy starts with *Traditional Foods: General and Consumer Aspects* edited by the undersigned and Jorge Oliveira. The book offers general descriptions of different traditional foods and topics related to consumers and sensory aspects. The second book in the trilogy is *Modernization of Traditional Food Processes and Products* edited by Anna McElhatton and Mustapha Missbah El Idrissi. The chapters are devoted to recent changes and modernizations of specific traditional foods with a focus on processing and engineering aspects. The third volume in the trilogy, *Functional Properties of Traditional Foods*, is devoted to functional and biochemical aspects of traditional foods and the beneficial effects of bioactive components found in some traditional foods.

The series will continue with several books including a textbook *Food Processing* intended for senior-level undergraduates and junior graduate students. This textbook, edited by the undersigned and Semih Ötles, will provide a comprehensive introduction to food processing. The book should also be useful to professionals and scientists interested in food processing both from the equipment and process approach as well as in the physicochemical aspect of food processing. The book will contain five sections starting with chapters on the basic principles and physicochemical properties of foods, followed by sections with chapters on conversion operations, preservation operations, and food processing operations with separate chapters on most common food commodities. The final section will be devoted to post-processing operations.

Applied Statistics for Food and Biotechnology, edited by Gerhard Schleining, Peter Ho, and Saverio Mannino, will be intended for graduate students and industry personnel who need a guide for setting up experiments so that the results will be statistically valid. The book will provide numerous samples and case studies on how to use statistics in food and biotechnology research and testing. It will contain chapters on data collection, data analysis and presentation, handling of multivariate data, statistical process control, and experimental design.

The book *Process Energy in Food Production*, edited by Winfried Russ, Barbara Sturm, and the undersigned, is being prepared for publication. The book will offer an introduction section on basic thermodynamics and an overview of energy as a global element. It will also cover environmental effects of energy provision and usage. This will be followed by chapters on the use of energy in various food processes such

as flour production, bakery, fish processing, meat processing, brewery and beverage production, direct and indirect heat integration in breweries, fruit juice, spray drying systems (milk powder), and chilling and storage of fresh horticultural products. There will also be chapters related to energy supply, thermal, solar, hydroelectric, energy distribution, insulation for energy saving, storage systems for heat and coldness, waste heat recovery, and energy management systems.

Three more books are being prepared. The textbook *Physical Chemistry for Food Scientists*, edited by Stephan Drusch and Kirsi Jouppila, will provide senior undergraduate and beginning graduate-level students an overview of the basic principles of physical chemistry of foods. The first part of the book will be devoted to fundamental principles of physical chemistry. The second part of the book will be devoted to the physical chemistry of food systems. The book *Consumer-Driven Development of Food for Health and Well-Being*, edited by the undersigned, Paola Pittia, Margarida Vieira, and Howard R. Moskowitz, is in preparation with chapters on general aspects of food development, the house of quality and Stage-Gate® process, consumer aspects of food development, mind genomics, conceptualization of well-being in the framework of food consumption, formulation of foods in the development of food for health and well-being, ingredients contribution for health and well-being, new trends on the extension of shelf life, nutritional aspects of the development of foods focusing on health and well-being, regulatory and policy aspects, and several case studies on product development with special emphasis on health and well-being. Finally, there is a *Book on Ethics in Food Production and Science* that will be edited by Rui Costa and Paola Pittia.

The ISEKI-Food series draws on expertise from universities and research institutions all over the world, and we sincerely hope that it may offer interesting topics to students, researchers, professionals, as well as the general public.

Reykjavík, Iceland Kristberg Kristbergsson

Series Preface Volumes 1–6

The single most important task of food scientists and the food industry as a whole is to ensure the safety of foods supplied to consumers. Recent trends in global food production, distribution, and preparation call for increased emphasis on hygienic practices at all levels and for increased research in food safety in order to ensure a safer global food supply. The ISEKI-Food book series is a collection of books where various aspects of food safety and environmental issues are introduced and reviewed by scientists specializing in the field. In all of the books, a special emphasis was placed on including case studies applicable to each specific topic. The books are intended for graduate students and senior-level undergraduate students as well as professionals and researchers interested in food safety and environmental issues applicable to food safety.

The idea and planning of the books originates from two working groups in the European thematic network ISEKI-Food an acronym for "Integrating Safety and Environmental Knowledge In to Food Studies." Participants in the ISEKI-Food network come from 29 countries in Europe, and most of the institutes and universities involved with food science education at the university level are represented. Some international companies and nonteaching institutions have also participated in the program. The ISEKI-Food network is coordinated by Professor Cristina Silva at the Catholic University of Portugal, College of Biotechnology (Escola) in Porto. The program has a web site: http://www.esb.ucp.pt/iseki/. The main objectives of ISEKI-Food have been to improve the harmonization of studies in food science and engineering in Europe and to develop and adapt food science curricula emphasizing the inclusion of safety and environmental topics. The ISEKI-Food network started on October 1 in 2002 and has recently been approved for funding by the EU for renewal as ISEKI-Food 2 for another 3 years. ISEKI has its roots in an EU-funded network formed in 1998 called FoodNet where the emphasis was on casting a light on the different food science programs available at various universities and technical institutions throughout Europe. The work of the ISEKI-Food network was organized into five different working groups with specific task, all aiming to fulfill the main objectives of the network.

The first four volumes in the ISEKI-Food book series come from WG2 coordinated by Gerhard Schleining at Boku University in Austria and the undersigned. The main task of the WG2 was to develop and collect materials and methods for teaching of safety and environmental topics in the food science and engineering curricula. The first volume is devoted to food safety in general with a practical and a case study approach. The book is composed of 14 chapters which were organized into three sections on preservation and protection, benefits and risk of microorganisms, and process safety. All of these issues have received high public interest in recent years and will continue to be in the focus of consumers and regulatory personnel for years to come. The second volume in the series is devoted to the control of air pollution and treatment of odors in the food industry. The book is divided into eight chapters devoted to defining the problem, recent advances in analysis, and methods for prevention and treatment of odors. The topic should be of special interest to industry personnel and researchers due to recent and upcoming regulations by the European Union on air pollution from food processes. Other countries will likely follow suit with more strict regulations on the level of odors permitted to enter the environment from food processing operations. The third volume in the series is devoted to utilization and treatment of waste in the food industry. Emphasis is placed on sustainability of food sources and how waste can be turned into by-products rather than pollution or landfills. The book is composed of 15 chapters starting off with an introduction of problems related to the treatment of waste and an introduction to the ISO 14001 standard used for improving and maintaining environmental management systems. The book then continues to describe the treatment and utilization of both liquid and solid wastes with case studies from many different food processes. The last book from WG2 is on predictive modeling and risk assessment in food products and processes. Mathematical modeling of heat and mass transfer as well as reaction kinetics is introduced. This is followed by a discussion of the stoichiometry of migration in food packaging, as well as the fate of antibiotics and environmental pollutants in the food chain using mathematical modeling and case study samples for clarification.

Volumes five and six come from work in WG5 coordinated by Margarida Vieira at the University of Algarve in Portugal and Roland Verhé at Gent University in Belgium. The main objective of the group was to collect and develop materials for teaching food safety-related topics at the laboratory and pilot plant level using practical experimentation. Volume five is a practical guide to experiments in unit operations and processing of foods. It is composed of 20 concise chapters each describing different food processing experiments outlining theory, equipment, procedures, applicable calculations, and questions for the students or trainees followed by references. The book is intended to be a practical guide for the teaching of food processing and engineering principles. The final volume in the ISEKI-Food book series is a collection of case studies in food safety and environmental health. It is intended to be a reference for introducing case studies into traditional lecture-based safety courses as well as being a basis for problem-based learning. The book consists of 13 chapters containing case studies that may be used, individually or in a series, to discuss a range of food safety issues. For convenience the book was divided into

three main sections with the first devoted to case studies, in a more general framework with a number of specific issues in safety and health ranging from acrylamide and nitrates to botulism and listeriosis. The second section is devoted to some well-known outbreaks related to food intake in different countries. The final section of the book takes on food safety from the perspective of the researcher. Cases are based around experimental data and examine the importance of experimental planning, design, and analysis.

The ISEKI-Food books series draws on expertise from close to a hundred universities and research institutions all over Europe. It is the hope of the authors, editors, coordinators, and participants in the ISEKI network that the books will be useful to students and colleagues to further their understanding of food safety and environmental issues.

Reykjavík, Iceland Kristberg Kristbergsson

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ISEKI_Food 3 and ISEKI_Mundus 2 were thematic networks on food studies, funded by the European Union through the Lifelong Learning and Erasmus Mundus programs as projects N° 142822-LLP-1-2008-PT-ERASMUS-ENW and 145585-PT-2008-ERA MUNDUS—EM4EATN, respectively. The ISEKI_Mundus 2 project was established to contribute for the internationalization and enhancement of the quality of the European higher education in food studies and work toward the network sustainability, by extending the developments undergoing through the Erasmus Academic Network ISEKI Food 3 to other countries and developing new activities toward the promotion of good communication and understanding between European countries and the rest of the world.

Preface

Functional Properties of Traditional Foods is the third book in the *Trilogy of Traditional Foods*, part of the ISEKI-Food series. The three books in the trilogy are devoted to different characteristics of traditional foods. The trilogy covers general and consumer aspects, modernization of traditional foods, and functional properties of traditional foods in a total of 74 chapters written by authors from all over the world.

Functional Properties of Traditional Foods is divided into four parts. The first is "General Functional Properties of Foods," with chapters on functional aspects of antioxidants and probiotics in traditional food. This part also includes chapters on the potential health benefits of Thai, Slovak, and Turkish traditional foods. The second part covers functional properties of some cereal-based foods in eight chapters by contributors from Europe, Mexico, and South America. Topics addressed in Part II include carob flour, products from Mexican chia, and the ancient grain cañahua, to name a few. The third part is devoted to fruits and other plant-based foods. The eight chapters in this part provide information on antioxidant properties of dates from Israel, medical properties of cactus products from Mexico, beneficial properties of mastic gum from the Greek island Chios, and the properties of argan oil from Morocco. The final part, "Honey and Beverages with Functional Properties," includes chapters on functional and nutritional properties of honey from Slovenia and Portugal. Other chapters in the part cover the functional properties of camellia tea, as well as the Spanish drink horchata de chufa. Some of the chapters, such as "The Potential Health Benefits of Traditional Thai Fermented Foods and Beverages" and "Boza a Traditional Cereal-Based Fermented Beverage," could fit in more than one part, and the editors hope that such trivial matters will not distract the reader from learning about and enjoying the functional properties of some of the traditional foods discussed in the book. All the chapters are written by practicing food scientists or engineers but are written with the interested general public in mind. The book should cater to the practicing food professional as well as the interested reader.

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Part I
General Functional Properties of Foods

Chapter 1
Functional Aspects of Antioxidants in Traditional Food

Anna Gramza-Michałowska and Dominik Kmiecik

1.1 Introduction

Antioxidants became one of the hottest topics in modern food industry, not only as food additives preventing oxidative changes, but also as food components acting in vivo after ingestion. Average antioxidants consumption with the diet is nearly 1000 mg, where about 50–200 mg of polyphenols per meal (Murkovic 2003). Daily contribution of fruits and vegetables was calculated as 450 mg of gallic acid equivalents (Ock et al. 2005).

1.2 Food Antioxidants

People have learned how to preserve food many centuries ago, but its proper definition is still a puzzle for scientists. Halliwell and Gutteridge (1995) have defined antioxidants as any substance present in low concentrations, when compared with oxidized substrate, that delays or inhibits oxidation of that substrate. According to Halliwell (1995) and Giese (1996), antioxidants are food additives, which are used to delay free radicals accumulation and hence strengthening oxidative stability of food. Lipid antioxidants however are defined as any substance that delays, retards, or prevents development in food of rancidity or other flavor deterioration due to oxidation (Frankel 1998).

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According to activity mechanism, antioxidants had been divided into two basic groups (Gordon 1990, 2001; Yanishlieva-Maslarowa 2001; Pokorny 2007; Choe and Min 2009):

1. Primary antioxidants or donators, called preventive antioxidants, which activity depends on inactivation of peroxides by annexation to free radicals of fatty acid—the antioxidant hydrogen interrupting the reaction sequence; eventually antioxidant loses its activity (e.g., phenolic compounds, tocopherols).
2. Secondary antioxidants or the acceptors, called chain-breaking antioxidants, protecting lipids by binding air oxygen or, delaying lipids oxidation as the result of processes different than breaking of autoxidation chain reaction:

   (a) Metal ions binding (e.g., ascorbic acid).
   (b) Scavenging oxygen (ascorbic acid).
   (c) Quenching of singlet oxygen (β-carotene).
   (d) Absorbing the UV radiation.
   (e) Primary antioxidants regeneration (ascorbic acid and thiol substances).
   (f) Synergists of proper antioxidants, meaning substances that increase their antioxidative potential when applied in mixture.
   (g) Peroxides and nonradical products decomposing (Maillard reaction products).
   (h) Creating protective border between oil and air surface (phospholipids).
   (i) Prooxidative enzymes inactivators (lipoxygenase).

There is basic division for antioxidants namely synthetic and natural ones. According to many researches, mostly used synthetic antioxidants are BHT, BHA, TBHQ, propyl gallate, ascorbic acid, and tocopherol, however recent trends show wider use of high potential natural antioxidants like phospholipids, rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), and tea extracts (*Camellia sinensis*) (Frankel et al. 1996; Nguyen et al. 1999; Pokorny 2007; Dreosti 2000). Concerning recent research it was shown that addition of synthetic antioxidants should be limited, because of their possible carcinogenic activity (Barlow 1990; Prior and Cao 2000a, b; Kaur and Kapoor 2001). Other important factor for addition of proper antioxidants is consumers awareness according to consumed products (origin, additives type, harvesting, or growing methods).

Functional food could be a natural source of dietary antioxidants; however, these substances are limited by many factors (Pokorny 1991; Frankel 1998; Honglian et al. 2001):

- Carrying specific flavor, taste, and color.
- Usually lower efficiency in relation to synthetic antioxidants.
- High solubility in aqua and rather low solubility in lipids.
- Low temperature and light resistance.
- High production cost.
- Questionable and unknown toxicity.

- Possible inverse activity when certain concentration is exceeded (e.g., tocopherols).
- Presence of possible impurities.

Besides negative influence of natural antioxidants use in food products, contradictionary sometimes it could be of great benefit. For example natural extracts could be used as food colorants (anthocyanins), flavor agents (specific sensory characteristics) or preservatives (antibacterial properties).

Recent years researches have turned their attention towards phenolic compounds. Polyphenols are secondary metabolites of plant world, not produced by animals (Parr and Bolwell 2000; Herrmann 1976; Prior and Cao 2000a, b). Today food industry needs to search for other, innovative sources of antioxidants, that consumed everyday would benefit healthier life. The biggest problem for researches seems to be finding the source of natural antioxidant that would be acceptable by the consumers, safe, high potential, cheap, and biologically active in low concentrations.

Research on antioxidant potential of substances of different origin didn't show a single antioxidant active in all possible food systems. Giese (1996) and Houlihan and Ho (1985) suggested that antioxidant potential is a result of many factors like e.g., mixing ability, affinity to different phases, and processing stability. Pokorny (2007) suggested that antioxidants, whether natural, synthetic, or identical to natural ones are applied to foods for two purposes: firstly to suppress oxidation of lipids and free radicals formation in food heated and stored for long time; second purpose is application in order to reduce free radicals concentration in vivo after food ingestion.

1.3 Antioxidants Sources

Food products are rich sources of strong antioxidants—phenolic compounds. Table 1.1 presents common sources of natural antioxidants. The most important antioxidants sources in the human diet are vegetables and fruits. Other sources are cereals and essential oils from *Labiatae* family. In Mediterranean countries very important source regularly consumed is olive oil, in other countries it is sunflower or rapeseed oil, significantly poorer in natural antioxidants, due to its production process. Mentioning the technological processing it is worth to notice that antioxidative potential of consumed food is highly dependent on preparation procedures. Cooking might release some compounds, but basically high temperatures result in polyphenols destruction. During cooking however other antioxidants could be produced, like e.g., Maillard reaction products. According to research of Nicoli et al. (1999) basic effects of food processing on the overall antioxidant potential are: loss of naturally occurring antioxidants or its improvement, formation of novel antioxidative and/or prooxidative compounds, interactions among different compounds; or in many cases no effect on antioxidant potential could be noticed.

Table 1.1 Common sources of antioxidants

Source	Example	Substance
Cereals	Whole wheat products, oat, rice, bran	Various lignin-derived compounds
Vegetables	Leaf vegetables, potatoes	Ascorbic acid, flavonoids, carotenoids
Fruits	Apples, bananas, berries, olives	Ascorbic acid, flavonoids, carotenoids
Oilseeds	Sesame seeds, hazelnuts, almonds	Tocopherols, tocotrienols, phospholipids
Legumes	Beans, peanuts, soybeans	Phenolic compounds, amino acids
Cocoa products	Chocolate	Phenolic compounds
Beverages	Tea, coffee, red wine, beer, fruit juices	Phenolic compounds
Herbs and spices	*Labiatae* plants (rosemary, sage, oregano)	Phenolic compounds

Source: Pokorny (1991, 2007)

1.4 Antioxidants as Bioactive Compounds

Plant polyphenols are bioactive compounds consisting of e.g., flavones, flavonols, flavan-3-ols, isoflavones, anthocyanidins, lignans, and many others (Kaur and Kapoor 2001). Research showed that those bioactive compounds are variably absorbed and metabolized in the human body. Potential mechanisms of bioactive compounds action is described with the reducing levels of circulating LDL, its oxidation inhibition as result of enhanced antioxidant activity, reduced platelet aggregation, and tumor development, anti-inflammatory effect and estrogen-like activity, helping to maintain healthy bones, breast, and increasing menopausal symptoms (Hooper and Cassidy 2006). It was suggested that regular consumption of plant products could significantly reduce many diseases occurrence, however further research are needed to specify the health effect of specific compound.

1.5 Natural Antioxidants Safety

Average consumer is convinced that every substance of natural origin should be safe. That persuasion could be tragic in some cases (e.g., toxic substances in edible mushrooms or fishes), which is why detailed research is needed. It is obvious that naturally occurring antioxidants, consumed normally within food products should be preferred by the industry, but as it was already mentioned there are many limitations. During the measuring of natural antioxidants, especially extracts toxicity, it must be remembered that usually it is a mixture of different substances, where possible synergistic and antagonistic effects could be noticed. Also single component toxicity is often not known, as it was always a part of human diet, so there is no guarantee of natural antioxidants safety. Summarizing consumers should think what to eat, so that daily consumed diet, rich in functional food, would be sufficient source of natural antioxidants.

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Chapter 2
Probiotics and Prebiotics in Traditional Food

Anna Gramza-Michałowska

2.1 Introduction

For centuries, probiotics and prebiotics have been used in foods for their technological aspects and beneficial health effect on human organism. Functional food is defined as food, or food ingredient, with positive effects on host health and/or well-being beyond its nutritive value (Huggett and Verschuren 1996). As the part of functional food, both probiotics and prebiotics should be well identified, characterized, and targeted on different populations. Since the colon bacteria are proved to be beneficial for human health it is very important to use the plant-derived prebiotics and probiotics in terms of colonic microflora composition manipulations. Special focus will be given to definitions, health aspects, and possible directions in food industry.

2.2 Human Colon

Human colon undertakes many of the physiological activities and is one of the body's most metabolically active organs. The human embryo has sterile gut, which is being colonized with aging by the indigenous bacteria (Mitsuoka 1990). During life, human gut is settled with bacteria responsible for undigested food fermentation. Microorganisms exist in human alimentary tract with population characteristic for the particular parts of the gut. Typical colonic transit is 48–70 h for the adults, which allows to establish the bacterial community in large intestine (Macfarlane and Gibson 1994). The colon pH and relatively low absorptive state encourage

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Table 2.1 Factors influencing human gut microflora composition

Factors
Diet and type of feeding
Amount, chemical composition, and availability of growth substrates
Presence of antimicrobial compounds
Availability of colonization sites
Immunological interactions
Intestinal transit time
Individual fermentation strategies by the bacteria
Gut pH
Production of bacterial metabolites
Redox potential
Availability of inorganic electron acceptors
Xenobiotic compounds
Host's age
Peristalsis

Source: Fooks et al. (1999)

microbial colonization (O'Sullivan 1996). However the main important factor influencing the gut bacteria is diet and food that has not been absorbed in the upper gastrointestinal tract (Fooks et al. 1999). Other factors influencing human gut microbiota composition presents Table 2.1. It is possible that any and food product reaching the colon, like carbohydrates, some peptides, proteins, and even lipids might be the candidate for prebiotic criteria. However main attention is directed at the nondigestible oligosaccharides (Mussatto and Mancilha 2007). Basic action of prebiotics is the stimulation of certain indigenous bacteria growth, rather than introducing new bacteria species as it is with the probiotics. It was established that the gut microflora management helps to resist to pathogens growth, lowers the blood lipids, and might have effect on gut tumors (Fooks et al. 1999).

2.3 Probiotics and Prebiotics

Definition of probiotic origins from Greek words meaning "for life". Firstly the probiotic definition symbolized microbial substances stimulating the growth of another organism (Lilley and Stillwell 1965). According to definition proposed by Fuller (1989) probiotics are living microbial feed supplement beneficially affecting the host animal by improving its intestinal flora balance. Modern market offers probiotics in the form of tablets, liquids, sprays, or powder. However most of human dedicated probiotic foodstuffs are in fermented dairy products or in capsules containing single or several species of bacteria or fungi. Today food technology uses limited number of microorganisms, among which the *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* are commonly used.

Probiotic effects on human body is beneficial in many cases, especially since the lactose malabsorption is common in human population, the lactose administration in yoghurt could be utilized more efficiently than from untreated milk. It was found that probiotics in yoghurt can produce β-galactosidase improving lactose tolerance (Fooks et al. 1999). Other probiotics benefits for human organism are: ability to treat intestinal infections, possible suppression of cancer, lowering the plasma cholesterol concentrations and incidence of coronary heart disease, helping in digestion, increasing the nutritional effect of dairy products (increased protein availability, immunological stimulation by the antibodies production (Cummings and Macfarlane 1991; McIntosh 1996; Gilliland et al. 1985; Anon 1997).

Different strains of lactobacilli and bifidobacteria are added to many fermented dairy products as the probiotics. Those bacteria are able to metabolize prebiotic carbohydrates in vivo, which in result is responsible for the enrichment of the selected bacteria in gastrointestinal tract and then production of short chain organic acids like lactic or acetic acid (Wang and Gibson 1993). However there are many factors influencing the balance of the gut microflora, where the beneficial bacteria might be choked and pathogens like *Salmonella*, *Escherichia coli*, *Listeria*, or *Campylobacter* could grow unlimitedly. Mainly the prebiotics activity is measured by the specific effects like growth rates of microbial populations, assimilation of a substrate, and production of short-chain fatty acids (Huebner et al. 2007).

Many plant-derived substances have received considerable attention due to its health benefits. According to the definition of Gibson and Roberfroid (1995) those plant substances so-called prebiotic carbohydrates are defined as nondigestible food ingredient or ingredients beneficially affecting host health by the selective stimulation of the growth and/or activity of one or a limited number of colon bacteria. Nowadays the prebiotics are defined as "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health" (Gibson et al. 2004). Resident colon bacteria mainly consist of various lactobacilli and bifidobacteria. These carbohydrates possess the ability to influence the gastrointestinal tract population, especially colonic microflora, by their selective utilization. It was found that prebiotics allow the bifidobacteria and lactobacilli to increase its populations (Macfarlane et al. 2006).

Lactobacilli are responsible for the digestion aid of lactose in lactose intolerant humans, helping to resist *Salmonella* infections, reduce the diarrhea (Manning and Gibson 2004; Saulnier et al. 2009). Bifidobacteria are responsible for the stimulation of the immune system, production of B vitamin, reducing the blood cholesterol and ammonia levels, and inhibit the pathogen growth (Gibson and Roberfroid 1995).

Recent research showed that the modifications of the intestinal microflora by the prebiotics can interact with the immunological substances and resulting not only protective effects, but also immune response of the gut (Fakuda et al. 2002; Sartor 2004). The immune system response may have significant effect in mucosal surfaces, like respiratory tract or the skin, leading to broader systemic benefits (Saavedra and Tschernia 2002). It was found that the potential of the prebiotic is effective depending on the susceptibility to selective fermentation and supporting specific microorganisms growth. Certain carbohydrates and polysaccharides reveal many

functional and health claims like: modulation of gastrointestinal tract transit time, improving the glucose tolerance and via bile acids binding reducing fat and cholesterol absorption. Ability to bind water helps to increase water content and volume of intestinal contents, as well as pH and ammonia production (Roberfroid 1996).

Food products are very well adapted to contain the so-called synbiotics, which mean the probiotics and prebiotics combination. The conjunction of microbial substrate and its specific nutritional source result both improved survival of the probiotic substance and directed fermentation of the growth factor that might not be used by competing organisms. Such approach is widely accomplished in food for infants and elderly.

2.3.1 Criteria for Probioticity and Prebioticity

There are wide range of substances fermented by the gut microflora that are not digested in the small intestine but are available for fermentation in the colon. Those substances delivered with the diet include dietary fiber, resistant starch, oligosaccharides, amino acids, and proteins (Douglas and Sanders 2008). Human diet is rich in prebiotics, since many fruits and vegetables contain oligosaccharides namely: banana, garlic, onion, asparagus, and chicory (Manning and Gibson 2004). Probiotic strains have been reported to possess a number of beneficial influences like (Ziemer and Gibson 1998):

- Reduction of lactose intolerance and enhancing lactose metabolism.
- Renovation of intestinal microflora, and improving the colonization resistance.
- Reduction of blood cholesterol and potential mutagen-inducing tumors.
- Immune system response enhancement.
- Improved calcium absorption, vitamins synthesis, and predigestion of proteins.

To be included in the group of probiotics, microflora needs to fulfill a number of the following properties (Lee and Salminem 1995):

- Must survive the acidic environment of the upper gastrointestinal tract, than proliferate and/or colonize the intestine.
- Its fermentation products or cell components must not be toxic, pathogenic, mutagenic, and carcinogenic to the host organism.
- Genetically stable and antagonistic towards carcinogenic microorganisms.
- Easily reproducible.
- Remaining viable during processing and storage.

Modern biotechnology divides bacteria according to influencing the flora composition in terms of health impact. According to Wang (2009) and Fooks et al. (1999) food ingredients classified as prebiotics must accomplish many goals:

- Beneficial to host's health and well-being.
- Selectively fermented by potentially beneficial for health and well-being bacteria in the colon.
- Not hydrolyzed and absorbed in the upper part of the gastrointestinal tract.

– Ability to withstand digestive processes before reaching the colon: resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption.
– Preferable persisting throughout the large intestine.
– Stable throughout the food-processing treatments.

2.3.2 *Food Applications*

Modern food technology uses a broad number of prebiotics and probiotics applications in food products. Food producers claim that the products with prebiotics addition are safe and support human well-being and health.

Prebiotics are of research interest because of their nutritional and technological properties. There are many prebiotics sources like vegetables or fruits, and can be received also from renewable materials. Prebiotics production includes (Crittenden and Playne 1996):

– Extraction procedures (e.g., inulin)
– Extraction followed by an enzymatic hydrolysis (e.g., oligofructose).
– Synthesis from mono- or disaccharides (sucrose—fructooligosaccharides, lactose—galactosaccharides).

Most recognizable ingredients considered as prebiotics are oligosaccharides, inulin-type fructans, lactulose, carbohydrates, and fructooligosaccharides (Losada and Olleros 2002; Rastal and Maitin 2002; Roberfroid 2002; Kanauchi 2003). Prebiotic substances for food usage should fulfill selected criteria, representing specific functional characteristic presented in Fig. 2.1.

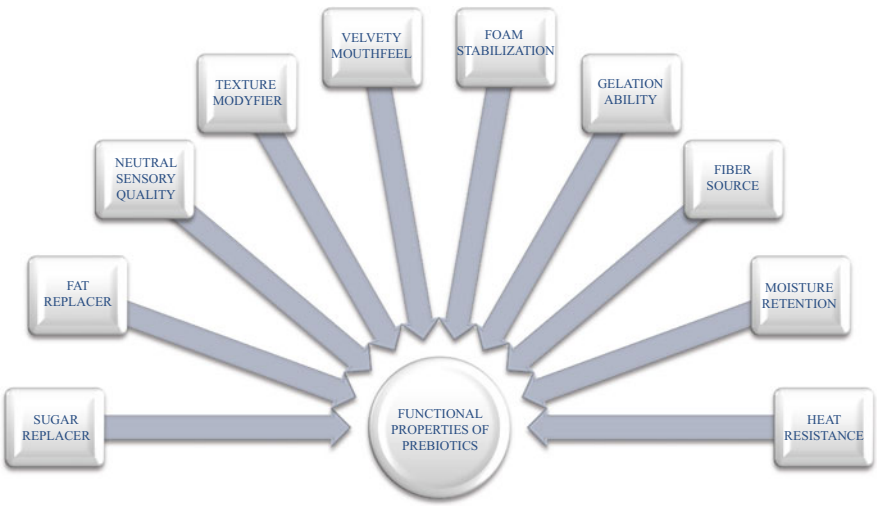

Fig. 2.1 Functional properties of prebiotics

Since the positive effect of probiotics and prebiotics use in food products is well recognized, food industry offers wide range of preparations for consumers. Food applications of prebiotics include many groups of products like:

- Dairy products: yoghurts, desserts, milk drinks, and cheeses.
- Beverages and drinks, soups, and sauces.
- Bakery products: cakes, biscuits, bread, and cereals.
- Chocolate and sugar confectionary.
- Baby food.
- Dietetic products.

2.3.3 Probiotics and Prebiotics Safety

Prebiotics are regarded as safe because of naturally sourced substances and historically nontoxic. However there is still a question for its safety that needs to be considered, which has been highlighted by many authors (Huggett and Schliter 1996; Huggett and Verschuren 1996). The highest concerns exist regarding use of probiotics which contain *Enterococci* recognized as opportunistic pathogens (Weese 2002).

Probably the best solution for positive influence and gastrointestinal health improvement is synbiotics, a combination of prebiotics and probiotics (Tuhoy et al. 2003). Summarizing, there is a need to increase our knowledge according to the probiotics use impact on gut microflora. Also very important is to provide the results on those plant-derived substances safety for human. Consumption of synbiotics would surely help gut microflora balance maintenance and restoring the equilibrium in the organisms that have been altered by diet, age, or disease.

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Chapter 3
Bioenrichment of Vitamin B_{12} in Fermented Foods

Busaba Yongsmith, Vichien Kitpreechavanich, Janpen Tangjitjaroenkun, and Warawut Krusong

3.1 Introduction

It has been over 80 years since Minot and Murphy first announced the remarkable efficiency of liver therapy in treating pernicious anemia. It is now well known that the antipernicious anemia principle present in significant amounts in liver extract is vitamin B_{12}. Commonly known as cobalamin, this vitamin is the water-soluble compound of the largest, and most complicated structure of any vitamin discovered hitherto. Although its biochemical role has not been completely elucidated, vitamin B_{12} is known to be indispensable for certain metabolic process in which the vitamin acts as a coenzyme: for normal blood formation, for maintenance of neural function and for normal growth. It is thus required by all higher animals, including humans and has not been found either in higher plants. The principal dietary sources of vitamin B_{12} in nature for human being are animal tissue and liver, in which the vitamin B_{12} is not synthesized but accumulated. People with a normal diet are unlikely to have a problem with vitamin B_{12} deficiency, while vegetarians would most likely have health problem due to vitamin B_{12} deficiency. It is well known that all vitamin

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B_{12} in nature is produced only by microorganisms, such as bacteria and algae. This exclusive microbial synthesis of vitamin B_{12} is unique among the other known vitamins. Evidence of vitamin B_{12} biosynthesis during food fermentations using various kinds of raw materials and microorganisms has been carried out by many scientists led by Steinkraus et al. since 1960. In this chapter, we discern the enrichment of fermented foods with this vitamin and trends to improve B_{12} enrichments using soybean substrate mainly as the model.

3.2 Vitamin B_{12} and its Significance

Vitamin B_{12} is essential for human diet, although a minimum daily requirement has not yet been conclusively established. A minimal dairy requirement of 1.0 μg of vitamin B_{12} was set by Darby et al. (1958), Sullivan and Herbert (1965), and Baker and Mathan (1981), while WHO (1980) recommended dietary intake of vitamin B_{12} for adult males or non-pregnant females to be 2 μg per day. However, the Committee on Dietary Allowances 1974 suggested that the requirement of this vitamin should be 3 μg per day. In general, vitamin B_{12} is not found in plant kingdom. An exclusive vegetarian diet consumed by man or animal leads to vitamin B_{12} deficiency. In man, synthesis of the vitamin B_{12} occurs through bacteria action in the large bowel, but it is not absorbed from this site.

3.2.1 Vitamin B_{12} Compounds in Microorganisms

Although it is undoubtedly possible to synthesize vitamin B_{12} in a purely chemical fashion, its chemical synthesis, developed by the group of Woodward and Eschenmoser, is significant only as the epoch-marking progress in the history of synthetic chemistry (Woodward 1972). Vitamin B_{12} production using microorganisms has continued to be more important industrially. Propionibacteria, clostridia, nocardia, streptomycetes, butyribacteria, and methane bacteria have been found to produce a high level of vitamin B_{12} compounds.

The industrial preparation describing vitamin B_{12} for medical purposes (Merck and Co., Inc.) started in 1948 immediately after the first publication of its isolation in pure form from microorganisms by the Falkers group. Since then the interest in commercial production of vitamin B_{12} has shifted from its use in medicine to the field of agriculture. The amount produced for animal feed is far higher than that of the crystalline form used for pharmaceutical purposes.

While over 100 fermentation processes have been described for the production of vitamin B_{12} in technical papers and patent descriptions, apparently only half a dozen have been used on an industrial scale. These include recovery of vitamin B_{12} as a by-product in streptomycin and aureomycin antibiotic fermentations, a process based on *Bacillus megaterium* fermentation, and direct fermentation using

Table 3.1 Vitamin B_{12} production by different strains using conventional carbon sources

Strain	Carbon source	Yield (mg/L)
Micromonospora sp.	Glucose	11.5
Nocardia rugosa	Glucose–cane molasses	14
Propionibacterium freudenreichii	Glucose	*25*
Propionibacterium shermanii	Glucose	23–29
Propionibacterium vanielli	Glucose	25
Pseudomonas denitrificans	Beet molasses	59
Streptomyces olivaceus	Glucose–lactose	8.5

Source: modified from Florent and Ninet (1979)

Streptomyces olivaceus, propionibacteria or *Pseudomonas* as shown in Table 3.1 (Florent and Ninet 1979). *Propionibacteria* and *Pseudomonas* are mainly used for the industrial production of vitamin B_{12} from carbohydrates. Both single-batch and continuous processes have been adopted. A number of culture media have been utilized for the growth of these organisms. Some of them contain a variety of materials of natural origin such as soybean oil meal, fishery wastes and fish meal, yeast preparations, meat extract, animal stick liquor, corn steep liquor, casein or hydrolysates, and residues from various other fermentation processes. In most cases, the vitamin B_{12} yields have been found to be correlated with cellular growth, and the inclusion in the media of these animal and plant extracts and fractions helped to increase the cell-mass production.

3.2.2 Vitamin B_{12} in Foods

The primary source of vitamin B_{12} in nature appears to result only from the metabolic activity of bacteria and algae; there is no convincing evidence for its elaboration in tissues of higher plants or animals.

3.2.2.1 Non-vegetarian Foods

Vitamin B_{12} has not been synthesized in foods of animal origins but it can be accumulated in such food as organs (kidney, heart, liver), other animal tissues, fish flesh, fish meal, stickwaters, and condensolubles of fresh water or marine fish (Tarr et al. 1950; Braekkan 1958). Areekul and Chantachum (1980) also found vitamin B_{12} present in some Thai foods including animal, poultry, eggs, milk, cheese, butter, fish, shellfish, and other marine products. They found kidney, meat and heart contained a high amount of vitamin B_{12} but still at a lower concentration than in the liver. Moreover, they found vitamin B_{12} concentration from cow product to be considerably higher than those from pigs and chickens. Oysters had the highest content of vitamin B_{12} among the seafood, while the foods that had an intermediate amount

of vitamin B_{12} were chicken, fish, shrimp, crab, egg yolk, and milk. A very low quantity of this vitamin was found in egg white. Therefore, it seems that those who eat normal diet should not have problems with vitamin B_{12} deficiency.

3.2.2.2 Vegetarian Foods and Their Problem with Vitamin B_{12} Deficiency

Vegetarianism involves the consumption of a diet composed dominantly of plant foods. Vegetarian diets may be based only on plant food sources (total vegetarians or vegans), plant foods plus dairy products (lacto vegetarians) or plant foods plus dairy products and eggs (lacto ovo vegetarians). Vegetarianism has grown more prevalent in the last decade for many reasons—philosophical, religious, health-related, cultural, and economics. However, a nutrient missing in most green vegetables, pulses, and nuts (Table 3.2) is vitamin B_{12}.

Vegetarians can meet their dairy requirements of this vitamin by drinking two glasses of milk or one glass of milk and consuming an egg (William 1979). However, a non-dairy vegetarian should supplement her diet to ensure sufficient intake of vitamin B_{12} (Gustafson 1984). It has been reported that the vitamin B_{12} intake recommended in infancy and childhood have been considerably increased since this vitamin is essential for normal blood cell formation and nerve function. Higginbottom et al. (1978) reported that vitamin B_{12} deficiency in infants in a vegan religious community whose mothers are non-dairy vegetarians resulted in the symptoms of severe megaloblastic anemia, coma, and hyperpigmentation of the extremities. The patients were often found to have methylmalonicaciduria and hemocystimuria. In addition, Davis et al. (1981); Shinwell and Gorodischer (1982), also reported that manifestations of this nutritional deficiency may include neurological symptoms, anemia, pancytopenia, hypothermia, and severe weight loss. Deficiency levels of serum vitamin B_{12} (45–193 pg/mL with an average value of 111 pg/mL as compared to normal value of 200–300 pg/mL) have been found in adults who have subsisted on vegetarian diets for extended periods (Wokes et al. 1955). Bindra et al. (1987) reported that

Table 3.2 Vitamin B_{12} content in cereals, pulses, nuts, vegetables, fruits, oil, and their products

No	Cereals	Pulse and nuts	Vegetables and fruits	Cooking oil
1.	Rice	Peanut	Papaya, unripe	Coconut oil
2.	Glutinous rice	Soybean	Papaya, ripe	Salad oil
3.	Enriched rice	Mungbean	Guava, unripe	Lard
4.	Home-pounded rice	Mungbean, sprout	Banana, unripe	Shell oil
5.	Rice noodle, raw	Soybean, sprout	Banana, ripe	Vegetable oil
6.	Egg rice noodle, raw	Geerlig's cheese	Chilli pepper	
7.	Corn	Soybean curd	Swamp cabbage, raw	
8.	Bread	Soybean paste	Shallot	
9.	Plain cake		Garlic	
10.	Chocolate cake		Chinese radish	

All samples contained no vitamin B_{12}

a large percentage of Punjabi subjects, especially females, also had serum folate and vitamin B_{12} levels below those observed for healthy omnivorous populations.

It was also reported that the vitamin B_{12} level in most Thai vegetarian foods is as low as 0.04 μg/100 g whereas that level is increased in Kapi je (vegetarian kapi, a kind of local fermented soybean). Nevertheless, 23 % of Thai male vegetarian subjects and 34 % of Thai female vegetarian subjects had serum vitamin B_{12} level below 150 pg/mL. However, these subjects showed no clinical B_{12} deficiency (Chitchumrunchokchai et al. 1988) the problem with vitamin B_{12} deficiency represents a scientific and medicosocial challenge to pediatricians and nutritionists. Therefore, vitamin B_{12} supplements or vitamin B_{12}-fortified foods should be included in the strict vegetarian diet to alleviate vitamin B_{12} deficiency.

This finding that supplementation is necessary may promote vegetarians who are aware of their health to consume more fermented foods. To avoid the unlikely possibility of vitamin B_{12} deficiency, a non-animal source of vitamin B_{12} (e.g., fermented soybean products) should be added to the vegetarian diet in certain situations. There is some evidence that vitamin pills should not be used for vitamin B_{12} supplementation since they might contain B_{12} breakdown products that inhibit B_{12} function in the nervous system and can actually cause a vitamin B_{12} deficiency (Anonymous 1978).

3.3 Bioenrichment of Vitamin B_{12} through Food Fermentation

The process of fermentation has been used to produce a variety of food products, for example: soysauce, natto, thua-nao, miso, and hamanatto products from soybean substrates (Wang 1987). Steinkraus (1977) reviewed the technology for improving food quality in these products. It included fermentation to produce flavors as in the Japanese soysauce (shoyu) and miso (a soysauce-flavored soybean cereal paste). Texture changes are also produced by some fungal fermentation, notably in the production of tempeh, an Indonesian soybean product in which the texture is due to the bending properties of *Rhizopus oligosporus* mycelia. In addition, this product also contains significant quantities of vitamin B_{12} from bacterial co-fermentation. Microbiological assays indicated the vitamin content of tempeh is higher than the original soybean substrate for some certain vitamins e.g., riboflavin, niacin and vitamin B_{12} (Steinkraus et al. 1961). Vitamin B_{12} is in fact considered to be the nutrient least available in the soybean diet if it is not fermented. Fermented soybeans in the forms of miso and tempe have been of considerable interest to the food industry in the United States in the past few decades. Truesdell et al. (1987) examined vitamin B_{12} content in these commercial products: pasteurized tempeh, pasteurized tempeh burger, unpasteurized dark rice miso, unpasteurized light rice miso, and unpasteurized barley miso. They found that all of the fermented soybeans investigated contained detectable amounts of vitamin B_{12} activity. When unpasteurized, miso are considered together, total vitamin B_{12} activity averaged 0.21 μg/100 g, comparable

to that of light yellow miso which contained 0.17 μg B_{12}/100 g as reported by Shurtleff (1983). Barley miso contained vitamin B_{12} concentration as high as 0.25 μg/100 g while light rice miso contained as little as 0.15 μg/100 g. Pasteurized tempeh contained 0.12 μg vitamin B_{12}/100 g food and a tempeh burger contained 0.06–0.11 μg vitamin B_{12}/100 g food. The variation in vitamin B_{12} activity found in these various products may be due to different conditions used or produced during fermentation. In addition, an increase in vitamin B_{12} during fermentation of tempeh-like products made from cowpeas (Djartoft and Nielsen 1983) and from *Lathyrus sativus* seeds (Table 3.3) (Moslehuddin and Hang 1987), from natto (Okada et al. 1983) and in kimchi (Lee et al. 1958; Ro et al. 1979) have been reported.

Thai-fermented foods also contain a considerable amount of vitamin B_{12} (Table 3.4). Fish sauce, fermented fish, and Nam Budu contained such relatively high vitamin B_{12} concentration as 0.68, 2.27 and 17.91 μg/100 g, respectively (Areekul and Chantachum 1980).

Fish sauce and fermented fish are important traditional food items used extensively in Thailand. Fish sauce is used as a flavoring material and sometimes as a substitute for salt. For fish sauce, the relationship between the vitamin B_{12} content and the price of the different grades are also shown in Tables 3.5 and 3.6.

Table 3.3 Vitamin B_{12} content of *Lathyrus sativus* seeds after various processing conditions (dry weight basis)

Treatments	Vitamin B_{12} (ng/100 g)[a]
1. Seeds soaked in water, washed and steamed	176
2. Seeds soaked in water, washed, steamed, and fermented	283
3. Seeds soaked in water, washed, steamed, autoclaved, and fermented	401

[a]Values represent an average of duplicate experiments
Source: Moslehuddin and Hang (1987)

Table 3.4 Vitamin B_{12} content in Thai-fermented foods

	No. of samples	Vitamin B_{12} content (μg/100 g)	
		Mean ± SD	Range
Fish sauce	10	0.68 ± 0.53	0.04–1.56
Fish paste	20	1.47 ± 0.98	0.33–3.16
Fermented fish	99	2.27 ± 1.18	0.33–6.43
Nam Budu	2	17.91	17.91–18.64
Soya sauce	35	0.14 ± 0.13	0.01–0.53
Fermented soybean	2	0.82	0.62–1.16
Fermented oyster	2	27.25	20.50–24.00
Fermented ark shell	2	3.39	2.15–4.63
Fermented salt water mussel	2	1.72	1.40–2.04

Source: Areekul and Chantachum (1980)

Table 3.5 The composition of fish sauce with the standard requirement set by the Department of Science, Ministry of Industry, Thailand

	Low grade	Medium grade	High grade	Standard requirement
Price (baht/bottle)	0.80–2.00	2.50–4.50	5.00–15.00	–
pH	5.4–5.9	5.4–5.9	5.4–5.9	5.0–6.0
Specific gravity	1.19–1.20	1.21–1.22	1.21–1.22	Over 1.20
Salt (g/L)	280–310	270–300	260–290	Over 230
Total nitrogen (g/L)	0.6–10.0	10.1–20.0	20.1–44.1	Over 19.0
Amino acid nitrogen				
% of total nitrogen	40–60	40–60	50–60	Over 50
Ammonical nitrogen				
% of total nitrogen	10–30	10–20	10–20	–
Iron (mg%)	0.46–0.55	0.78–1.56	1.44–2.51	–
Iodine (mg/L)	0.19–0.23	0.38–0.67	0.54–0.92	–
Niacin (mg%)	1.34–1.67	2.16–6.51	4.42–11.20	–
Vitamin B_{12} (μg%)	0.20–1.76	0.32–1.80	0.87–3.35	–

Source: Areekul et al. (1972)

Table 3.6 The vitamin B_{12} concentration in various grades of fish sauce examined

		Vitamin B_{12} content (μg/L)	
Price[a] (baht/bottle)	No. samples examined	Mean ± SD	Range
1.50	14	0.58	0.20–1.38
2.00	30	0.66	0.13–1.76
2.50–3.00	13	1.08	0.47–1.80
4.00–6.00	8	1.74	0.88–2.67
7.00–8.50	16	2.08	0.88–3.35
12.00–15.00	3	2.41	1.71–3.22

[a]25 baht equal to US$1

Source: Areekul et al. (1972)

3.4 Case Study: Relationship of Fermented Fish to Deficiency Anemias in Thailand (Sundharagiati 1957)

The common causes of deficiency anemias are iron deficiency, hook worm infestation, and macrocytic anemia which is a deficiency of protein factor or extrinsic factor. Sundharagiati (1957) has set up an extensive study of blood in the Thai population and discovered that macrocytic anemia is exceedingly rare, especially among the poor people of the Northeastern part of the country even though consume a rather low amount of animal protein in their diet which consists largely of glutinous rice, fermented fish (plaraa), red pepper and salt. Plaraa is commonly consumed in the Northeastern and Northern parts of the country; about 15 g of plaraa per person is estimated to be eaten daily. However, in the Central and Southern parts of the country, fish sauce (nampla) is more commonly used in daily cooking.

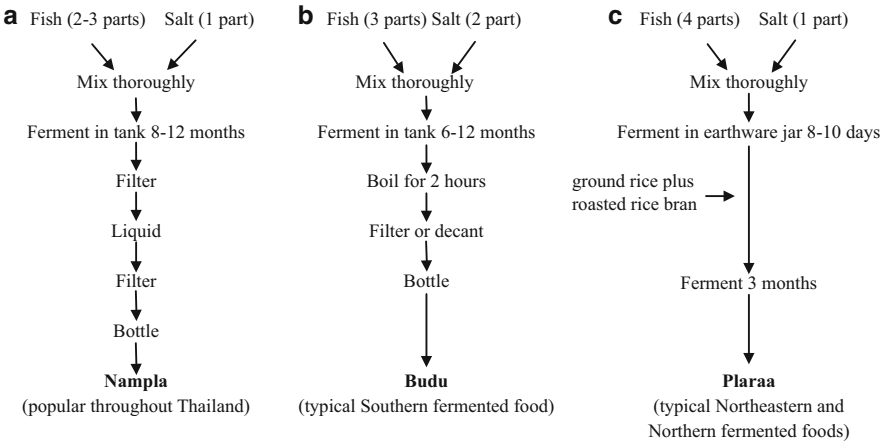

Fig. 3.1 Flow diagram: traditional production of Thai-fermented fish products (**a**) Nampla (**b**) Budu (**c**) Plaraa

According to the flow diagram demonstrating the processing of different fermented fish products (Figure 3.1), both plaraa and budu (a fish product prepared in the Southern Thailand) have a greater concentration of "fish soluble" than fish sauce. "Fish soluble" is thus known to be one of the sources of vitamin B_{12}, for plaraa and budu contain 42 and 16 μg vitamin B_{12} per kg, respectively. However, fish sauce products such as nampla are known to certain 0.3–39 μg vitamin B_{12} per kg depending on the quality. High-grade nampla contains more fish soluble, resulting in a better taste and better odor, while the lower grade which is less expensive of produced for commercial purposes, contains more salted water.

Because of the great effectiveness of vitamin B_{12} in treating macrocytic anemia, the maintenance dose in its treatment is as low as one microgram daily. The following case study by Sundharagiati (1958) was the first to report the use of the fish sauce to successfully treat macrocytic anemia. Two weeks after delivery of a normal baby, a hematological study revealed a mixed type of microcytic anemia in the mother. A bone marrow study showed normoblastic and megaloblastic hyperplasia.

The microcytic (iron deficiency) anemia was first treated with 18 g of ferrous sulfate daily for 22 days (Table 3.7) and after 22 days the treatment was discontinued. After this iron therapy, the treatment of macrocytic type of anemia was then initiated with the administration of 25 g of fish sauce daily, the amount which contains 1 μg of vitamin B_{12}. The fish sauce treatment was discontinued on the 26th day (Table 3.8). The results of the treatment are as follows:

The fish sauce used in this study was diluted one to four times with tap water to reduce the salty taste, before it was given to the patient. After withdrawal of the administration of ferrous sulfate, and with the fish sauce therapy alone, the erythrocyte (red blood cells), hemoglobin, and hematocrit remarkably increased while the MCV gradually declined (Table 3.8). This study support Sundhagiati's contention that one of the many factors involved in rarity of macrocytic anemia is the consumption of fermented fish of fish sauce by the Thai population.

Table 3.7 The treatment of microcytic anemia with 18 g of ferrous sulfate daily for 22 days

	Oct. 22	Oct. 25	Oct. 26	Oct. 27	Oct. 28	Oct. 31	Nov. 4	Nov. 12
Reticulocyte	1.2 %	8	11	6.6	5.4	4	2.6	0.8 %
Red blood cells	1.3 million	ND	ND	ND	ND	ND	2.0	2.3 million
Hemoglobin	5.4 g %	ND	ND	ND	ND	ND	8.4	9.4 g %
Hematocrit	15 %	ND	ND	ND	ND	ND	24	30 %
MCV	115 μm³	ND	ND	ND	ND	ND	120	130 μm³

ND not determined
Source: modified from Sundharagiati (1958)

Table 3.8 The treatment of macrocytic anemia with 25 g of fish sauce daily for 25 days

	Nov. 12	Nov. 13	Nov. 14	Nov. 15	Nov. 18	Nov. 20	Nov. 30	Dec. 16
Reticulocyte	0.8 %	1.6	1.0	1.0	0.9	ND	ND	ND
Red blood cells	2.3 million	ND	ND	ND	ND	2.7	3.3	3.9
Hemoglobin	9.4 g %	ND	ND	ND	ND	10.4	11.4	12.2
Hematocrit	30 %	ND	ND	ND	ND	33	35	38
MCV	130 μm³	ND	ND	ND	ND	122	106	97

ND not determined
Source: modified from Sundharagiati (1958)

3.5 Development and Future Trends

Inoculation of indigenous fermented foods with certain vitamin B_{12} producing bacterial strains of the genera *Propionibacterium*, *Klebsiella*, and *Bacillus* spp. has been evaluated as a vitamin enrichment method. The utilization of plant origins such as the lime fruit waste products as the vitamin B_{12} fermentation by propionibacteria has been reported (Perez-Mendoza and Garcia-Hernendez 1983). Soybean paste when it was inoculated with *B. megaterium* and then fermented was found to have increased vitamin B_{12} content (Choe et al. 1963; Ke et al. 1963; Yongsmith et al. 1999). The following samples are the practical examples of the development of vitamin B_{12} enrichment in various indigenous fermented foods.

3.5.1 Vitamin B_{12} Bioenrichment Using **Propionibacterium** *spp.*

It is interesting to find that three kinds of indigenous fermented foods: tempe, kefir, and kimchi can be practically developed for vitamin B_{12} bioenrichment using propionibacteria. Propionibacteria are the Gram-positive, non-motile, catalase positive, non-spore forming, facultative anaerobes first isolated from Swiss (Emmenthaler)

cheese. These microorganisms play an important role in several industrial processes. In western countries, the fermentation of lactic acid to propionic acid and carbon dioxide results in the characteristic sharp flavor and the unique "eye" of Swiss cheese. In addition, since they synthesize relatively large amounts of vitamin B_{12}, propionibacteria can be utilized for commercial production of the vitamin. This group of bacteria can utilize a wide range of carbon and nitrogen sources. Some growth factors like amino acids are beneficial but not essential for the growth of propionic acid bacteria. The main products of the propionic acid bacteria fermentation are propionic acid, acetic acid, and carbon dioxide. The ratio of propionic acid to acetic acid varies according to the species, the nitrogen sources and certain other growth factors (Yongsmith and Passattayangul 1984). Yongsmith and Kittipornpanich (1987) reported the investigation of a successful process for increasing vitamin B_{12} levels in soybean solid residues from a tofu factory using mixed-type fermentation involving lactic acid bacteria and propionic acid bacteria. The tofu solid residues were divided into three portions: the first portion was used for natural lactic acid fermentation, the second portion was used for propionic acid fermentation and then both were mixed together as the third portion and incubated for 3 days. This study indicated that using sequential fermentation of lactic acid bacteria and propionic acid bacteria resulted in an increase in the production of vitamin B_{12} in the finished fermented soybean masses from less than 1 μg per kg of the original soybean substrate to 100 μg per kg of fermented mass.

3.5.1.1 High Vitamin B_{12} Tempeh

Later on, Krusong (1990) found that *Lactobacillus casei* did not affect the vitamin B_{12} production of *P. shermanii* 1250 in tempeh when added during the soaking of the beans and also when added with *P. shermanii* 1250 during fermentation. Then Krusong et al. (1991) had developed successfully the vitamin B_{12}-enriched tempeh using non-sequential mixed fermentation of *R. oligosporus* with *P. shermanii* 1250 process on laboratory or bench scale as shown in Figure 3.2. In general, the optimum conditions used for bench scale production: 55 % initial moisture content of the beans, a mixed culture of 5.0 % *P. shermanii* 1250 and 0.5 % *R. oligosporus* inoculum powder and an incubation temperature of 35 °C for 24–48 h. Bench scale production of high vitamin B_{12} tempeh, performed in stainless steel trays (30 cm×45 cm×3 cm), yielded 297.47 ng vitamin B_{12} per 100 g of mixture at optimum conditions of 2 cm thickness of sterilized soybean mass (1400 g per tray) covered with non-perforated aluminum foil within 24 h incubation (Table 3.9). Krusong method (1990) was the first to elucidate the non-sequential mixed fermentation with *R. oligosporus* and *P. shermanii* 1250 to enhance the high vitamin B_{12} production in tempeh.

There are several advantages in Thai method of vitamin B_{12}-enriched tempeh developed by Krusong. First, besides the production of vitamin B_{12} in tempeh, there is a reduction in the sporulation of *Rhizopus* that affected the texture and appearance of tempeh. Secondly, the strong ammonia smell which normally occurs in traditional tempeh and makes the product unacceptable, especially to westerners is reduced during fermentation. Finally, since *Propionibacterium* is normally used as

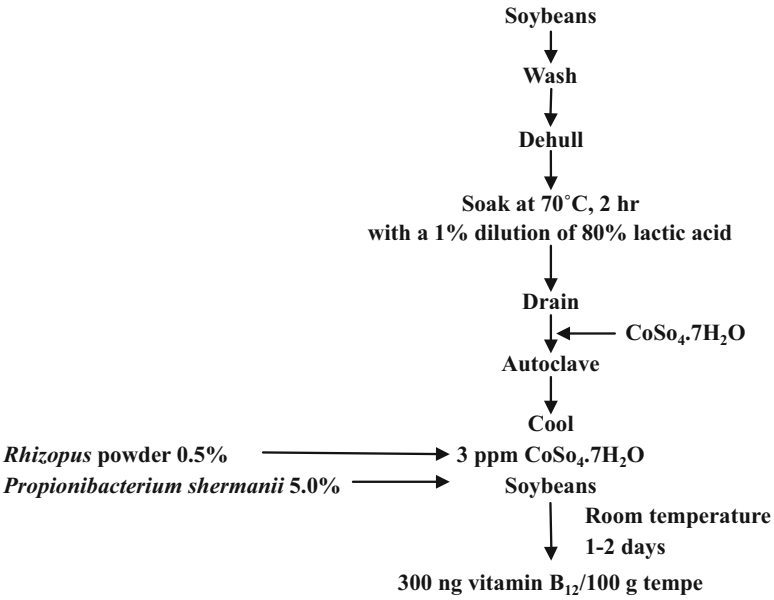

Fig. 3.2 Flow diagram of vitamin B_{12}-enriched tempeh production with propionibacteria

Table 3.9 Yield of vitamin B_{12} in tempeh produced by the Krusong bench-scale method under optimum conditions

Fermentation time (hour)	Vitamin B_{12} (ng/100 g)
Control	36
0	138.1
24	297.5
48	328.1
72	396.0

Source: Krusong (1990)

a starter in cheese making, it provides a favorable flavor suitable to Non-Asians. Thus this product is acceptable to both Asians and Westerners because of the mixed fermentation process.

3.5.1.2 High Vitamin B_{12} Kefir

Another possible utilization of propionibacteria is to enrich vitamin B_{12} in the fermented milk products, yoghurt or kefir by Karlin since 1961. Kefir has been called the yoghurt of the twenty-first century (Gorski 1994). It belongs to a class of acid and alcoholic-fermented milk that had its origin in the Caucasian mountain, Russia, Tibetor Mongolia, many centuries ago. Traditional kefir contains 70 % lactobacilli, 20 % streptococci, and 5 % yeast. It is prepared by adding kefir grains to boiled milk (cow, sheep, or goat). Incubation is carried out at 23–25 °C overnight until acidity

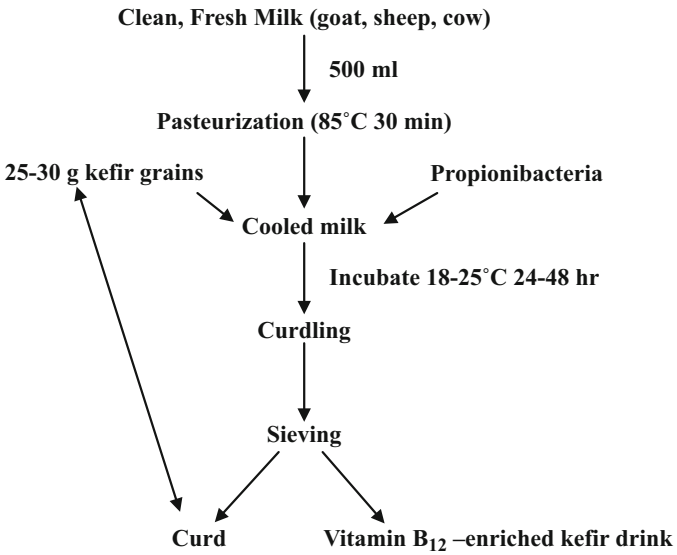

Fig. 3.3 Flow diagram of vitamin B_{12}-enriched kefir production with propionibacteria

reaches 0.85–1.0 % (as lactic) and the pH may drop below 3.0. Carbon dioxide is produced, making the product effervescent. Generally, less than 1 % ethanol is produced. All these changes produce desirable organoleptic quantities. In addition, the benefits of consuming kefir in the diet are numerous, as it is reported to have the antibacterial (Zarcconi et al. 1995), immunological (Furukawa et al. 1990), antitumoral (Furukawa et al. 1991) and hypocholesterolemic effects (Tamai et al. 1996). It should be noted that the presence of propionibacteria in kefir fermentation (Fig. 3.3) results in a marked increase in vitamin B_{12} levels. In another work, Kruglova (1963) prepared vitamin-enriched curds from pasteurized cow's milk by fermenting the milk with equal parts of cultures of lactic acid and propionic acid bacteria (2.5 % each). These curds had approximately ten times more vitamin B_{12} than those produced in the usual way with lactobacilli.

3.5.1.3 High Vitamin B_{12} Kimchi

Our last vitamin B_{12} bioenrichment using propionibacteria is kimchi. A typical winter kimchi was used by Ro et al. (1979) because it is the major and most popular type among Koreans as well as worldwide. The ingredients and their proportions of kimchi are as follows: 81 % (by weight) cabbage, 9 % reddish roots, 27 % green onion, 1.8 % salt shrimp, 0.8 % red pepper, 0.7 % garlic, and 0.5 % ginger (Ro et al. 1979). A flow diagram of vitamin B_{12}-enriched kimchi production is shown in Figure 3.4. The increase in vitamin B_{12} was obtained after 1 week and 5 weeks incubation.

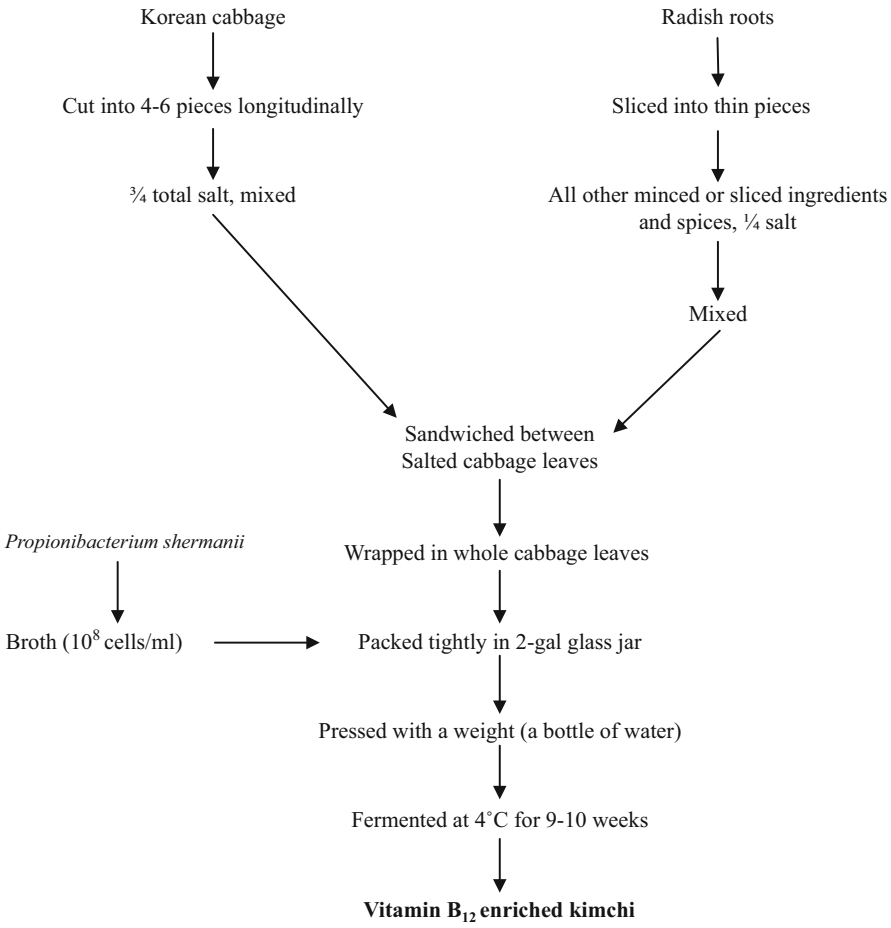

Fig. 3.4 Flow diagram of vitamin B_{12}-enriched kimchi production with propionibacteria

The per capita consumption of kimchi in Korea ranges from 200–300 g (about 1–1½ cups) per day in the winter season which would provide about 0.6 μg of vitamin B_{12} after one week of fermentation or 50 % of the daily requirement (Ro et al. 1979).

3.5.2 Vitamin B_{12} Bioenrichment Using Klebsiella *spp.*

3.5.2.1 High Vitamin B_{12} Tempeh

Steinkraus et al. (1960) reported that the only essential microorganism in tempeh fermentation was the mould, *R. oligosporus*. Steinkraus et al. (1961) examined vitamin analysis in commercial tempeh and found vitamin B_{12} present in all samples, but they also discovered that tempeh made with the pure mould contains no vitamin

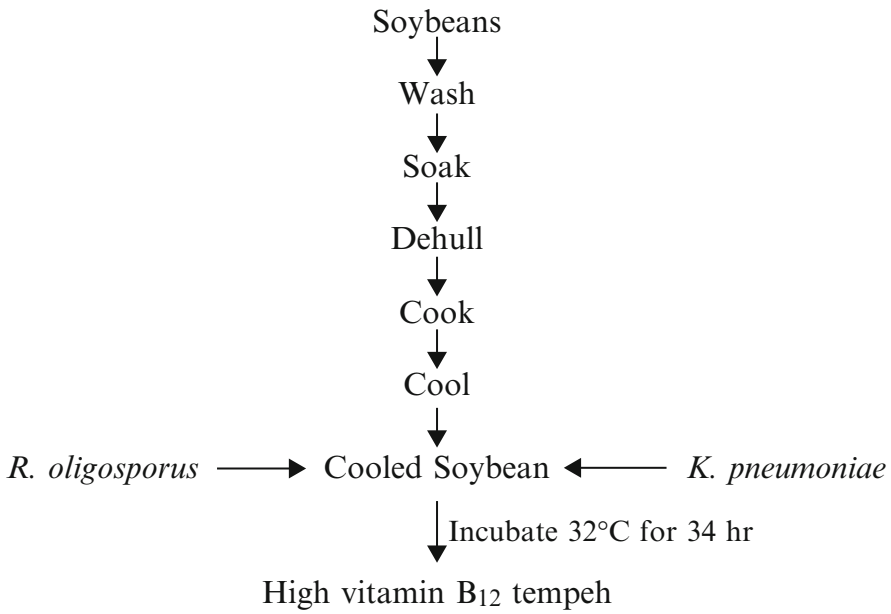

Fig. 3.5 Flow diagram of vitamin B_{12}-enriched tempeh production with *K. pneumoniae*

B_{12}. It was later discovered that the source of vitamin B_{12} in tempeh is a Gram-negative rod (Liem et al. 1977) which is a non-pathogenic strain of *Klebsiella pneumonia*. Since tempeh has become popular among the vegetarians worldwide, the vitamin B_{12} bioenrichment using *Klebsiella* spp. should be considered. Keuth and Bisping (1993; 1994) using PCR screened high vitamin B_{12} producing *Klebsiella pneumonia* and *Citrobacter freundii* from Indonesian tempeh samples and found that neither of them posed the genes encoding the enterotoxins Shiga-like toxin SLT IIA, heat-labile enterotoxins LT Ih, and heat-stable enterotoxins ST Ih. This finding suggested their use to produce vitamin B_{12} during tempeh fermentation. The following diagram (Fig 3.5) illustrates the method of the vitamin B_{12} bioenrichment in tempeh using *Klebsiella pneumonia.*

3.5.2.2 High Vitamin B_{12} Thua-nao

Our group at Kasetsart University has screened a total of 121 isolates of vitamin B_{12} producing thermotolerant *Klebsiella* spp. from various soybean processing sources including soybean soaking drains, soybean curd (tofu) whey, soy sauce drains, and fermented soybean paste (tao-jiew or Thai miso). A strain, *Klebsiella pneumonia* isolated from tofu whey and producing the highest yield of vitamin B_{12} was selected (Phuangpetch 2004) and subsequently used for vitamin B_{12} bioenrichment in thua-nao.

Thua-nao is a Thai traditional non-salted product fermented by *Bacillus* spp. It is produced in Northern Thailand and utilized as a substituent for shrimp (Sundhagul

et al. 1972). To make traditional thua-nao, soybeans are washed, soaked overnight, cooked thoroughly until soft, drained, and fermented naturally without inoculation on banana leaf-lined baskets for 3 or 4 days until the surface of the beans are covered with a sticky, viscous colorless gum and have developed a pungent, ammonia-like odor (Steinkraus 1996). *Bacillus* spp. strains were found to be the dominant microflora of thua-nao. Using soybean as substrates, they cannot produce vitamin B_{12} which is essential for vegetarian diets.

Based on soybean-fermented source and their capability to utilize oligosaccharide (raffinose), three potent microbial isolates having different characteristics to improve thua-nao fermentation were selected. They are *Bacillus amyloliquefaciens*, which is a highly proteolytic, *Klebsiella* sp.KB2 which produces a high level of vitamin B_{12}, and yeast, *Candida* sp.KB1 which produces a pleasant aroma.

In fermentation with monoculture of *Bacillus* sp.B4, there was an increase in proteolytic enzyme activity (825.40 units/gdw) and soluble protein (25.59 %) but not on vitamin B_{12} production. In contrast, monoculture of *Klebsiella* sp.KB2 was essential for vitamin B_{12} production in fermented beans (10.15 μg/100 gdw). Coculture of *Klebsiella* sp.KB2 to Thua-nao fermented by *Bacillus* sp.B4 improved the vitamin B_{12} production (15.45 μg/100 gdw), proteolytic activity (1255.08 units/gdw), and soluble protein (25.87 %). During Thua-nao fermentation by this coculture, *Bacillus* sp.B4 exhibited an increase of proteolytic enzyme as well as soluble protein while *Klebsiella* sp.KB2 was essential for vitamin B_{12} production.

In our investigation we determined optimum conditions for the production of vitamin B_{12} using this model of mixed cultures. As shown in Figure 3.6. The best conditions involved using 8 % of a 10^8 cells/mL inoculums of *Klebsiella* sp.KB2 with the addition of vitamin B_{12} precursors in the following concentrations: 0.3 mg/mL $CoSO_4.7H_2O$ and 0.2 mg/mL 5,6-dimethylbenzimidazole (DBI). This resulted in a vitamin B_{12} concentration of 91.43 μg/100 gdw in the thua-nao. Thus vitamin B_{12} production was correlated with inoculum size and the optimum content of cobalt and 5,6-dimethylbenzimidazol. The vitamin B_{12} content of thua-nao fermented by *Bacillus* sp.B4 and *Klebsiella* sp.KB2 under the optimum condition was nine times higher than the control which was fermented with only *Klebsiella* sp.KB2.

When the fermenting mass was inoculated with *Candida* sp.KB1 which had been isolated from traditional thua-nao, it demonstrated as oligosaccharide-utilizing capacity. In addition, the presence of *Candida* sp.KB1 had a detectable effect on the reduction of strong ammonia smell of the thua-nao product but no interferent effects on vitamin B_{12} enrichment.

Thua-nao sold in the market often has an unpleasant smell and even a stronger and persistent smell of ammonia. This smell could be detected when soybeans were fermented with either a monoculture of *Bacillus* sp.B4 or the mixed cultures of *Bacillus* sp.B4 and *Klebsiella* sp.KB2. However, the strong smell was remarkably reduced when the fermenting mass was co-inoculated with *Candida* sp.KB1 and showed no interference on the growth of *Bacillus* sp.KB4, *Klebsiella* sp.KB2, proteolytic activity, and vitamin B_{12} in the product (Tangjitjaroenkun et al. 2004).

Foods derived from plants are generally thought to be completely absent of vitamin B_{12}. In most cases, soybeans or vegetables would not be expected to contain vitamin B_{12}. Because its biosynthesis is exclusively limited in nature to certain

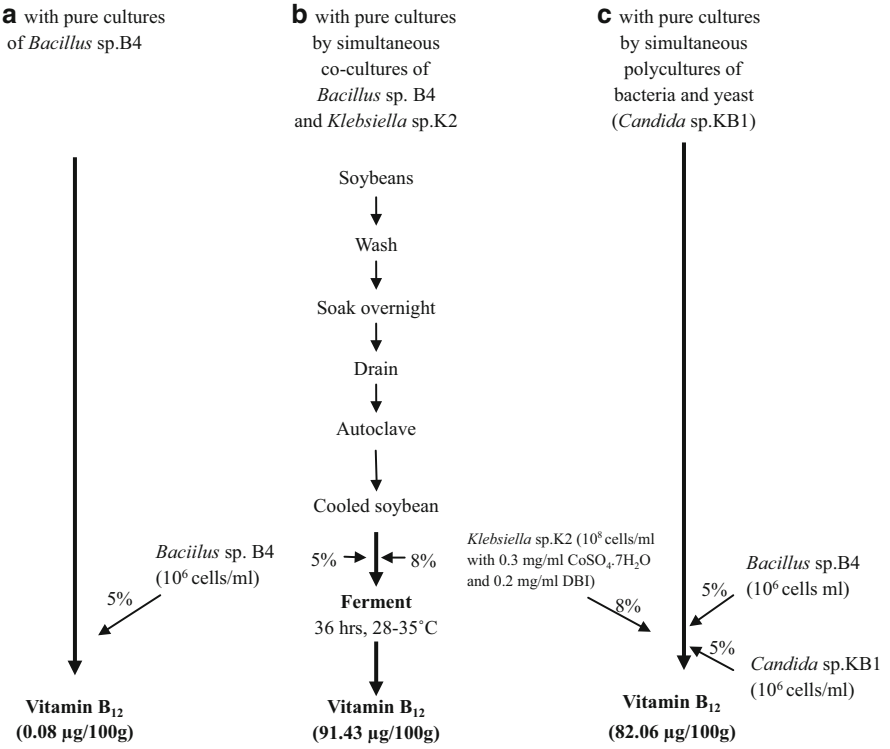

Fig. 3.6 Kasetsart University method of vitamin B_{12}-enriched thua-nao production

bacteria, e.g., *Propionibacteria*, *Pseudomonas*, *Clostridium*, *Streptomyces*, or *Bacillus megaterium*. It is suggested that low levels of vitamin B_{12} in some plants could be detected due to the presence of vitamin B_{12} producing accompanying bacteria (Shurtleff 1983; Steinkraus 1961; Liem et al. 1977; Truesdell et al. 1987). Wang (1984) states that microorganisms used in fermentation of tempeh or miso are not known to synthesize vitamin B_{12}. However, vitamin B_{12} activity in tempeh has been preliminarily detected due to an accompanying *Klebsiella pneumoniae*, which Steinkraus (1983) suggests is a desirable if not essential microorganism in the natural fermentation process. Besides essential lactic acid bacteria in kimchi fermentation, an accompanying *B. megaterium* had been detected as a vitamin B_{12} producer in the product (Kim and Chung 1962). Some developing processes using proper safe bacteria accompanying the fermentation could achieve greater vitamin B_{12} bioenrichment of fermented vegetarian foods, as shown in Table 3.10. It may become feasible for people to get their daily requirement of vitamin B_{12} by these fermented vegetarian foods.

For example, 91.43 µg vitamin B_{12}/100 gdw was produced in thua-nao product with accompanying *Klebsiella* sp.KB2, which was equivalent to about 0.32 µg vitamin B_{12}/100 g wet weight of fermented beans and therefore sufficient for the daily dietary requirement of 1 µg (Herbert 1987, 1994) if we consume 3–4 g wet weight

Table 3.10 Vitamin B_{12} bioenrichment in vegetarian fermented products

Fermented product	Substrate	Essential microbial producer	Accompanying Bacteria	Fermentation Vitamin B_{12} content (μg/100 gdw)		References
				Before	After	
Tempeh	Soybean	*R. oligosporus*	–	None	None	Liem et al. (1977)
			+	0.015	0.5	Steinkraus et al. (1961)
			+	0.1	6.0	Liem et al (1977)
			+	ND	0.12	Truesdell et al (1987)
			+ (Propionibacteria)	0.036	0.4	Krusong et al. (1991)
Miso	Soybean and wheat	*Asp. oryzae*	+	ND	0.15–0.26	Truesdell et al. (1987)
		Yeast				
		Lactic acid				
		Bacteria				
Thua-nao	Soybean	*B. amyloliquefaciens*	+ (*Klebsiella* sp.)	0.08	91.43	Tangjitjaroenkun et al. (2004)
Kimchi	Vegetables	Lactic acid bacteria	+ (Propionibacteria)	0.06	0.186	Ro et al. (1979)

ND not determined

of thua-nao. In another experiment, when the concentration of B_{12} is increased to 60 ng/g (6 μg/100 g) of tempeh (Liem et al. 1977), it becomes feasible for the consumers to get their daily requirement of vitamin B_{12} by consumption of approximately 20 g of tempeh. Moreover, tempeh is one of the first vegetarian foods shown to contain a nutritionally important amounts of vitamin B_{12} essential for proper formation of erythrocytes and prevention of pernicious anemia (Steinkraus et al. 1961; van Veen and Steinkraus 1970). Similar results were also obtained when Sundharagiati (1958) used nampla (fermented fish sauce) to treat macrocytic anemia among disadvantaged people in Thailand.

3.6 Summary

Food fermentation is a method of food preservation and food development that makes a food looks better, tastes better, and give it more nutrients such as enzymes, amino acids, and vitamins. Vitamin B_{12} is a vitamin that is important for nutrition and health. Plants and animals cannot synthesize the vitamin by themselves but a small amount of vitamin B_{12} can be accumulated in animal tissue. A food fermentation process can increase the amount of vitamin B_{12} a great deal. For example, vegetarian foods such as tempe, soy sauce, and soybean as well as non-vegetarian foods such as fish or oysters, where they undergo fermentation, demonstrate an increase in vitamin B_{12} and up to ten times its original concentration. In Thailand, where fermented foods is consumed as a daily flavor enhancing ingredient in the form of fish sauce, the consumption of an average of 15 mL/person/day can prevent Thais from developing vitamin B_{12} deficiency. Therefore since the type of bacteria is so crucial to the production of vitamin B_{12} bioenrichment food. It is apparent that Thailand cannot compete with developed countries in generating pure vitamin B_{12} from her own resources and with available technology, but Thailand could consider the alternative of using certain types of bacteria which are both safe and do not adversely affect food flavor to enrich fermented food products with vitamin B_{12} for the sake of nutrition and health benefits for the people.

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Chapter 4
The Potential Health Benefits of Traditional Thai-Fermented Foods and Beverages

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4.1 Introduction

Fermented foods are globally widely found and consumed. Many kinds of traditional fermented foods which play an important role as seasoning ingredient or food in everyday diet are available in Thailand. There are over 60 traditional Thai-fermented food products that are consumed nationwide (Phithakpol et al. 1995). These traditional fermented food products are made from various local agriculture products and most of traditional fermented foods are produced at household and small-scale industrial levels with relatively simple techniques and equipments. However, a few of traditional fermented foods have undergone industrial development and are now manufactured on a large scale. Fish sauce (nam-pla), fermented fish (pla-ra), and fermented pork (nham) have become well-known examples of Thai traditional fermented foods which are manufactured industrially and commercialized globally. Fermented food products have become of interest and consequently provide new subjects for intellectual creation in these few years. While traditionally fermented foods may pose a potential health concern to nontraditional

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consumers due to the reported therapeutic properties of fermented food. Advanced scientific knowledge on food fermentation and the involved microbial agents have increasingly revealed a great number of beneficial effects, which lead to new applications beyond food preservation, safety, and sensory appreciation.

In the past, due to the lack of scientific and technological know-how, traditional Thai-fermented food products were generally evaluated on the basis of qualitative attributes, such as flavor, aroma, texture, and taste. Nowadays, Thais, like the other consumers worldwide, pay an increased attention to the relation of food consumed and health. Additionally, the market for foods with health-promoting properties, also known as functional foods, has shown a remarkable growth over the last few years. These are significant driving factors for scientific investigations with increased interest in the research and development in the area of functional food. A lot of attention is currently focused on fermentation technologies and their products with the objective to tap into potentially associated health benefits. In this chapter, some selected traditional Thai-fermented food and beverages are reviewed in relation to the availability of current scientific data and the associated proposed functionalities to communities.

4.2 Classification of Traditional Thai-Fermented Foods and Beverages

Fermented food products can be classified into many different ways in accordance to the diverse viewpoints of authors (Yokotsuka 1982; Kuboye 1985; Campbell-Platt, 1987, Odunfa and Komolafe 1988; Dirar 1993) (Table 4.1). The fermented foods and beverages produced in Thailand are generally classified in accordance with the raw materials used, the major fermentation type, the product usage, and the production technology. Tanasupawat and Visessanguan (2008) present the classification of Thai-fermented food products which is based on commodity, while Kraidej (2003) proposed a segregation into two main categories; non-salted and salted-fermented food products (Caplice and Fitzgerald 1999).

4.3 Microorganisms Involved in Traditional Thai-Fermented Food and Beverages

Traditional food fermentation normally involves a number of specific microorganisms, which are highly variable and strongly dependant on raw materials, ingredients, processing conditions, and further environmental and handling circumstances. A variety of microorganisms, including lactic acid bacteria, aerobic bacteria, yeasts, and molds are present in many traditional fermented food products. The microorganisms perform physio-biochemical modifications of the initial materials and other activities, resultant in the contribution of microbial safety, at least one organoleptic,

Table 4.1 Classification of fermented foods and beverages

Classification	Group	References
Microorganisms	1. Alcoholic beverages (yeast)	Yokotsuka (1982)
	2. Fish or meat (enzymes and *Lactobacillus* spp.)	
	3. Milk products (*Lactobacillus* spp.)	
	4. Plant proteins (molds with or without *Lactobacillus* spp. and yeasts)	
	5. Pickles (*Lactobacillus* spp.)	
Commodity	1. Cassava-based products	Kuboye (1985)
	2. Cereals	
	3. Legumes	
	4. Beverages	
	1. Beverages	Campbell-Platt (1987)
	2. Cereal products	
	3. Dairy products	
	4. Fish products	
	5. Fruit and vegetable products	
	6. Legumes	
	7. Meat products	
	8. Starch crop products	
	9. Miscellaneous products	
	1. Alcoholic beverages	Odunfa and Komolafe (1988)
	2. Animal protein	
	3. Cereals	
	4. Starchy roots	
	5. Vegetable proteins	
	1. Fermented grains and cereal products (khao-mak: Thai-fermented glutinous rice, khanom-jeen: Thai-fermented rice noodle, sa-to, nam-khao, kra-chae, and ou: Thai rice wines, thua-nao: Thai natto)	Vachanavinich et al. (1994), Tanasupawat and Visessanguan (2008)
	2. Fermented fish products (nam-pla: Thai fish sauce, ka-pi: Thai shrimp paste, bu-du, pla-ra, pla-som, pla-chai, som-fug, pla-chom, and pla-paeng-daeng)	
	3. Fermented meat products (nham: Thai-fermented pork sausage, sai-krog-prieo: Thai sausage, mum: Thai-fermented beef or pork sausage or beef/pork liver)	
	4. Fermented plant products (naw-mai-dong: Thai-fermented bamboo shoot, phak-gad-dong: Thai pickle green mustard, miang: Thai-fermented tea leaves, hom-dong: Thai-fermented green onion, phak-sian-dong)	
Functional basis	1. Akil-munasabat (food for special occasions)	Dirar (1993)
	2. Kissar (staples)	
	– porridges and breads such as aceda and kissar	
	3. Marayiss (30 types of opaque beer, clear beer, date wines, meads (honey wines), and other alcoholic beverages)	
	4. Milhat (sauces and relishes for the staples)	

(continued)

Table 4.1 (continued)

Classification	Group	References
Microbial metabolism	1. Acetic fermentations (apple cider, palm wine vinegars, coconut water vinegars, Kombucha, nata de pine, and nata de coco)	Steinkraus (2002), Astawan (2010)
	2. Alcoholic fermentations (wines, Indian chichi, sugarcane wines, Japanese sake, Thai rice wine, Chinese lao-chao, and Mexican pulque)	
	3. Alkaline fermentations (African iru, Nigerian dawadawa, Japanese natto, ogiri, Indian kenima, and Thai thua-nao	
	4. Lactic acid fermentations (sauerkraut, Korean kimchi, cucumber pickle, yogurts, Russian kefir, cheeses, sour-dough bread, Egyptian kishk, Greek trahanas, Indian idli, Ethiopian enjera, Sudanese kisra, Philippine puto, and Thai nham: fermented pork)	
	5. Leavened breads (yeast and sour-dough breads)	
	6. Meat-flavored-amino acid/peptide-generating fermentations (Chinese soy sauce, Japanese shoyu, Japanese miso, nuocmam: Vietnamese fish sauce, patis: Philippine fish sauce, bagoong: Philippine fish paste, nam-pla: Thai fish sauce, mam: Vietnamese fish paste, and ka-pi: Thai shrimp paste)	
	7. Protein meat substitute-producing fermentation (Indonesian tempe and ontjom)	
Other classifications	1. Ready for consumption (yogurt, bread, and salami)	Sahlin (1999)
	2. Ready for consumption but mostly used as ingredient (crème fraiche)	
	3. Only used as ingredient (soy sauce and dawadawa)	
	1. Containing viable starter culture (yogurt and cheese)	Sahlin (1999)
	2. Not containing viable starter culture (soy sauce, bread, beer, and wine)	
	3. Microorganisms used in an early step of the production (cocoa, coffee, and cassava products)	
	1. Lactic acid bacteria-fermentation	Sahlin (1999)
	2. Mold-fermentation	
	3. Yeast-fermentation	
	4. Other bacteria-fermentation	
	5. Enzymatic fermentation	

(continued)

Table 4.1 (continued)

Classification	Group	References
Other classifications	1. Non-salted-fermented foods	Kraidej (2003)
	– Alcoholic fermentation (khao-mak, Thai fruit wine, and nam-tan-mao)	
	– Non-alcoholic fermentation (khanom-jeen and thua-nao)	
	2. Salted-fermented foods	
	– Fermented vegetables (phak-gad-dong and phak-sian-dong)	
	– Fermented fruits (kra-thorn-dong, ma-kam-dong, ma-yom-dong, and ma-nao-dong)	
	– Fermented beans (tao-huu-yee, tao-cheow, and see-iu)	
	– Fermented meats (nham and sai-krog-prieo)	
	– Other fermented meat products (khai-kemp)	
	– Fermented fish (bu-du, khem-mak-nat, nam-pla, pla-jom, pla-paeng-daeng, pla-ra, pla-som, som-fug, and tai-pla)	
	– Fermented shrimp (ka-pi, koong-jao, koong-jom, and koong-som)	
	– Other fermented aquatic invertebrates (hoi-kraeng-dong, hoi-ma-laeng-poo-dong, and hoi-siap-dong)	
	– Other (hoi-som, nam-poo, and poo-khem)	

Adapted from Stienkraus (1997), Sahlin (1999), and Kraidej (2003), Sumpavapol et al. (2010)

nutritional or health advantage in the fermented food products. Examples of the significant microorganisms responsible for the fermentation of traditional Thai-fermented foods and beverages are summarized below.

4.3.1 Bacillus *spp.*

Bacillus spp. are Gram-positive, strict or facultative aerobe, and endospore-forming bacteria. A variety of *Bacillus* spp. (i.e., *B. subtilis*, *B. laterosporus*, *B. pumilus*, *B. brevis*, *B. macerans*, *B. licheniformis*, *B. polymyxa*, and *B. coagulans*) are naturally present in raw materials, especially grains and cereals. Most varieties of *Bacillus* spp. are not harmful to humans and generally known to be safe (GRAS, generally recognized as safe) microorganisms (Kramer and Gilbert 1989; Denner and Gillanders 1996). Such microorganisms have been found to be involved in various traditional fermented cereal and legume foods with the main activity during the

fermentation process to be enzymatic proteolysis of the starting raw materials (Ogbadu et al. 1990). Additionally, *B. subtilis* and *B. licheniformis* are found in plara and participate in protein degradation (Hong-tongdaeng 1979). *Bacillus* proteases are very efficient in breaking down proteins into smaller peptides or amino acids. Furthermore, fermentation by *Bacillus* spp. can provide products of high nutrient value (Kiers et al. 2000). Among *Bacillus* spp., *B. subtilis* is well-known to be the dominant isolate in fermented soybeans (Chantawannakul et al. 2002; Sarkar et al. 2002; Ouoba et al. 2004) and has a long history of application in enzyme production and food fermentation.

4.3.2 Lactic Acid Bacteria

Lactic acid bacteria (LAB) are Gram-positive, non-spore forming, catalase-negative coccus, or rod-shaped bacteria which are aerotolerant or microaerophilic. LAB can be categorized into two groups (homo- or hetero-fermentative microorganisms) based on the production of organic acids or other metabolites subsequent to the utilization of available sugars. LAB include the genus *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, and *Weissella*. The group of LAB has a long and safe history in the production of fermented foods and beverages and the consumption thereof. LAB cause rapid acidification of the raw materials through the production of organic acids, mainly lactic acids. Moreover, their production of acetic acid, ethanol, aromatic compounds, bacteriocins, exopolysaccharides, vitamins, and several useful enzymes is of importance. Therefore, they play a crucial role in the development of the desirable organoleptic and hygienic quality of the end products. Many studies revealed various LAB to be involved in the production of traditional Thai-fermented foods. *Lb. farciminis*, *Lb. acidipiscis*, *P. halophilus*, and *W. thailandensis* have been found in fermented fish products, such as pla-ra, while *Lb. fermentum*, *Lb. pentosus*, *Lb. plantarum*, *Lb. sakei*, and *P. pentosaceus* have been found to be present in pla-som and som-fug (Hong-tongdaeng 1979; Tanasupawat et al. 1998, 2000; Kopermsub and Yunchalard 2010). The lactobacilli including *Lb. pentosus*, *Lb. plantarum*, and *Lb. vaccinostercus* are usually found in miang, Thai-fermented tea leaves (Okada et al. 1986; Tanasupawat et al. 1992).

4.3.3 Yeasts and Molds

Yeasts are unicellular microorganisms which reproduce asexually by budding. Yeasts occur in a wide range of traditional Thai-fermented foods and beverages, made from raw materials of both plant as well as animal origin. The most beneficial yeasts in terms of desirable food fermentation are from the *Saccharomyces* genera, especially *S. cerevisiae*. Other genera include *Pichia* spp., *Saccharomycopsis* spp., *Endomycopsis* spp., *Candida* spp., *Hansenula* spp., *Rhodotorula* spp., and *Torulopsis*

spp. (Batra and Millner 1974). In general, yeasts play an important role in the food fermentation as they produce enzymes which favor desirable biochemical reactions, such as the production of alcohol, aroma, and flavor (Aidoo et al. 2006). In addition to yeasts, molds are also the main microorganisms involved in the fermentation of traditional Thai food products, such as soy sauce, tao-cheow, and rice wines (Nout and Aidoo 2002). *Aspergillus* spp., *Rhizopus* spp., *Mucor* spp., *Actinomucor* spp., *Amylomyces* spp., *Neurospora* spp., and *Monascus* spp. are dominant mold species and widely used in the production of fermented food products in Thailand

Loog-pang (commonly well-known as Chinese yeast cake) is taken the form of a dry power or dry tablets which contain mixed starter cultures of amylolytic molds, fermenting yeasts, and sometimes lactic acid bacteria grown on rice or other cereals. Various natural herbs or spices are added. Each recipe of loog-pang is normally different in composition and generally kept secret. Loog-pang is used for the production of traditional fermented products from various kinds of starchy raw materials, especially rice. Thai loog-pang is commonly categorized into 3 types (loog-pang khao-mak; starters for alcoholic sweetened rice snack, loog-pang-lao; starters for lao (rice wine) and loog-pang nam-som-sai-chu; starters for nam-som-sai-chu (vinegar) (Lotong 1998). Previous study elucidated on the diversity of yeasts present in loog-pang khao-mak and loog-pang lao (Limtong et al. 2002). The major predominant yeast in both types of loog-pang is *Saccharomycopsis fibuligera*, which shows strong amylolytic activity, but which is revealed to be alcohol producer. Other isolates include *Pichia anomala*, *P. fabianii*, *P. mexicana*, *P. heimii*, *P. burtonii*, *Issatchenkia orientalis*, *Candida rhagii*, *C. glabrata*, *Torulaspora globosa*, *T. delbrueckii*, *Rhodotorula phylyla*, *S. cerevisiae*, and *Trichospora faecale*. These have low amylolytic activity, but exhibit high or moderately high alcoholic fermentation. Limtong et al. (2005) reported that the two most isolated molds present in loog-pang khao-mak are of the genus *Amylomyces* and *Rhizopus*. The remaining isolates are of the genera *Actinomucor*, *Aspergillus*, *Mucor*, *Monascus*, and *Penicillium*. As for loog-pang-lao, the two most isolates of molds belong to the genus of *Rhizopus* and *Amylomyces*. Most isolates of *Amylomyces* and *Rhizopus* exhibited a relatively high amylolytic activity, which indicated these two mold genera to play a significant role in the hydrolytic breakdown of starch in glutinous rice (the starting raw material for production of khao-mak and lao).

4.3.4 Other Important Bacteria

Some other bacteria can be found in many traditional Thai-fermented products. Such bacteria may be exerted with the quality of the products. *Micrococcus* spp., *S. aureus*, and *S. epidermis* are found both in pla-ra and pla-som. *Halobacterium piscisalsi* is also found in pla-ra (Yachai et al. 2008). Certain isolates of *Staphylococcus* spp. are found to produce proteinase, lipase, and gelatinase, which are thought to be important for flavor development in fermented pork (Khieokhachee et al. 1997). Tanasupawat et al. (2006, 2007a, b, 2008) have recently reported

Lentibacillus halophilus, *Piscibacillus salipiscarius*, and *Enterococcus thailandicus* can be isolated from nam-pla, pla-ra, and mum (fermented beef sausage mixed with liver). Moreover, *Lactobacillus thailandensis*, *Lb. camelliae*, and *Pediococcus siamensis* have been identified to be present in miang (Thai-fermented tea leaves) (Tanasupawat et al. 2006, 2007a, b).

Nearly all food fermentations are the result of activity of microorganisms, either working together or in a sequence. For example, Thai sweet-low alcoholic snack (khao-mak) is a joint effort between mold and yeast. Rice is rich in starch and thus not utilizable by yeast. Therefore, the amylolytic mold present in loog-pang hydrolyzes starch to simpler sugars, which subsequently can be used by the yeast present to produce alcohol. Yeasts and molds exhibit dissimilar preferences of conditions of growth, which are set within narrow limits. A microorganism that initiates fermentation grows there until its by-products inhibit further growth and activity. During this initial growth period, other microorganisms develop which are ready to take over when the conditions become intolerable for the former ones.

4.4 Selected Traditional Thai-Fermented Foods and Beverages

The majority of traditional Thai-fermented products is produced by using traditional processes and methodologies at both the cottage and small-scale levels (Valyasevi and Rolle 2002). Main raw materials for the preparation of fermented foods are normally derived from local agricultural products, such as fish, meat, shrimp, soybean, rice, or vegetables. In addition, other ingredients may be added to improve the unique characteristic and quality of the final product. The relevant microorganisms play an active role in changes of the physical, nutritional, and organoleptic properties of the starting raw materials and ingredients during the spontaneous fermentation (Table 4.2). This section illustrates the general process steps for the production of selected traditional Thai-fermented foods and beverages.

4.4.1 Fermented Minced Fish Cake (Som-fug)

Som-fug (Thai: ส้มฟัก) is a low salt-fermented minced fish cake and sometimes known as nham-pla. Som-fug constitutes a combination of minced fish flesh, streamed rice, salt (2.5–5 %), minced garlic (4 %), and sugar. The mixture is kneaded until its appearance becomes gel-like and forms a sticky and elastic paste (Riebroy et al. 2004, 2005, 2008). The paste is divided into small portions and subsequently packed and tightly wrapped in either banana leaves or plastic bags. The raw som-fug is left to ferment for 3–5 days at ambient temperatures (Saisithi et al. 1986) (Fig. 4.1). Changes in physical, microbiological, and biochemical during fermentation involve fish tissue as well as microbial enzymes. These changes are influenced

Table 4.2 Some traditional Thai-fermented foods and beverages and their associated microorganisms

Local name	Main raw materials and ingredients	Microorganisms	Typical characteristics	References
Nham	Ground red pork meat, pork rind, garlic, steamed rice, salt, pepper, chili	*Lb. plantarum*, *Lb. pentosus*, *Lb. sakei*, *Lb. curvatus*, *Lb. cellobiosus*, *Lb. acidophilus*, *Lb. paracasei*, *Lb. delbruckii*, *Lb. brevis*, *P. acidilactici*, and *P. pentosaceus*	Pink with a sour and slightly salty taste and firm texture	Tanasupawat and Daengsubha (1983), Tanasupawat et al. (1992), Valyasevi et al. (1999), Valyasevi et al. (2001), Visessanguan et al. (2006)
Som-fug	Minced fish, minced garlic, ground steamed rice, and salt	*Lb. plantarum*, *Lb. brevis*, *Lb. fermentum*, and *P. entosaceus*	Pinkish in color, slightly sour and salty with garlic flavor and a relatively firm and springy texture	Saisithi et al. (1986), Tanasupawat et al. (2000)
Khanom-jeen	Rice and water	*Lb. plantarum*, *Lb. cellobiosus*, *Lb. fermentum*, *Leuco. lactis*, and *P. acidilactici*	Sour taste and flavor, sticky and tenderized texture, and yellowish rice noodle	Tanasupawat and Komagata (1995), Sribuathong et al. (2004), Sribuathong and Trevanich (2005)
Khao-mak	Glutinous rice and loog-pang	*A.rouxii*, *Rhizopus* spp., *Mucor* spp., *S. cerevisiae*, *Hansenula*, *P. anomala*, and *P. burtonii*	Semisolid, sweet, low alcoholic rice paste with mild-sour taste	Kofi et al. (1996), Boon-Long (1986)
Phak-gad-dong (fermented mustard leaf or mustard leaf pickle)	Green mustard leaf, salt, pickle sauce (water, soy sauce or fish sauce, vinegar, sugar, additives such as chili, herbs, etc.)	*Lb. plantarum*, *Lb. pentosus*, *Lb. fermentum*, *Lb. pentosaceus*, *Leuco. mesenteroides*, *Enterococcus* spp., and *Pediococcus* spp.	Yellowish, crispy and sour or salty in taste	Tanasupawat and Komagata (1995), Srilaong (2007)
Miang (fermented tea leaves)	Tea leaf (*Camellia sinensis*)	*Lb. plantarum*, *Lb. fermentum*, *Lb. pentosus*, *Lb. vaccinostercus*, *E. casseliflavus*, *Lb. thailandensis*, *and P. siamensis*	Pickle flavor, sour and flowery	Okada et al. (1986), Tanasupawat and Komagata (1995), Tanasupawat et al. (2007a, b), Klayraung et al. (2008a, b)

(continued)

Table 4.2 (continued)

Local name	Main raw materials and ingredients	Microorganisms	Typical characteristics	References
Nam-pla	Fish (freshwater or marine fish) and salt	*Halophilic and holotolerant bacteria* (i.e., *Lentibacillus halophilus*, *Salinivibrio siamensis*)	Clear, amber to reddish brown liquid with sharp and salty taste and cheesy and meaty aroma	Wongkhalaung (2004), Tanasupawat et al. (2006), Tapingkae et al. (2008), Chamroensaksri et al. (2009), Namwong et al. (2011)
Naw-mai-dong (fermented bamboo shoots)	Bamboo shoots, salt, and water (rice wash water)	*Lb. pentosus* and *Lb. brevis*	Sour taste with pickled flavor	Tanasupawat and Komagata (1995)
Sa-to (rice wines)	Rice, water, and loog-pang-lao	*S. cerevisiae*, *S. fibuligera*, *A. oryzae*, *Amylomyces rouxii*, *Rhizopus* spp., and *Mucor* spp.	Cloudy yellow liquid with alcohol	Sukhumavasi et al. (1975), Lotong et al. (1985)
Thua-nao	Soybean	*B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. megaterium*, *B. amyloliquefaciens*, and *Klebsiella* spp.	Grayish brown color of soybean seeds of a slight mucilaginous substance in appearance and a strong pungent ammonia-like odor	Leejeerajumnean et al. (2001), Chantawannakul et al. (2002), Tangjitjaroenkun et al. (2003), Petchkongkaew et al. (2008), Chukeatirote et al. (2010)

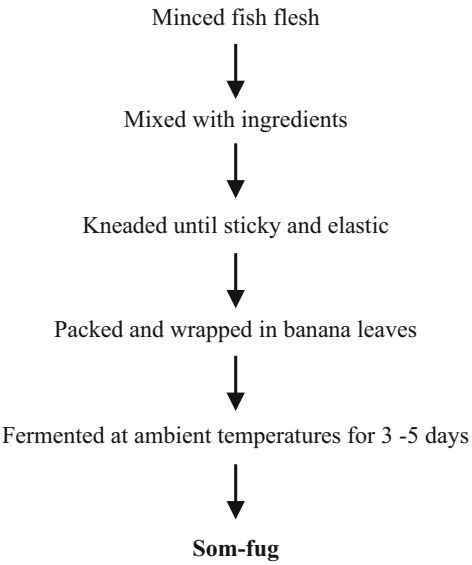

Fig. 4.1 Process for the production of som-fug

by the process conditions. The fermentation process depends on different parameters, such as the proportions and the quality of the raw materials, the use of starter cultures, temperatures and relative humidity, and mixed bacteria naturally present in the raw materials including *Lb. plantarum*, *Lb. fermentum*, *Lb. paracasei*, *Lact. lactis*, *P. pentosaceus*, and coagulase-negative *Staphylococcus* occupy a major role in the spontaneous fermentation of som-fug. Som-fug, which is slightly sour and salty in flavor and of firm and springy texture, is an excellent source of protein (11.4–16.2 %). Som-fug can be either served as a main dish or as a condiment with vegetables.

4.4.2 Fermented Pork Sausage (Nham)

Nham (Thai: แหนม) is one of the most popular traditional fermented pork sausages, which is widely consumed throughout Thailand. It is made from fresh ground pork, boiled and sliced pork rind, steamed rice, garlic, ground black pepper, chili, sugar, salt, and sodium or potassium nitrite. The mixture is packed and tightly wrapped in either fresh banana leaves or airtight cylindrical plastic bags. The product is left to ferment at ambient temperatures for 3–4 days (Fig. 4.2). The final pH level of raw nham is typically in the range of 4.5–4.6. Lactic acid bacteria including *Lb. plantarum*, *Lb. pentosus*, *Lb. sakei*, *P. acidilactici*, and *P. pentosaceus* are the main bacteria responsible for the nham fermentation (Wiriyacharee 1990; Tanasupawat and Komagata 1995; Krairojananan et al. 1997). Acid production by these LAB contributes to both intrinsic quality and safety in the fermentation of nham. Lactic acid produced is mainly responsible for the sour taste, typical fermented aroma and firmness

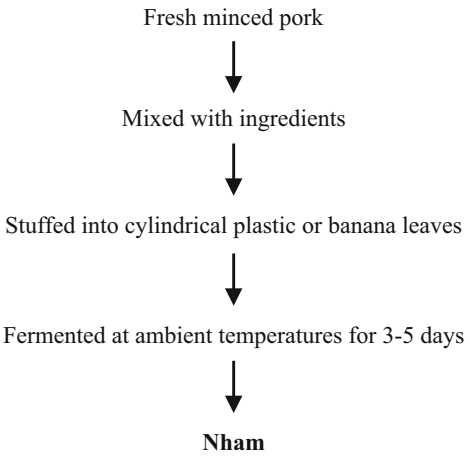

Fig. 4.2 Process for the production of nham

of texture and can inhibit the growth of acid-sensitive pathogenic bacteria. In addition, *Micrococcus varians* (*Kokuria varians*) is also found to be important in conversion of nitrite to nitroso-haemoglobin by means of nitrite reductase, thus, renders nham to be of pink color. However, yeasts, molds as well as non-lactic acid bacteria are also found during the initial stages of nham fermentation (Khieokhachee et al. 1997).

Nham is considered to be ready to eat and usually consumed raw. However, some studies revealed nham to be occasionally contaminated with diverse pathogenic bacteria (e.g., *Salmonella* spp., *Listeria monocytogenes*, and *S. aureus*) and parasites (e.g., *Trichinella spiralis* and *Taenea solium*) (Somathiti 1982). Therefore, nham should be cooked on its own or as a part of a main dish such as fried rice with nham. At present, nham is developed to full-scale commercialization by using defined starter culture. Vanusnun nham, for example, has with the application of mixed starter cultures including *Lb. plantarum* and *P. cerevisiae* as well as *Micrococcus varians*. Further potential LAB are likely to be used as new starter cultures in nham fermentation in the future because their properties potentially renders a certain added value to the finished product.

4.4.3 Fermented Rice Noodle (Khanom-jeen)

Khanom-jeen (Thai: ขนมจีน) is the oldest traditionally fermented rice noodle of Thailand. Khanom-jeen is popularly consumed among most Thai people because of its unique characteristics, for instance sour taste and flavor, sticky and tenderized texture, and yellowish color. Khanom-jeen can be eaten with various curries, such as red curry with fish and green curry with chicken (Tanasupawat and Komagata 1995). Khanom-jeen is traditionally only made from rice, fermented with microorganisms naturally present in the starting raw materials and/or processing environments. The simple process of khanom-jeen fermentation is shown as in Fig. 4.3.

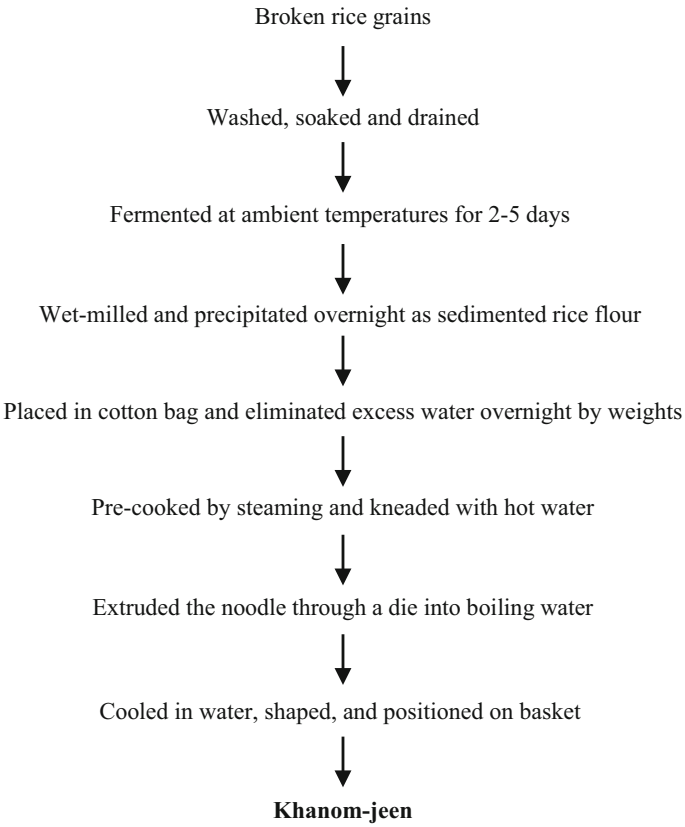

Fig. 4.3 Process for the production of khanom-jeen

Khanom-jeen is classified into two types: fermented and non-fermented rice noodles. As for the fermented type, the broken rice grains are washed and soaked in water both with and without change of water and fermented naturally without a starter at ambient temperatures for 2–5 days. The resultant material is wet-milled and precipitated overnight. The sedimented rice flour is transferred into a cotton bag and excess water is extracted by means of weights overnight. Rice flour is pre-cooked by streaming and kneaded with the addition of appropriate volume of hot water. The kneaded rice flour is extruded through a die into boiling water and the rice noodle is cooled in water. The rice noodle is shaped and lined on either a plastic sheet or banana leaf-covered bamboo or plastic basket. Lactic acid bacteria are the main microorganisms involved in the fermentation of khanom-jeen. These include *Lactobacillus* spp., *Leuconostoc* spp., and *Pediococcus* spp. The non-ferment type is made by soaking of rice grains in water for one night. Next morning, the wet-grains are immediately milled and the subsequent flour paste is weighed, processed, and cooked similar as the fermented type. Khanom-jeen is widely produced on a cottage or small scale. Therefore, the quality of the product depends on the skills of the manufacturer and processing conditions present. Moreover, the

process of the production of khanom-jeen in Thailand varies plant to plant and region to region.

4.4.4 Fermented Soybean (Thua-nao or Thai natto)

Thua-nao (Thai: ถั่วเน่า), a natto-like product, is the best-characterized-fermented soybean in Thailand. This food has been mainly produced by the rural people in the northern part of Thailand. Processes and equipment apply differ in different areas and communities. However, the conventional production process of thua-nao is summarized as shown in Fig. 4.4.

Briefly, soybeans are washed, soaked in water overnight, and steamed for approximately 3–4 h until soft. The steamed soybeans are cooled and packed in bamboo-lined baskets and covered with banana leaves. The fermentation proceeds outdoors exposed to sunlight, resultant in a dried form of thua-nao (thua-nao-kab), which can be kept for several months. Alternatively, soybeans are allowed to ferment spontaneously for 2–3 days at ambient temperatures, resulting in a fresh thua-nao. Fresh thua-nao can be consumed after steaming or roasting while the dried products are important ingredients in a variety of local Thai dishes. Moreover, thua-nao is appropriate for vegetarians. Similar fermented soybean products are in some Asian countries, e.g., kinema in India, schuidouchi in China, and natto in Japan. Similar to the Japanese-styled fermented natto, the dominant microorganism found in the

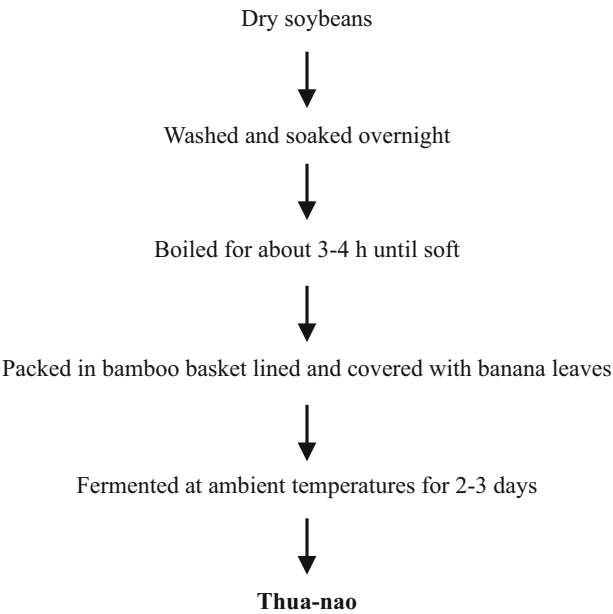

Fig. 4.4 Process for the production of thua-nao

Thai traditional thua-nao was isolated and identified as *B. subtilis* (Chantawannakul et al. 2002). This bacterium exhibits a relatively strong proteolytic activity which causes protein hydrolysis and a release of ammonia. The release of ammonia creates alkaline condition and thus thua-nao is often known as alkaline-fermented soybeans. However, the viscosity of thua-nao is less apparent than that of Japanese natto, probably due to the activity of the γ-polyglutamic acid-degrading enzyme (γ-glutamyl hydrolase) from *Bacillus* spp.

4.4.5 Fermented Mustard Leaf or Mustard Leaf Pickle (Phak-gad-dong)

Phak-gad-dong (Thai: ผักกาดดอง) is one of the traditional salted-fermented leafy vegetables in Thailand. The fresh mature green leaf mustard is cut, left to sun-dry for 1 day, trimmed, and packed tightly layer by layer in a fermentation tank or other container. Dry salt or salt solution (approximately 20 % brine solution) is subsequently added into the layered vegetable and covered with a heavy plastic sheet secured with weights. The vegetable is allowed to ferment naturally at ambient temperatures for a certain period, which depends on the desired characteristic of the finished product and salt concentration (Srilaong 2007). Additionally, the salt concentration used affects the nutritional changes of the product during fermentation (Lee et al. 1985). The general manufacturing process of leaf mustard pickle is shown in Fig. 4.5.

Lactic acid bacteria are the main microorganisms involved in the fermentation of leaf mustard. These include *Leuco. mesenteroides*, *Lactobacillus* spp., and

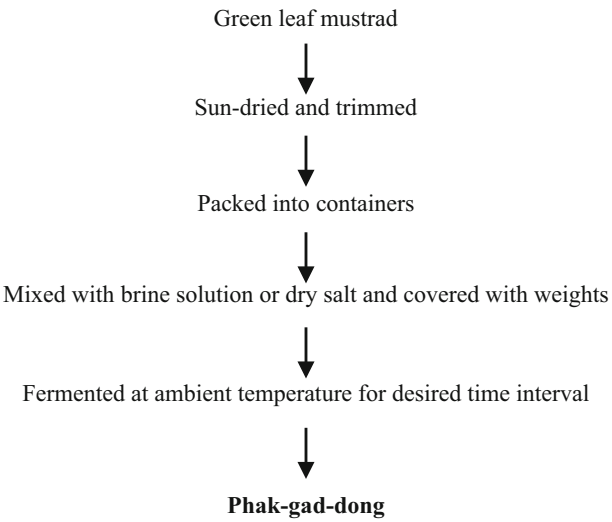

Fig. 4.5 Process for the production of phak-gad-dong

Pediococcus spp. The populations of such LAB vary widely as affected by salt concentration and fermentation time intervals. LAB play an important role in the sensory changes of the vegetables and inhibitory effects on growth of spoilage and pathogenic microorganisms. In general, the resultant phak-gad-dong is yellowish with a crispy texture and a sour or salty taste (pickled flavor). Phak-gad-dong can be displayed uncovered in the market or hermetically sealed and sterilized in tin cans with easy to open lids and retort pouches for domestic and overseas marketing. Phak-gad-dong is popularly consumed on its own without cooking or used as ingredients for food preparation.

4.4.6 Rice Wines

Unlike in Europe and the United States, traditional alcoholic beverages in south-east Asia are primarily produced primarily from cereals, especially rice grains (Law et al. 2011). Rice wines or lao are a group of traditionally well-known alcoholic beverages found in Thailand. The varieties of Thai rice wines depend on the raw material used, inoculums, and fermentation processes. The most popular rice wines are sa-to (Thai: สาโท), nam-khao (Thai; น้ำขาว), kra-chae (Thai; กระแช่), and ou (Thai; อุ). Sa-to is the Thai rice wine with an alcohol content of 7–8 % (by

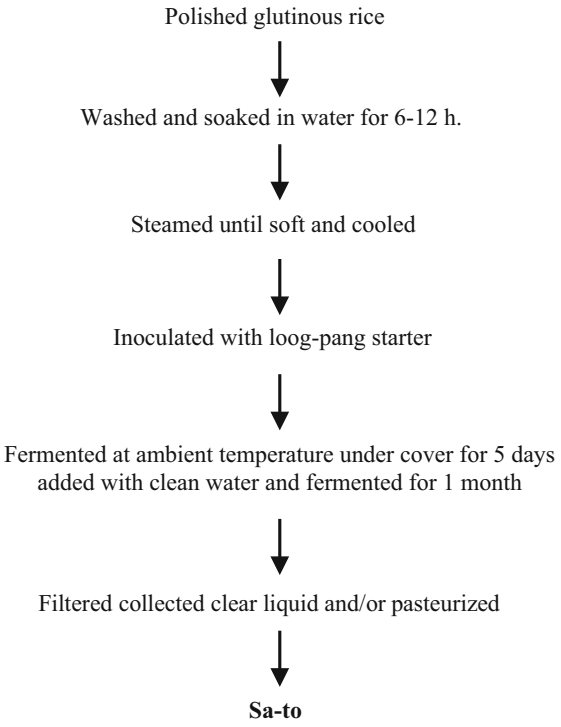

Fig. 4.6 Process for the production of sa-to

volume) or beyond and its appearance is a cloudy yellow. It is believed that sa-to is served as an important source of nutrients. Generally, the traditional method of sa-to production can be defined as follows (Fig. 4.6). The polished glutinous rice (either white or black) is washed, soaked in water for a few hours, and then steamed. The streamed glutinous rice is spread out on cheesecloth to cool to room temperature and the powdered starter (loog-pang-lao) is dispersed on the steamed rice. The mixture is transferred into a wide-mouthed earthen jar for the saccarifying process (first stage fermentation) by molds at ambient temperatures for 3–5 days. Clean water is added into moromi (nam-toy) in an earthen jar for continuous fermentation at ambient temperatures for 1 month (second stage fermentation for alcoholic production). A combination of the functional enzymes from yeasts and molds present in loog-pang-lao is beneficial for the fermentation process and the development of flavor of the product. Sa-to is traditionally drunk through a long fine bamboo straw without filtration. Sa-to is a popular alcoholic beverage with certain local Thai people since ancient times. Additionally, sa-to can be also used as an ingredient of meals to relieve odor or stench and to enhance appetite. Recently, the sa-to production process under hygienically controlled conditions has already developed into industrial scale. Sa-to is filtered and pasteurized prior to filling and packaging into clean bottles or other forms of containers (Ritiplang 2006; Sirisantimethakom et al. 2008).

4.4.7 Sweet Alcoholic Rice Paste or Snack (Khao-mak)

In Thailand, alcoholic fermentation has been used for the production of paste-type rice products. Khao-mak (Thai: ข้าวหมาก), is a traditionally sweet, low-alcoholic rice paste, which is prepared from streamed glutinous rice fermented with loog-pang

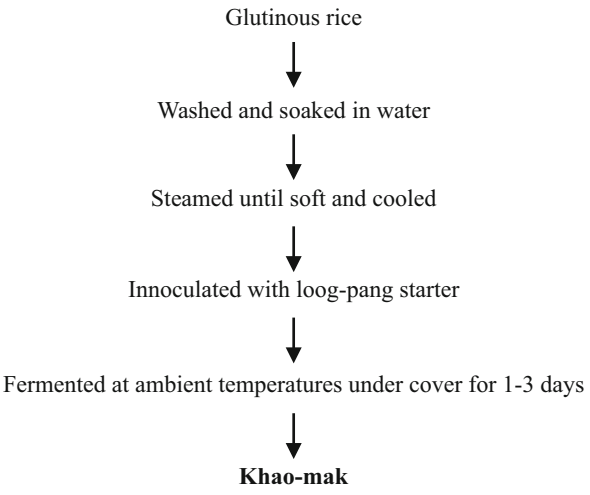

Fig. 4.7 Process for the production of khoa-mak

khao-mak. Figure 4.7 outlines the general process for the manufacture of khao-mak in Thailand. The glutinous rice is washed, soaked, and streamed until soft, followed by cooling, inoculating with powdered loog-pang khao-mak and fermentation under cover for 1–3 days at ambient temperatures. The microorganisms majorly responsible for khao-mak fermentation are yeasts and molds. These include *A. rouxii*, *Rhizopus* spp. *Mucor* spp., *Endomycopsis* spp., *P. anomola*, *P. burtonii*, *S. cerevisiae*, and *Hansenula* spp. (Boon-Long 1986; Ko 1986). Additionally, LAB may be involved in the fermentation of lactic acid. Therefore, khao-mak has a unique flavor with a sweet, little sour, and alcohol taste. After fermentation, khao-mak is consumed as a sweet dish without cooking.

4.5 Potential Health Benefits of Several Traditional Thai-Fermented Foods and Beverages

Fermentation has been proven to be beneficial in the production of food products of superior aroma and flavor. It is now known that fermentation process leads to production of valuable products including enzymes; vitamins; amino acids; soluble fiber; antioxidants, and other products of biosynthetic process, such as lactic acid (Shi et al. 2005). In addition to the enhancement of food safety and food preservation, such bioactive substances may impart health benefits for the consumers. Such benefits include improvements of gastrointestinal health, reduction of allergies, improvement of cardiovascular health, reduction of cancer risk, boosting of the immune defense system, antimicrobial activity, and further benefits. The bioactive compounds also present the unique characteristics of the product in aspect of flavor, taste, texture, and color. Thus, it would be reasonable to suggest that traditional Thai-fermented food products and beverages may contain various functional active compounds which either originate from the raw materials and ingredients used or from activities of microorganisms during the fermentation process. Only a few of the proposed beneficial health effects of related microorganisms involved in the fermentation processes of traditional Thai-fermented food products and beverages are known at present. Therefore, this section reviews the studies with scientific research backup for such claims and the current status of traditional Thai-fermented foods and beverages in respect to health benefits.

4.5.1 Beneficial Health Effects from Microbial Products

The health benefits associated with traditional fermented foods are mainly a result of the activities of microorganisms. Microorganisms involved in fermentation process are known to release various enzymes, metabolites, and other bioactive substances which potentially play a significant role in the promotion of the consumer's health.

4.5.1.1 Microbial Enzymes

Previous researches have revealed that *Bacillus* spp. from fermented soybean products including Japanese natto, Thai thua-nao, and African dawadawa exhibit high proteolytic activities (Chantawannakul et al. 2002). Most of *Bacillus* spp. were identified as *B. subtilis*. Comparison to *B. subtilis* from natto, little information on the beneficial effects of *B. subtilis* present in thua-nao has been established. Petchkongkaew et al. (2008) first reported that in addition to the inhibitory effects on growth of aflatoxin or ochratoxin A-producing *Aspergillus* strains, two isolates of *Bacillus* strains (*B. subtilis* MHS 13 and *B. licheniformis* CM 21) isolated from fresh thua-nao collected from the Maehongson and Chiangmai provinces also possess the ability to reduce aflatoxin B_1 (AFB_1) and ochratoxin A (OTA). *B. subtilis* MHS 13 was observed to merely remove AFB_1, while a further *Bacillus* strain was proven to be able to efficiently detoxify both aforementioned mycotoxins (decrease of 74 % Of AFB_1 and 92.5 % of OTA). The mechanism of AFB_1 removal by both *Bacillus* strains remains unknown, but OTA detoxification by *B. licheniformis* CM 21 is probably caused by a carboxypeptidase A activity which was shown to convert OTA to the much less toxic ochratoxin product (ochratoxin α). Similar results on OTA detoxification were reported by Wegst and Lingens (1983), Hwang and Draughon (1994), and Varga et al. (2000), who described bacteria and molds to be able to convert the parent compound OTA to products of lesser toxicity. It is possible to use both *Bacillus* strains for quality control of thua-nao fermentation in order to reduce health risks of AFB_1 or OTA for consumers. Furthermore, *B. licheniformis* CM 21 has the potential to be a significant source of efficient enzyme for the detoxification of OTA in foods or feeds.

Two of proteolytic *B. subtilis* strains (DB and SR) from traditional Thai-fermented food products (wheat flour dough of steam stuffed bun (sa-la-pao) and soaked rice used for khanom-jeen processing) exhibited high-potential proteolytic activity in the degradation of various allergenic proteins (Phromraksa et al. 2008). The crude enzyme of *B. subtilis* DB has the potential to digest both allergenic gliadin (wheat flour allergen) and β-lactoglobulin (allergenic protein from cow milk), while the enzyme of *B. subtilis* SR is likely to hydrolyze allergenic gliadin. Additionally, Phromraksa et al. (2008) reported that *B. subtilis* DB and SR have the potential to reduce allergenicity of the 16 kDa rice protein according to their strong protease activities in the degradation of the allergenic rice protein. The protease enzymes can hydrolyse allergen proteins in rice into smaller units, which are likely to have no allergenicity to sensitive consumers. In addition, *B. subtilis* SR has the potential to hydrolyze other rice proteins into smaller units, e.g., amino acids, which lead to the increase of free amino-acid production such as glutamic acid, valine, and leucine. The possibility to reduce allergenic rice protein by *B. subtilis* SR is of great interest for the development of hypoallergenic fermented rice noodles of high-nutrient availability. Moreover, Phromraksa et al. (2008) proposed that *B. subtilis* DB and SR have the potential for application in the production of hypoallergenic fermented wheat flour or fermented milk products.

Tanasupawat and Visessanguan (2008) and Namwong et al. (2009) recently elucidated *Bacillus* spp. isolated from some of traditional Thai-fermented products,

for instance fermented shrimp paste (ka-pi), fermented crab (poo-dong), tao-hu-yee, tao-cheow, and pla-chao, is probably capable to produce fibrinolytic enzymes. Such enzymes have a potential for application in thrombolytic therapy and prevention of cardiovascular diseases.

4.5.1.2 Antimicrobial Substances

A number of species within the LAB genera isolated from some traditional Thai-fermented foods are known to produce many different antimicrobial substances, such as bacteriocins (Cleveland et al. 2001; Chen and Hoover 2003). Lactic acid helps not only in the alteration of pH levels of food products to not favor growth of pathogenic microorganisms, but also a decrease in the pH levels of the intestinal contents which leads to inhibition of the development of invasive pathogens, such as *Salmonella* spp. or *Escherichia coli* (Mallett et al. 1989; Mack et al. 1999). Hwanhlem et al. (2011) reported *Streptococcus salivarius* LD219, *Enterococcus faecalis* LPS04, *E. faecalis* LPS17, and *E. faecalis* LPS18 isolated from Thai-fermented fish (pla-som) to exhibit strong antimicrobial activity against foodborne pathogenic bacteria (*Salmonella* spp., *Staphylococcus aureus*, and *E. coli*). The aforementioned research concluded the inhibitory effect against pathogenic bacteria tested to mainly come from organic acid.

Bacteriocin are proteinaceous in nature and bacteriocidal against both pathogenic and food spoilage bacteria (Nettles and Barefoot 1993; Jack et al. 1995). *Lac. lactis* WNC 20 and *Lb. plantarum* N014 isolated from nham produce nisin Z and antilisteria bacteriocin, respectively (Paik and Oh 1996; Noonpakdee et al. 2003; Rattanachaikunsopon and Phumkhachorn 2006). Similar to nizin, nisin Z from *Lac. lactis* WNC 20 is heat-stable and active over a wide pH range (2–10). This bacteriocin exhibits antagonistic activity against *Bacillus cereus*, *Clostridium perfringens*, *Listeria monocytogenes*, and *S. aureus (Oscáriz and Pisabarro 2001)*. Due to the relative sensitivity of *Lac. lactis* WNC 20 to acid, the strain plays potentially a minor role in nham fermentation. However, it is possible to use it as starter culture at the early stages of fermentation. The bacteriocin of *Lb. plantarum* N014 is heat- and pH-stable peptide with a molecular mass of approximately 8 kDa and able to kill several pathogenic bacteria including *L. monocytogenes* and *B. cereus*. Use of *Lb. plantarum* N014 as starter culture in nham fermentation is potentially beneficial for the improvement of both safety and quality of products (Phumkhachorn et al. 2007). Weissellicin 110 is produced by *Weissella cibaria* 110 isolated from a Thai-fermented fish product (pla-som) (Srionnual et al. 2007). This bacteriocin was defined as a newly discovered bacteriocin because of the absence of similarity to other known bacteriocins. Weissellicin 110 had no antagonistic activity against *L. monocytogenes*. This property may limit its use in fermented foods. *Lb. plantarum* PMU33 isolated from som-fug also produces plantaricin W. *Enterococcus faecium* NKR-5-3 isolated from Thai-fermented fish (pla-ra) produce two-synergistic bacteriocin peptides (Enterocin NKR-5-3A and enterocin NKR-5-3B) which reveal strong antimicrobial activity against *B. cereus* (Wilaipun et al. 2004).

4.5.1.3 γ-Aminobutyric Acid

γ–Aminobutyric acid (GABA) is well recognized to be produced by various microorganisms, particularly lactic acid bacteria (Kono and Himeno 2000). GABA is a non-protein amino acid that is widely distributed in nature. Some of LAB isolated from traditional Thai-fermented foods have an ability to produce GABA from glutamic acid by glutamate decarboxylase activity. Ratanaburee et al. (2010) reported that 64 out of 124 LAB isolates from traditional fermented food products in the southern part of Thailand produced GABA. Most of LAB isolates responsible for GABA (44 isolates) are from fermented vegetables, such as kra-tium-dong (fermented garlic), phak-gad-dong (fermented mustard leaf), naw-mai-dong (fermented bamboo shoot) (Chen et al. 2010), sa-taw-dong (fermented stink bean), tang-kwa-dong (cucumber pickle), and ka-lum-plee-dong (cabbage pickle). Moreover, two strains of *Lactobacillus* (LSF8-12 and LSF8-13) isolated from pla-som exhibits high productivity of GABA (Sukontasing et al. 2007). Microbial GABA has recently been applied in various functional food products, such as chocolate, coffee, candy, and chewing gum. Traditional fermented foods which contain GABA-producing LAB represent an alternative to attain beneficial health effect from GABA.

4.5.1.4 Antioxidative Activity

Much attention has currently been paid to the beneficial health effects derived from the presence of natural antioxidants in food. This is particularly related to antioxidative protection against heart diseases and cancer (Lovegrove et al. 2000). Antioxidative activities associated with the fermentation of traditional Thai-fermented products have been reported. Aside from the potential as an important source of proteins, ka-pi (highly salted-fermented shrimp paste), ja-loo (salted-fermented krill), and koong-som (salted-fermented small shrimp) contain large amounts of small peptides which result from the breakdown of raw material proteins by microbial or indigenous proteases during fermentation (Faithong et al. 2010). These in situ released peptides act as an effective natural antioxidant with high stability over wide pH and temperature ranges, resultant in an increase in the free radical-scavenging activity. Due to size and composition of the free amino acids and small peptides which affect the free radical-scavenging activity (Wu et al. 2003), further investigation on isolation and characterization of antioxidative peptides and amino acids from these fermented shrimp and krill products is called for (Binsan et al. 2008). Although more information on in vivo activity of bioactive peptides from fermented shrimp and krill products is required, some scientific studies reported peptides derived from animal or plant proteins in fermented food products to have various regulatory functions in the human body, e.g., lowering blood pressure and cholesterol levels, antioxidant, and antithrombotic activities (Okamoto et al. 1995; Ichimura et al. 2003; Hartmann and Meisel 2007). Therefore, typical Thai-fermented shrimp and krill products potentially constitute apposite sources of natural antioxidants and nutrients which help to enhance health benefits for consumers.

Table 4.3 Potential probiotic lactic acid bacteria from selected Thai traditional fermented foods

Product	Potential probiotic lactic acid bacteria	Reference
Khanom-jeen	*Lb. plantarum* PD110	Sribuathong and Trevanich (2005), Sribuathong et al. (2007)
	Lb. cellobiosus RE33	
	Leuco. lactis PD128	
Nham	*P. pentosaceus* LA 6	
	Lb. casei LA 13	
	Lb. plantarum LA 71	
	Lb. fermentum 3007	Klayraung et al. (2008a, b)
	Lb. fermentum 3010	Klayraung et al. (2008a, b)
Pla-ra	*Lb. plantarum* LA 102	
Koong-som	*Lb. delbrueckii* LA 198	
Miang	*Lb. fermentum* 2311	Klayraung et al. (2008a, b)

4.5.2 Health Benefits as a Result of Probiotics and Prebiotics

Probiotics have been widely defined as living microorganisms with beneficial effects on the health of hosts (Kalanzopoulos 1997; Holzapfel and Schillinger 2002; Saito 2004; Grajek et al. 2005; De Vrese and Schrezenmeir 2008). Most probiotics fall into the group of lactic acid bacteria and are normally found in fermented milks or other fermented food products (Pianpumepong and Noomhorm 2010). Lactic acid bacteria are considered to have some beneficial physiological effects, such as improvements of gastrointestinal health, enhancements of the immune defense system, increases of the bioavailability of nutrients, reductions of the symptoms of lactose tolerance, decreases of the prevalence of allergy in susceptible individuals, and a decrease in the risk of certain cancers. The mechanisms by which probiotics exert their effects are largely unknown, but are thought to involve alterations of the pH levels in the gastrointestinal tract, inhibition of pathogens by means of production of antimicrobial substances, and competing for the pathogen adherence to intestinal tract (Ouwehand et al. 2002; Helland et al. 2004; Saito 2004; Parvez et al. 2006). Traditional Thai-fermented food products are a plentiful source of microorganisms and some of which show probiotic properties (Nuphet 2003) (Table 4.3). Sribuathong and Trevanich (2005) isolated and identified the dominant lactic acid bacteria from fermented broken rice. These include *Lb. plantarum* PD110, *Lb. cellobiosus* RE33, and *Leuco. lactis* PD128, which show inhibitory effects against foodborne pathogenic bacteria, such as *E. coli*, *S.* Typhimurium, and *S. aureus*. In addition, *Lb. plantarum* PD110 is found to be acid tolerant and exhibits relatively high survival under in vitro gastrointestinal tract model. Sribuathong and Trevanich (2005) also reported all three isolates to show anti-adherence capacities on human intestinal epithelial cell lines (CaCo-2) in vitro against multidrug-resistant *Salmonella* Typhimurium. Five of LAB with probiotic properties, which were isolated from nham, pla-ra, and koong-som were identified as *P. pentosaceus* LA 6, *Lb.*

Table 4.4 Potential health benefits of selected traditional Thai-fermented foods and beverages

Specific types of fermented foods or beverages	Potential health benefits and associated actions	Health-promoting agents or known active component	References
Ka-pi, pla-chao, poo-dong (fermented crab), tao-cheow, and tao-huu-yee	Therapeutic effects against cardiovascular diseases	Fibrinolytic enzymes produced by *Bacillus* spp.	Chamroensaksri et al. (2009, 2010), Tanasupawat and Visessanguan (2008)
Ka-pi and Ja-loo	Antioxidative activity and bioavailability	Small peptides produced by proteolytic bacteria	Faithong et al. (2010)
Khanom-jeen	Gastrointestinal tract effect of probiotics	Bacteriocin-like substance and organic acids produced by *Lb. plantarum* PD128, *Lb. cellobiosus* RE33, *and Leuco. lactis* PD128	Sribuathong and Trevanich (2005)
		In vitro studies demonstrate some LAB (*Lb. plantarum* PD128, *Lb. cellobiosus* RE33, and *Leuco. lactis* PD128) isolated from Khanom-jeen inhibit adherence of *Salmonella* to intestinal epithelial cells via various mechanisms	
	Reduction of allergenicity to susceptible individuals	Protease enzymes from *Bacillus subtilis* SR in soaked rice used for fermented Khanom-jeen	Phromraksa et al. (2008)
	Increases in the micronutrient synthesis and bioavailability	*Lactobacillus* isolated from Khanom-jeen process has the ability to produce large amounts of functional components, such as GABA (γ-aminobutyric acid).	Kobayashi et al. (2007)
		Bacillus subtilis SR can digest rice proteins resulted in the increase of glutamic acid, which is substrate for the production of GABA.	Phromraksa et al. (2008)
	Therapeutic effects against hypertension	GABA-producing LAB isolates	Ratanaburee et al. (2010)
Koong-som	Antioxidative activity and bioavailability	Small peptides produced by proteolytic bacteria	Faithong et al. (2010)
	Therapeutic effects against hypertension	GABA-producing LAB isolates	Ratanaburee et al. (2010)
Miang	Gastrointestinal tract effect of probiotics	*Lb. fermentum* 2311strain	Klayraung et al. (2008a, b)

Table 4.4 (continued)

Specific types of fermented foods or beverages	Potential health benefits and associated actions	Health-promoting agents or known active component	References
Nham	Inhibition of foodborne pathogenic bacteria	Antilisteria bacteriocin from *Lactobacillus plantarum* N014 and nisin Z from *Lactococcus lactis* WNC20	Phumkhachorn et al. (2007)
	Therapeutic effects against hypertension	GABA-producing LAB isolates	Ratanaburee et al. (2010)
	Gastrointestinal tract effect of probiotics	*Lb. fermentum* 3007 and 3010 strains	Klayraung et al. (2008a, b)
Nam-pla	Increases in the micronutrient synthesis and bioavailability	Microorganisms associated with fish sauce fermentation produce relatively high concentration of cyanocobalamin (vitamin B12) and free amino acids	Areekul and Chantachum (1980)
Phak-sian-dong	Increases in the micronutrient synthesis and bioavailability	LAB found in these fermented food products possess glutamic acid decarboxylase and produces GABA	Ratanaburee et al. (2010)
Pla-ra	Increases in the micronutrient synthesis and bioavailability	*Lactobacillus* found in pla-ra possess glutamic acid decarboxylase and produces GABA	Ratanaburee et al. (2010)
	Inhibition of foodborne pathogenic bacteria	Enterocins NKR-5-3A and B produced *Enterococcus faecium* NKR-5-3 inhibited *B. cereus*	Wilaipun et al. (2004)
Pla-som	Therapeutic effects against hypertension	*Lactobacillus* found in pla-som has glutamic acid decarboxylase and produces GABA	Ratanaburee et al. (2010)
	Inhibition of foodborne pathogenic bacteria	Weissellicin 110 produced by *Weissella cibaria* 110	Srionnual et al. (2007)
		Organic acid produced by *Streptococcus salivarius* LD219, *Enterococcus faecalis* LPS04, *E. faecalis* LPS17, and *E. faecalis* LPS18 could inhibit *Salmonella* spp., *Staphylococcus aureus*, and *E. coli*	Hwanhlem et al. (2011)
	Therapeutic effects against hypertension	*Lactobacillus* (LSF8-12 and LSF8-13) isolated from pla-som exhibits high productivity of GABA	Sukontasing et al. (2007)

Specific types of fermented foods or beverages	Potential health benefits and associated actions	Health-promoting agents or known active component	References
Sa-to	Reduction of toxic alcohol formation	Low pectin content in raw material used for sa-to production	Sirisantimethakom et al. (2008)
Som-fug	Inhibition of foodborne pathogenic bacteria	Plantaricin W produced by *Lactobacillus plantarum* PMU33	Jumriangrit (2003)
Thua-nao	Increases in the micronutrient synthesis	Microorganisms associated with soybean fermentation produce vitamin B_{12} and free amino acids	Yongsmith et al. (1999), Tangjitjaroenkun et al. (2003), Chunhachart et al. (2006), Chukeatirote et al. (2010), Dajanta et al. (2011)
	Reduction of mycotoxigenicity by detoxifying mycotoxins	*B. licheniformis* isolated from fresh thua-nao exhibits efficient enzyme activity for removal of aflatoxin B_1 and ochratoxin A	Petchkongkaew et al. (2008)
	Bioavailability, antioxidant and antiproliferative activity against human cancer cell	Isoflavone aglycones (daidzein and genistein) produced by *B. subtilis* TN51	Chukeatirote et. al. (2010)
	Antioxidant and antiproliferative activity against human cancer cells	Isoflavone aglycones produced by *Aspergillus oryzae*	Chaiyasut et al. (2010)

casei LA 13, *Lb. plantarum* LA 71, *Lb. plantarum* LA 102, and *Lb. delbrueckii* LA 198 (Unknown). Klayraung et al. (2008a, b) found that three strains of *Lb. fermentum* (2311, 3007, and 3010) isolated from nham and miang (fermented tea leaves) exhibit the probiotic characteristics and functions, such as adhesive potential, acid resistance, bile tolerance, antimicrobial activity against some foodborne pathogenic bacteria, and antibiotic susceptibility. The resultant data of further research would be additional matters for consideration in the selection of safe and functional probiotics for the use as starter cultures. As with other traditionally fermented beverages, unpasteurized khao-mak contains viable LAB and yeast/mold between $1.5\times10^5-2.3\times10^6$ and $2.4\times10^4-6.2\times10^6$ CFU/mL, respectively (Khumweera 2004), which potentially include microorganisms of beneficial effects on the intestinal ecosystem of consumers. No reports on the probiotic efficacy of typical khao-mak LAB and yeasts are presently known, and effects beneficial to health of such microorganisms still needs to be demonstrated. However, some studies revealed yeast glucan to show anticancer (Bohn and Be Miller 1995), immunomodulating (Sandula et al. 1999), and cholesterol-lowering activities (Bell et al. 1999). Additionally, yeast cells or yeast glucan are shown to absorb various mycotoxins and can be used for the removal of such substances from beverages (Yiannikouris et al. 2004; Bejaoui et al. 2004).

4.5.3 Micronutrient Synthesis and Bioavailability

The actions of microorganisms during the production of traditional Thai-fermented food products have been shown to improve the quantity, availability, and digestibility of various dietary nutrients. Nam-pla is a good source of vitamin B12 and a daily consumption of approximately 15–30 mL per person is sufficient to protect Thais from B12 deficiency, which is a cause for megaloblastic anemia (Areekul et al. 1974; Thongthai and Gildberg 2005). Apart from the activity of the endogenous fish enzymes, proteolytic enzymes from halophilic bacteria involved in the fermentation process of fish sauce are thought to enhance the bioavailability of proteins and increase the production of small peptides and free amino acids. Moreover, Thai fish sauce contains a relatively high amounts of lysine, which plays an important role in human bone metabolism and growth (Fini et al. 2001).

Thua-nao-kab contains a relative high protein of about 36 % and thus constitutes a good source for digestible protein-rich supplements (Visessanguan et al. 2005; Chukeatirote and Thakang 2006). This change is possibly due to several extracellular enzymes produced by microorganisms during fermentation (Chukeatirote et al. 2006). Additionally, a variety of essential amino acids are found in thua-nao including tryptophan, glutamic acid, cysteine, lysine, and leucine (Dajanta et al. 2011). Tryptophan and lysine are considered to act as antioxidants (Saito et al. 2003). In addition, thua-nao contains higher amounts of aglycone

compounds than unfermented cooked soybeans and thua-nao obtained from the pure starter culture, *B. subtilis* TN51, exhibits the highest content of daidzein and genistein (Dajanta et al. 2009). These isoflavone aglycones are of great interest due to their beneficial effects on human health, such as their bioavailabilty, high rates of absorption, and strong antiproliferative activity against human breast cancer cell lines (Peterson et al. 1998; Izumi et al. 2000). Chaiyasut et al. (2010) also reported fermented soybeans with *Aspergillus oryzae* to show higher isoflavone contents and antioxidation activity than non-fermented soybeans. Moreover, the isoflavone aglycone contents are increased with extended periods of fermentation.

Traditional fermented vegetables are generally well-known to be low in calories but rich in edible fiber products. The contents of water-soluble vitamins including riboflavin, thiamine, and niacin are more elevated in leaf mustard pickles fermented with 15 and 18 % sodium chloride than in those fermented with 9 and 12 % (Lee et al. 1985). The use of yeast in fermentation of traditional foods or beverages is believed to contribute to the improvement of the nutritional value (Mai et al. 2002) because yeast has a relatively high content of proteins, lipids, and micronutrients (e.g., B vitamins; thiamine, riboflavin, biotin, and cyanocobalamin, trace elements; selenium and chromium, glycans). Sirisantimethakom et al. (2008) reported the methanol concentrations in all Thai sa-to samples tested to be in the range of 0.96–5.15 mg/L, which is not in excess of the maximum acceptable level (420 mg/L) of fruit wines in Thailand. Interestingly, these concentrations were much lower than those found in sherry fino wines (68.6–80.1 mg/L), Mencia wines (121–138 mg/L), kiwifruit wines (485–768 mg/L), fruit wines, and fruit distillates (371.3–1980.7 mg/L) (Hernandez-Gomez et al. 2003; Perez-Prieto et al. 2003; Soufleros et al. 2004; Cabaroglu 2005; Calleja and Falque 2005; Moreno et al. 2005; Peinado et al. 2006). The low concentration of methanol in sa-to is due to the low pectin contents in glutinous rice used for fermentation. Methanol is not a usual product of alcoholic beverage fermentation. The control of methanol contents in alcoholic beverages is particularly important due to its negative impact on the health of consumers. High concentrations of methanol can cause blindness or even death (Cortes et al. 2005).

4.6 Conclusion

It is well accepted that the development of fermentation technology plays an important role in the improvement of quality and safety of products. In addition, new aspects of functionality are becoming more important for exploitation. There is now a broad acceptance that a wide variety of microorganisms, for instance yeasts and molds, are known to be significantly involved in traditional Thai food products. Although the occurrence of various microorganisms has been reported, knowledge and comprehension of the beneficial impacts of such microorganism on human health remains to be

attained. In comparison to fermented food products in developed countries in terms of health benefits, only little scientific information on traditional Thai-fermented food products or beverages appears to be available, especially data on the composition of bioactive substances and their effectiveness and safety for direct human consumption is scattered. Therefore, more scientific fundamental and applied researches relevant to nutritional quality, therapeutic effects against diseases as well as other health benefits are still needed to attain at better insight of such relationships.

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Chapter 5
Traditional Slovak Products as Functional Foods

Dezider Toth, Jan Brindza, Elena Panghyova, and Stanislav Silhar

5.1 Introduction

Inhabitants of the present Slovakia territory are exploiting traditional technologies to prepare many products of plant and animal origin. Several such products could be marked as original and unique not only from technological point of view, but also for phytotherapeutic reasons as well of view. It makes them attractive not only for the consumers, but for experimental study too. In the submitted chapter are presented some historical facts and knowledge on two original products—the sugarless plum jam and a special cheese known in Slovakia as "bryndza".

5.2 Plum Jam Without Sucrose

The term "jam" is a name of a fruit matter conserved by boiling. Generally the jam is produced from raw fruit material with addition of sugar in needed proportion, and the sugar role is on one side to gain a product with desired taste quality and energetic content, but on the other side the main purpose is the final product conservation. Sometimes the jams are conserved by addition of some chemical agents to a mass of ready product (Drdák et al. 1996).

Plum jam is a traditional product made of washed assorted fruits, pitted, crushed, and passed through a sieve. Preferred are the fruits of plum land varieties. The preliminary processed plum mass of jelly to pulpy consistence is further thickened using traditional technology—boiling without addition of any sucrose.

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5.2.1 Basic Characteristic of Plum Land Varieties Fruit

Traditional plum jam is prepared from the plum land varieties called "Bystrická" and "Gemerská", which are grown in some regions of Slovakia. Basic fruit traits were experimentally evaluated and the results are presented in Table 5.1. It is apparent, that there are no significant differences between two tested plum land varieties.

Fruits of the Gemerská variety population prevailingly (75 %) are of ellipsoidal shape and partly narrowly ellipsoidal, globular or ovoid. In the case of Bystrická variety the major shape of fruits is elipsoidal (53 %) or narrowly elipsoidal (40 %). Globular form was found only with minority of 7 % evaluated fruits. The fruit shape variability is documented in Fig. 5.1.

With the Gemerská variety is dominating the typical conical (58 %) to slightly conical (36 %) shape of the basal fruit end, while with the Bystrická variety is more characteristic the curved (60 %) or flat basal fruit end (Fig. 5.1).

The apical fruit end of the Gemerská variety is prevailingly round (33 %) or flat (33 %), while the same traits of the Bystrická variety are of round (54 %) or pointed shape (46 %) (Fig. 5.1).

Fruit skin of the Gemerská plum variety is blue to dark blue, the plum of Bystrická variety is violet to dark violet. Differences in the flesh color of any variety could not be always taken as a typical trait of the given genotype, because this coloration depends on the fruit technological ripeness degree. The fully ripened fruits of both tested land varieties were from yellow-green to yellow-orange (Fig. 5.2).

Table 5.1 Variability of traits on fruits of plum land varieties Bystrická and Gemerská

Evaluated traits	Land variety collection	*n*	Min	Max	$\bar{x}$	*V*%
Fruit height (mm)	Gemerská	1744	26.68	50.31	37.61	8.55
	Bystrická	200	24.88	44.53	37.25	7.11
Fruit width (mm)	Gemerská	1744	20.24	40.20	27.64	8.68
	Bystrická	200	21.36	31.32	26.66	7.09
Fruit height/width ratio	Gemerská	1744	0.90	1.95	1.36	5.94
	Bystrická	200	0.98	1.64	1.40	5.05
Fruit thickness (mm)	Gemerská	1744	18.41	33.31	25.58	9.77
	Bystrická	200	21.63	31.75	26.10	7.19
Fruit weight (g)	Gemerská	1742	7.55	29.38	16.36	21.31
	Bystrická	200	9.27	24.58	15.61	20.03
Fruit stalk length (mm)	Gemerská	1581	7.05	26.07	15.49	16.96
	Bystrická	133	8.77	23.99	17.82	13.47
Refractometric dry weight (%)	Gemerská	93	14.00	27.40	20.65	11.13
	Bystrická	59	15.00	28.60	21.83	13.28
Flesh ratio to whole fruit (%)	Gemerská	1743	90	97	94	1.41
	Bystrická	200	93	97	95	0.79

Fig. 5.1 Comparison of the Gemerská (*upper row*) and Bystrická (*lower row*) varieties in fruit shapes (Photo: Alexej Oravec, Valéria Müllerová 2006)

Fig. 5.2 Comparison of the fruit flesh color of the Gemerská (*upper row*) and Bystrická (*lower row*) varieties (Photo: Alexej Oravec, Valéria Müllerová 2006)

5.2.2 Traditional Production Technology of Jam Without Added Sucrose

In the past the production of plum jam has been taken in the concerned family as an extraordinal social event. Actually all family members were engaged in the preparatory activities. First of all—it was necessary to collect great amount of fully matured and healthy fruits. The plums were washed and left for drying under natural condition. Day before the jam boiling the fruits were manually unstoned by the family members. Next day in early morning on the yard was prepared the copper boiler, which should be lined with a lard. The boiler was placed over the open fireplace. The unstoned plums were gradually filled into boiler with a wooden stirrer and mixed under continual boiling. The whole boiling process lasted around 10–12 h (Fig. 5.3).

Fruit flesh is gradually boiled to sodden liquefied mass, which is followingly due to water evaporation changing the consistency into thick pulpy state. Finally the product is stored in 1.5–2 L earthen vessels. The jam surface is covered by lard, because when conserved in such a way, could be stored all year even in heated room. This product represented an important part of many traditional dishes for several Slovak region inhabitants.

Fig. 5.3 Basic activities sequence during the preparative activities and boiling of plum jam in copper boiler with wooden stirrer (Photo: Alexej Oravec, Valéria Müllerová 2006)

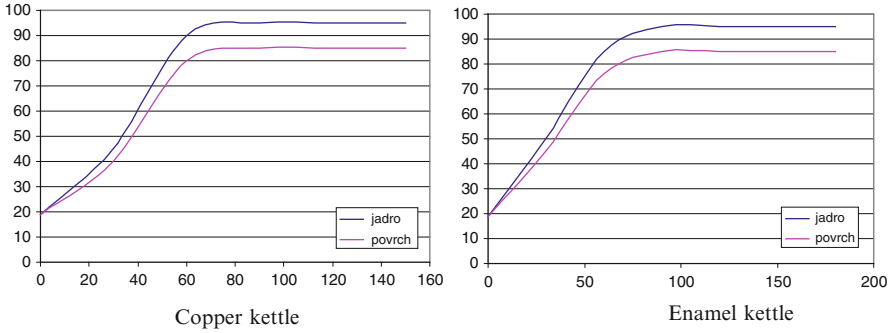

Fig. 5.4 Temperature course (in °C: *red*—surface, *blue*—core part) during the fruit flesh boiling process of Gemerská plum variety

Principal advantage of plum jam is the fact that it consists only of fructose and glucose and high proportion of fiber, polyphenols, antioxidants, and other nutritionally effective components. It could be marked as a natural food product with functional effects.

In our experiments has been verified the traditional approach to production technology of plum jam. The input material consisted of two land varieties—Gemerská and Bystrická fruits, collected by local inhabitants of the Krivoklát and Hradište villages.

The first experimental series was devoted to jam preparation in both, the copper and enamel kettles, where were applied unstoned fruits of Gemerska plum variety. During the boiling process were measured temperatures on the surface and in the core part of boiled biomass. As documented in Fig. 5.4, in both type of kettles was kept the same temperature regime. In any case, the temperature of boiled plum flesh does not passed the value of 100 °C. On the surface of boiling mass the temperature was slightly lower—around 85 °C, what is highly important, as such is prevented the caramelization of fructose and glucose. If this condition is not kept, the product acquires brown color and bitter taste, but when the temperature values stay below 90 °C, jam will be dark red, with pleasant odor and delicate sourish taste.

As shown in Fig. 5.5 the temperature regime during the plum variety Bystrická fruit flesh boiling process in a copper kettle is similar as with Gemerska variety, the only difference in the sinusoid features is caused by the gradual addition of unstoned fruit fractions to the boiling mass in kettle.

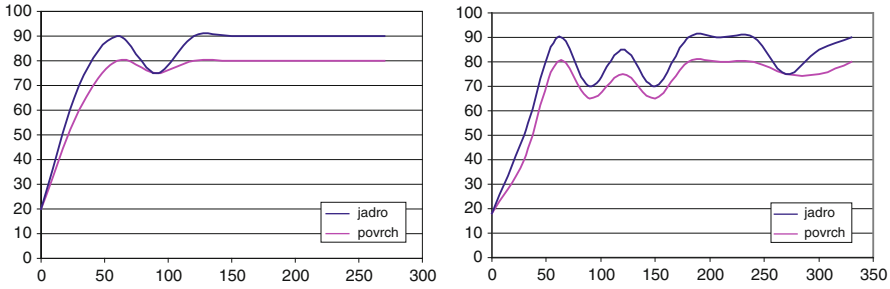

Fig. 5.5 Temperature course (in °C: *red*—surface, *blue*—core part) during the fruit flesh boiling process of Bystrická plum variety

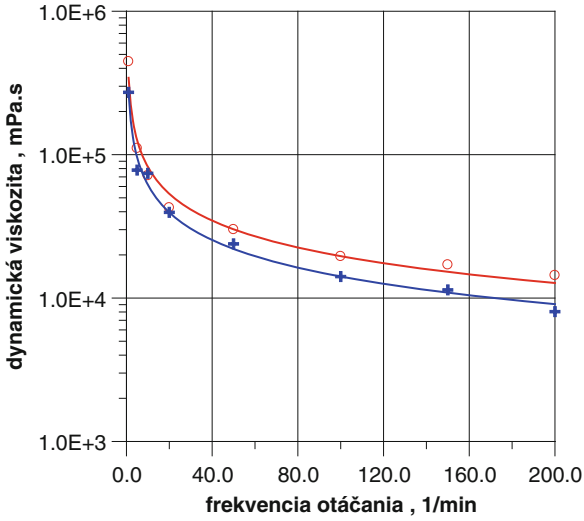

Fig. 5.6 Plum jam dynamic viscosity dependence on the frequency of spindle rotation in copper (*open circle*) and enamel (*plus sign*) kettles

In the second experimental cycle the samples dynamic viscosity based on rheological behavior was determined. Results are shown in Fig. 5.6, documenting that the plum jam behavior is different from the Newton materials. Its dynamic viscosity is changing during the movement, what is typical for tixotropic (non-crawling) materials. After finishing a movement the material viscosity returns slowly to the original value. From the fluidity point, these materials are time-depending. As the viscosity decreases with the deformation speed increase or with the growth of tangential tension, respectively (owing to structural changes inside the material), the tested material could be classified as a pseudoplastic one. From the curves position is apparent, that the boiler (kettle) affected the rheological properties of the final product. Therefore plum jam boiled in copper kettle achieved higher viscosity.

Medium values of dry weight in jams produced in the copper and enamel kettles were determined in a range from 29.60 to 38.56 %. From 53 kg of pitted fruits has been gained 21.8 kg of plum jam, what corresponds with the yield of 42 %.

5.3 Importance of Plum Jam for Human Health

Plum fruits are taken as quite important plant product owing to their nutritional and phytotherapeutic importance. Plums consumption in fresh or processed forms provides for human organism important components like vitamin C, beta-caroten, beta-sitosterol (Spiller 1996), chlorogenic acid, flavoxanthin (Harborne and Baxter 1983), lutein, phytosterols (Spiller 1996) quinic and tartaric acids (Harborne and Baxter 1983), further there are around with fructose and glucose different sugars like galactose, mannose, xylose, and rhamnose (Harborne and Baxter 1983). Total phenolic contents of different plum cultivars have been reported between 282 and 922 mg/100 g of fruit (Siddiq 2006). Phenolic compounds of prunes consist mainly of chlorogenic acid, neochlorogenic acid, caffeic acid, coumaric acid, rutin (Donovan et al. 1998), and proanthocyanidin (Kimura et al. 2008).

Drying process increases the antioxidant activity due to nonenzymatic reaction products, called melanodins. In plums, polyphenols contribution in antioxidant activity of prunes is only about 23 % of the total antioxidant activity (Madaru et al. 2010).

Plums and their products are highly reputed in folk medical practices for nutritive, laxative, and digestive properties and used for the treatment of hypertension, diabetes, jaundice, and fever. The recent studies showed that it has antioxidant, anticancer, antihyperglycemic, anti-hyperlipidemic, antihypertensive, anti-osteoporosis, laxative and hepatoprotective activities. Prunes contain dietary fibers, carbohydrates, amino acids, vitamins, minerals, and antioxidant polyphenolic phytochemicals. Therapeutically active constituents and their possible mechanisms of actions are also discussed (Jabeen and Aslam 2011).

P. domestica is used for lowering blood glucose in Elazing Central District of Turkey (Cakilcioglu and Turkoglu 2009). It is also indicated for the treatment of dysmenorrhea, leucorrhea, miscarriage, asthma, and fever (Duke et al. 2002).

Prune extract and juice inhibit oxidation of isolated human LDL (Donovan et al. 1998). Ethanol fraction of prune juice has been shown to suppress proliferation and induce apoptotic changes in human colon carcinoma cells (Fujii et al. 2006).

Many people may have avoided consuming dried plum due to reported laxative effects. But studies demonstrated that consumption of prunes in daily diet up to 100 g by men and postmenopausal women did not significantly change their bowel habits (Tinker et al. 1991; Lucas et al. 2004).

5.4 Slovak Sheep Cheese (*bryndza*)

Slovak sheep cheese commonly called "*bryndza*" is a soft maturing cheese. In a traditional way is made of the sheep lumpy cheese or as a mixture of sheep and cow lumpy cheeses where the sheep cheese fraction should be minimally 50 %.

5.4.1 *The* bryndza *History*

There are two versions of the sheep cheese production history on the present Slovakia territory. One is connected even with the ancient Roman empire, but more probable is the second version, that the *bryndza* was brought to us by the celtic tribes of Valachs (their name id derived from the Carpathian tribe of Wallachians/Valasi). These people are still forming some community named as "*Valasi*" living in the Central Slovakia mountains, in the Morava region of the Czech Republic and Podhale region of Poland. This version is based on the facts, that owing to several climatic and other disasters the sheep numbers and the local population in our region substantially dropped. Introduction of new sheep herds coming from the territory of present Romania has been led by shepherds belonging to *Valasi* community. They stayed in our country, shortly followed by their families. They had brought new know how of the sheep breeding including the "technology" of using the rennet gained from the calf or lamb bellies to sheep milk processing. From the cheese lumps was produced a new product called "*brandža*" (brandja) from which our predecessors developed the word *bryndza*. There is no written document on the time when the *bryndza* phenomenon started its existence and the production was spread in all mountainous regions. Anyway, the present technology of "*bryndza*" production in Slovakia and in Romania is different.

The Slovak *bryndza* is mixed up with salt, therefore the sensory traits are completely distinct. In the time period of 1690–1740 this product was made only for the needs of owners family. The *bryndza* business trade was started by two families—Slezáček and Roháčik living in village Stara Tura. They organized the bulk buying of the salted processed durable sheep cheese and sold it in Pressburg (presently Bratislava), Vienna and Budapest. The first *bryndza* manufacture arised in 1787. It was founded by Ján Vagač, who improved the ripening process, achieved a more fatty cheese consistence which could be better spread (e.g., on the slice of bread) and what is important, he substantially prolonged the product durability. He compiled the technological scheme of the *bryndza* production, defined not only the manufacturing procedure but elaborated a scheme for the thermal profile necessary for the best mode to keep the cheese quality and product durability. Further he introduced the cheese lumps sensory evaluation and the principles which were adapted by all *bryndza* producing families. In his technological approach was included the cheese pressing aiming at water content reduction, what further increased the product stability. The formerly used manual crumbling of matured cheese was replaced by crushing on grinding mill and this process combined with addition of 3–6 % of salt. The thoroughly mixed up *bryndza* was pressed into small barrels called *geletka*, which from inside were tapped by spruce-wood veneers (their task was to draw the water liberated by temperature changes or by inner bedewing).

In the eighteenth century the Slovak sheep cheese sale has covered the whole Europe (Demo et al. 2001). The traditional mode of *bryndza* production is preserved up to now in the Slovak sheepcotes, which are prevailingly located on hillsides above mountain villages. Slovak Republic applied at the Commission of the EU

with regard to Council Regulation (EC) No.510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs to register the *Slovenská bryndza* as typically Slovak product. Romania objected to Slovakia's use of the *bryndza* designation, but failed, because the Slovak experts came to European Commission with argument, that the Romanian word *brinza* covers the group name of all salted cheeses. Followingly, *Slovenská bryndza* has been registered in the EU's Register of protected designations of origin and protected geographical indications on the 16 July 2008 as Protected Geographical Indication (PGI). This registered product must contain more than 50 % sheep cheese and can be produced year-round in the hilly Central and Northern Slovakia. Bryndza production is historically bound on this territory representing 80 % of the Slovak Republic, as documented in many written references. In the *Codex alimentarius Austriakus* (1917) is described the specific microflora of bryndza, that time called "Karpathenkokkus", later these microorganisms were identified as bacterial species belonging to the genera of *Lactobacillus, Streptococcus,* and *Enterococcus.* Professor Laxa called another microorganism "*Oidium Lactis*" (Laxa 1907), which was later identified as a beneficial fungal species—*Geotrichum candum.* As defined in the EU's Register, the "Slovak bryndza is a natural cheese of white to mildly yellowish color (not clearly yellow), spreadable with isolated grits, it should be of sandy, gum, or grease consistence. Its odor should be pleasantly sour, taste mildly piquant, and salty. Lump cheese weight share must be over 50 % calculated from the dry matter. Basic raw material is the lumpy cheese or a mixture of both, the sheep and cow lump cheeses, and the third variant is based on the sheep lump cheese kept in barrels for aging under specific conditions".

5.4.2 Production Technology

(a) Precipitation and forming of sheep lump cheese

Freshly milked sheep milk is adjusted under constantly mixed to temperature of 28–32 °C, and then added the liquid rennet. Approximately in half an hour the milk should be curdled. The gained coagulated milk is mixed by curd rake and cut by curd knife to get curd particles sized from 5 to 10 mm. After sedimentation the crushed curdled material is manually pressed and formed into lumpy shape. Using the curd sack, the sheep cheese lump is separated from whey and then left to allow the draining. In all steps of the described technological processes, the milk and/or curd temperature should be kept above 27 °C. The curdling is done in a wood vessel called *putera* or in double-bottomed anticorrosive vessel. The needed temperature is stabilized by addition of hot water directly into the vessel containing the processed material. Weight of the sheep lump cheese after the water separation is from 3 to 7 kg.

(b) Sheep lump cheese fermentation

The sheep lump cheese drained (in approximately 10 h) and then the solidified cheese is transferred on wooden or anticorrosive shelf to start the fermenta-

tion process. Within the fermentation it is transposed and turned over—the whole procedure lasts from 2 to 3 days at temperature 21–25 °C. During the fermentation are rapidly multiplied various microorganisms transforming lactose to lactic acid and the sheep cheese acidity is decreasing at least to pH 5.2.

(c) Sheep lump cheese ripening

Ripening of the sheep lump cheese is conducted at temperatures between 8 and 20 °C, depending on specific conditions of given sheepfold. During the maturation process the cheese is put on wooden or anticorrosive shelves and in time period lasting 4–6 days they are from time to time turned to allow better trickling of the whey, and the cheese is wiped with dry towel. In the same time the fermentation process continues and the pH value is lowered to 4.2–4.8. Fully matured cheese does not liberate whey, it is deformable, the taste is pleasant with milky acidity.

(d) Cheese sorting and ripening

The sheep lump cheese is transported from the sheepfolds to *brindza* manufactures and according the maturation degrees they are placed into the curd maturing vat. During the maturation process is needed to keep temperature in a range of 8–20 °C for 3–5 days. The right ripeness is important for the quality and durability of the Slovenska bryndza product.

(e) Cheese fusing

Matured lump cheese is thoroughly cleaned by scraping and trimming of dried crust. It is important to clean up the folding parts of the lump crust. Then is the cleaned cheese placed into fusing vat and 2 days pressed by 0.5–4 bar.

(f) Cheese crushing and milling

The next treatments are crushing and milling of the processed cheese. For crushing is used the screw crusher producing particles with diameter in the range of 20–50 mm, which are continually transported into curd mill, where the cheese particles are milled and grinded down. There is a different speed of the mill rollers and their distance is adjusted in such a way, to achieve fine consistency of the product—without clods, anyway it could be slightly gritty.

(g) Cheese mixing

In the mixing device the cheese is thoroughly mixed up with added salt or saturated salt solution until the prescribed salt concentration and dry weight of the final product is achieved.

(h) Cheese packing

The well-mixed sheep cheese is packed manually or using automatic wrapping machine into 5 kg wooden or synthetic buckets. For retail consumers it is packed in smaller amounts ranging from 125 to 250 g into multilayer combined aluminum foils, or in roll-form into wooden veneers, eventually into synthetic cylindrical casings.

(i) Bryndza storage

Packed Slovenska bryndza is kept at temperature 2–6 °C.

5.5 Importance of Bryndza for Human Health

Composition of sheep milk quantitatively differs from the cow milk. Dry weight of the sheep milk is in average around 18.2 %, while the relevant value of cow milk is 12.4 %. Similarly, from the standpoint of nutritional value the sheep milk contains nearly twofold more proteins—medium values 3.3 g 100 g^{-1} (for cow milk) and more than 5.7 g 100 g^{-1} (sheep milk), more fats 3.8 g 100 g^{-1} (cow milk), 6.9 g 100 g^{-1} (sheep milk), and more saccharides 4.3 g 100 g^{-1} (cow milk), 5.0 g 100 g^{-1} (sheep milk). When comparing the vitamins, the sheep milk has markedly higher amount of vitamin C (4.3 mg 100 g^{-1} for sheep and 1.7 mg 100 g^{-1} in cow milk). Vojtašáková et al. (2000) found in sheep milk essentially more calcium than in the cow milk.

Biological importance of the sheep milk is increased owing to conjugated fatty acids (CLA) content, which positively affect the metabolic syndrome by supporting body weight reduction, changing the fatty substances composition, lowering the LDL cholesterol, decreasing blood tension and reducing the risk of diabetes mellitus II type inception. Amount of fatty acids in sheep milk depends on the season and pasture qualities, but the concentration of biologically active substances is doubled in comparison with the cow milk (Michalcová 2007).

Bryndza is a source of important proteins, mineral substances, and vitamins. Proteins in the fermentation process are transformed to peptides, which are more easily digested, and simultaneously is degraded lactose as well, what makes bryndza acceptable for consumers with lactose intolerance or those suffering allergy on milk proteins. Probiotic bacteria naturally occurring in bryndza form owing to lactic acid production and acid environment in the intestinal tract, what inhibits multiplication of pathogens, and so the sorption of calcium, magnesium, and iron is facilitated. Some of the bacteria are able to produce vitamin B12. Around with the lactic acid other short-chain-acids like acetic, propionic, and butanoic acids have positive effects on the intestinal functions, especially as energy sources for the epithelial tissue. Important functions are connected with the sodium-butyrate, which is supporting the inhibition of colorectal cancer cells differentiation, stimulates apoptose, inhibits cytokines inflammation activities and supports the mucous membrane regeneration. Probiotics are forming microbicidal substances, some synthesized proteins or peptides are able to inhibit specific pathogenous microbial species, while other probiotics are bounding carcinogenic substances and then remove them from organism (Chebenova et al. 2010).

Natural microflora of bryndza, prepared under best basic hygienic rules, is guaranteedly securing the hygienical safety of consumers—what places this product of Slovakia among the microbial phenomenon (Keresteš et al. 2008). There is a mixture of microorganisms, which is highly complex consisting of a large number of species—with the dominance of bacterial genus *Enterococcus*. Enterococci optimally are multiplying at 35 °C, but able to grow in a wide temperature range of 10–45 °C. Even the concentration of 6.5 % of NaCl in a cultivation medium does not stop their proliferation. In a small intestine survive many species of this genus

in the presence of bile salts. These bacteria are securing the proteolytic and lipolytic activities during the cheese maturation phase, causing the typical odour formation. Citrate metabolism pathway of enterococci leads to production of diacetyl, acetaldehyde, acetoin and 2,3-butanediol, which are strongly influencing the maturing cheese organoleptic qualities (Giraffa 2003, Keresteš et al. 2008). Among important enterococci abilities should be named the production of bacteriocines—an antibacterial tool against Gram-positive bacteria and against such food pathogens like *Listeria* and *Clostridium.* Enterococci biomass in Slovenska bryndza is higher than in other Mediterranean cheese types. Enterococci counts in bryndza in a range of 10^6–10^8 CFU g^{-1} (colony-forming units in 1 g of cheese). Dominant species is E*nterococcus faecium*, further species determined are usually *Enterococcus durans* and *Enterococcus faecalis.* Determination of the bryndza microflora by PCR method showed, that from 308 isolates were the following bacterial counts per species—177 *Enterococcus faecium,* 59 *E. durand,* 41 *E. faecalis,* 13 *E. mundtii* and 11 *E. casseliflavus* (Sarantinopoulos et al. 2002, Jurkovic et al. 2006, Belicova et al. 2011). These authors studied the bacteriocines production by isolated enterococci and found that some enterococci are able to produce enterocines, which failed to affect the growth of tested lactobacilli, but inhibited the tested potential pathogens *Listeria innocua, Staphylococcus lentus, Enterococcus faecalis V583*, and *Staphylococcus enterica.* These bacteriocines are responsible for an effective defence against potentially harmful microflora of bryndza (Giraffa 2003, Belicova et al. 2011).

The bacteria involved in the milk fermentation represent another group of microorganisms important for the bryndza quality. Sulo et al. (2009) isolated and identified *Lactobacillus plantarum, L. paracasei, L. brevis,* and *L. fermentum*. Lactobacilli are highly important and responsible for the cheese ripening owing to their ability to produce lactic acid and to successive staphylococci devitalization. Bryndza made of raw milk contains a huge amount of microorganisms. Their exact ranking into genera and species is not known exactly, therefore bryndza has been taken as an interesting object for microbiological research trying to find new unknown bacteria with specific properties important not only for their influence on the sensory traits but on the positive effects of Slovenska bryndza on the consumer's health.

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Chapter 6
Functional and Nutritional Properties of Some Turkish Traditional Foods

Fahrettin Göğüş, Semih Ötles, Ferruh Erdoğdu, and Beraat Özçelik

6.1 Introduction

Traditional foods are defined as "the products forming as a result of mutual interaction between ecological socio-cultural environment with the historical period and representing the traditional food culture peculiar to societies" by Sagdıç et al. (2010). Turkey has numerous types of traditional foods such as ayran, tarhana, bulgur, kefir, yogurt containing functional food components like phenolic compounds, vitamin, minerals, essential oils, etc. In addition, many of them are fermented products enriched with possible probiotic lactic acid bacteria. Therefore; beyond their role to reflect Turkish culture, traditional Turkish foods have some potential benefits including reducing risk of cardiovascular disease and cancer, lowering cholesterol and LDL levels, regulating intestinal flora, and exhibiting antioxidant and antimicrobial activity.

This chapter highlights the functional and nutritional properties of some popular meat-based (pastirma, sucuk), dairy-based (ayran, kefir), legume-based (tarhana, bulgur), vegetable- and fruit-based (kayısı pestili, şalgam suyu), and sweet (baklava, lokum) Turkish traditional foods.

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6.2 Turkish Traditional Foods

6.2.1 Meat-Based Traditional Products

6.2.1.1 Pastirma

Identification

Pastirma is the most popular traditional dry-cured meat product consumed in Turkey (Fig. 6.1). It is produced from whole muscle obtained from various parts of beef and water buffalo carcasses. It can be stored at room temperature since it is classified as intermediate moisture food (Aksu et al. 2002). It does not require any preparation step, and it can be thought as a ready-to-eat food. It can be thought as a good source of pure meat, and protein which does not contain any additive pastirma. Pastirma is famous in Kayseri region of Turkey. Composition and nutritive value of pastirma is shown in Table 6.1.

Production

After removing the exterior fat and connective tissue, meat is cured (at 10 °C for 2 days, 80–90 % RH), dried (at 15 °C for 3 days, 75–85 % RH), and pressed (1.0 kg/cm^2, 20 h, 10 °C) at first. Then, drying (2 days at 22 °C, 65–70 % RH) and pressing (1.0 kg/cm^2, 4 h, 25 °C) are repeated before coating with cement paste. Cement paste, made from fresh mashed garlic, red pepper, paprika, and ground fenugreek (*Trigonella foenum graecum*) seeds, gives the characteristic flavor and aroma of pastirma. Finally, the meat strips covered with cement paste are dried (6 days at 15 °C, 60 % RH) for the last time (Gök et al. 2008).

Fig. 6.1 Pastirma

Table 6.1 Composition and nutritive value of pastirma (Aksu et al. 2005)

Component	Content (%)
Moisture	40 (max.)[a]
Salt	8.5 (max. on dry basis)[a]
Fat	3.4
Protein	42.9

[a]Turkish Food Codex

Consumption

Pastirma is sliced 1–2 mm thick and consumed without cooking.

Functional Properties and Health Effects

Beside improving appearance, color, taste, and flavor; ingredients of cement paste such as garlic, red pepper, paprika, and ground fenugreek (*Trigonella foenum graecum*) seeds exhibit hypoglycemic effect, antioxidant activity, and antimicrobial activity (Dixit et al. 2005). Moreover, pastirma is a very good source of protein.

6.2.1.2 Sucuk

Identification

Turkish style sausage, sucuk, is one of the most popular traditional fermented dry meat products consumed in Turkey (Fig. 6.2). Sucuk is a very similar product like semi-dried fermented meat products in Europe and the USA (Soyer et al. 2005). Water buffalo sucuk is very famous in Afyonkarahisar region in Turkey. Composition and nutritional value of sucuk is shown in Table 6.2.

Production

Sucuk is made from ground sheep and/or beef meat (80 % lean), tail fat, salt, sugar, clean dry garlic, spices such as black pepper, red pepper, cumin, and cinnamon. While traditional fermentation is employed by natural microflora before ripening under environmental conditions; commercial fermentation is carried out by addition

Fig. 6.2 Sucuk

Table 6.2 Composition and nutritive value (Coskuner et al. 2010, Yildiz-Turp and Serdaroglu 2008)

Component	Content (%)
Moisture	40 (max.)[a]
Fat	40 (max.)[a]
Protein	18–33
Salt	1.8–2.5

[a]Turkish Food Codex

of starter culture such as *Lactobacillus sake*, *Lactobacillus plantarum*, *Lactobacillus curvatus*, and *Lactobacillus brevis before* ripening under controlled atmospheric conditions (Bozkurt et al. 2006).

The ingredients are mixed and stuffed into natural or artificial casing with or without addition of starter culture. Sucuks are hung for fermentation at 20–22 °C and 90 % RH for 3 days. After repining and drying at 18 °C and 75–85 % RH for 3 days, sucuks are stored at 18 °C with 65–75 % RH. In contrast to traditional production, additives such as nitrate, ascorbic acid, and tocopherol are used as ingredients, and heat treatment is applied in ovens at about 60 °C for 1 h for commercial sucuks to obtain the color and aroma after fermentation (Erkmen et al. 2004; Bozkurt et al. 2006). However, consumers prefer naturally fermented sucuks (Soyer et al. 2005).

Consumption

Sucuk is generally consumed as grilled at breakfasts and picnics and after removing its casing and slicing. It is also preferred to add in some traditional Turkish meals.

Functional Properties and Health Effects

Sucuk is a fermented product and carries probiotic lactic acid bacteria which exhibit effects on improving immune response modulation, promoting ingestion system, etc. However, it should be consumed carefully since it contains high amounts of saturated animal fats. Therefore, studies related to decrease animal fat content of sucuk were conducted by addition of fiber or replacing saturated fat with olive oil in last years.

6.2.2 Dairy-Based Traditional Products

6.2.2.1 Ayran

Identification

Ayran is one of the traditional drinks of Turkish culture dating back to Central Asia (Fig. 6.3). It is a yogurt-based beverage commonly consumed in Turkey, especially in summer season, although it is popular in other regions of the Central Asia, Middle East, and Balkans as well. Ayran is white and its consistence is creamy Anonymous (2011a).

Fig. 6.3 Ayran

Table 6.3 Nutritive value (approximate per serving) of Ayran

Component	Content
Energy	90 cal
Protein	7.0 g
Fat	2.7 g
Carbohydrate	9.6 g
Iron	0.2 mg
Phosphorus	194 mg
Cholesterol	18 mg
Zinc	1 mg
Sodium	710 mg
Vitamin A	190 IU
Thiamin (B1)	0.06 mg
Riboflavin (B2)	0.30 mg
Niacin	0.16 mg
Vitamin C	2 mg

Composition and Nutritive Value

Nutritive value of ayran is shown in Table 6.3.

Production

Ayran is traditionally manufactured by addition of water at a level of 30–50 % and salt at a maximum level of 1 % to yogurt. In the industrial manufacture of ayran, milk with adjusted dry matter content is fermented using exopolysaccharide producing cultures and the viscous curd obtained is further diluted with salt-containing water. Ayran is separated from other fermented milk beverages being a yogurt drink with salt and without any fruit flavoring. Commercially made ayran are mostly sold fresh in tightly sealed plastic cup containers, plastic bottles in liters, and single serving tetra packs.

Consumption

In Turkey, Ayran is so popular that it is readily available in most fast food restaurants, and it is a common offering in the summer, when people view ayran as a refreshing drink in the heat. Some stores sell ayran pre-made, but it is also very easy to make: mix 100 mL of plain cold yogurt with 100 mL of cold water and a coffee tablespoon of salt, and mix it with the blender. Serve cold.

Functional Properties and Health Effects

Health benefits of yogurt are undeniably obvious whether you eat it or drink it as ayran. One of the health benefits of ayran is that the process of fermentation sees milk's natural sugar, lactose, broken down, used as food by the bacteria. This makes digestion of drink easier than its predecessor, milk. Its other nutritional benefits come from their abundant minerals: calcium, potassium, phosphorus, magnesium, and vitamins riboflavin, B_{12}, A, D, and K.

6.2.2.2 Kefir (*Keefir*, *Kephir*, *Kewra*, *Talai*, *Mudu Kekiya*, *Milkkefir*, *Búlgaros*)

Identification

Kefir is a fermented milk drink that originated with shepherds of the Caucasus region, who discovered that fresh milk carried in leather pouches would occasionally ferment into an effervescent beverage (Fig. 6.4). The world of kefir is said to have originated from the Turkish word "Keyif" which means "good feeling." Anonymous (2011b).

Composition and Nutritive Value

Kefir contains vitamins, minerals, and essential amino acids that help the body with healing and maintenance functions. Kefir is rich in Vitamin B_1, B_{12}, calcium, amino acids, folic acid, and Vitamin K. It is a good source of biotin, a B vitamin that aids the body's assimilation of other B vitamins, such as folic acid, pantothenic acid, and B_{12}. The numerous benefits of B vitamins are regulation of the kidneys, liver, and nervous system to helping relieve skin disorders, boost energy, and promote longevity. Kefir has the complete proteins that are partially digested, and in this respect the body easily utilizes them. Tryptophan is one of the essential amino acids in kefir that is well known for relaxing effect on the nervous system and calcium and magnesium are abundant in kefir, which are important minerals for a healthy nervous system. Kefir is also a good source of phosphorus, which is the second most abundant mineral in our bodies, helps utilize carbohydrates, fats, and proteins for cell growth, maintenance, and energy.

Fig. 6.4 Kefir

Production

The traditional method of making kefir is occurred by directly adding kefir grains. The raw milk is boiled and cooled to 20–25 °C and inoculated with 2–10 % (generally 5 %) kefir grain. After a period of fermentation, 18–24 h at 20–25 °C, the grains are separated from the milk by filtering with a sieve and can be dried at room temperature and kept at cold temperature for being used in the next inoculation. Kefir is stored at 4 °C for a time then is ready for consumption.

In industrial process of kefir, different methods can be used but basically up on the same principle. The first step is to homogenize the milk to 8 % dry matter and held by heat treatment at 90–95 °C for 5–10 min. Then, cooled at 18–24 °C and inoculated with 2–8 % kefir cultures (bacterial starters) in tanks. Fermentation time is changed for 18–24 h. The coagulum is separated by pomp and distributed in bottles. After maturing at 12–14 or 3–10 °C for 24 h, kefir is stored at 4 °C.

Consumption

Traditional kefir can be made from any type of milk, cow, goat, or sheep. People who prefer not to use dairy may also make kefir from plant or nut milks such as soy or almond milk. The beverage is typically made at room temperature, and it takes around 18 h to mature fully, although in cold climates this may take longer.

Functional Properties and Health Effects

Kefir has antimutagenic and antioxidant properties. Regular kefir consumption can help relieve all intestinal disorders, promote bowel movement, reduce flatulence, and create a healthier digestive system. It cleans effectively the whole body that

helps to establish a balanced inner ecosystem for optimum health and longevity and however easily digested, provides beneficial bacteria and yeast vitamins and minerals and complete proteins, is a nourishing food to contribute a healthy immune system, and has been used to help patients suffering from AIDS, chronic fatigue syndrome, herpes, and cancer.

6.2.3 Legume-Based Traditional Products

6.2.3.1 Tarhana

Identification

Tarhana is a popular and widely consumed dried, fermented food product in the Middle East and Turkey (Dalgic and Belibagli 2008). It is both produced commercially and in domestic ways (Fig. 6.5).

Composition and Nutritive Value

It has a high nutritional value with its higher protein content (≈15 %) (Daglooglu 2000; Kose and Cagindi 2002). It has a sour taste with yeasty flavor. Lactic acid bacteria are usually responsible for the acid formation during fermentation, and *Saccharomyces cerevisiae* is used to increase the characteristic flavor with the formation of CO_2, alcohol, organic acids, aldehydes, ketones, and other fermentation products (Tarakci et al. 2004). The proximate composition of commercial tarhana is listed in Table 6.4.

Fig. 6.5 Tarhana

Table 6.4 Proximate composition of commercial tarhana (adapted from Ozdemir et al. 2007)

Component	Content (%)
Moisture	6–10
Crude protein	12–20
Crude fat	1–9
Crude carbohydrate	40–75
Crude ash	1.5–4
Titratable acidity	1.5–2.5
pH	3.5–5.0

Production

Tarhana is produced by mixing cereal flour, yogurt, baker's yeast (*Saccharomyces cerevisiae*), vegetables (tomatoes, onions, green and red pepper), salt, and spices (mint, thyme, dill, tarhana herb, etc.) followed by a 1–6 days of fermentation, drying, and fine grinding. Due to its low moisture content (6–8 %) and lower pH (3.8–4.4), the storage time up to 2–3 years is possible. Tarhana might also is locally consumed as snack after being dried as thin layer or nugget (Erbas et al. 2005).

Consumption

It is served a hot soup after the reconstitution process with hot water (Dalgic and Belibagli 2008). Tarhana soup is highly flavored and thick–creamy with its improved digestibility (Ozdemir et al. 2007).

Functional Properties and Health Effects

Tarhana is a good source of B vitamins, minerals (Ca, Mg, K, Zn, Mn, Fe, Cu, and Na) with favorable bioavailabilities, organic acids, and free amino acids, and it is healthy for children, the elderly, and patients with its contents (Ozdemir et al. 2007). As reported in the literature, flavonoids are considered to be significant anticarcinogens and antioxidants with their various biochemical and antiallergic properties. One of the food-derived flavonoids, quercetin, was the major flavonoid found in tarhana (Ozdemir et al. 2007). With these properties, tarhana might be considered a functional food.

6.2.3.2 Bulgur

Identification

Bulgur (in general produced from *Triticum durum*) is a yellow color, precooked wheat product (Fig. 6.6). It has been produced and consumed widely in Turkey as well as in the world. The annual consumption of wheat bulgur is about 12 kg/person in Turkey. It is extremely huge in Syria, Iraq, Iran, Israel, Lebanon, and Arabia (25–35 kg/person) (Kahyaoglu et al. 2010).

Fig. 6.6 Bulgur

Table 6.5 Nutrients in bulgur (per 100 g) USDA National Nutrient Database for Standard Reference, Release 24 (2011)

Component	Content
Energy	342 kcal
Carbohydrates	75.87 g
Protein	12.29 g
Fat	1.33 g
Thiamin	0.23 mg
Riboflavin	0.12 mg
Niacin	5.11 mg
Vitamin B_6	0.34 mg
Pantothenic acid	1.05 mg
Phosphorus	300 mg
Potassium	410 mg
Magnesium	164 mg
Calcium	35 mg
Iron	2.46 mg
Zinc	1.93 mg

Composition and Nutritive Value

The nutritive value of bulgur is similar to that of the wheat from which it is made. Nutrient composition of bulgur is listed in Table 6.5.

Production

In general, wheat is cleaned, washed, parboiled, dried, dehulled, ground, and sifted to produce bulgur. After choosing the wheat according to standards, mechanical cleaners remove weeds, other parts of plants, stones, soil, foreign seeds, and legumes grains. Then, the kernels are washed several times in washing pans to take away soil and dust. Pressure parboiling technique is used for cooking, wheat is steamed at 12.5 kg/cm^2 for 10 min in a specially developed vessels and then steamed for 10–15 min with a constant pressure of 1.4 kg/cm^2 (gauge) for 20–25 min. The wheat is then dried to 12.5–14.0 % moisture, dehulled, and milled (Bayram 2003).

Consumption

Bulgur can be used in pilafs, soups, bakery goods, or as stuffing. In Turkey, bulgur is prepared (using *pilavlık bulgur*) as pilaf in chicken stock, with or without sauteed noodles, or cooked with tomatoes, onions, and red pepper. The fine grind (*köftelik bulgur*) is used for making kısır, a bulgur salad similar to tabbouleh, prepared with tomato paste, fresh tomatoes, cucumbers, parsley, olive oil, and a variety of other salad ingredients to personal taste. A variety of mezes and main dishes are also prepared with *köftelik bulgur*, such as çiğ köfte, içli köfte, and ezogelin soup. It also forms the base of a soup, tarhana.

Functional Properties and Health Effects

Bulgur is a natural food in that no chemicals or additives are used in processing the product. Many of the naturally occurring vitamins and minerals are allowed to permeate into the kernel during cooking thus maintaining more nutritive content than other forms of processed wheat products (Bayram 2006).

So bulgur

- Increases nutrient absorption due to high mineral and cellulose and prevents constipation and intestinal cancer risk.
- Is suitable for vegetarian nourishment.
- Is found in nutritional pyramid in the world.
- Is a good food material for pregnant and baby due to its folic acid content.
- Has unsaturated lipids, and it is important for healthy life due to its low fat content.
- Meals can be prepared in home easily due to cooking operation during its production.
- Is consumable individually due to its special nutritional properties.

6.2.4 Vegetable- and Fruit-Based Traditional Products

6.2.4.1 Pekmez

Identification

Pekmez (Fig. 6.7a, b) is one of the most common mulberry/grape products in Turkey. Approximately 37 % of the grapes produced is used in pekmez production (Kaya and Belibagli 2002). Pekmez might be defined as a concentrated and shelf-life extended form of mulberry or grape juice produced by boiling without adding sugar or any other food additives (Yogurtcu and Kamisli 2006).

Composition and Nutritive Value

Proximate composition of the grape and mulberry pekmez is summarized in Table 6.6.

Production

Pekmez processing generally varies depending upon the origin of the fruit used in the production, but a general outline of the process consists of boiling of the water–fruit mixture with a calcareous substance (pekmez earth—containing 90 % calcium

Fig. 6.7 Pekmez

Table 6.6 Proximate composition of grape and mulberry pekmez (adapted from Ustun and Tosun 1997)

Component	Content	
	Grape pekmez	Mulberry pekmez
Total dry matter (%)	72	61–76
Total sugar (per dry matter)	83.73	52.93–70.89
Inver sugar (per dry matter)	82.223	35.07–61.48
Protein (%)	0.627	0.26–1.28
Ash (%)	1.864	1.50–2.05
Total acidity (g/kg)	4.8	0.18–0.71
pH	5.05	5.35–6.03

carbonate) under continuous stirring, cooling, pressing, and filtering to obtain a clear product. The use of calcareous substance lowers the acidity caused by naturally existing tartaric and malic acids by precipitating them in the form of calcium tartarate and calcium malate (Kaya and Belibagli 2002). The boiling process might be carried out in open vessels and under vacuum. Depending upon the production, the liquid pekmez with its 65–68° Brix might also be mixed with 5–15 % previously prepared crystal seed to produce a solid pekmez with its pasty form.

Consumption

Besides its direct consumption, tahin (sesame paste)-pekmez blend is a traditional way of consumption in breakfast especially in winter season due to the higher energy content (Arslan et al. 2005).

Functional Properties and Health Effects

Pekmez included high amounts of natural sugars (glucose, fructose, and galactose), mineral and organic acids, and with its contents, it is important in human nutrition (Demirozu et al. 2002; Ustun and Tosun 1997). It was reported to have a significant effect on brain as energy source conferring approximately 293 kcal/100 g (Tosun and Ustun 2003). Grape pekmez also contains iron (0.005–0.01 %) and calcium (0.084–0.086 %) (Bozkurt et al. 1999; Arslan et al. 2005). With its iron content, it is also recommended for anemia patients (Arslan et al. 2005).

6.2.4.2 Shalgam (Salgam) Drink

Identification

Shalgam (salgam) is a traditional, red-colored, cloudy, and sour soft drink (Fig. 6.8) produced in mainly south provinces of Turkey with lactic acid fermentation of black carrot, turnip, rock salt, sourdough, bulgur flour, and drinkable water (Tanguler and Erten 2011).

Fig. 6.8 Shalgam drink

Table 6.7 The proximate composition of shalgam (adapted from Erten et al. 2008)

Component	Minimum	Maximum	Average
Total acidity as lactic acid (g/L)	5.94	8.91	7.11
pH	3.33	3.67	3.49
Lactic acid (g/L)	5.18	8.05	6.81
Volatile acidity as acetic acid (g/L)	0.57	1.16	0.89
Alcohol (g/L)	1.32	6.41	3.64
Total solids (g/L)	22.9	29.2	26.0
Protein (g/L)	0.88	1.83	1.25
Ash (g/L)	14.6	20.65	17.25
NaCl (%)	1.37	1.97	1.63
CO_2 (g/L)	0.44	0.79	0.74
Color index (D520)	71	131	102
Anthocyanin as cyanidin-3-glycoside (g/L)	88.3	134.6	114.1

Composition and Nutritive Value

The proximate composition of shalgam is given in Table 6.7.

Production

The traditional production methodology consists of first dough fermentation stage, performed for enrichment of lactic acid bacteria and yeast, and second or main fermentation stage (Tanguler and Erten 2012). In the first stage, mixture of

bulgur flour, salt, sourdough, and water is left for fermentation for 3–5 days, and the resulting extract of the first fermentation stage is then combined with copped black carrot, sliced turnip, and water for the second stage, which takes 3–10 days at ambient temperature (Tanguler and Erten 2012). In the direct applied method, however, only the second fermentation stage is carried out, where the chopped black carrots, salt, if available sliced turnip, bakers' yeast (*Saccharomyces cerevisiae*) or sourdough, and adequate water are allowed to ferment at ambient temperature (Erten et al. 2008).

Consumption

Shalgam is highly popular in the Turkish south provinces, especially in Adana, Mersin, Hatay, and Kahramanmaras. It is widely consumed with food and as a refreshing beverage.

Functional Properties and Health Effects

Shalgam was reported to be a good source of the minerals, containing potassium (300–1000 mg/L), phosphorus (10.6–22.2 mg/L), calcium (89–173 mg/L), iron (0.2–2.9 mg/L), and some other minerals (Erten et al. 2008) with its distinctive anthocyanin content which are known to have positive health benefits like reduction of the coronary heart disease risks and visual acuity, antioxidant activities, and anti-carcinogenic properties (Inceday et al. 2008). It also includes thiamin and riboflavin. Shalgam has been known to impart a positive effect on the digestive system with its probiotic effects and to act as an appetizer (Turker et al. 2007). Black carrot anthocyanins, derivatives of cyanidins having a high ratio of monoacylated anthocyanins, are responsible for the purplish red color of shalgam (Turker et al. 2007).

6.2.5 Sweet Traditional Products

6.2.5.1 Lokum (Turkish Delight)

Identification

Turkish delight or lokum is a family of confections based on a gel of starch and sugar (Fig. 6.9). It is like a jelly candy. It has a soft, elastic texture, and coated with powder sugar or coconut. There are hundreds of variations, so lokum can be seen in various colors, sizes, shapes (in cubes or rolls), and flavors. Some are softer; some are thicker; some are flavored with fruit, rosewater, or gum mastic; some have pistachios, hazelnuts, or walnuts; and some are plain. But their main ingredients are the same: sugar, starch, water, and citric acid. Component for plain type lokum is indicated as Table 6.8.

Fig. 6.9 Lokum

Table 6.8 Composition and nutritive value of lokum (Turkish Food Codex, Notification No: 2004/24)

Component	Content %
Moisture	15 (max.)
Total sugar (as invert sugar in dry solid) %	85 (min.)

Production

The first step in lokum production is cooking of sugar, starch, and water in a copper or stainless steel pan. The powdered sugar is completely dissolved in water, then defined amount of starch is added, and they are cooked together for 2 h. In order to provide the conversion and solidification of mixture, some amount of citric acid is added during the cooking. Then, some flavoring compounds, dried fruit particles, or some nuts are added. This mixture is allowed to cooling, after that mixture is poured into the trays and allowed to rest between 3 and 4 h to 1 day. Then, they are cut with special knife to give desired shape (Doyuran et al. 2004).

Consumption

People would celebrate every important event with lokum in Turkey. When they had important guests, they would serve lokum after dinner. Also, it is still the greatest treat to serve with Turkish coffee.

Functional Properties and Health Effects

Lokum is one of the traditional foods consumed in Turkey. It is a natural and healthy food source and is believed to have many benefits. Especially plain types, as a carbohydrate source, are advised to people who have kidney disease. Additionally, lokum is used to treat wound and carbuncle in some region of Turkey (Anonymous 2004).

6.3 Conclusion

Traditional Turkish foods have great potential to exhibit health benefits beside their nutritional and sensorial properties due to containing bioactive components like phenolic compounds, vitamin, minerals, essential oils, etc. or probiotic lactic acid bacteria.

In this chapter, majority of them such as pastirma, sucuk, ayran, kefir, tarhana, bulgur, kayısı pestili, şalgam suyu, baklava, and lokum are considered in terms of their product characteristics, nutritive value, production method, consumption style, functional properties, and health effects. However, there is a need for available data on nutrient and bioactive composition and health benefits of traditional foods. In addition, novel processing techniques should be studied to keep the bioactive components and nutritional value of the traditional foods.

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Part II
Functional Properties of Cereal and Flour Foods

Chapter 7
Functional Aspects of Carob Flour

Maria Cecilia Puppo and Daniel Pablo Ribotta

7.1 Carob Pod as Food Ingredient

The carob tree (*Ceratonia siliqua* L. Fabaceae) is a native evergreen plant of the Mediterranean area including West and South Anatolia (Turkey) (Ayaz et al. 2007). In many Arabian countries, the carob fruit, named as carob pod, is utilized for making beverages and typical sweets. In western countries, carob seeds are removed and flour with the external pod is prepared. This flour is grinded and toasted and used as cacao substitute (Yousif and Alghzawi 2000). Seeds are exploited in food industry for their gum (Avallone et al. 1997) and fiber (Bravo et al. 1994) content.

Chemical composition of carob pods were studied by several authors (Binder et al. 1959; Calixto and Canellas 1982). Carob is a good food ingredient due to its high content of sugar (approximately 50 %). Carob pod flour (Avallone et al. 1997) contains 45 % of carbohydrates, 3 % of protein, and 0.6 % of lipids; while carob germ and carob seed flours are enriched in protein and lipids and present lower contents of carbohydrates. Toasted carob flour, used as cacao substitute with the advantage of not containing exciting substances as theobromine and caffeine, has low contents of lipids and high amounts of fiber and natural sugars. This flour also contains vitamins (A, B1, B2, D) and minerals (iron, calcium, phosphorous, and magnesium) (Ayaz et al. 2007). Carob germ flour composition is of 46 % of protein rich in lysine and arginine, 25 % of carbohydrates, 5 % of fiber, and 7 % of soluble sugars. Carob enzymatic hydrolysates with a protein content higher than 85 %, high levels of glutamine (18 %) (Candow Darren et al. 2001), and arginine

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(12 %) (Flynn et al. 2002) were also obtained. These amino acids constitute a great contribution to nutrition of sporty peoples and immune-depressed patients.

Carob flours are generally used in light foods because of their high fiber content (Dakia et al. 2007) or in gluten-free food destined to celiac people, due to the lacking of prolamins (Feillet and Roulland 1998).

7.2 *Prosopis* Species and Traditional Foods

The pods of various species of semi-arid adapted nitrogen-fixing trees of various *Prosopis* species were an important component in the diets of the indigenous peoples in the deserts of North America, Peru (D'Antoni and Solbrig 1977; Felker et al. 2003), and Argentina (Burkart 1976). These species were named as "American carob" by Spanish explorers, due to their similarities with carob trees. *Ceratonia* and *Prosopis* belong to the same family (Fabaceae) although to different genus: the European tree belongs to *Ceratonia* sp., while American crop belongs to *Prosopis* sp. The later is a very ancient genus with almost 45 species distributed along America, Africa, and Asia. With 27 species throughout all the territory, Argentina constitutes the region with the greatest worldwide diversity (Felker 2009).

Prosopis pod contains high amounts of sugar. The mesocarp, which is the spongy pulp between the pod exterior and the leathery capsule (endocarp) which surrounds the seeds, was the fraction consumed by indigenous peoples. Probably, this fraction was consumed as the sucrose content is typically about 45 % (the pods are often 30–35 % sugar on whole pod basis) (Grados and Cruz 1996; Felker et al. 2003) and does not contain much fiber. Despite *Prosopis* flour is used in pastries, as coffee substitutes and to produce syrups for use in flavoring applications due to its excellent sensory properties (taste and aroma), is not generally used in any commercial application at significant scale (Felker et al. 2003).

Prosopis represents an important productive alternative for the economy of rural families of the Argentinean North West (Noroeste Argentino, NOA), giving to them raw material for different uses (Juarez et al. 2003). The wood is useful for furniture and several rural constructions, and the resin is utilized as black ink for "chaguares," wool or cotton. The fruit is used in the NOA region mainly as food for goats. Honey can also be obtained from *Prosopis* flours and pods are appreciated for their flavor and nutritional properties. For this reason, pods and seeds from *Prosopis alba* are used in the NOA in different traditional foods (Pantanelli 2001).

A regional non-fermented beverage called "añapa" can be obtained from pods after grinding them with water. Fermenting this mix another beverage is able to be produced, the "aloja." Heating a water suspension of dried, grinded, and sieved fresh pods allow after concentration obtaining a syrup product like honey, the "arrope."

Roasted flour, used as cacao substitute, can be obtained by roasting, grinding, and sieving of dried pods. Non-roasted flour is prepared only by drying pods. The Argentinean Food Codex (Código Alimentario Argentino, CAA) defines carob flour as the product obtained by milling cleaned, healthy and dried seeds of white

carob (*Prosopis alba* Griseb), and/or black carob (*Prosopis nigra* (Grisebach) Hieronymus) (CAA 1995, Art. 681). European carob flour is also defined (CAA 1995, Art. 681 bis) as the product obtained by fine milling of decorticated, healthy, cleaned, and dried pods of fruits of *Ceratonia siliqua* L. American carob flours contains low amounts of proteins due to these flours are obtained from the whole fruit.

The CAA also defines "patay" (CAA 1995), a pressed circular cake from the ground *Prosopis* pods commonly sold in bus stations in interior villages of provinces of Santiago del Estero and Tucuman.

7.3 Functional and Nutritional Carob-Based Foods

Carob pod (*Ceratonia siliqua*), due to its high content of sucrose, is an ideal raw material for chocolate succedaneum and bakery products like cakes. This fruit also contains high content of minerals, mainly calcium, iron, manganese (Ayaz et al. 2007), and fiber. Therefore, carob consumption is recommendable in cases of malnutrition, low weight, decalcification, constipation, among other diseases. Carob flour is also enriched in unsaturated fatty acids like oleic and linoleic (Maza et al. 1989). It also contains tannins, useful as diarrhea preventers, acting as inhibitor of bacterial toxic substances. Polyphenols act as antioxidants retarding free radicals formation and decreasing oxidation rate (Kumazawa et al. 2002), increasing their antioxidant activity when they are linked to fiber (Saura-Calixto et al. 2007; Pérez-Jiménez et al. 2008).

The carob seed, and more particularly the endosperm fraction of this seed, is already widely valorized in the food industry since constitutes a source for the production of locust bean gum (LBG), which has a wide application in foods as a thickening agent and stabilizer (Bouzouita et al. 2007). Adjacent to this endosperm fraction is the germ which is obtained in large quantities as a by-product during the isolation of LBG. This germ has a substantial protein concentration which is capable of being valorized as functional protein. This protein system from carob germ, which has been called caroubin by some authors (Feillet and Roulland 1998; Wang et al. 2001), consists of a mixture of different proteins with a molecular weight ranging from several thousands Da to more than one million (Feillet and Roulland 1998).

Gums (galactomannans) derived from *Prosopis* species were also widely studied due to its application as food additives (Bravo et al. 1994; Ibáñez and Ferrero 2003). *Prosopis* sp. gum (mesquite gum) is used in elaboration of diet foods and foods for diabetic people. In addition, mesquite gum is used as a substitute of Arabic, guar, and LBG in gum candies and for micro-encapsulation of essential oils and active molecules.

Several authors have characterized different species of *Prosopis* spp. Meyer et al. (1986) obtained different fractions of Mesquite pods (*Prosopis* spp.). One fraction was the exo–mesocarp powder that contained most of the pod sugar and flavor components which would be useful as a starting material for sugar syrup and as an ingredient in breads and crackers. Endocarp hulls comprised mainly fiber.

Form mucilage that covered seed a gum fraction with a galactose to mannose ratio and food applications similar to guar gum was obtained. The protein-rich seed cotyledon was separated as other fraction and found to have typical uses of bean protein. This protein is nutritionally limiting in tyrosine and methionine–cysteine. The protein efficiency ratio (PER) of uncooked pods and seeds was found to be 0.71 and 0.69, respectively. Both pods and seeds had reduced PER'S and metabolizable energy (chick feeding studies) values after cooking, probably due to the presence of seed gum (Meyer et al. 1986).

Different parts of Peruvian "algarrobo" (*Prosopis pallida* and *Prosopis juliflora*) fruits were also analyzed by Grados and Cruz (1996). These researchers studied different fractions: exocarp, mesocarp or pulp, endocarp and also the episperm, endosperm, and cotyledon from the seeds. Sucrose was the main sugar (46 % in weight) in the pulp. In the endosperm, the polysaccharide was a galactomannan, with a 1:1.36 galactose/mannose ratio. Among the important amino acids of seed cotyledon proteins were glutamic acid, arginine, aspartic acid, leucine, proline, and serine. In the pulp, vitamin C, nicotinic acid, and calcium pantothenate were also found. In the seed, the content of vitamin C and E were significant. The dietary fiber of the pulp and endocarp hulls was basically insoluble dietary fiber.

Ceratonia and *Prosopis* pods, therefore constitutes a valuable food. Flours and pod derivates result essential ingredients with high potential application in several kinds of foods, destined to cover specific requirements of different target people.

More refined flour (germen flour), obtained from *Prosopis* seeds with an optimized elaboration process, would improve organoleptic (discarding toasted flavor) and nutritional (high protein content) characteristics of raw material. Carob germen flour (*Ceratonia siliqua*) was found to contain high amounts of proteins with high nutritional value (Bengoechea et al. 2008b). Flour enriched in protein obtained from *Prosopis* would enhance protein performance (quality and quantity) of different type of Latino American foods like bakery products and goods like gels, foams, and emulsion. This will achieve added value to the flour and increase their marketing potential, contributing to the development of the region.

Little information is available for functional properties of American carob (*Prosopis* sp.) flours. Although not too many studies can be found, European carob flour (*Ceratonia siliqua*) has been more studied. Both species belong to the same leguminous family; therefore, a similarity in flour composition and in some functional properties of the developed food is expected. This is the reason of the following review of results obtained for different authors on functional properties of carob proteins.

7.4 Carob Protein Isolate

The protein system from carob germ, named caroubin by some authors (Feillet and Roulland 1998; Wang et al. 2001), consists of a mixture of different proteins with a molecular weight ranging from several thousands Da to more than one million (Feillet and Roulland 1998).

Bengoechea et al. (2008b) applied the procedure of alkaline solubilization followed by isoelectric precipitation and obtained an isolate with protein content higher than 95 % from carob flour. The isolate presented, as in flour, high content of aspartic and glutamic acids, and arginine amino acids. The limiting amino acids were methionine + cysteine and phenylalanine + tyrosine. The isolate proteins had low electrophoretic mobility and were mainly stabilized by high molecular mass aggregates. These aggregates were formed by the 131 and 70 kDa subunits linked by non-covalent bonds and other peptides strongly bounded by disulfide interactions. Aggregates were formed mainly by 100 and 48 kDa monomers linked by disulfide bonds. The 70 kDa subunit was a dimmer composed by the 48 and 20 kDa polypeptides, also connected by S–S. A considerable content of high molecular mass peptides strongly bounded were also found. These peptides were not dissociated by the combined effect of the sodium dodecyl sulfate (SDS) and dithiothreitol (DTT). No differences in the nature of proteins present in the acid or alkaline isolates were observed. Nevertheless, proteins presented higher denaturation temperature and became more denatured at acid pH (pH 2) than at pH 10.

Results from different authors showed that carob is a legume whose composition, protein nature, and protein thermal properties varied from other leguminous crops as soybean, pea, and lupine. The knowledge about nature and thermal properties of proteins that form carob materials like germ flours and isolates would be important from the point of view of the application of this crop as ingredient in formulated foods.

7.5 Emulsions and Gels from Carob Ingredients

The effect of protein concentration (0.125–1 wt%) on linear viscoelastic properties and microstructure of O/W emulsions stabilized by carob flour (CF) or carob protein isolate (CPI) was studied by Bengoechea et al. (2008a). All the emulsions showed a significant linear viscoelastic range, which increased with protein concentration. Linear and nonlinear viscoelastic properties of concentrated O/W emulsions containing sunflower oil (75 wt% O), water, and a vegetable protein (carob, gluten, or soya, 1 wt%) were also compared.

Carob may be considered a valuable protein source for the manufacture of highly concentrated emulsions, in view of the low costs and functional properties of carob protein raw materials. A closed-packing of droplets with a broad distribution of sizes leading to the formation of a three-dimensional flocculated network was obtained when CF or CPI were used as emulsifiers. Both type of emulsions showed an evolution of the elastic and loss modulus with protein concentration that was qualitatively similar to those obtained for wheat gluten and soybean protein isolate-based emulsions (Bengoechea et al. 2006). CPI gave rise to thinner continuous phases than CF leading to higher transport rates to the O/W interface during emulsification, smaller droplet sizes, stronger interactions among droplets, and more elastic flocculated networks that showed wider linear viscoelasticity ranges.

It was found that emulsions stabilized by CPI showed higher values for linear and nonlinear rheological parameters (i.e., dynamic viscoelastic functions, relaxation modulus, and flow properties) than those prepared with soybean or gluten proteins (Bengoechea et al. 2008a). This behavior is in concordance with the amount of available protein molecules in the continuous phase which may be related to an enhancement in extensive flocculation.

A factorable nonlinear viscoelastic constitutive equation, the Wagner model, that uses a memory function calculated by the generalized Maxwell model and a Soskey–Winter damping function, has been used to reproduce experimental results from stress growth tests after inception of steady shear flow. Experimental values and model predictions matched fairly well for the emulsions stabilized either by CPI, soybean protein isolate, or wheat gluten. This behavior may be at least partially attributed to a more accurate reproduction of linear viscoelasticity data by using the generalized Maxwell model.

Gelling properties of CPI at different concentration levels (10, 20, 30 % w/v) and pH values (native, 2, 10) were studied by Bengoechea et al. (2009). CPI gels were thermally processed at 95 °C for 30 min. Only gels containing a percentage of protein higher than 20 % could be formed.

Gels prepared at a pH closer to the isoelectric point of the protein isolate (p$I \sim 4$) presented the lowest solubility in water. Gels of pH 10 were those that presented the greatest amount of protein in the supernatant resulting from the dispersion of the gel. In all cases, protein solubility increased when SDS was used in the extraction media, as the hydrogen bonds are disrupted. Solubility increased even more after using a reducing agent, DTT, which cleaves disulphide bonds. These effects were more important in the case of CPI dispersions previous to thermal treatment, particularly when only physical interactions are being disrupted (by SDS).

Fracturability and hardness of CPI gels prepared at pH 10 were high. Those gels presented the higher content of soluble protein, and also a high water holding capacity. The gels of fine-stranded matrix were harder and retained more water than those of more open matrices (particulate gels). Moreover, electrostatic interactions, which are more important far from the isoelectric point (i.e., pH 10), tend to form linear aggregates that favor formation of fine-stranded gels. Evolution of G' and G'' along time during thermal treatment applied to a 30 % CPI dispersion pH 2 showed a higher strengthening effect of the structure at pH 2 than at pH 5.9 or 10. The steep increase of the elastic modulus (G') observed for pH 2 gels during the cooling stage has been related to the formation of hydrogen bonds.

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Chapter 8
Salvia hispanica: Nutritional and Functional Potential

Maira Rubí Segura-Campos, Luis Antonio Chel-Guerrero, and David Abram Betancur-Ancona

8.1 Introduction

Chia (*Salvia hispanica* L.) is an annual summer herb, and a member of the *Labiatae* family (Fig. 8.1). It was a basic food in several ancient Mesoamerican civilizations, for example, 5000–15,000 tons of chia arrived annually in the Aztec capital Tenochtitlan as tribute from conquered nations. For thousands of years before European contact, chia was important for its seeds, flour, and oil which fulfilled medicinal, nutritional, artistic, and religious ends. After contact, chia was replaced by crops of European origin and practically disappeared for the following 500 years (Cahill 2003).

8.2 Composition and Functional Properties

8.2.1 Chia Seed Proximate Composition

Chia seed protein content is higher than in other food grains such as corn (14 %) and rice (8.5 %) (Table 8.1). Unlike most other cereals, chia is not limiting in any of the essential amino acids (Bushway et al. 1981). It also stands out for its high levels of ω-3 linolenic acid, a fatty acid essential in nutrition and effective in reducing cardiovascular conditions (Hernández-Gómez and Miranda-Cólin 2008).

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Fig. 8.1 (**a**) *Salvia hispanica* L.; (**b**) *Salvia hispanica* L. seeds and a seed pod

Table 8.1 Chia seed proximate composition

Component	%
Water	6.25
Protein ($N \times 6.25$)	18.65
Oil	33.00
Crude fiber	28.38
Ash	4.35
Carbohydrates	9.37

Almost 90 % of the chia seed carbohydrate fraction is fiber and the remainder is starch, with no sugar content. Most of the fiber is soluble and has high molecular weight (mucilage) and an extraordinary water holding capacity. In fact, when chia seed comes into contact with water or any other aqueous environment, it increases in weight more than 16 times than linseed, creating a sticky gel texture. When seeds are ingested, they enter the stomach acid and form a gel which acts as a physical barrier between consumed carbohydrates and digestive enzymes. This barrier slows carbohydrate digestion, leading to more gradual and sustained conversion to glucose. This is beneficial in dietary treatment of diabetes because it prevents abrupt peaks in blood glucose after eating (Reyes-Caudillo et al. 2008).

8.2.2 Fatty Acid Composition

Chia seed oil content ranges from 29.8 to 32.0 %. Of this proportion, 97 % are neutral lipids and 57 % of these are linolenic acid. This unsaturated fatty acid, also known as omega-3, can help to significantly reduce the risk of cardiovascular

Table 8.2 Antioxidant concentration in chia seed extracts

Compound	Concentration (mol/kg of seed)
Caffeic acid	6.6×10^{-3}
Chlorogenic acid	7.1×10^{-3}
Myricetin	3.1×10^{-3}
Quercetin	0.2×10^{-3}
Kaempferol	1.1×10^{-3}

disease. The remaining oils are 17 % linoleic acid, 9.3 % oleic acid, and 1 % saturated fatty acids. The importance of ω-3 lays in its status as a precursor of eicosapentaenoic and docosahexaenoic acids, both vital to blood clotting and reduction of platelet aggregation and blood triglyceride levels (Ayerza 2011). Chia's fatty acid composition makes it promising as a food or a functional ingredient source. For example, fatty acid composition in poultry products can be modified by adding whole chia seed flour to poultry diets, without affecting animal productivity or health (Salazar-Vega et al. 2009). Including chia in functional foods can provide them a better fatty acid composition, potentially preventing undesirable changes in consumer plasma lipids and lipoprotein levels and enhancing product appeal.

8.2.3 Antioxidant Capacity

The water and methanol extracts produced by oil extraction from chia seed exhibit strong antioxidant activity. This is due primarily to their chlorogenic acid, caffeic acid, and flavonols contents (Table 8.2). These help to maintain stability in seed lipid composition which is why chia products such as oil or flour require no added antioxidants for preservation.

An additional benefit of consuming chia seeds is that consumption of foods and beverages with high flavonol content may protect against cardiovascular disease, stroke, lung, and stomach cancer (Ayerza and Coates 2001).

8.3 Conclusions

The nutrient composition of *Salvia hispanica* seeds has clear potential functional effects. Interest has consequently increased in this raw material. More thorough research into this promising food resource could help to promote its cultivation worldwide.

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Chapter 9
Cañahua: An Ancient Grain for New Foods

Gabriela T. Pérez, M. Eugenia Steffolani, and Alberto E. León

9.1 Introduction

Cañahua (*Chenopodium pallidicaule*) is a remarkably nutritious grain of the high Andes area that is cultivated in small plots in Bolivia, Ecuador, and Peru and grows well at altitudes of up to 4400 m in the extreme highland environment where wheat, rye, nor corn cannot grow and where even quinoa cannot yield well at the altitudes where cañahua grows.

Depending on the region where it grows, cañahua has different names: kañawa, kañiwa, or cañihua. In this chapter interchangeably kñiwa and cañahua be used.

This Andean grain is perhaps the strongest crop due to its resistance to frost, drought, salt, and pests. At the time of the Conquest, cañahua grain was an important food in the high Andes. Nowadays, it is cultivated in the Peruvian and Bolivian Andean plateau, consumed by local population and bought in Andean markets.

Cañahua is an erect or semiprostrate, highly branched plant that grows from 25 to 60 cm high and varies in precocity; one kind matures in only 95 days from the date of sowing although the preferred varieties take about 150 days before they are harvested (Gade 1970). The plant is not completely domesticated, and it often grows almost like a weed, reseeding itself year after year.

Agronomic classifications have been devised based on plant shape and seed color. There are two "ecotypes": an erect plant (*saihua*) with 3–5 basal branches and determinate growth, and a semierect type (*lasta*) with more than 6 basal branches

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and indeterminate growth. Each of these types is further classified by the black or brown color of the seed.

The erect types usually grow faster for about 70 days, at which time dry-matter production ceases and the plants flower. The semierect types continue to grow throughout the season, and eventually produce more stems and dry matter than the erect types. Some 380 accessions have been collected and are under evaluation in Puno, Peru.

9.2 Grain Characteristics and Chemical Composition

9.2.1 Grain Characteristics

Cañahua has hermaphrodite flowers because at fertility the flower is closed, and it almost exclusively pollinates itself.

The numerous seeds (achenes) are approximately 1–1.2 mm in diameter (about half the size of quinoa grains) and have a clasping, papery pericarp. Most seed coats range in color from chestnut brown to black (Fig. 9.1). Compared with conventional grains, the embryo is large in relation to the seed size.

9.2.2 Chemical Composition

Although Cañahua produces a cereal-like seed, it is not a true cereal, its grains are very small but their protein and lipid content are higher than wheat. However, different ecotypes present a wide range of protein and lipid content. Comparing four Bolivian ecotypes the total protein values varied from 12 to 17.5 % and lipids from

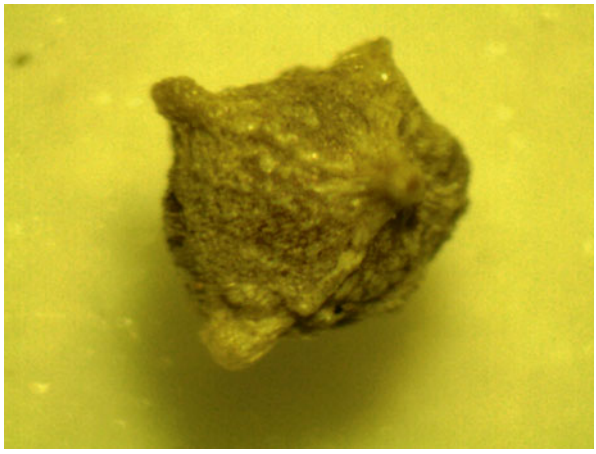

Fig. 9.1 Cañahua seed

Table 9.1 Chemical composition of cañahua, quinoa, and wheat

	Protein (%)	Carbohydrate (%)	Ash (%)	Lipids (%)	Crude fiber (%)
Cañahua	18.8	63.4	4.1	7.6	6.1
Quinoa	14.4	72.6	2.9	6.0	4.0
Wheat	10.5	78.5	1.8	2.6	2.5

De tales harinas, tales panes (2007)

Table 9.2 Sequential protein extraction from cañahua ecotypes

Ecotype	Albumin (%)	Globulin (%)	Prolamins (%)	Glutelin (%)	Insol. glutelin (%)
Local	26.7	24.3	6.0	11.4	31.5
81	18.3	19.3	4.2	11.0	47.1
300	18.8	20.3	4.2	9.3	47.2
381	17.4	19.9	4.7	10.9	47.2

9.9 to 11.7 % (Ramos et al. 2007). In ecotypes from Peru, the protein content varied from 13.3 to 18.3 % (Repo-Carrasco et al. 2010).

The chemical composition of Andean crops (cañahua and quinoa) and wheat are shown in Table 9.1.

9.2.2.1 Proteins

The importance of cañahua proteins is more related to quality than quantity. Regarding solubility, cañahua proteins can be classified as albumin, globulin, prolamin, and glutelin, according to Osborne extraction procedure. Approximately 40–50 % of total cañahua proteins are mainly soluble in water and saline solution, albumin and globulin; however, insoluble proteins can reach approximately 45–50 % (Repo-Carrasco 1998; Ramos et al. 2007). Insignificant amounts of protein are present in the prolamin fraction. Cañahua does not contain gluten, so it can be eaten by people who have celiac disease as well as by those who are allergic to wheat. The percentage of protein distribution according to solubility showed significant variations in different ecotypes (Table 9.2). The content of essential amino acids, especially lysine, is higher in soluble proteins than in insoluble proteins.

The limiting amino acid compared to the values recommended by the FAO for schoolchildren was threonine (81 ecotype, chemical value = 0.90; 300 ecotype, chemical value = 0.89).

In terms of essential amino acid, the ratio of essential to total amino acid (E/TN) in kañiwa protein isolates was higher than 0.44 in two ecotypes, which had higher value than the minimum E/TN ratio (0.36) suggested by FAO/WHO/UNU (WHO, 1985) thus cañahua protein could be considered as a high quality of natural protein (Table 9.3).

Table 9.3 Essential amino acid content and score from quinoa and cañahua

Amino acid	Quinoa: a.a. g/16 N	Amino acid score	Cañahua: a.a. g/16 g N	Amino acid score
Isoleucine	3.9	1.00	3.2	1.00
Leucine	6.9	1.00	6.5	0.98
Lysine	6.3	1.00	5.7	0.98
Methionine + Cisteyne	3.7	1.00	2.9	1.00
Phenylalanine + Tyrosine	7.2	1.00	6.8	1.00
Threonine	3.4	1.00	3.1	0.91
Tryptophan	1.1	1.00	1.2	1.00
Valina	4.6	1.00	4.0	1.00
Protein score		1.00		0.91
Limiting amino acid		–		Treonin

From Repo-Carrasco (1991)

9.2.2.2 Carbohydrates

Among carbohydrates, polysaccharides, such as starch, and simple sugars (glucose, fructose, sucrose, and maltose) are present in cañahua grain. Like cereals, starch is the most abundant carbohydrate in cañahua, although it contains more simple sugars than cereals (Ahamed et al. 1998).

9.2.2.3 Starch

Kañiwa starch granules present a bimodal size distribution since they form aggregated which are typical of most starches that consist of small granules. The size range of kañiwa starch granules could be estimated from the first peak of the bimodal curve. Cañahua starch granules are smaller than 2.0 μm and their shape is irregular, as they have the polygonal and angular shape typical of most small granule starches (Fig. 9.2). The relation amylose/amylopectin is lower than wheat; cañahua contains approximately 15 % of amylose and 85 % amylopectin, although different ecotypes can present from 14 to 18 % of amylose (Steffolani et al. 2013).

X-ray diffractometry can be used to evaluate the presence and characteristics of the crystalline structure of starch granules. Starch from kañiwa shows the A-type crystalline arrangement pattern that is associated with quinoa and is also typical of cereal starches. Starch functional behavior depends on thermal and pasting properties, which are strongly related to the food uses of starch. Regarding the pasting properties measured by Rapid Viscoanalyzer (RVA), cañahua starch presents low viscosity (peak viscosity, PV) and high pasting temperature compared to quinoa and cereal starches, while high granule stability is observed, as evidenced by its low breakdown value. The increase in viscosity during cooling of a paste (setback, SB) is a measure of retrogradation produced by reassociation of starch molecules, especially amylose, which results in the formation of a gel structure (Biliaderis 2009). Starches from cañahua have similar setback values to wheat (Table 9.4).

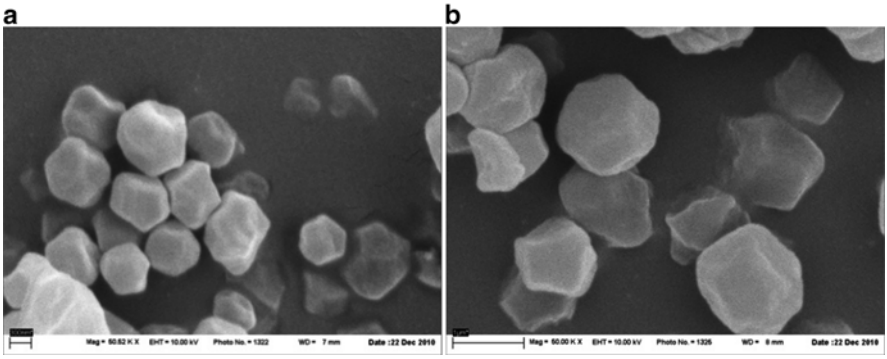

Fig. 9.2 Starch granules from Cañahua (**a**) and Quinoa (**b**)

Table 9.4 Pasting properties from cañahua, quinoa, and wheat starch

Starch	PV (cp)	BD (cp)	FV (cp)	SB (cp)	P. temp (°C)
Cañahua	2227.8	397.0	2983.0	1152.3	72.8
Quinoa	3932.0	781.0	3978.3	827.3	62.7
Wheat	2553.6	655.2	3057.6	1159.2	66.5

PV peak viscosity, *BD* breakdown, *FV* final viscosity, *P. temp* peak temperature

According to their thermal properties, cañahua starch has good potential as food ingredient for food exposed to heat treatment at high temperature and mechanical stirring.

9.2.2.4 Dietary Fiber

Cañahua has higher dietary fiber content than common cereals, such as wheat, rye, and barley. Nyman et al. (1984) reported a total dietary fiber content of 12.1 %, 16.1 %, and 18.8 % for wheat, rye, and barley, respectively. Cañahua presents approximately 25 % of total dietary fiber, although most of it corresponds to insoluble fiber, and only 3 % is soluble fiber (Repo-Carrasco et al. 2009). According to this study, cañahua cannot be considered a good source of betaglucans because the content of this compound is very low (0.04–0.07 %). The lignin content ranges from 6.8 to 8.0 % depending on the ecotype (Repo-Carrasco et al. 2009). This content is relatively high compared to other cereals: 2.0 %, 2.1 %, 3.5 %, 2.5 %, 3.9 %, and 1.4 %, for wheat, rye, barley, sorghum, rice, and corn, respectively (Nyman et al. 1984).

9.2.2.5 Lipids

Cañahua grain is especially rich in lipids. Different ecotypes can contain from 7 to 11 % (Repo-Carrasco et al. 2010; Ramos et al. 2007; Steffolani et al. 2013); for this reason, cañahua is a potential source of oil. However, its physicochemical oil properties and fatty acid composition have not been studied enough.

Table 9.5 Mineral composition of cañahua and rice

	Cañahua[a]	Rice
Fe (mg/100 g)	4.91	1.8
Ca (mg/100 g)	29.8	15
Zn (mg/100 g)	2.2	2.8

[a]Values taken from Repo-Carrasco et al. (2010)

Cañahua oil presents a 1.47 refraction index and approximately 73 % content of unsaturated acids, where 42 % corresponds to linoleic acid (omega 6), 25 % to oleic acid (omega 9), and 6 % to linolenic acid (omega 3). Cañahua oil has 788 and 721 ppm of γ and α tocopherol, respectively, which is higher than corn oil content (Repo-Carrasco et al. 2003). Tocopherols are components of E vitamin with a potent antioxidant activity that improve oil quality and oil conservation; besides, high contents of tocopherols increase the nutritional value of cañahua.

9.2.2.6 Minerals

Andean crops are rich in minerals. Cañahua contains more Zn, Ca, and Fe than rice (Table 9.5), but during the boiling process iron and zinc content significantly decrease (Repo-Carrasco et al. 2010). The roasting process does not modify mineral content.

The bioavailability of minerals is defined as the amount of mineral that is absorbed in the gastrointestinal tract and utilized for metabolic functions. Minerals in plant sources are less available than in animal sources because of the presence of certain compounds, such as dietary fiber, phytate, and oxalate, which have negative effects on mineral absorption. The processing of raw grains can modify the bioavailability. Repo-Carrasco et al. (2010) reported that in cañahua boiling increased the dialyzability of minerals (Zn, Ca, and Fe) although this process decreased the total content of Zn and Fe.

9.2.2.7 Bioactive Compounds

"Bioactive compounds" are extranutritional constituents that typically occur in small quantities in foods. They are being intensively studied to evaluate their effects on health. These compounds vary widely in chemical structure and function and are grouped accordingly. Phenolic compounds, including their subcategory, flavonoids, are present in all plants and have been studied extensively in cereals, legumes, nuts, olive oil, vegetables, fruits, tea, and red wine. Many phenolic compounds have antioxidant properties, and some studies have demonstrated their favorable effects on thrombosis and tumorogenesis and promotion. Andean grains are rich in bioactive compounds with antioxidant activity. The total content of polyphenolic compound of cañahua was about 2.5 mg GAE/g (Repo-Carrasco et al. 2009), this content is

higher than in oat (Gorinstein et al. 2007), buckwheat, quinoa, and rice (Pasko et al. 2009). In addition, flavonoid content determined for cañahua was exceptionally high, varying from 46.2 to 144.3 mg/100 g. Berries have been considered excellent sources of flavonols, but their level in flavonoid-rich berries is 5–10 times lower than in cañahua seeds. When compared on a dry weight basis, flavonoid contents in berries and cañahua are of the same magnitude. Cañahua seeds have excellent potential as sources of health promoting bioactive compounds.

9.3 Cañahua Uses in Food Production

9.3.1 Traditional Uses in Local Food

Cañahua was used as food by Andean people before the Spanish conquest (Gade 1970), and nowadays it is used in Bolivia and Peru mainly as *cañihuaco* or *pito*, which is the toasted and milled grain. This toasted flour is mixed with sugar, milk, water, or fruit juice for breakfast. Local people take it on their long travels because of its high caloric and protein value. Some varieties of cañahua expand when toasted and can be included in sweets and snacks. Cañahua can also be used in soups and baby food mixtures.

In a study performed by Repo-Carrasco and Li Hoyos (1993), a dietary mixture was formulated: quinoa-cañahua-beans with high nutritional value, with protein efficiency ratio value of 2.36, very close to casein. The extrusion process can be applied to cañahua. An extrudate with good functional properties, high gelatinization degree, low water absorption, and high water solubility was obtained, although the nutritional quality was reduced during the extrusion process (Repo-Carrasco et al. 2009).

Cañahua flour is also used to make small cookies called *quispiños* and is added to wheat flour to make bread and pastry products (Gade 1970).

9.3.2 Potential Uses of Cañahua in Global Food

Because of the high nutritional value and the composition of cañahua seed, its flour has been used to partially replace wheat flour in baked goods and pasta, improving the nutritional quality of baking products. However, the inclusion of flour other than wheat in bread or pasta making will modify the protein–starch matrix responsible for such quality parameters as volume and texture in bread and cooking behavior in pasta.

Rosell et al. (2009) evaluated the breadmaking potential of cañahua replacing wheat with cañahua flour from 10 to 100 %. The breads obtained with the different substituting levels showed a range of loaves with different sensory and technological characteristics.

Bread with 12.5 % cañahua flour had a higher specific volume, probably due to more fermentable sugars that favor yeast fermentation and gas production. When

Fig. 9.3 Bread slice from wheat and wheat-local cañahua blends. *W* 100 % wheat flour, *W-CL 10* 90 % wheat—10 % local cañahua flour, *W-CL 20* 80 % wheat—20 % local cañahua flour

Fig. 9.4 Pasta made with 10, 20, and 30 % of cañahua flour

the substitution level was 25 %, the SV was similar to pure wheat. A 50 % substitution produced a strong drop of volume due to the reduction of the gluten content in the dough. Crumb color and hardness were negatively affected by 50 % substitution. Breads made from blends of up to 25 % of cañahua flour gave acceptable sensory scores.

In another study, breadmaking performance of different ecotypes of Bolivian cañahua was evaluated (Ramos et al. 2007). Bread made with 10 and 20 % local ecotype flour did not show a significant difference in specific volume compared with control wheat (Fig. 9.3), but other ecotypes affected bread quality attributes negatively. Cañahua flour ecotypes presented differences in protein, lipid content, and gelatinization behavior that could explain the differential functional properties in breadmaking. Bread staling velocity during store was negatively affected by all cañahua flour inclusion (Ramos et al. 2007).

Cañahua flour was used to produce pasta enriched in dietary fiber and protein (Bustos 2012). Pasta was made with wheat flour substituted at different levels (10, 20 or 30 %) for cañahua flour (Fig. 9.4), and its cooking properties were evaluated.

Table 9.6 Cooking properties of wheat and wheat–cañahua pasta

Pasta sample	OCT (min)	Water absorption (g kg^{-1})	Swelling index	Cooking loss (g kg^{-1})
Control	10	1360 a	1.94 a	44.0 a
LC-10 %	7	1670 c	2.23 b	59.0 b
LC-20 %	7	1730 d	2.33 c	60.5 b
LC-30 %	7	1810 e	2.60 d	68.8 c

Control: 100 % wheat flour, LC-10 %, LC-20 %, LC-30 %: substitution level with local cañahua ecotype. Different letters indicate significantly differences ($p < 0.05$)

Table 9.7 Protein and dietary fiber content of raw and cooked pasta

	Protein (g kg^{-1})		Dietary fiber (g kg^{-1})		
	Raw pasta	Cooked pasta	Raw pasta	Cooked pasta	Lost Fiber
Control	130.5	144.8	57.0	53.9	3.1
L1-10 %	128.4	142.8	67.6	63.9	3.7
L1-20 %	126.3	143.4	78.2	71.4	6.8
L1-30 %	124.2	144.7	88.8	82.8	6.0

Pasta made with cañahua–wheat flour blends showed lower optimum cooking time (OCT) than wheat pasta, but it did not show significant differences between samples with different cañahua substitution levels. Water absorption and swelling indexes increased in relation with the content of cañahua in the pasta (Table 9.6). The addition of whole cañahua flour disrupted the gluten matrix for the presence of perigonium particles, providing a path for water absorption into the wheat–cañahua pasta that reduced cooking time and increased water absorption. The greater the level of cañahua contents, the higher the cooking loss values. Low cooking loss is a desirable property of good quality pasta and a commonly used predictor of spaghetti cooking performance (Tudorica et al. 2002). Values lower than 80 g/kg are considered acceptable for good quality pasta (Dick and Youngs 1988); so, pasta of up to 30 % substitution level can be considered of acceptable quality.

Protein and dietary fiber contents were measured in cooked pasta to determine some lost during cooking process. A slight protein increment observed in cañahua-cooked pasta can be translated in a higher protein quality of this product because of its high lysine content. Cañahua flour improved the dietary fiber content of raw and cooked wheat pasta and the level of fiber lost during cooking was low. The dietary fiber content of a normal serving (dry weight = 100 g) of 20 % substitution level pasta would correspond to 20 % in men or 30 % in women of the daily total fiber intake recommended (The National Academies Press 2005) (Table 9.7).

Finally, the replacement of 20 % or 25 % of wheat flour by cañahua flour in pasta and bread, respectively, is a viable option to improve the nutritional value of wheat products for quantitative and qualitative protein composition, high dietary fiber content, and bioactive components. Ramos Diaz et al. (2013) showed that it was

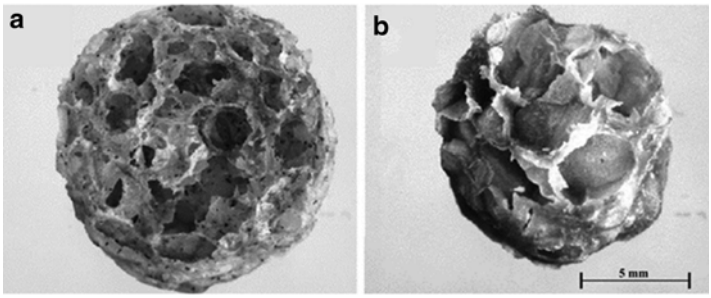

Fig. 9.5 Cross-sectional area of corn-based extrudates containing 20 % kañiwa (**a**) and 100 % corn (**b**) (Ramos Diaz et al. 2013)

Table 9.8 Technological quality of wheat and wheat-kañiwa cookie

Cookie sample	Cookie factor	Fracture hardness (gf)	*L**	*a**	*b**
Control	4.74	7222	68.81	9.02	31.38
81	5.22	5472	53.60	6.15	22.05
300	4.94	7449	56.64	6.09	22.05
381	4.98	5314	53.94	4.55	21.33
Local	5.13	6037	53.86	5.96	22.24

possible to prepare expanded gluten-free corn-based extrudates containing kañiwa flour (20 % of solids).

Extrudates containing kañiwa presented the lower hardness as compared with pure corn extrudates, on the contrary, sectional expansion index (SEI, ratio between the cross sectional area of the extrudate and the area of the die) was high in extrudates containing kañiwa and low in pure corn extrudates.

Extrudates containing kañiwa presented small, irregular and poorly defined pores. Pure corn extrudates had a rigid structure with bigger pores and thicker walls (Fig. 9.5). It is plausible that fiber present in kañiwa caused severe bubble disruption leading to lower radial expansion and higher number of pores.

In other work (Steffolani et al. 2013), kañiwa flour was used to elaborate cookies in order to make them healthier. The cookies were prepared by substitution of wheat flour by 25 % kañiwa flour from four ecotypes (Local, 300, 81, 381).

In general, except the C300 ecotype, kañiwa flour significantly increased the cookie factor (the ratio between the width and height of four cookies randomly picked) as compared to the control (100 % wheat flour) and only the kañiwas C081 and C381 had significantly lower fracture hardness (Table 9.8). In addition, the cookies prepared with kañiwa presented lower values of L*, a* and b* as compared to control cookie, this result is related to the dark pigmentation of crop kañiwa. Finally, cookies kañiwa had a greater number of cracks (roughness) on the surface, parameter related to good quality (Fig. 9.6). Kañiwa flour (Local, 81 and 381 ecotypes) could be used to develop healthier cookies since it greatly improves the technological quality and increased dietary fiber content and protein quality and content.

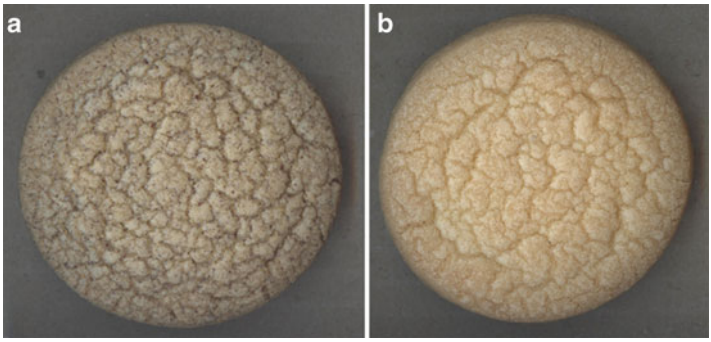

Fig. 9.6 Cookie containing 25 % kañiwa (**a**) and 100 % wheat (**b**)

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Chapter 10
Chemical Characterization of Mexican Chia (*Salvia hispanica* L.) Flour

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10.1 Introduction

Chia species (*Salvia* sp.) are native to Mexico. All (*S. columbaria Benth*, *S. hispanica* L., *S. polystachya*) belong to the Lamiaceae family, although *S. hispanica* L. is the most widely distributed (Alvarez-Chavez et al. 2008). Chia was widely used in ancient Mesoamerica for millennia. Along with other staple grains such as corn, amaranth, and beans, chia seed was an important crop in the Aztec culture. The Aztecs roasted chia seeds and mixed them with water to form a gruel for eating or ground them into flour for baking. They also used chia seed oil in body paints and as an ointment and emollient. A paste made from the mucilaginous moistened seeds was utilized as a poultice for wounds and to remove dirt from the eye. However, because of its association with indigenous medicinal and religious practices chia cultivation was banned by the Spanish conquerors. Chia is still consumed today in the form of a beverage known as *agua de chia*.

Chia seeds contain approximately 19–23 % protein, a much higher protein concentration than in wheat (14 %), corn (14 %), rice (8.5 %), oats (15.3 %), and barley (9.2 %) (Monroy-Torres et al. 2008). Its proteins also have a good essential amino acid balance. Chia seeds are approximately 25–38 % oil by weight, with the highest known α-linolenic acid (60 %) proportion among vegetable crops, and a high linoleic acid content (20 %).

Chia seeds are consumed in Mexico, the southwestern United States and South America, but are not widely used in other parts of the world. Its high oil, protein,

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antioxidant, and dietary fiber contents have led to continued research into chia's properties. Chia seed oil is receiving increasing attention as a natural, plant-based source of ω-3 fatty acids and antioxidants, with potential applications in human nutrition (Ixtaina et al. 2011). The chapter objective was to chemically characterize chia oil, and protein- and fiber-rich fractions (PRF, FRF) from defatted chia flour to assess chia's potential as a value-added source of nutraceutical compounds.

10.2 Chia Seeds and. Production of Flour

10.2.1 Chia Seeds

Chia (*S. hispanica* L.) seeds were obtained from the February 2000 harvest at Tapachula, Chiapas, Mexico (14°54′N, 92°56′W). All chemicals were reagent grade from J.T. Baker (Phillipsburg, NJ), and enzymes were from Sigma (Sigma Co., St. Louis, MO, USA).

10.2.2 Chia Flour

The flours were produced by first removing all impurities and damaged seeds from 10 kg of seed and milling the remaining sound seeds (Thomas-Wiley, Model 4, Thomas Scientific, USA). Oil was extracted from the milled seeds with hexane in a Friedrich system, using four refluxes of 80 min each. The remaining fraction was milled until it passed through 1 mm screen, a second oil extraction done, and the remaining fraction milled until it passed through a 0.5 mm screen.

10.2.3 Protein and Fiber-Rich Fractions

The PRF, FRF were separated by dry fractionation of the defatted flour according to Otto et al. (1997). Briefly, 500 g flour was sifted for 20 min using a Tyler 100 mesh (140 μm screen) and a Ro-Tap® agitation system (Laval Lab In, Quebec, Canada). This separated the high fiber-content fraction (retained in screen) from the high protein-content fraction (passed through screen).

10.2.4 Proximate Composition

Standard AOAC (1997) procedures were used to determine nitrogen (method 954.01), fat (method 920.39), ash (method 925.09), crude fiber (method 962.09), and moisture (method 925.09) contents of the chia flours (whole, and defatted) as

well as the PRF, FRF (AOAC 1997). Nitrogen (N_2) content was quantified with a Kjeltec Digestion System (Tecator, Höganäs, Skåne län, Sweden) using cupric sulfate and potassium sulfate as catalysts. Protein content was calculated as nitrogen × 6.25. Fat content was obtained from a 1 h hexane extraction. Ash content was calculated from sample weight after burning at 550 °C for 2 h. Moisture content was measured based on sample weight loss after oven-drying at 110 °C for 2 h. Carbohydrate content was estimated as nitrogen-free extract (NFE) by difference from the sum of the protein, fat, ash, and crude fiber content.

10.2.5 Fiber-Rich Fraction Characterization

Chemical composition of the FRF was determined by quantifying total dietary fiber (TDF), insoluble dietary fiber (IDF), soluble dietary fiber (SDT) (Prosky et al. 1988), neutral detergent fiber (NDF) (van Soest 1994), acid detergent fiber (ADF) (van Soest and Wine 1967), acid detergent lignin (ADL) (Goering and Van Soest 1975), cellulose and hemicellulose (Tejada 1992).

10.2.6 Protein-Rich Fraction Characterization

Chemical composition of the PRF was determined by extracting and characterizing the soluble protein fractions (Gallegos-Tintore et al. 2004) and generating an amino acid profile (Alaiz et al. 1992).

10.2.7 Chia Oil Characterization

Chia oil chemical composition was characterized by determining the acidity index (NMX-F-101-1987, Pereira 1988), saponification index (NMX-F-174-S-1981, Pereira 1988), non-saponifiable matter content (Hart and Fischer 1991), iodine index (NMX-F-152-S-1981, Kirk et al. 1996), and peroxides index (NMX-F-154-1987).

10.2.8 Statistical Analysis

All determinations were done in triplicate. Statistical analyses were run to calculate the data's central tendency and standard deviation using the Statgraphics plus 5.1 computer software.

10.3 Composition of Chia Seed Flours

10.3.1 Proximate Composition of Flour

The whole chia flour contained 211 g/kg protein and 305.9 g/kg crude fiber, while the defatted chia flour contained 322.4 g/kg protein and 265.0 g/kg crude fiber (Table 10.1).

10.3.2 Fiber-Rich Fraction Characterization

The FRF contained 29.56 g/kg crude fiber (Table 10.2) and 56.46 g/kg total dietary fiber. Of the total dietary fiber, 3.01 g/kg was soluble dietary fiber and 53.45 g/kg was insoluble dietary fiber with higher cellulose, hemicellulose and lignin levels (Table 10.3).

10.3.3 Protein-Rich Fraction Characterization

The PRF contained 44.62 g/kg crude protein (Table 10.2), which consisted mainly of globulins (64.86 %) and glutelins (20.21 %). Protein recovery was 74.22 % (Table 10.4). The PRF amino acid profile showed high levels of essential sulfur amino acids and nonessential amino acids, with deficiencies in tryptophan and lysine (Table 10.5).

Table 10.1 Proximate composition of whole and defatted chia flour

Component	Whole flour (g/kg d.b.)	Defatted flour (g/kg d.b.)
Moisture	$(44.2 \pm 0.2)^a$	$(68.7 \pm 1.0)^b$
Protein (N × 6.25)	211 ± 1.1^a	322.4 ± 1.7^b
Crude fiber	305.9 ± 3.7^a	265 ± 2.8^b
Fat	259.8 ± 1.0^a	4.5 ± 0.2^b
Ash	48.6 ± 0.3^a	70.9 ± 0.2^b
NFE	174.7 ± 3.2^a	337.2 ± 4.9^b

[a,b]Values with different letters in the same row denote significant difference ($p < 0.05$)

Table 10.2 Proximate composition of fractions obtained by dry processing defatted chia (*Salvia hispanica* L) flour

Component	Fraction >140 μm (g/kg d.b.)	Fraction <140 μm (g/kg d.b.)
Protein (N × 6.25)	28.14^a	44.62^b
Crude fiber	29.56^a	11.48^b
Fat	0.46^a	0.54^a
Ash	6.51^a	8.84^b
NFE	35.33^a	34.52^a

[a,b]Values with different letters in the same row denote significant difference ($p < 0.05$)

Table 10.3 Chemical composition of chia (*Salvia hispanica* L) fiber-rich fraction (FRF)

Component	FRF (g/kg d.b.)
Total dietary fiber	56.46 ± 0.35
Insoluble	53.45 ± 1.62
Soluble	3.01 ± 1.28
Neutral detergent fiber	54.51 ± 0.44
Acid detergent fiber	45.43 ± 0.44
Acid detergent lignin	20.27 ± 0.46
Cellulose	25.16 ± 0.02
Hemicellulose	9.08 ± 0.00

Table 10.4 Protein solubility distribution of chia protein-rich fraction

Fraction	Protein extracted (g/kg)	Protein recovery (%)	Osborne fractions (relative %)
Albumins	33.86 ± 0.43	8.07	10.89
Globulins	201.55 ± 4.02	48.41	64.86
Prolamins	12.57 ± 1.45	3	4.04
Glutelins	62.79 ± 3.39	15	20.21
Total	310.77	74.22	100

Table 10.5 Amino acid composition of defatted chia flour and protein-rich fraction (PRF)

Amino acid	Defatted flour (g/kg d.b.)	PRF (g/kg d.b.)
Lys	5.00 ± 0.0	5.00 ± 0.0
Trp	0.95 ± 0.1	0.80 ± 0.0
Phe	5.15 ± 0.1	5.05 ± 0.1
Tyr	2.30 ± 0.0	2.90 ± 0.0
Met	1.30 ± 0.0	3.10 ± 0.0
Cys	1.90 ± 0.0	2.40 ± 0.0
Thr	3.90 ± 0.0	3.90 ± 0.0
Leu	7.20 ± 0.0	6.95 ± 0.1
Ile	3.30 ± 0.0	3.20 ± 0.0
Val	4.60 ± 0.0	4.60 ± 0.0
Asx	10.25 ± 0.1	9.35 ± 0.1
Glx	19.95 ± 0.1	19.20 ± 0.0
Ser	6.45 ± 0.1	6.30 ± 0.0
His	2.55 ± 0.1	2.70 ± 0.0
Arg	10.25 ± 0.1	10.60 ± 0.0
Ala	5.10 ± 0.0	5.00 ± 0.0
Pro	4.00 ± 0.1	4.05 ± 0.2
Gly	5.90 ± 0.0	4.95 ± 0.1

Table 10.6 Chemical properties of chia oil

Parameter	Chia oil
Acidity index (mg KOH/g oil)	2.053 ± 0.03
Saponification index (mg KOH/g oil)	222.66 ± 0.29
Non-saponifiable matter (g/kg)	0.839 ± 0.10
Iodine index (g I absorbed/100 g oil)	193.45 ± 0.54
Peroxides index (mEq O_2/kg oil)	13.57 ± 0.01

10.3.4 Chia Oil Characterization

The chia oil acidity index and non-saponifiable matter content were within reported ranges (Codex Stan 210 2003; Codex Stan 33 1989). The acidity index indicated acidity to be normal, with 1.032 % oleic acid and 1.017 % linolenic acid (Table 10.6). The saponification index was higher than reported for unsaturated oils such as soy and linseed oil. These results suggest that chia lipids have a lower molecular weight than common vegetable oils. Non-saponifiable matter content was lower than reported for soy, olive, and sunflower oils (≤15 g/kg), suggesting low impurities levels. The iodine index value (193.45 g I absorbed/100 g oil) indicated a high insaturation level similar to linseed oil (Hosseinian et al. 2004). These results suggest chia oil has a high refraction index and density levels, as well as high polyunsaturated fatty acid (PUFA) content. PUFAs such as ω-3 and ω-6 are considered essential because the human body is unable to synthesize them. Including them in human diets is vital to maintaining a healthy organism because oils rich in ω-3 FA are fundamental to prevention and treatment of coronary artery diseases, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders, and cancer. Chia seed oil is receiving increased attention since it can benefit human nutrition by providing a natural, plant-based source of ω-3 FA and antioxidants. The peroxides index values indicated there to be no rancidity, indicating good handling and storage practices.

Chia (*Salvia hispanica* L) oil and the fiber- and protein-rich fractions from defatted chia seed flour clearly contain compounds that could make them potential functional food ingredients. The fiber- and protein-rich fractions could also have industrial applications.

Conclusion

Chemical characterization of chia oil and fiber- and protein-rich fractions extracted from defatted chia flour showed this seed to have potential as a functional food and a value-added source of nutraceutical components. The results reported here constitute useful data for determining the suitability of chia seed as a regular ingredient in human diets, as well as use of chia oil in prepared foods and new food products with possible health benefits.

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Chapter 11
Functional and Beneficial Properties of Corn Tortilla

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11.1 Tortilla: An Ancient Staple

Tortilla is the main staple of people living in Mexico and Guatemala as well as Mexican people living in the United States. Tortilla represents an important source of calories, proteins, calcium and dietary fibre (Campas-Baypoli et al. 1999; Billeb de Sinibaldi and Bressani 2001). Tortilla production in Mexico is approximately 11 million tons, of which 23 % is from dry corn flour and the remaining 77 % comes from commercial fresh masa produced by the traditional nixtamalisation process in small factories named "tortillerias" or traditional self-preparation in rural areas (Serna-Saldívar et al. 1990; Paredes-López et al. 2000).

Maize is deficient in essential amino acids such as lysine and tryptophan, and it should be prepared under determined conditions to increase the nutritional value. In this sense, any population that uses maize as the principal food tends to suffer from some degree of malnutrition. Populations that did not use cooking of maize also did not use compounds such as calcium hydroxide or wood ashes, because they had to adopt other nutrition models with supplements in addition to maize.

Based on this information, it is hypothesised that the populations where maize was the basis of nutrition also used alkaline cooking of maize. To confirm this hypothesis, the information about 51 populations that lived in America was studied. It was found that out of 27 populations of the United States, 14 used alkaline cooking of maize; however, only Tewa and Zuñi Indians in the southwest used maize as principal food. On the other hand, of the 18 populations of South America analysed in this study, only Páez Indians of Colombia harvested maize, but this cereal was not the sole basis of their nutrition.

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Archaeological evidences indicate that maize was introduced to Colombia from Mesoamerica (south of the United States, Mexico and Central America), and alkaline cooking was introduced with the crop. Populations of North America and Colombia used wood ashes for maize cooking, while in Mexico and Central America, calcium hydroxide was used. The information that calcium hydroxide was used by populations of Mexico supports the origin of nixtamalisation. How did the Aztecs and Mayas select the nixtamalisation process? Probably the process conditions were selected by trial and error, or perhaps, the nixtamalisation process was selected due to their observations of appearance or disappearance of some physiological disorders such as pellagra produced by niacin deficiency.

The word nixtamalisation is derived from the Nahuatl words *nixtli*, meaning ashes, and *tamalli*, meaning "masa", and this process has been transmitted from generation to generation. Nowadays, in Mexico this technique is used in the same way as it was used by the Aztecs. Nixtamalised maize is used to produce "masa", which is moulded and baked. This product was named *tlaxcalli* by the Aztecs and, thereafter, tortilla by Spanish people. Currently, as part of the ethnic food trend, table corn tortillas are highly popular in industrialised countries and are often consumed as snacks or during the main meal (Yau et al. 1994).

11.2 Nixtamalisation

11.2.1 Traditional Process

The traditional nixtamalisation process starts with the addition of two parts of calcium hydroxide (1 % w/w) to one part of maize grain. The blend is cooked for about 50–90 min, depending on the endosperm type of the grain, followed by steeping of maize in the cooking water for 14–18 h, and then the cooking solution ("nejayote") is discarded. The resulting "nixtamal" is washed three or four times with tap water to remove bran and excess lime. The "nixtamal" with approximately 45 % of moisture content is ground into a "masa" using a stone grinder with tap water addition. The moisture content of the "masa" lies between 48 and 55 %. "Masa" is then pressure-moulded and extruded into thin circles to make tortillas 1 mm thick. Finally, the tortillas are baked in a gas-fired oven. "Masa" is the raw material to make "totopos", "tostadas", "tamales", etc., all are traditional dishes in the Mexican culture.

11.2.2 Novel Processes

Nowadays, table tortillas are highly popular in the United States and, to some extent, in Canada and several European countries (Yau et al. 1994) principally due to the Mexican people who continue the tradition of consuming maize tortilla. The expanded consumption of tortillas has led to industrialisation of the nixtamalisation process for nixtamalised corn flour (NCF) production. Different methods that use batch

cooking, steeping and stone grinding and their effects on flour quality have been reported (Almeida-Dominguez et al. 1996). However, "masa" production at the industrial level does not exactly follow the traditional nixtamalisation conditions, resulting in tortillas that exhibit lower quality texture and decreased stability during storage as compared to traditionally made tortillas. In dry "masa" production, the flour is obtained by hammer milling utilising a low moisture "nixtamal", which does not allow the release of starch granules from other components. Consequently, particles of NCF are rather different from those of fresh "masa". Fresh "masa" particles contain significant quantities of free starch granules but low protein levels, while NCF particles contain starch and protein levels similar to those present in the original endosperm (Gómez et al. 1991). The main advantage of NCF use is that a tortilla producer might simply rehydrate the flour with water, obtaining the "masa" which is then sheeted, moulded and baked. This greatly reduces labour and capital investment for processing equipment, problems associated with maize acquisition and wastewater generation during nixtamalisation. The disadvantages in the use of NCF are its high price and important differences in flavour and texture in comparison with products made with fresh "masa". However, the big companies that produce NCF have been able to improve the processes to obtain NCF that produces tortillas with better functionality and sensory characteristics. Little by little, "tortillerias" do not produce "nixtamal" or the amount produced is minor, due to the fact that NCF is used to make tortillas. Fresh "masa" is often blended with NCF to make tortillas with the objective of preventing consumers from detecting the difference in the traditional flavour and taste of tortilla. The traditional machines used to make tortillas have been modified to use NCF, because this flour needs to be hydrated to make "masa" and tortillas.

The traditional nixtamalisation process generates high amount of wastewater, which has an important ecological impact due to the presence of high levels of organic material. An alternative process is where cooking of the maize grain is carried out with calcium hydroxide and injecting steam into an extruder. Additionally, the use of alternative energy source as infrared, microwave and ohmic heating for tortilla baking results into a process that is friendly with the environment (Figueroa et al. 1997).

11.3 Characteristics of Tortilla

11.3.1 Physical

Recently baked tortilla (fresh tortilla) is soft in texture, and depending on the maize used for its preparation, it has a white, yellow, blue, black, green or other colours. In Mexico, people prefer fresh tortilla due to that soft texture which permits rolling or bending of tortilla that enables putting of foods inside such as meat, beans, cheese, etc. Fresh tortillas roll easily without cracking, whereas aged tortillas are rigid and brittle and crack when they are rolled (Suhendro et al. 1998). During rolling, tortillas are subjected to some extensibility before they crack or break (Suhendro et al. 1999). Parameters of force, modulus, work of deformation

Table 11.1 Textural characteristics of fresh tortillas made with three different types of endosperm

	Extensibility		Puncture
Endosperm type	Rupture force (N)	Distance (mm)	Force (N)
Floury	4.18	3.81	1.44
Intermediate	4.39	3.88	1.72
Vitreous	3.16	2.11	2.04

Source: Osorio-Díaz et al. (2011)

and force and distance to rupture are measured by extensibility methods (Bejosano et al. 2005).

Tortilla prepared from nixtamalised maize flour and immediately analysed presented a force to puncture of 1.01 N (Bejosano et al. 2005) and 1.2 N (Román-Brito et al. 2007). Recently, the endosperm type of maize used to make tortilla was evaluated; tortilla of floury endosperm had the lowest force to puncture the sample and that of vitreous endosperm the highest one (Table 11.1). Tortillas from vitreous endosperm were firmer than those from floury and intermediate endosperms.

Tortillas made from floury and intermediate endosperm had similar rupture force, which was higher than those prepared from vitreous endosperm. This pattern indicates that tortilla from vitreous endosperm has a more rigid structure.

The distance of the extension before the rupture of tortillas presented similar patterns as the force to rupture; tortillas of vitreous endosperm were harder and more brittle. The distance of extensibility for tortilla elaborated with maize of floury and intermediate endosperms was similar, and these values were higher than the distance of extensibility for tortilla with vitreous endosperm. Tortilla with vitreous endosperm had similar distance of extensibility than that determined in fresh tortilla made with commercial nixtamalised maize flour (Román-Brito et al. 2007).

Nixtamalisation of maize, grinding of nixtamal and cooking of tortilla produce gelatinisation of starch that is the main component of the grain. Gelatinised starch is composed of structures, which are thermodynamically unstable. When tortillas are cooling, starch molecules may be reorganised. The ability of starch chains to form ordered structures during storage is often called retrogradation, which greatly influenced the texture of tortilla. One problem with tortillas is that staling occurs after preparation (1–2 h), which increases rigidity and affects palatability. In order to consume stored tortillas, they are reheated and soft texture is produced, but this soft texture is maintained for a very few minutes only. Texture of stored tortillas showed that the force to penetrate was similar between control tortilla and that stored for 3 days, but an increase in this parameter was assessed at longer storage times. Fresh tortillas were softer and more extensible than aged tortillas, which were harder and more brittle as indicated by the smaller distance of extensibility before rupture. The distance of extensibility in fresh tortillas was 2.25–2.75 mm, and it decreased in the tortilla stored for 3 days, but at longer storage time the values did not change. Laboratory-prepared fresh tortillas had a distance extensibility value of 26.31 mm, decreasing when storage time increased from 3 h (9.36 mm) to 240 h (5.25 mm) (Suhendro et al. 1999).

Table 11.2 Chemical composition of maize tortilla (%)

Sample	Moisture	Fat[a]	Protein[a]	Ash[a]	Ref.
Commercial flour	Nd	3.15–3.97	8.28–9.33	1.14–1.49	Agama-Acevedo et al. (2004)
Tortilla of commercial flour	Nd	2.75–4.10	7.51–8.63	1.28–1.54	
Tortilla of commercial masa	Nd	3.15–3.97	8.05–9.17	1.47–1.67	Agama-Acevedo et al. (2005)
White tortilla	38.3	2.6	8.03	1.36	Hernández-Uribe et al. (2007)
Blue tortilla	34.74	4.47	9.56	1.60	
Maize tortilla	47.6	4.27	9.1	1.31	Rendón-Villalobos et al. (2009a, b)
Tortilla + 10 % flaxseed	43.8	8.23	10.87	1.54	
Tortilla + 15 % flaxseed	44.1	9.98	11.60	1.44	
Tortilla + 20 % flaxseed	44.13	12.0	12.93	1.64	
Maize/amaranth tortilla (80/20)	Nd	4.81	10.60	1.52	Islas-Hernández et al. (2007)
Whole-grain corn tortilla prepared using the ecological method of nixtamalisation	Nd	3.06	7.96	1.14	Maya-Cortes et al. (2010)
Tortilla prepared by the traditional method of nixtamalisation	Nd	2.63	7.48	1.31	

[a]Dry matter
Nd not determined

11.3.2 Chemical

The proximate composition of tortillas is shown in Table 11.2. Moisture content in fresh tortillas ranged between 35 and 48 %, and this value depends on the parameters used in nixtamalisation process such as temperature, water content, steeping time and mixed. Additionally, the maize variety plays an important role in determining this characteristic of tortilla. Fat content in corn tortillas ranged between 2.6 and 4.5 %, a parameter that depends on the variety and nixtamalisation conditions, because more lipids from the germ of the grain can be solubilised during the thermal treatment or during the steeping of the cooked grain. The addition of amaranth resulted in a slight increase in the fat content, and the flaxseed flour increased this value up to 100 %. The presence of fat content in tortillas can prevent the starch retrogradation and the staling of tortilla due to the formation of amylose-lipid complexes; it has recently been postulated that amylose-lipid complexes produce RS (Hasjim et al. 2010). The average value of protein content of corn tortilla is 8.5 %. It is well known that tortilla is deficient in essential amino acids (lysine and tryptophan), for this reason some new varieties of maize have been developed with a higher amount of these two essential amino acids and are named quality protein maize (QPM). Recently, we prepared tortillas with QPM and the protein content was 8.8 %, a value close to average protein content of corn tortilla, because the

elaboration of tortilla with QPM variety increased the availability of both amino acids but not the total amount of protein. The addition of amaranth and flaxseed flours at different levels increased the protein content of corn tortilla. Ash content in corn tortilla ranged between 1.1 and 1.6 %, and the addition of other flours in the formulation did not modify this parameter. In the ash content, mineral components such as calcium used in the nixtamalisation process are quantified.

11.3.3 Thermal Properties

To evaluate the organisation of the diverse components (starch, lipids, proteins) in the matrix of corn tortilla, differential scanning calorimetry (DSC) was used. Diverse studies show the use of this analytical technique in the raw materials (nixtamal and masa), fresh and stored tortillas (Table 11.3). The raw materials had higher peak temperature (average gelatinisation temperature) but lower enthalpy than native maize starch; this pattern is due to the fact that during nixtamalisation, the starch granules in the periphery of the endosperm are gelatinised, but those inside of the endosperm suffer a rearrangement of the structure (annealing), thus increasing the average gelatinisation temperature, but with a decrease in the enthalpy due to the partial gelatinisation of the starch. Fresh corn tortilla presented lower average gelatinisation temperature and enthalpy, showing that the baking of tortilla

Table 11.3 Average temperature (Tp) and enthalpy (ΔH) of gelatinisation of raw maize and nixtamalised samples, measured by differential scanning calorimetry

Sample	Tp (°C)	ΔH (J/g)	Reference
Raw maize starch	67.9	13.7	Méndez-Montealvo et al. (2007)
Nixtamal	83.0	6.3	Campas-Baypoli et al. (1999)
Masa	80.1	6.0	
Fresh tortilla	61.5	2.0	
White tortilla	51.54	0.75	Hernández-Uribe et al. (2010)
Blue tortilla	52.72	0.86	
Dried nixtamalised maize masa	80.5	11.4	Aguirre-Cruz et al. (2005)
Dried masa with CMC 0.2 %	81.2	10.3	
Dried masa with CMC 0.5 %	81.5	9.8	
Dried masa with xanthan 0.2 %	81.4	8.2	
Dried masa with xanthan 0.5 %	81.6	5.4	
Stored tortilla (72 h) 4 °C	66.0	3.2	Campas-Baypoli et al. (1999)
Stored tortilla (72 h) at room temperature	63.5	3.3	
Stored tortilla (48 h) at 4 °C	86.9	7.6	Rendón-Villalobos et al. (2002)
Stored tortilla (72 h) at 4 °C	87.2	7.8	
Carboxymethyl cellulose			

disorganises the starch components, but some interactions (starch-lipids and/or starch-protein) are maintained in the product due to the low enthalpy values. The storage of corn tortilla at a low temperature produced an increase in the average gelatinisation temperature and enthalpy due to reorganisation (retrogradation) of starch components. This is an issue that is important from the nutritional point of view due to formation of resistant starch with the physiological benefits as is pointed out in the next sections.

11.4 Nutritional Aspects

11.4.1 Vitamins and Minerals

Vitamins and minerals are both important micronutrients for different metabolic functions. Tortillas supply B complex vitamins such as folic acid, and some varieties of maize are a source of carotenoids (Table 11.4). There is no wide variation in the vitamin content in tortillas elaborated with different varieties of white and yellow maize, both of which are traditionally used in tortilla preparation at commercial level. The presence of carotenoids in yellow tortillas and anthocyanins in pigmented tortillas (red, blue, black) has been important due to the antioxidant capacity that produces a nutraceutical effect. Vitamins are in low

Table 11.4 Vitamins in corn tortillas

Tortilla	B1	B2	B3	B6	Folic acid	Total carotenoids	Reference
Tortilla of white corn	0.1–0.19	0.04–0.06	0.96–1.01	Nd	Nd	Nd	FAO (1993)
Tortilla of yellow corn	0.11	0.05	1.01	Nd	Nd	0.41	Cravioto et al. (1945)
Tortilla	0.2	0.06	1.0	0.04	0.06	Nd	Figueroa-Cárdenas (2004) on line
Tortilla with traditional process	0.28	0.12	1.52	Nd	0.044	Nd	Figueroa-Cárdenas et al. (2001)
Tortilla supplemented with vitamins	0.6	0.71	2.8	Nd	0.045	Nd	
Tortilla added with soy flour	0.56	0.61	2.02	Nd	0.062	Nd	
Tortilla added with vitamins and soy flour	0.72	0.78	2.92	Nd	0.07	Nd	

Nd not determined

amount in tortillas, for this reason there is interest to increase this micronutrient with addition of vitamin supplements and soy flour. The addition of both sources increases up to 50 % B complex vitamins, but no change in the folic acid level was determined. The presence of carotenoids (precursor of vitamin A) in tortilla in snacks elaborated from tortilla of two different maize varieties was evaluated (Coutiño-Estrada et al. 2008). Both snacks had lutein content between 6.7 and 7.7 μg g^{-1} of product, but one variety showed contents of β-criptoxantina and β-carotene around 300 % lower.

Diverse minerals such as Ca, Fe, Zn, K and Na have been determined in flours and tortillas (Table 11.5). The role of tortilla in the supply of calcium is well recognised; however, wide variation is reported in the concentration of this mineral in tortilla and flours which depends on the salt type and concentration used in the nixtamalisation process that is retained in the final product. The addition of other ingredients in the tortilla such as soy flour and vitamins supplement did not change the calcium amount. Potassium is found at higher level than calcium; however, the role of potassium in the metabolic function is minor than calcium. Fe and Zn are found at low levels in tortilla, but the supply of these micronutrients can be complementary to those present in other foods of the diet. The role of Fe and Zn in the human metabolism is minor and only low amounts are necessary.

11.4.2 Proteins

Tortilla is considered as a source of protein for people with low economical resource in the suburban and rural regions of Mesoamerican countries. It is well known that maize and consequently tortilla are deficient in two essential amino acids (lysine and tryptophan), but during the nixtamalisation process, the availability of protein is more for the human organism and this helps to equilibrate this deficiency; however, these two amino acids can be supplied by other grains such as beans. Tortillas made with fresh masa (obtained by extrusion with 25 % calcium hydroxide) showed higher amount of protein (8.5 %) and dietary fibre (14.5 %) than tortillas elaborated with fresh masa obtained with the traditional nixtamalisation process (8.2 % protein and 7.4 % dietary fibre) (Martínez-Flores et al. 2002). Due to the importance of the maize in the human nutrition and the deficiency in lysine and tryptophan, there was the development using traditional genetic techniques named QPM. QPM can be an alternative for improving the nutritional quality of tortilla, which was developed from opaque2 maize. QPM shows higher lysine (34–60 g kg^{-1} g of protein) and tryptophan (8–12 g kg^{-1} of protein) content than regular maize (Serna-Saldívar and Rooney 1994). In order to evaluate the protein efficiency ratio (PER), tortillas with QPM and other varieties were elaborated. QPM tortilla presented the highest PER value for tortillas, around up to 80 % of those obtained in tortillas made with other maize varieties (Table 11.6).

Table 11.5 Minerals in corn nixtamalised flour and tortillas

	Ca (mg g-1)	K (mg g-1)	Na (mg g-1)	Fe (mg g-1)	Zn (mg g-1)	Reference
Industrial nixtamalised corn flour	97	250	Nd	2.53	1.9	Bressani et al. (2001)
Corn nixtamalised flour	122.5	254	Nd	Nd	Nd	Cornejo-Villegas et al. (2010)
Corn nixtamalised flour added with 2 % of prickly pear	205.5	250	Nd	Nd	Nd	
Corn nixtamalised flour added with 10 % of prickly pear	489	269	Nd	Nd	Nd	
Tortilla	202.65	317.25	19.25	Nd	Nd	Bressani et al. (1990)
Tortilla of quality protein maize	210.0	291.7	24.2	Nd	Nd	
Tortilla of nixtamal	114.0	Nd	Nd	1.2	1.8	Figueroa-Cárdenas et al. (2001)
Tortilla added with vitamins	131.3	Nd	Nd	4.5	2.0	
Tortilla added with soybean flour	212.5	Nd	Nd	3.3	2.3	
Tortilla added with vitamins and soybean flour	199.9	Nd	Nd	5.2	Nd	
Integral tortilla added with soybean flour	153.7	Nd	Nd	5.0	1.7	
Corn tortilla	87–214	Nd	Nd	1.2–4.5	1.1–1.4	Ranhotra (1985); Saldana and Brown (1984); Cravioto et al. (1945)

Nd not determined

Table 11.6 Protein quality of tortillas of different maize cultivars

Cultivar	Average weight gain (g)	Average food intake (g)	Protein efficiency ratio (average)
Quality protein maize (Nutricta)	66	355	2.12
White tropical	28	238	1.4
Xetzac	25	250	1.12
Azotea	25	247	1.41
Santa Apolonia	18	211	0.98
Casein	126	392	2.63

Source: Bressani et al. (1990)

11.5 Starch Digestibility

Nutritionally, carbohydrates can be classified as digestible and nondigestible, or glycaemic and nonglycaemic. The starch rate digestion and absorption of glucose in the small intestine determine the glycaemic index of starchy foods (O'Dea et al. 1981; Englyst et al. 1996, 1999). In this sense, starch has been classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) to differentiate its digestion properties in food products (Englyst et al. 1992). RDS is rapidly digested and absorbed in the duodenum and proximal regions of the small intestine leading to a rapid elevation of blood glucose and usually a subsequent episode of hypoglycaemia. SDS may prolong satiety and be beneficial in products that are utilised by athletes, as SDS would provide a longer, more consistent source of systemic glucose with a low glycaemic response (Wolf et al. 1999). SDS improves overall blood glucose control in patients with diabetes mellitus (Brand et al. 1991) and attenuates total serum cholesterol levels in hyperlipidaemic patients (Jenkins et al. 1987).

Recently, it was reported that RS could be used for battling obesity (Hendrich 2010). The main classification of RS was proposed by Englyst et al. (1992) based on the nature of the starch and its environment in the food; RS1 corresponds to physically inaccessible starch entrapped in a cellular matrix, as in legume seeds (Tovar et al. 1992); RS2 are native uncooked starch granules in foods such as raw potato or unripe banana. Crystallinity makes them less susceptible to hydrolysis (Englyst and Cummings 1987; Faisant et al. 1995a, b); and RS3 are retrograded starches formed in cooked foods that are kept at low or room temperature (Noah et al. 1998). RS is not digested in the upper gastrointestinal tract, but it is fermented by microorganisms in the colon that produce short-chain fatty acids (SCFAs), and these SCFAs provide additional energy to the body along with a high proportion of butyrate that is beneficial to colonic health (Topping and Clifton 2001).

The rate and extent of starch digestion and, therefore, the RS content of foods will affect a number of physiological functions and have different effects on health including reduction of the glycaemic and insulinemic, hypocholesterolaemic effects and protective effects against colorectal cancer (Asp et al. 1996; Carmona-García et al. 2007; De Deckere et al. 1995; Jenkins et al. 1987). Among the factors affecting

the rate and extent of starch digestion, the most important are botanic origin of the starchy foods, food processing type and storage conditions after processing (temperature and time). RS2 starch in raw foods is barely digestible. However, during processing (usually cooking), starch is gelatinised and made available, although a fraction of this available starch is retrograded on cooling and becomes resistant to enzymatic digestion (RS3) (Asp et al. 1996; Björck et al. 1994; Bravo et al. 1998; Snow and O'Dea 1981).

Starch digestibility of corn tortilla immediately elaborated and analysed (fresh tortilla) has been reported (Table 11.7). Variation in the available starch (AS) content was observed in corn tortillas, which ranged between 65.2 and 76.5 %. The AS in fresh tortilla can be considered high, but other components in tortilla (such as dietary fibre) can modulate the glycaemic response of this staple. With the idea to reduce the AS content of corn tortilla, it was added with flaxseed, amaranth and bean flours. Flaxseed and bean decreased the AS content approximately to 25 %, but amaranth did not. Low amount of RS content was determined in fresh tortilla (between 0.98 and 3.10 %), and this value increased with the addition of flaxseed

Table 11.7 Available starch (AS), resistant starch (RS), retrograded resistant starch (RRS) and predicted glycaemic index (pGI) of corn commercial flour and tortillas (%)

Sample	AS[a]	RS[b]	RRS[c]	pGI[d]	Reference
Commercial flour A	76.5	1.38	Nd	Nd	Agama-Acevedo et al. (2004)
Commercial flour B	72.2	0.98	Nd	Nd	
Commercial flour C	75.0	1.29	Nd	Nd	
Commercial flour D	75.0	1.80	Nd	Nd	
Laboratory-made tortilla	73.0	3.10	1.10	108.5	Bello-Pérez et al. (2006)
Tortilla of commercial masa	74.0	1.40	1.10	102.6	
Tortilla of commercial flour	70.9	2.30	1.90	102.0	
Commercial tortilla	65.3	2.20	1.10	Nd	
Laboratory-made corn tortilla	68.5	1.92	Nd	101.41	Rendón-Villalobos et al. (2009a, 2009b)
Tortilla + 10 % flaxseed	61.6	2.77	Nd	88.24	
Tortilla + 15 % flaxseed	58.1	3.25	Nd	80.93	
Tortilla + 20 % flaxseed	55.9	5.08	Nd	73.64	
Corn/amaranth tortilla (80/20)	73.3	1.40	0.58	Nd	Islas-Hernández et al. (2007)
Commercial corn tortilla	65.2	2.14	1.05	75[e]	Sáyago-Ayerdi et al. (2005)
Commercial corn tortilla-bean mixture 60/40 (Taco)	52.6	3.93	3.14	51	
Tortilla with nixtamalised transgenic maize flour	Nd	2.4	1.63	Nd	Ayala-Rodríguez et al. (2009)

A Comercial Mexicana, B MASECA, C MINSA, D CICATA-IPN

Nd not determined

[a]Using method of Holm et al. (1986)

[b]Using method of Goñi et al. (1996)

[c]Using method of Saura-Calixto et al. (1993)

[d]The glycaemic index was calculated from the equation proposed by Goñi et al. (1997)

[e]The glycaemic index was calculated from the method proposed by Granfeldt et al. (1992)

and bean. In some corn tortillas, retrograded resistant starch (RRS) was determined, and this can be due to starch reorganisation during cooling of tortilla. This pattern shows that starch reorganisation occurs in some minutes while the food is cooked and cooled, but this characteristic depends on the food.

Due to the metabolic importance of the carbohydrate consumption, the prediction of glycaemic index (pGI) in fresh corn tortilla was tested using two procedures. In general, fresh corn tortilla had high pGI when the Goñi's equation was used, and a low pGI value was calculated with the Granfeldt's procedure based on chewing. The addition of flaxseed and bean decreased the pGI value, indicating that other components (dietary fibre and proteins) have an important role in the absorption of glucose during digestion of the food.

The effect of storage time on starch digestibility was evaluated in raw materials (nixtamal and masa) and corn tortilla (Table 11.8). The AS content decreased when storage time was increased, a pattern observed due to the formation of RS content (RRS). This effect was more notorious in tortilla. The addition of commercial hydrocolloids did not change the RRS content, preventing the starch retrogradation and the staling of corn tortilla.

11.6 Nutraceutical Aspects

Some years ago, in Mexico, the people with diabetes and obesity problems avoided the consumption of corn tortilla because it was believed that high amount of glucose is being supplied to blood. Thereafter, it was found that the dietary fibre content of tortilla decreased the supply of glucose or this is slowly liberated after the consumption of tortilla. Thereafter, the studies of starch digestibility of fresh and stored tortillas showed the presence of RS. In this sense, the nutraceutical characteristics of starchy foods with low or slow starch digestion are related with a reduction in the availability of starch to digestive enzymes (amylolytic enzymes) that decreased postprandial blood glucose and insulin response. Foods containing RS present lower caloric contributions than those with digestible glucose-based carbohydrates. This low caloric contribution (low glucose supply) produces low insulin response with increased insulin sensitivity. Some factors are related with this low glucose supply such as decrease in the fat accumulation in the body with low adipocyte volume. The consumption of foods containing RS is associated with increase in the lipid oxidation at the expense of carbohydrate oxidation and decreased lipid production. These physiological properties are considered important for weight control and to reduce obesity (Birkett and Brown 2008).

Diverse factors may be related with the decrease of the metabolic response of starchy foods such as a reduced rate of gastric emptying, a reduced motility of the luminal content and a reduced rate of diffusion of starch hydrolysis products to the small intestinal mucosa (Björck et al. 1994).

RS and dietary fibre present in corn tortilla are substrate for beneficial microorganisms present in the large intestine named probiotics. In this sense, both indigestible car-

Table 11.8 Available starch (AS) and resistant starch (RS) content in cold-stored samples at 4 °C (g/100 g dry matter)

Sample/storage time	AS[a]	RS[b]	RRS[c]	Ref.
Maize	73.85	1.99	Nd	Rendón-Villalobos et al. (2002)
Nixtamal				
0	75.89	2.18	0.85	
24 h	71.31	2.47	0.82	
48 h	70.42	2.58	0.81	
72 h	70.2	2.6	0.72	
Masa				
0	79.64	2.05	0.65	
24 h	78.93	2.14	0.72	
48 h	76.99	2.25	0.85	
72 h	75.57	2.27	0.86	
Tortilla				
0	72.92	3.12	1.06	
24 h	72.83	3.27	1.79	
48 h	73.13	3.45	1.82	
72 h	70.97	3.87	1.84	
Tortilla of commercial flour[d]				Rendón-Villalobos et al. (2006)
0	70.03	2.74	1.66	
7 days	68.38	5.04	2.83	
14 days	66.91	5.23	3.05	
Tortilla[a] with WG[e]				
0	67.42	2.49	1.71	
7 days	64.17	3.55	1.23	
14 days	62.55	3.08	1.54	
Tortilla[a] with TC-1[e]				
0	68.00	3.01	1.69	
7 days	66.44	3.18	1.71	
14 days	64.77	3.38	1.76	
Tortilla[a] with TC-20[e]				
0	65.69	3.01	1.46	
7 d	64.22	3.33	1.37	
14 d	63.20	3.27	1.48	

Nd not determined
[a]Using method of Holm et al. (1986)
[b]Using method of Goñi et al. (1996)
[c]Using method of Saura-Calixto et al. (1993)
[d]MASECA flour
[e]Commercial hydrocolloids

bohydrates are called prebiotics because they maintain the viability and the number of these bacteria in the large intestine avoiding the proliferation of pathogen microorganisms that produce infections. This pattern is produced when starchy food with RS and probiotics are consumed together, due to which a symbiotic effect is initiated. When RS and the probiotics are present in the large intestine, then the RS may initiate its role as substrate for a portion of the probiotic organism (Topping et al. 2003).

Beneficial intestinal flora produces the fermentation of indigestible carbohydrates present in corn tortilla to produce short-chain fatty acids (SCFA), and the main SCFA produced from the fermentation of RS is butyrate (Sharp and Macfarlane 2000). Butyrate is known as the principal nutrient for the colonocytes and produces a healthy state of these cells, because its lack would increase the risk of some colonic diseases such as colon cancer. Additionally, when the beneficial microflora is actively growing, the metabolic products associated with this activity have a potential beneficial influence on immune function (Brouns et al. 2002). A balance of the beneficial microflora is necessary to maintain a good function of the large intestine, in this sense, the effect of RS ingest on stool weight might be related with the increase of microflora masa during fermentation. The production of SCFA modifies the pH of the large intestine, and it was suggested that some types of RS (RS2) would improve calcium and magnesium absorption by enhancing mineral solubility (Schulz et al. 1993 and Younes et al. 1996).

Other nutraceutical characteristic of tortilla is the calcium that is partially retained in the cooked grain or nixtamal. Tortilla supplies approximately 60 % of the calcium requirements of Mexican's diet. Calcium is necessary to avoid osteoporosis.

11.7 Final Remarks

The ancient nixtamalisation process of maize improves the protein digestibility due to the fact that this food crop is deficient in lysine and tryptophan. Additionally, the products elaborated with nixtamalised maize supply important calcium content. The consumption of tortilla was traditionally related with high supply of glucose to blood and, hence, also related to the nutritional and metabolic problems associated with the ingestion of glycaemic carbohydrates. Now, the dietary fibre and resistant starch contents of tortilla and the beneficial characteristics of both these compounds are well known. The low prevalence of colon cancer in the Mexican population, associated with tortilla consumption, supports the beneficial properties of tortilla. Although this staple is the most popular in the Mexican diet, many people, due to unknown reasons, have eliminated the tortilla from their daily diet. However, with the increase in the numbers of overweight and obese people in Mexico that resulted in increased interest among the people and government to reduce the incidence of both these public health problems, tortilla consumption is regaining its lost power.

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Chapter 12
Boza, a Traditional Cereal-Based Fermented Beverage: A Rich Source of Probiotics and Bacteriocin-Producing Lactic Acid Bacteria

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12.1 Introduction

Fermentation of food is a very old technology, with earliest records dating back to 6000 B.C. (Fox et al. 1993). The methodologies and knowledge associated with the manufacturing of fermented products were handed down from generation to generation within local communities (Caplice and Fitzgerald 1999). These communities needed products to be produced in small quantities for distribution in or around the immediate area. However, the population increase in towns and cities, due to the industrial revolution by the middle of the nineteenth century, resulted in a need for these products to be produced in larger quantities. This led to commercial

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production of fermented food. Furthermore, the blossoming of microbiology as a science from the 1850s onwards and the development of pasteurization by Louis Pasteur towards the end of the nineteenth century had a major impact on our understanding of the biological basis of fermentation. According to Caplice and Fitzgerald (1999), milk, meat, cucumber, and cabbage are the main substrates used in the production of most familiar fermented products.

Large-scale production required products with consistent quality. Characterization of microorganisms responsible for the fermentation of various fermented products led to the isolation of starter cultures, which could be produced on a large scale to supply factories involved in the manufacturing of these products. Defined starter cultures replaced undefined starters traditionally used in manufacturing and ensured reliable fermentation and consistent product quality (Caplice and Fitzgerald 1999). The intensive use of starter cultures has some drawbacks and can lead to unsatisfactory strain performance (Ross et al. 2000). In the case of lactococcal fermentation, bacteriophage proliferation can affect the performance of cheese starter cultures (Klaenhammer and Fitzgerald 1994).

The digestibility, nutritional value, organoleptic qualities, and shelf life of food are increased by fermentation (Hancioglu and Karapinar 1997). A number of lactic acid bacteria (LAB) used as starter cultures in fermented food have probiotic properties and may confer potential health benefits to the consumer.

LAB are Gram-positive, rods, cocci, or coccobacilli, i.e., oval-shaped, non-spore-forming bacteria (Leroy and De Vuyst 2004). They all produce lactic acid under microaerophilic conditions and are catalase and oxidase negative. Some strains exhibit pseudocatalase activity when grown on media rich in heme, such as blood agar (Singh et al. 2007). Carbohydrates are fermented via the Embden-Meyerhof-Parnas pathway to lactic acid and the pentose phosphate pathway to lactic acid, CO_2, and ethanol, depending on the presence of aldolase or phosphoketolase.

LAB produce various antimicrobial compounds, including organic acids, hydrogen peroxide, carbon dioxide, diacetyl, bacteriocins, and low molecular weight antimicrobial substances (Todorov 2009) that may have a positive influence on the microbial quality and extended shelf life of the fermented food products.

12.1.1 LAB in Cereal-Based Fermented Products

Cereal and cereal-legume-based fermented products are consumed in almost all parts of the world (Table 12.1) and form a major part of the diet in most countries. Cereals are cultivated on more than 73 % of agricultural soil and contribute to over 60 % of the world's food production, providing vitamins, proteins, dietary fiber, energy, and minerals (Charalampopoulos et al. 2002). It is therefore important to study the nutritional value and basic composition of these products. Many cereal-based products are boiled or steamed, e.g., porridges, rice, pasta, and cookies. In many cases the same product is fermented, e.g., pancakes and flatbreads in Asia,

Table 12.1 Cereal and cereal-legume-based fermented food and beverages from different regions of the world

Product	Country	Substrate	Microorganism	Form in which consumed
Adai	India	Cereal/legume	*Pediococcus* sp., *Streptococcus* sp., *Leuconostoc* sp.	Breakfast or snack food
Anarshe	India	Rice	Lactic acid bacteria	Breakfast, sweetened snack food
Ang-kak (anka, red rice)	China, Southeast Asia, Syria	Rice	*Monascus prupureus*	Dry red powder as colorant
Atole	Southern Mexico	Maize	Lactic acid bacteria	Porridge based on maize dough
Bagni	Caucasus	Millet	Unknown	Liquid drink
Banku	Ghana	Maize or maize and cassava	Lactic acid bacteria, molds	Dough as staple
Bhattejaanr	India, Sikkim	Rice	*H. anomala*, *Mucor rouxianus*	Sweet sour alcoholic paste
Bogobe	Botswana	Sorghum	Unknown	Thick, acidic
Bouza	Egypt	Wheat, malt	Lactic acid bacteria	Alcoholic thin gruel
Boza	Albania, Turkey, Bulgaria, Romania	Wheat, millet, maize, and other cereals	*L. acidophilus*, *L. coprophilus*, *L. brevis*, *L. plantarum*, *L. fermentum*, *Le. mesenteroides*, *Le. mesenteroides* subsp. *dextranicum*, *Le. raffinolactis*, *L. rhamnosus*, *L. coryniformis*, *L. paracasei*, *L. pentosus*, *L. sanfrancisco*, *Lc. lactis* subsp. *lactis*, *P. pentosaceus*, *Le. oenos* (reclassified to *Oenococcus oeni*), *Weissella confusa* and *Weissella paramesenteroides*, *S. cerevisiae*, *C. glabrata*, *C. tropicalis*, *G. penicilatum*, *S. carlsbergensis*, *S. uvarum*, *C. diversa*, *C. pararugosa*, *Issatchenkia orientalis*, *Pichia fermentans*, *Rhodotorula mucilaginosa*, *C. inconspicua*, *Torulaspora delbrueckii*, *Pichia guilliermondii*, *and Pichia norvegensis*	Thick, sweet, slightly sour beverage
Braga	Romania	Millet	Unknown	Liquid drink
Brem	Indonesia	Rice	Unknown	Cake

(continued)

Table 12.1 (continued)

Product	Country	Substrate	Microorganism	Form in which consumed
Brembali	Indonesia	Rice	*Mucor indicus*, *Candida* sp.	Dark brown alcoholic drink
Burukutu	Nigeria, Benin, Ghana	Sorghum	*S. cerevisiae*, *Le. mesenteroides*, *Candida* sp.	Alcoholic beverage of vinegar-like flavor
Busa	Syria, Egypt, Turkestan	Rice or millet	*Lactobacillus* sp., *Saccharomyces* sp.	Liquid drink
Busaa	Nigeria, Ghana	Maize	*L. helveticus*, *L. salivarius*, *L. casei*, *L. brevis*, *L. plantarum*, *L. buchneri*, *S. cerevisiae*, *Penicillium damnosus*	Alcoholic beverage
Bussa	Kenya	Maize, sorghum, malt, finger millet	*C. krusei*, *S. cerevisiae*, *L. helveticus*, *L. salivarius*, *L. plantarum*	Food refreshment drink
Chee-fan	China	Soybean wheat curd	*Mucor* sp., *Aspergillus glaucus*	Cheese-like product, eaten fresh
Chicha	Peru	Maize	*Aspergillus*, *Penicillium*, yeast, bacteria	Spongy solid eaten with vegetables
Chikokivana	Zimbabwe	Maize and millet	*S. cerevisiae*	Alcoholic beverage
Chinese yeast	China	Soybeans	*Mucoraceous* molds, yeast	Solid eaten fresh with rice
Chongju	Korea	Rice	*S. cerevisiae*	Alcoholic clear drink
Dalaki	Nigeria	Millet	Unknown	Thick porridge
Darassum	Mongolia	Millet	Unknown	Liquid drink
Dhokla	Northern India	Rice or wheat and Bengal gram	*Le. mesenteroides*, *St. faecalis*, *Torulopsis candida*, *T. pullulans*	Steamed cake for breakfast or snack food
Doro	Zimbabwe	Finger millet malt	Yeast and bacteria	Colloidal thick alcoholic drink
Dosa	India	Rice and Bengal gram	*Le. mesenteroides*, *Streptococcus faecalis*, *Torulopsis candida*, *T. pullulans*	Griddled cake for breakfast or snack food
Enjera	Ethiopia	Tef or other cereals	*Le. mesenteroides*, *P. cerevisiae*, *L. plantarum*, *S. cerevisiae*	Pancake

Gari	Nigeria	Cassava	*Leuconostoc*, *Alcaligenes*, *Corynebacterium*, *Lactobacillus* sp.	Staple, cake, porridge
Hamanatto	Japan	Wheat, soybeans	*A. oryzae*, *Streptococcus* sp., *Pediococcus* sp.	Raisin-like, soft, flavoring agent for meat and fish, eaten as snack
Hopper	Sri Lanka	Rice and coconut water	Yeast, lactic acid bacteria	Stake-baked pancake
Hulumur	Sudan	Red sorghum	*Lactobacillus* sp.	Clear drink
Idli	South India, Sri Lanka	Rice grits and black gram	*Le. mesenteroides*, *St. faecalis*, *Torulopsis* sp., *Candida* sp., *Trichosporon pullulans*	Steamed cake for breakfast food
Ilambazi lokubilisa	Zimbabwe	Maize	Lactic acid bacteria, yeast, and molds	Porridge as weaning food
Injera	Ethiopia	Sorghum, tef, maize, or wheat	*C. guilliermondii*	Bread-like staple
Jaanr	India, Himalayas	Millet	*H. anomala*, *Mucor rouxianus*	Alcoholic paste mixed with water
Jalebies	India, Nepal, Pakistan	Wheat flour	*S. bayanus*	Pretzel-like syrup-filled confection
Jamin-bang	Brazil	Maize	Yeast, bacteria	Bread, cake-like
Kaanga-Kopuwai	New Zealand	Maize	Yeast	Soft, slimy eaten as vegetable
Kachasu	Zimbabwe	Maize	Yeast	Alcoholic beverage
Kaffir	South Africa	Malt of sorghum, maize	Lactic acid bacteria	Beer
Kaffir beer	South Africa	Kaffir corn	Yeast, lactic acid bacteria	Alcoholic drink
Kanji	India	Rice and carrots	*H. anomala*	Liquid added to vegetables
Kecap	Indonesia	Wheat, soybeans	*A. oryzae*, *Lactobacillus* sp., *Hansenula*, *Saccharomyces*	Liquid flavoring agent

(continued)

Table 12.1 (continued)

Product	Country	Substrate	Microorganism	Form in which consumed
Kenkey	Ghana	Maize	*L. fermentum*, *L. reuteri*, *Candida* sp., *Saccharomyces* sp., *Penicillium* sp., *Aspergillus* sp., and *Fusarium* sp.	Mush, steamed eaten vegetables
Khanomjeen	Thailand	Rice	*Lactobacillus* sp., *Streptococcus* sp.	Noodle
Khaomak	Thailand	Rice	*Rhizopus* sp., *Mucor* sp., *Saccharomyces* sp., *Hansenula* sp.	Alcoholic sweet beverage
Kichudok	Korea	Rice	*Le. mesenteroides*, *S. faecalis*, yeast	Steamed cake
Kichudok	Korea	Rice, takju	*Saccharomyces* sp.	Steamed cake
Kishk	Egypt, Syria, Arabian countries	Wheat and milk	*L. plantarum*, *L. brevis*, *L. casei*, *B. subtilis*, and yeasts	Solid, dried balls, dispersed rapidly in water
Kisra	Sudan	Sorghum, millet	*Lactobacillus* sp., *Acetobacter* sp. *S. Cerevisiae*	Pancake
Koko	Ghana	Maize	*Enterobacter cloacae*, *Acinetobacter* sp., *L. plantarum*, *L. brevis*, *S. cerevisiae*	Porridge as staple
Kurdi	India	Wheat	Unknown	Solid, fried crisp, salty
Kwunu-Zaki	Nigeria	Millet	Lactic acid bacteria, yeast	Paste used as breakfast dish
Lao-chao	China, Indonesia	Rice	*Rhizopus oryzae*, *R. chinensis*, *Chlamydomucor oryzae*, *Saccharomycopsis* sp.	Paste, soft juicy, glutinous consumed as dessert or combined with eggs or seafood
Mahewu	South Africa	Maize and wheat flour	*Lactobacillus* sp.	Sour drink
Mangisi	Zimbabwe	Millet	Unknown	Sweet/sour nonalcoholic drink
Mantou	China	Wheat flour	*Saccharomyces* sp.	Steamed cake
Mawe	South Africa	Maize	Lactic acid bacteria, yeast	Basis for preparation of many dishes
Mbege	Tanzania	Malted millet acidic banana juice	Unknown	Food, refreshment drink

Me	Vietnam	Rice	Lactic acid bacteria	Sour food ingredient
Merissa	Sudan	Sorghum and Millet	*Saccharomyces* sp.	Alcoholic drink
Minchin	China	Wheat gluten	*Paecilomyces* sp., *Aspergillus* sp., *Cladosporium* sp., *Fusarium* sp., *Syncephalastum* sp., *Penicillium* sp., and *Trichothecium* sp.	Solid as condiment
Mirin	Japan	Rice, alcohol	*A. oryzae*, *A. usamii*	Alcoholic liquid seasoning
Miso	Japan, China	Rice and soy beans or rice other cereals such as barley	*A. oryzae*, *Torulopsis etchellisii*, *Lactobacillus* sp.	Paste use as seasoning
Mung bean starch	China, Thailand, Korea, Japan	Mung bean	*Le. mesenteroides*, *L. casei*, *L. cellobiosus*, *L. fermentum*	Noodle
Munkoyo	Africa	Kaffir corn, millet, or maize plus roots of munkoyo	Unknown	Liquid drink
Mutwiwa	Zimbabwe	Maize	Lactic acid bacteria, bacteria, and molds	Porridge
Nan	India, Pakistan, Afghanistan, Iran	Unbleached wheat flour	*S. cerevisiae*, Lactic acid bacteria	Solid as snack
Nasha	Sudan	Sorghum	*Streptococcus* sp., *Lactobacillus* sp., *Candida, S. cerevisiae*	Porridge as a snack
Ogi	Nigeria	Maize, sorghum, or millet	*L. plantarum*, *Corynebacterium* sp., *Acetobacter*, yeast	Sour porridge, baby food, main meal
Ogi	Nigeria, West Africa	Maize, sorghum or millet	*L. plantarum*, *S. cerevisiae*, *C. mycoderma*, *Corynebacterium* sp., *Aerobacter* sp., *Rhodotorula* sp., *Cephalosporium* sp., *Fusarium* sp., *Aspergillus* sp., and *Penicillium* sp.	For breakfast or weaning food for babies
Otika	Nigeria	Sorghum	Unknown	Alcoholic beverage
Papadam	India	Black gram	*Saccharomyces* sp.	Breakfast or snack food
Pito	Nigeria, Ghana	Maize, sorghum, maize, and sorghum	*G. candidum*, *Lactobacillus* sp., *Candida* sp.	Alcoholic dark brown drink

(continued)

Table 12.1 (continued)

Product	Country	Substrate	Microorganism	Form in which consumed
Pozol	Southeasters Mexico	Maize	Lactic acid bacteria, *Candida* sp.	Spongy dough formed into balls, basic food
Puto	Philippines	Rice, sugar	*Le. mesenteroides*, *Streptomyces faecalis*, yeasts	Solid paste as seasoning agent, snack
Rabdi	India	Maize and buttermilk	*P. acidilactici*, *Bacillus* sp., *Micrococcus* sp.	Semisolid mash eaten with vegetables
Rye bread	Denmark	Rye	Lactic acid bacteria	Sandwich bread, bread
Sake	Japan	Rice	*Saccharomyces* sp.	Alcoholic clear drink
Seketeh	Nigeria	Maize	*S. cerevisiae*, *St. chevalieri*, *St. elegans*, *L. plantarum*, *Lc. lactis*, *B. subtilis*, *A. niger*, *A. flavus*, *Mucor rouxii*	Alcoholic beverage
Shaosinghjiu	China	Rice	*S. cerevisiae*	Alcoholic clear beverage
Shoyu (soy sauce)	Japan, China, Taiwan	Wheat and soybeans	*A. oryzae*, *Lactobacillus* sp., *Zygosaccharomyces rouxi*	Liquid seasoning
Sierra rice	Ecuador	Rough rice	*A. flavus*, *A. candida*, *B. subtilis*	Brownish-yellow dry rice
Sorghum beer	South Africa	Sorghum, maize	Lactic acid bacteria, *L. plantarum*, yeast	Liquid drink, acidic, weakly alcoholic
Sour bread	Germany	Wheat	Lactic acid bacteria, yeast	Sandwich bread
Soybean milk	China, Japan	Soybeans	Lactic acid bacteria	Drink
Takju	Korea	Rice, wheat	Lactic acid bacteria, *S. cerevisiae*	Alcoholic turbid drink
Talla	Ethiopia	Sorghum	Unknown	Alcoholic drink
Tao-si	Philippines	Wheat and soybeans	*A. oryzae*	Seasoning
Taotjo	East India	Roasted wheat meal or glutinous rice and soybeans	*A. oryzae*	Condiment
Tapai pulut	Malaysia	Rice	*Chlamydomucor* sp., *Endomycopsis* sp., *Hansenula* sp.	Alcoholic dense drink
Tape ketan	Indonesia	Rice or cassava	*S. cerevisiae*, *Hansenula anomala*, *Rhizopus oryzae*, *Chlamydomucor oryzae*, *Mucor* sp., *Endomycopsis fibuligera*	Soft, alcoholic solid staple

Tapekekan	Indonesia	Glutinous rice	*Aspergillus rouxii*, *E. burtonii*, *E. fibuligera*	Sweet/sour alcoholic paste
Tapuy	Philippines	Rice, glutinous rice	*Saccharomyces* sp., *Mucor* sp., *Rhizopus* sp., *Aspergillus* sp., *Leuconostoc* sp., *L. plantarum*	Sweet/sour alcohol
Tapuy	Philippines	Rice	*Saccharomyces* sp., *Mucor* sp., *Rhizopus* sp., *Aspergillus* sp., *Leuconostoc* sp., *L. plantarum*	Sweet/sour alcoholic drink
Tarhana	Turkey	Parboiled wheat meal and yogurt (2:1)	Lactic acid bacteria	Solid powder, dried seasoning for soups
Tauco	West Java (Indonesia)	Cereals and soybeans	*R. oligosporus*, *A. oryzae*	Seasoning
Tesgüino	Northern and North Western Mexico	Maize	Bacteria, yeast and molds	Alcoholic beverage
Thumba	Eastern India	Millet	*E. fibuligera*	Liquid drink
Tobwa	Zimbabwe	Maize	Lactic acid bacteria	Nonalcoholic drink
Torani	India	Rice	*H. anomala*, *C. quilliermondii*, *C. tropicalis*, *G. candidum*	Liquid as seasoning for vegetables
Uji	Kenya, Uganda, Tanzania	Maize, sorghum, millet, or cassava flour	*Le. mesenteroides*, *L. plantarum*	Sour porridge, main meal
Vada	India	Cereal/legume	*Pediococcus* sp., *Streptococcus* sp., *Leuconostoc* sp.	Breakfast or snack food

A. Aspergillus, *B. Bacillus*, *C. Candida*, *E. Endomycopsis*, *G. Geotrichum*, *H. Hansenula*, *L. Lactobacillus*, *Lc. Lactococcus*, *Le. Leuconostoc*, *P. Pediococcus*, *R. Rhizopus*, *S. Saccharomyces*, and *St. Streptococcus*

sourdough bread in Europe, and a variety of fermented dumplings, porridges, and alcoholic and nonalcoholic beers in Asia and Africa (Salovaara 2004).

By definition, fermentation is the process in which a substrate is subjected to biochemical modification resulting from the activity of microorganisms and their enzymes (Gotcheva et al. 2000). Yeast, LAB, fungi, or mixtures of these are mainly responsible for natural cereal-based fermentation. Carbohydrate metabolism is mainly performed by yeast and LAB, while bacteria show proteolytic activity (Chavan and Kadam 1989). Fermentations by yeast and lactobacilli change the biochemical composition of fats, minerals, and vitamins contained within the cereal.

Yeasts are predominantly responsible for the production of ethanol (e.g., beers and wines), while lactic acid bacteria produce mainly lactic acid (e.g., cereals and fermented milk products). Acetic acid fermentation, responsible for the conversion of alcohol to acetic acid in the presence of excess oxygen, is mainly conducted by *Acetobacter* spp. (Blandino et al. 2003). Alkali fermentation is commonly associated with the fermentation of fish and seeds, widely used as condiment (McKay and Baldwin 1990).

12.1.2 Boza, a Cereal-Based Fermented Beverage

Boza is a traditional nonalcoholic cereal-based fermented beverage from Bulgaria (Todorov and Dicks 2004). The beverage is also consumed in other countries of the Balkan region such as the Former Yugoslavian Republic of Macedonia, Serbia, Turkey, Albania, and Romania (Gotcheva et al. 2000). Its origin is believed to be from the ancient populations that lived in pre-Ottoman Turkey. The Ottomans were responsible for spreading the recipe over the countries they conquered. Furthermore, the Ottoman Empire was known to feed their army with boza due to its richness in carbohydrates and vitamins A, B, C, and E (http://www.veja.com.tr/english/index1.html). In Turkey, boza is served with cinnamon and roasted chickpeas and is enjoyed mainly during the winter months, whereas Bulgarians consume this beverage all year round, mainly at breakfast. Boza is light to dark beige and viscous and has a sweet to sour bread-like taste (Gotcheva et al. 2000; Gotcheva et al. 2001). Different cereals such as millet, wheat, rye, or combinations of these are used to produce boza. These grains are composed of an embryo (germ), an endosperm enclosed by the epidermis, and a seed coat (husk) (Gotcheva et al. 2001). The endosperm is filled with granulated starch (Hoseney 1992). Enzymes and most of the nutrients, such as amino acids, lipids, minerals, sugars, and vitamins, are located in the embryo. Cellulose, minerals, pentosans, and pectins are found in the husk (Nikolov 1993). Cereal grains generally contain a range of indigenous microflora, including enterobacteria, aerobic spore formers, and molds (Salovaara 2004).

Boza is produced according to traditional family recipes. Various raw materials, at different concentrations and different fermentation processes, are used, leading to differences in quality (Zorba et al. 2003). Further variations in the quality and stability

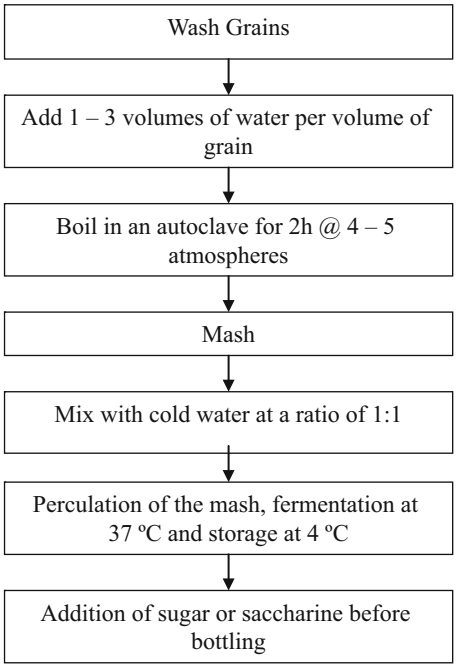

Fig. 12.1 Diagram summarizing the production process of boza

may occur because of the interactions between microorganisms that cannot be controlled during fermentation. To avoid such variations, it is necessary to use starter cultures (Zorba et al. 2003). Little is known about the physical and biochemical changes that occur during boza fermentation, and, therefore, future studies should focus on these variables. According to Genc et al. (2002), there is a growing interest in producing boza on a large scale, and the product has to be properly characterized. The industrial preparation of boza is illustrated in Fig. 12.1.

Fermentation occurs by natural combinations of yeast and LAB (Todorov and Dicks 2004). Only a few papers have been published on the microflora of boza. LAB isolated from boza have been identified as *Lactobacillus acidophilus*, *Lactobacillus coprophilus*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, *Leuconostoc mesenteroides* subsp. *dextranicum*, *Leuconostoc raffinolactis*, *Lactobacillus rhamnosus*, *Lactobacillus coryniformis*, *Lactobacillus paracasei*, *Lactobacillus pentosus*, *Lactobacillus sanfrancisco*, *Lactococcus lactis* subsp. *lactis*, *Pediococcus pentosaceus*, *Leuconostoc oenos* (reclassified to *Oenococcus oeni*), *Weissella confusa*, and *Weissella paramesenteroides* (Gotcheva et al. 2000; Arici and Daglioglu 2002; Todorov and Dicks 2005b; Todorov and Dicks 2006b; Von Mollendorff et al. 2006). The yeasts thus far isolated are *Saccharomyces cerevisiae*, *Candida glabrata*, *Candida tropicalis*, *Geotrichum candidum* (Gotcheva et al. 2000), *Geotrichum penicillatum*, *Saccharomyces carlsbergensis*, *Saccharomyces uvarum* (Gotcheva et al. 2000; Arici and Daglioglu 2002), *Candida diversa*, *Candida pararugosa*,

Issatchenkia orientalis, *Pichia fermentans*, *Rhodotorula mucilaginosa*, *Candida inconspicua*, *Torulaspora delbrueckii*, *Pichia guilliermondii*, and *Pichia norvegensis* (Botes et al. 2007). *Candida tropicalis*, *Geotrichum penicillatum*, *C. inconspicua*, *P. norvegensis*, and *R. mucilaginosa* are considered opportunistic human pathogens (Botes et al. 2007). Some of the lactic acid bacteria identified have been shown to exhibit probiotic properties and to produce bacteriocins (antimicrobial peptides) active against various Gram-positive and Gram-negative bacteria, emphasizing the importance of developing them as starter cultures. A number of bacteriocins have been described for lactic acid bacteria isolated from boza (Table 12.2).

12.2 The Beneficial Properties of Lactic Acid Bacteria

LAB are well known for their widespread occurrence in nature and application in several traditional and industrial fermentation processes, where they may have a positive or negative impact. Several LAB are evaluated as probiotics and may have a beneficial effect for the humans and animals.

Lactobacillus spp. and *Bifidobacterium* spp. are considered the genera containing the most probiotic strains (Corcoran et al. 2004). Probiotics can be defined as "live microorganisms of benefit to the host by improving its intestinal microbial balance when administered in adequate amounts" (FAO/WHO 2002). The microbial balance is subjected to various unfavorable factors, such as stress, diet, and other diseases, which may lead to a decrease in the presence of viable lactobacilli and bifidobacteria in the gastrointestinal tract (Fuller and Gibson 1997).

This decrease may result in the successive uncontrolled proliferation of pathogenic bacteria that may contribute to various clinical disorders (Fooks et al. 1999). In vitro studies and clinical trials with animals have shown that probiotic bacteria reduce symptoms related with irritable bowel syndrome (O'Mahony et al. 2005), diarrhea (Isolauri et al. 1991), lactose intolerance, colon cancer, allergies, and cholesterol (Gilliland 1990; Salminen et al. 1998; Fooks et al. 1999; Kalliomaki et al. 2001). De Vrese et al. (2005) found that it also reduces the duration of the common cold. As a consequence, probiotic LAB are being used as a preventative treatment alternative of different cases of diarrhea provoked by *E. coli*. The inhibitory activity of LAB against enterotoxigenic *E. coli* (Wellock et al. 2009), enteropathogenic *E. coli* (Parassol et al. 2005), enterohemorrhagic *E. coli* (Medellin-Peña et al. 2007), enteroinvasive *E. coli* (Resta-Lenert and Barrett 2003), and diffusely adherent *E. coli* (Liévin-Le et al. 2002) has been widely studied. *Lactobacillus* strains of intestinal microbiota origin, such as *L. acidophilus* LB (Liévin-Le et al. 2002), *Lactobacillus plantarum* 299v (Michail and Abernathy 2002), *Lactobacillus casei* DN-114 001 (Parassol et al. 2005), and *Lactobacillus helveticus* R0052 (Sherman et al. 2005), have previously been shown to inhibit infection by enterohemorrhagic *E. coli* or enteropathogenic *E. coli* within the intestinal epithelial barrier.

Table 12.2 Activity spectra of bacteriocins produced by lactic acid bacteria isolated from boza

Bacteriocins	Strain	Molecular mass (kDa)	Activity spectra	References
Pediocin ST18	*Pediococcus pentosaceus* ST18	ND	*Bacillus* spp. (*1/3*)*, *Carnobacterium piscicola* (*1/1*), *Carnobacterium divergens* (*1/1*), *Enterococcus faecalis* (*1/1*), *Lactobacillus amylophilus* (*1/1*), *Lactobacillus brevis* (*1/1*), *Lactobacillus delbrueckii* subsp. *bulgaricus* (*1/1*), *Lactobacillus fermentum* (*1/1*), *Lactobacillus helveticus* (*2/2*), *Lactobacillus plantarum* (*7/9*), *Leuconostoc mesenteroides* (*5/10*), *Listeria innocua* (*2/2*), *Listeria monocytogenes* (*1/1*), *Pediococcus damnosus* (*1/1*), *Pediococcus pentosaceus* (*2/2*), *Staphylococcus aureus* (*1/1*), *and Streptococcus thermophilus* (*1/1*)	Todorov and Dicks (2005b)
Mesentricin ST99	*Leuconostoc mesenteroides* subsp. *dextranicum* ST99	ND	*Bacillus* spp. (1/4), *E. faecalis* (1/1), *L. amylophilus* (1/1), *L. brevis* (1/1), *L. casei* subsp. *casei* (2/2), *L. helveticus* (2/2), *L. plantarum* (9/9), *Lactococcus lactis* subsp. *cremoris* (1/1), *L. innocua* (2/2), *L. monocytogenes* (1/1), *P. pentosaceus* (2/2), *S. aureus* (1/1), and *S. thermophilus* (1/1)	Todorov and Dicks (2004)
ST194BZ	*Lactobacillus plantarum* ST194BZ	3.0 and 14.0	*Enterobacter cloacae* (*1/2*), *Enterococcus faecalis* (*2/2*), *Escherichia coli* (*1/2*), *Lactobacillus casei* (*1/1*), *Lactobacillus delbrueckii* subsp. *bulgaricus* (*1/1*), *Lactobacillus sakei* (*1/1*), and *Pseudomonas* spp. (*1/4*)	Todorov and Dicks (2005a)
ST242BZ	*Lactobacillus paracasei* ST242BZ	10.0	*E. cloacae* (*1/2*), *E. faecalis* (*2/2*), *E. coli* (*1/2*), *Klebsiella pneumoniae* (*1/1*), *Lactobacillus casei* (*1/1*), *Lactobacillus delbrueckii* subsp. *bulgaricus* (*1/1*), *L. sakei* (*1/1*), *Pseudomonas* spp. (*2/4*), and *S. aureus* (*7/8*)	Todorov and Dicks (2006b)
ST284BZ	*Lactobacillus paracasei* ST284BZ	3.5	*E. cloacae* (*1/2*), *E. faecalis* (*2/2*), *E. coli* (*2/2*), *K. pneumoniae* (*1/1*), *L. casei* (*1/1*), *L. delbrueckii* subsp. *bulgaricus* (*1/1*), *L. sakei* (*1/1*), *Pseudomonas* spp. (*3/4*), and *Streptococcus* spp. (*1/7*)	Todorov and Dicks (2006b)
ST414BZ	*Lactobacillus plantarum* ST414BZ	3.7	*E. cloacae* (1/2), *E. faecalis* (1/2), *E. coli* (1/2), *K. pneumoniae* (1/1), *L. casei* (1/1), *L. curvatus* (1/1), and *Pseudomonas* spp. (1/4)	Todorov and Dicks (2006b)
ST461BZ	*Lactobacillus rhamnosus* ST461BZ	2.8	*E. faecalis* (2/2), *E. coli* (1/2), *K. pneumoniae* (1/1), *L. casei* (1/1), *L. curvatus* (1/1), *Pseudomonas* spp. (3/4), and *Streptococcus* spp. (1/7)	Todorov and Dicks (2006b)
ST462BZ	*Lactobacillus rhamnosus* ST462BZ	8.0	*E. faecalis* (2/2), *E. coli* (1/2), *L. casei* (1/1), *L. delbrueckii* subsp. *bulgaricus* (1/1), *L. sakei* (1/1), and *Pseudomonas* spp. (2/4)	Todorov and Dicks (2006b)

(continued)

Table 12.2 (continued)

Bacteriocins	Strain	Molecular mass (kDa)	Activity spectra	References
ST664BZ	*Lactobacillus plantarum* ST664BZ	6.5	*E. faecalis* (2/2), *E.* (1/2), *L. casei* (1/1), *L. delbrueckii* subsp. *bulgaricus* (1/1), *L. sakei* (1/1), and *Pseudomonas* spp. (1/4)	Todorov and Dicks (2006b)
ST712BZ	*Lactobacillus pentosus* ST712BZ	14.0	*E. faecalis* (1/2), *E. coli* (1/2), *K. pneumoniae* (1/1), *L. casei* (1/1), *L. curvatus* (1/1), and *Pseudomonas* spp. (1/4)	Todorov and Dicks (2006b)
JW3BZ	*Lactobacillus plantarum* JW3BZ	ND	*E. faecalis* (4/6), *Enterococcus mundtii* (1/1), *E. coli* (0/1), *K. pneumoniae* (0/2), *L. casei* (1/1), *L. curvatus* (0/1), *L. paracasei* subsp. *paracasei* (0/1), *L. plantarum* (0/3), *L. sakei* (2/2), *L. salivarius* (0/1), *L. lactis* subsp. *lactis* (1/1), *L. innocua* (2/2), *Pseudomonas* sp. (0/1), *S. aureus* (0/1), *Streptococcus caprinus* (0/1), *Streptococcus* sp. (0/1)	Von Mollendorff et al. (2006)
JW6BZ	*Lactobacillus plantarum* JW6BZ	ND	*E. faecalis* (3/6), *Enterococcus mundtii* (1/1), *E. coli* (0/1), *K. pneumoniae* (0/2), *L. casei* (1/1), *L. curvatus* (0/1), *L. paracasei* subsp. *paracasei* (0/1), *L. plantarum* (0/3), *L. sakei* (2/2), *L. salivarius* (0/1), *L. lactis* subsp. *lactis* (1/1), *L. innocua* (1/2), *Pseudomonas* sp. (0/1), *S. aureus* (0/1), *S. caprinus* (1/1), *Streptococcus* sp. (1/1)	Von Mollendorff et al. (2006)
JW11BZ	*Lactobacillus fermentum* JW11BZ	ND	*E. faecalis* (1/6), *Enterococcus mundtii* (0/1), *E. coli* (0/1), *K. pneumoniae* (0/2), *L. casei* (1/1), *L. curvatus* (0/1), *L. paracasei* subsp. *paracasei* (0/1), *L. plantarum* (0/3), *L. sakei* (2/2), *L. salivarius* (0/1), *L. lactis* subsp. *lactis* (1/1), *L. innocua* (0/2), *Pseudomonas* sp. (0/1), *S. aureus* (0/1), *S. caprinus* (1/1), *Streptococcus* sp. (1/1)	Von Mollendorff et al. (2006)
JW15BZ	*Lactobacillus fermentum* JW15BZ	ND	*E. faecalis* (4/6), *Enterococcus mundtii* (1/1), *E. coli* (0/1), *K. pneumoniae* (1/2), *L. casei* (1/1), *L. curvatus* (0/1), *L. paracasei* subsp. *paracasei* (0/1), *L. plantarum* (0/3), *L. sakei* (2/2), *L. salivarius* (0/1), *L. lactis* subsp. *lactis* (1/1), *L. innocua* (2/2), *Pseudomonas* sp. (0/1), *S. aureus* (0/1), *S. caprinus* (1/1), *Streptococcus* sp. (1/1)	Von Mollendorff et al. (2006)
Bozacin 14	*Lactococcus lactis* subsp. *lactis* B14	5.0	*E. coli* (2/2), *Lactobacillus alimentarius* (1/1), *L. brevis* (1/3), *L. casei* (6/8), *L. curvatus* (1/2), *L. delbrueckii* subsp. *delbrueckii* (1/1), *L. plantarum* (9/18), *L. lactis* (3/3), *Leuconostoc dextranicum* (3/3), *L. mesenteroides* (3/7), *L. innocua* (2/2), *L. monocytogenes* (1/1), and *P. pentosaceus* (1/2)	Kabadjova et al. (2000)

Numbers in parentheses: number of strains inhibited/number of strains tested (*ND* not determined)
*sensitive/total number of tested strains

Prescott and Björkstén (2007) address to the effects of probiotic bacteria on immune development and their role in the treatment and prevention of allergic diseases.

Several studies indicate that consumption of certain cultured dairy and non-dairy products could lower total plasma cholesterol and low-density lipoprotein cholesterol. Mann and Spoerry (Mann and Spoerry 1974) observed that consumption of *Lactobacillus* spp. containing fermented milk has cholesterol lowering effect. Hypocholesterolemic effect of *Lactobacillus* spp. was observed in humans (Agerbck et al. 1995), pigs (De Rodas et al. 1996), and mice (Chiu et al. 2005; Liong and Shah 2005).

Hernandez-Mendoza et al. (2009) explored the application of LAB probiotics in the reduction of the levels of aflatoxin B1. It has been proposed that the consumption of LAB capable of binding or degrading food-borne carcinogens would reduce human exposure to these deleterious compounds. In the study of Hernandez-Mendoza et al. (2009), the ability of eight strains of *Lactobacillus casei* (isolated from cheese, corn silage, human feces, fermented beverage) to bind aflatoxin B1 in aqueous solution was investigated. However, the effect of addition of bile salts to the growth medium during culturing *L. casei* strains on aflatoxin B1 binding was assessed by Hernandez-Mendoza et al. (2009).

One of the main selection criteria for probiotic LAB is their ability to adhere to epithelial cells or the intestinal mucosa. Adhesion is important as it is considered to play a vital role in persistence and stimulation of the immune system, enhanced healing of the damaged mucosa, and antagonism against pathogenic bacteria (Isolauri et al. 1991; Salminen et al. 1996; Rolfe 2000; Reid and Burton 2002). Other criteria include the ability to survive at low pH and high bile salt concentrations (Mattila-Sandholm et al. 1999; Bezkorovainy 2001).

12.3 Benefits of the LAB in the Cereal-Based Fermented Foods: Probiotic Lactic Acid Bacteria as Starter Cultures

The use of probiotic LAB, especially *Lactobacillus* spp. and *Bifidobacterium* spp. as starter cultures, either alone or in combination with traditional starter cultures in various fermentation processes, is gaining significant interest. Formulated probiotic food may present consumers with a healthy dietary component at a considerable low cost (Goldin 1998). Furthermore, it was reported that LAB may contribute to microbiological safety and/or provide one or more technological, nutritional, and organoleptic advantages to a fermented product, through production of ethanol, acetic acid, aroma compounds, exopolysaccharides, bacteriocins, and several enzymes (Leroy and De Vuyst 2004).

Different developments over the years led to the concept of using starter cultures. The earliest fermented food products relied on natural fermentation through microflora present in the raw material. The load and spectrum of microorganisms populating raw material have a definite effect on the quality of the end product.

Backslopping, i.e., inoculation of the raw material with a small quantity of a previously performed successful fermentation, was used to optimize spontaneous fermentation. In this case the best-adapted strain dominates. The dominant strains can be seen as a starter culture that shortens the fermentation process and reduce the risk of fermentation failure (Leroy and De Vuyst 2004). Backslopping is still used in developing countries and even in the industrialized countries for production of sauerkraut and sourdough (Harris 1998). The use of starter cultures in large-scale production of fermented foods has become important for industries in the Western countries as it resulted in a control over the fermentation process and a consistent end product. However, some disadvantages do occur due to the fact that commercial starter cultures were not selected in a rational way, but rather on phage resistance and rapid acidification of the raw materials (Leroy and De Vuyst 2004). With regard to the functionality and desired properties of the end product, these starters are not very flexible. Furthermore, it is believed that commercial starter cultures adapted to the food matrix led to a loss in genetic material (Leroy and De Vuyst 2004). This may have contributed to the limited biodiversity of commercial starter cultures. Moreover, this leads to a product that lacks the uniqueness and characteristics that made the original product popular (Caplice and Fitzgerald 1999).

Wild-type LAB that originates from the environment, raw material, or process apparatus serves as a natural starter culture in many of the traditionally fermented foods (Böcker et al., 1995; Weerkamp et al. 1996). Recent studies focused on the use of wild-type strains isolated from traditional products for use as starter cultures (Hébert et al., 2000; Beukes et al. 2001; De Vuyst et al. 2002). When considering LAB as a starter culture, the following factors have to be taken into account: (1) not all LAB strains have the same practical and technical importance in food fermentations; (2) *Lactobacillus* (*L. fermentum*, *L. plantarum*, *L. reuteri*), *Leuconostoc*, and, to a lesser extent *Lactococcus*, *Enterococcus*, *Pediococcus*, and *Weissella* spp. are usually present in traditional fermented foods; (3) not all strains of the same species are suitable as starter cultures; and (4) various industrial lactic acid fermentation processes are well controlled despite the fact that they are spontaneous (Holzapfel 2002). Some of these lactic acid bacteria may be classified as functional starters, due to their contribution to food safety, organoleptic properties, and other nutritional advantages (Table 12.3).

LAB are known to produce antimicrobial substances (e.g., bacteriocins), polymers, sugars, sweeteners, nutraceuticals, aromatic compounds, and various enzymes. This leads to a higher flexibility and wider application of LAB as starter cultures. It also represents a way by which chemical additives can be replaced by natural compounds and thus provide the consumer with new, appealing food products (Leroy and De Vuyst 2004). Bacteriocins produced by LAB may prevent food spoilage, e.g., late spoilage of cheese by clostridia (Thomas et al. 2002). Some probiotic strains may also be used as functional starters or cocultures in fermented food (Chandan 1999; Ross et al. 2000; Jahreis et al. 2002).

However, when considering the use of probiotic strains as functional starters or cocultures, it is important that they do not enhance the acidification during the shelf life of the product nor have adverse effects on the aroma or taste of the product (Heller 2001).

Table 12.3 Examples of *Lactobacillus* spp. as functional starters or cocultures and their role in the food industry

Advantage	Role	*Lactobacillus* spp.	References
Food preservation	Production of bacteriocins		
	– Fermented meats	*L. curvatus*	Vogel et al. (1999)
		L. sakei	
	– Fermented olives	*L. plantarum*	Ruiz-Barba et al. (1994)
Organoleptic	Production of exopolysaccharides	Several lactobacilli	De Vuyst and Degeest (1999; De Vuyst and Marshall 2001; De Vuyst et al. 2001)
	Production of amylase	Several lactobacilli	Mogensen (1993)
Technological	Prevention of overacidification in yogurt	*L. delbrueckii* subsp. *bulgaricus*	Mollet (1996)
Nutritional	Production of nutraceuticals		
	– Low-calorie sugars	*L. plantarum*	Wisselink et al. (2002)
	– Production of B-group vitamins	*L. delbrueckii* subsp. *bulgaricus*	Hugenholtz and Kleerebezem (1999)
	– Reduction of toxic and anti-nutritional compounds		
	– Reduction of phytic acid content, amylase inhibitors, and polyphenolic compounds	*L. plantarum*	Sharma and Kapoor (1996)
		L. acidophilus	

Uncertainty still exists whether multifunctional strains possessing all desirable metabolic features would result from modern techniques and selection procedures. Therefore, recent studies focus on the improvement of selected strains through the application of recombinant DNA technology. Application of DNA technology improves certain advantage features, e.g., health-promoting properties, accelerated acid production, wholesomeness, and overproduction of specific enzymes or bacteriocins (Holzapfel 2002). Gene disruption may be used to eliminate undesirable properties such as antibiotic and mycotoxin production by food-grade molds (Hammes and Vogel 1990; Geisen and Holzapfel 1996). A large array of these optimized cultures is available but is not used because of regulation (Holzapfel 2002).

12.3.1 Cereal-Based Probiotic Foods

The concept of probiotic foods has been developed to quite an extent since its introduction to clinical nutrition and food science during the 1980s (Fuller 1989; Shortt 1999). Most probiotic foods available today are milk based, while a few attempts have been made using cereals. Cereal grains have a high nutritive value

and are distributed worldwide, making them a very suitable raw material for the development of various fermented functional foods (Angelov et al. 2006). Togwa, a lactic acid-fermented maize and sorghum gruel, inhibits the growth of some enterotoxin-producing bacteria in children under 5 years old. This suggests that togwa may possess probiotic properties (Kingamkono et al. 1998). Vogel et al. (1999) found that the LAB present in various lactic-fermented foods, such as sourdough, are similar or in some cases identical to species found in the gastrointestinal tract of humans and animals. *Lactobacillus plantarum*, indigenous to a variety of cereal-based fermented food, is also associated with the gastrointestinal tract of humans (Ahrné et al. 1998; Molin 2001). Colonization of the intestinal mucosa with strains of *L. plantarum* isolated from sourdough has also been reported (Johansson et al. 1993).

Barley and oats contain β-glucan (Angelov et al. 2006), a prebiotic that reduces the levels of LDL-cholesterol by 20–30 % and thus also the risk of cardiovascular disease (Stark and Madar 1994; Wrick 1994; Gallaher 2002). For a polysaccharide or oligosaccharide to be characterized as a prebiotic, it should withstand digestion in the upper part of the gastrointestinal tract, be hydrolysable, soluble, and stimulate the growth and activity of beneficial microflora in the gut (Gibson and Roberfroid 1995). The low glycemic index of oats and barley is quite beneficial to diabetes in the gastrointestinal tract after ingestion as it alters the level of fat imulgation and reduces lipase activity (Angelov et al. 2006). Furthermore, β-glucan stimulates the growth of beneficial bacteria associated with the colon of animals and humans (Jaskari et al. 1998; Wood and Beer 1998).

To increase the number of beneficial bacteria in the gut, large numbers of probiotic bacteria have to be taken in by means of capsules or by using food as vector. Incorporating suitable dietary polysaccharides or oligosaccharides to the capsules may even be more effective. The latter is referred to as the prebiotic concept. Arabinoxylan is another prebiotic compound commonly found in wheat and rye (Jaskari et al. 1998; Crittenden et al. 2002; Karppinen 2003).

A number of LAB with probiotic properties have been isolated from boza (Todorov et al. 2008). Strains *L. plantarum* ST194BZ, ST414BZ, and ST664BZ, *L. rhamnosus* ST461BZ and ST462BZ, *L. paracasei* ST242BZ and ST284BZ, and *L. pentosus* ST712BZ survived low pH conditions (pH 3.0), grew well at pH 9.0, and were not affected by the presence of 0.3 % (w/v) ox bile. Cytotoxicity levels of the bacteriocins, expressed as CC_{50}, ranged from 38 μg/mL for bacteriocin produced by *L. plantarum* ST194BZ to 3776 μg/mL for bacteriocin ST284BZ. Bacteriocins ST284BZ, ST461BZ, and ST462BZ were the least cytotoxic. Bacteriocin produced by *L. paracasei* ST284BZ revealed high activity (EC_{50} = 735 μg/mL) against the virus HSV-1 that causes encephalitis and orofacial and genital lesions. The growth of *Mycobacterium tuberculosis* was repressed by 69 % after 5 days of incubation in the presence of bacteriocin ST194BZ. Various levels of auto (self) aggregation between the probiotic bacteria and co-aggregation with *L. innocua* LMG 13568 were observed. Adhesion of strains ST194BZ, ST284BZ, ST414BZ, ST461BZ, ST462BZ, and ST664BZ to HT-29 cells ranged from 18 to 22 %, which is similar to that reported for *Lactobacillus rhamnosus* GG. Adherence of strains ST194BZ,

Table 12.4 Possible applications of cereals or cereal constituents in functional foods

Application
Serving as fermentable substrate for growth of probiotic bacteria, particularly lactobacilli and bifidobacteria
As dietary fiber, promoting several beneficial physiological effects [e.g., laxation and blood cholesterol attenuation and blood glucose attenuation (Bijlani 1985)]
As prebiotics due to the presence of certain nondigestible carbohydrates
As encapsulation material (vector) to enhance the stability of probiotics

Adapted from Charalampopoulos et al. (2002)

ST242BZ, and ST712BZ to Caco-2 cells ranged between 7.0 and 9.0 % and is similar to values reported for *L. rhamnosus* GG. High hydrophobicity readings were recorded for most of the probiotic strains. Strain ST712BZ revealed only 38 % hydrophobicity, but 63 % of cells adhered to HT-29 cells, compared to 32 % adherence recorded for *L. rhamnosus* GG. Growth of strains ST194BZ, ST242BZ, ST284BZ, ST414BZ, ST461BZ, ST462BZ, ST664BZ, and ST712BZ was inhibited by only seven of 24 medicament substances tested. Although these properties are all characteristic of a good probiotic, in-depth in vivo studies will have to be performed to determine the composite influence of all conditions in the GI tract (Todorov et al. 2008).

Incorporation of probiotic strains in cereal-based fermented foods is possible. One such product is Yosa, a yogurt-type snack made of cooked bran fermented with LAB bacteria and bifidobacteria (Blandino et al. 2003). The cooked bran acts as a substrate for probiotic bacteria. This snack exhibits the postulated beneficial effects of bran and probiotic bacteria serving as an alternative to soy-based and milk-based yogurts (Salovaara 1996; Salovaara and Simonson 2003). Oats is a suitable substrate for fermentation with probiotic lactic acid bacteria after appropriate processing (Johansson et al. 1993; Salovaara 1996; Salovaara and Simonson 2003; Marklinder and Lonner 1992). Cereals are high in nutrition and confer specific health benefits (Table 12.4).

12.4 Benefits of the LAB in the Fermented Cereal Foods: Antimicrobial Compounds Produced by LAB

LAB produce various antimicrobial substances during fermentation, such as organic acids, hydrogen peroxide, carbon dioxide, diacetyl, low molecular weight antimicrobial substances, and bacteriocins (Blom and Mörtvedt 1991). These specific antimicrobial compounds act as biopreservatives in food, with records dating back to approximately 6000 B.C. (Pederson 1971; De Vuyst and Vandamme 1994).

The antimicrobial substances are not produced for human convenience but rather for one bacterium gaining advantage over another that competes for the same energy source (Ouwehand and Vesterlund 2004).

12.4.1 Organic Acid, Acetaldehyde, and Ethanol

Various heterofermentative LAB produce equimolar amounts of lactic acid, acetic acid, ethanol, and CO_2 upon hexoses fermentation. Homofermentation results in the formation of lactic acid alone (Caplice and Fitzgerald 1999). The antimicrobial effect of these organic acids formed during lactic acid fermentation is well known (Davidson 1997). The organic acids, dissociated and undissociated, are believed to disrupt the mechanisms responsible for maintaining the membrane potential, thereby inhibiting active transport (De Vuyst and Vandamme 1994; Sheu et al. 1972; Eklund 1989).

12.4.2 Hydrogen Peroxide

LAB produce hydrogen peroxide in the presence of oxygen through the action of NADH oxidases, flavoprotein-containing oxidases, and super oxide dismutase (Ouwehand and Vesterlund 2004; Condon 1987). LAB lack true catalase, and therefore it is believed that hydrogen peroxide may accumulate and act inhibitory to the growth of some microorganisms (Condon 1987). However, it is argued that hydrogen peroxide is decomposed by flavoproteins, pseudocatalases, and peroxidases in vivo and therefore does not accumulate to significant amounts (Nagy et al. 1991; Fontaine et al. 1996). Anaerobic environments can form due to some hydrogen peroxide-producing reactions scavenging oxygen (Ouwehand and Vesterlund 2004). Hydrogen peroxide production is important for the colonization of lactobacilli in the urogenital tract. This reduces the acquisition of gonorrhea, HIV, and urinary tract infections (Vallor et al. 2001). The antimicrobial effect of hydrogen peroxide in vivo is being questioned (Nagy et al. 1991; Fontaine et al. 1996).

12.4.3 Carbon Dioxide

Carbon dioxide is produced by heterolactic fermentation and contributes to an anaerobic environment that is toxic to various aerobic food microorganisms. Furthermore, carbon dioxide in itself has an antimicrobial activity (Lindgren and Dobrogosz 1990). The mechanism involved in this activity is not known, but it is believed that carbon dioxide accumulates in the lipid bilayer due to the inhibition of enzymatic decarboxylations (King and Nagel 1975), causing dysfunction of membrane permeability (Lindgren and Dobrogosz 1990). Low levels of CO_2 have been found to promote the growth of certain microorganisms, whereas high concentrations led to growth inhibition (Lindgren and Dobrogosz 1990).

12.4.4 Diacetyl

Diacetyl is produced from the fermentation of citrate and is responsible for the unique aroma and buttery flavor of various other fermented milk products (Lindgren and Dobrogosz 1990; Cogan and Hill 1993). Diacetyl is produced by many LAB, including the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* (Jay 1982). Gram-positive bacteria are less sensitive to its antimicrobial activity than Gram-negative bacteria, molds, and yeast. The mechanism responsible for this activity is the action of diacetyl on the arginine-binding protein of Gram-negative bacteria leading to interference with arginine utilization (Jay 1982; Motlagh et al. 1991; De Vuyst and Vandamme 1994).

12.4.5 Low Molecular Weight Antimicrobial Substances

Several studies have focused on the production of low molecular weight antimicrobial substances by lactic acid bacteria (Reddy and Shahani 1971; Hamdan and Mikolajcik 1974; Shahani et al. 1977a, b; Reddy et al. 1983; Silva et al. 1987). These substances share several characteristics, in addition to having a low molecular weight, such as being active at a low pH, soluble in acetone, thermostable, and displaying a broad spectrum of activity (Axelsson 1990). However, more in-depth studies need to be done to gain detailed information on these substances. Thus far, three low molecular weight antimicrobial substances have been properly characterized, i.e., reuterin and reutericyclin, both produced by *L. reuteri*, and 2-pyrrolidone-5-carboxylic acid, produced by *L. casei* subsp. *casei*, *L. casei* subsp. *pseudoplantarum*, and *Streptococcus bovis* (Chen and Russell 1989; Huttunen et al. 1995).

12.4.6 Bacteriocins

Bacteriocins are ribosomally synthesized peptides produced by various bacteria and exhibit a bacteriostatic or bactericidal activity against genetically closely related bacteria (Caplice and Fitzgerald 1999; Ross et al. 2000; Ouwehand and Vesterlund 2004; Chen and Hoover 2003). Although bacteriocins display antibiotic properties, they differ from antibiotics in that they are synthesized ribosomally and exhibit a narrow spectrum of activity, and the organisms responsible for their production have immunity against them (Cleveland et al. 2001). Most bacteriocins from Gram-positive bacteria are produced by lactic acid bacteria (Nes et al. 1996; Ennahar et al. 2000). Previous studies have reported the antimicrobial activity of bacteriocins produced by LAB against Gram-negative bacteria (Todorov and Dicks 2004; Todorov et al. 2005; Todorov and Dicks 2006b; Von Mollendorff et al. 2006).

Bacteriocins are of great importance to humans as they can play a considerable role in food preservation and human therapy (Richard et al. 2006). They can be used as an alternative or replacement to various antibiotics (Richard et al. 2006). This can limit the use of antibiotics and thus reduce the development of antibiotic resistance (Ouwehand and Vesterlund 2004). Furthermore, bacteriocins are more easily accepted by health-conscious consumers, because they are naturally produced compared to chemically synthesized preservatives (Ouwehand and Vesterlund 2004). According to Deegan et al. (2006) the ongoing study of existing bacteriocins and discovery of new bacteriocins look promising for application in the food industry.

12.5 Classification

Bacteriocins are divided into four main classes: (1) Class I, lantibiotics; (2) Class II, small non-modified heat-stable peptides; (3) Class III, large heat-labile proteins; and (4) Class IV, bacteriocins with a complex structure and glyco- and/or lipid moieties (Table 12.5). Class I and II bacteriocins are considered the most important due to potential commercial applications.

Table 12.5 Classification of bacteriocins produced by LAB

Group	Subgroups	Characteristics
Class I (lantibiotics)	Ia	Lantibiotics, peptides containing lanthionine and β-methyl lanthionine. Molecular weight 2–5 kDa. Undergo posttranslational modifications. Flexible elongated molecules. Peptide with slight positive or negative charge or highly negative net charge
	Ib	Globular molecules with no net charge or net negative charge
Class II (Non-lantibiotics, unmodified bacteriocins)	IIa	Small heat-stable peptides (<10 kDa), synthesized as inactive prepeptides to get activated by posttranslational cleavage of the N-terminal leader peptide. Have a consensus sequence of YGNGV in the N-terminal
	IIb	Two-peptide bacteriocins. Two different peptides required to form an active poration complex
	IIc	Other bacteriocins. Bacteriocins produced by the cell's general *sec*-pathway
Class III (non-lantibiotics, large heat labile bacteriocins)		Large molecules (>30 kDa) sensitive to heat
Class IV		Complex bacteriocins containing lipid or carbohydrate moieties

Adapted from Klaenhammer (1993) and Nes et al. (1996)

12.5.1 *Bacteriocins of the Genus* Lactobacillus

Most of the bacteriocins produced by lactobacilli belong to either Class I, Class II, or Class III (Ouwehand and Vesterlund 2004). This review will mainly focus on Class II bacteriocins. Class I bacteriocins are divided into two subgroups Ia and Ib (Table 12.5). Class II bacteriocins are divided into three subgroups (a, b, and c). Of these, Class IIa is the most common (Table 12.5). Class IIa bacteriocins are small (<10 kDa) heat-stable peptides and do not contain modified amino acids. They all contain a conserved amino-terminal sequence (YGNGVXC) (Ouwehand and Vesterlund 2004). Furthermore, they are of great interest for medical and industrial applications because of the exceptional properties they display, such as antiviral activity of enterocin CRL35 and bacteriocin ST4V and strong antilisterial activity of all Class IIa bacteriocins (O'Sullivan et al. 2002; Wachsmann et al. 2003; Todorov et al. 2005). The strong antilisterial activity of Class IIa bacteriocins make them even more promising for industrial applications than that of Class I, as they have a narrow spectrum of activity and may not be active against starter cultures (O'Sullivan et al. 2002).

Some Class II bacteriocins are composed of two separate peptides. Bacteriocins displaying this property are known as the Class IIb bacteriocins (Klaenhammer 1993). Class IIc bacteriocins used to include those activated by thiol and secreted by means of the sec-dependent pathway. However, studies on bacteriocins formerly characterized as Class IIc show that they can act with their cysteine residues being oxidized and thus can use the sec-dependent secretion pathway (Ennahar et al. 2000). Therefore, Class IIc is now used to group the other non-lantibiotic bacteriocins and the bacteriocins that do not belong to either Class IIa or IIb (Ouwehand and Vesterlund 2004).

Class III bacteriocins are large (>30 kDa) heat-labile proteins. Therefore, it has been proposed that this class may include bacteriolytic enzymes such as hemolysins and muramidases, which are able to imitate the physiological activities of bacteriocins (Jack et al. 1994). This group is the least well characterized and so far has only been isolated from the genus *Lactobacillus* (Klaenhammer 1993).

12.5.2 *Production and Modeling of Bacteriocins*

Consumers of food and beverages are more health conscious than in the past, and the need for these products to be minimally processed and free from chemical preservatives is imperative (Ross et al. 2000). This observation directed research to exploit the occurrence of natural preservatives and their application (Chen and Hoover 2003). In contrast to chemical preservatives, the use of LAB and/or their metabolites are generally more accepted by health-conscious consumers as "natural" and "health promoting" (Montville and Winkowski 1997; Rodríguez et al. 2003). Class IIa bacteriocins received extensive interest as food preservatives, due

to their bactericidal effect against various food-borne pathogens. Biopreservation can be defined as "the use of antagonistic microorganisms or their metabolic products to inhibit or destroy undesired microorganisms in foods to enhance food safety and extend shelf-life" (Chen and Hoover 2003). The efficiency of bacteriocin-producing LAB in fermented foods is usually limited by various factors such as low production, genetic instability, regulatory systems, inactivation, and occurrence of resistance among target bacteria (Ennahar et al. 2000). For the use of bacteriocins or bacteriocin-producing cultures in food, one needs to optimize their efficiency. Recent studies focus on the heterologous expression of bacteriocins form various LAB strains to overcome these obstacles (Papagianni 2003). Alternatively, media optimization or chemical modifications can be performed to optimize the yield of bacteriocin production (Chen and Hoover 2003, Ennahar et al. 2000).

Heterologous expression systems are generally implemented for clarifying the role of recombinant proteins and peptides, assisting in the transcriptional/translational control of recombinant gene expression, and attaining higher production levels than those of native sources (Papagianni 2003, Makrides 1996). Some LAB species, for example, *Lactococcus lactis*, are extremely versatile in the stabilization of gene maintenance, and the control of expression renders them useful for potential use as heterologous hosts (Venema et al. 1999). Constitutive production and overexpression of Class-IIa-bacteriocin genes have been reported when cloned and expressed in host organisms, therefore overcoming the regulation systems of bacteriocins (Fremaux et al. 1995; McCormick et al. 1996; Biet et al. 1998; Horn et al. 1998). Various species and strains of LAB are food grade, making them potentially useful as hosts for production of defined bacteriocins based on their properties relevant to specific food systems (Rodríguez et al. 2003). This provide a method by which bacteriocin-producing LAB can be developed that are adapted to a specific type of food, thus preventing or decreasing problems with colonization and bacteriocin production (Ennahar et al. 2000). In a study by Chikindas et al. (1995), pediocin PA-1 was transformed with pMC117, a plasmid containing the *ped* operon under control of the lactococcal promoter P32, in *Pediococcus pentosaceus* PPE1.2. Production and secretion were achieved with production being fourfold higher than that reported for the natural producer *Pediococcus acidilactici* PAC1.0. Coproduction of enterocin A and pediocin PA-1 in *Lactococcus lactis* IL 1403 has also been reported (Martínez et al. 2000). Other methods can also be implemented to achieve heterologous production of bacteriocins, namely, (1) exchanging the leader peptides and/or dedicated ABC secretion and processing systems and (2) by addition of signal peptides recognized by general secretory pathways (Rodríguez et al. 2003). Heterologous production of bacteriocins by LAB may have some drawbacks. In some cases low production levels of bacteriocins have been reported. Furthermore, the use of genetically modified organisms (GMOs) for the in situ production of these peptides may receive disapproval from industries and health-conscious consumers (Rodríguez et al. 2003).

Many studies have focussed on optimization of media and growth conditions for increased bacteriocin production. Verellen et al. (1998) and Todorov and Dicks (2005a; 2006a) reported higher bacteriocin production levels for *Lactobacillus*

plantarum ST194BZ, *Lactobacillus plantarum* ST13BR, *Lactobacillus plantarum* ST23LD, *L. plantarum* ST341LD, and *L. plantarum* 423 in optimized growth media.

Chemical modification of bacteriocins offers the possibility for peptides to be developed with improved stability and activity. A reduction in activity of Class IIa bacteriocins is generally associated with sequence modifications, including single-residue substitutions, compared to native bacteriocins (Fleury et al. 1996; Quadri et al. 1997; Chen et al. 1997; Montville and Chen 1998). However, in a study by Miller et al. (1998), pediocin PA-1 activity increased significantly after substitution of a Glu residue for Lys-11. Therefore, it is of interest to further explore the field of bacteriocin engineering.

12.6 Conclusion

Lactic acid bacteria have a long history in the preparation of traditional fermented food products. Most of them have a well-accepted GRAS status. Together with their importance as a starter culture in the fermentation process, LAB may be in same time a good bacteriocin producer and a potential probiotic. Traditional fermented food products need to be reevaluated in view on the potential source of probiotic LAB and a valuable source of their delivery to the humans. For centuries traditional medicine proved that these products have health benefits to the consumers, and modern medicine recommended them as accompanying treatment for several diseases.

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Chapter 13
Nutraceutical Properties of Amaranth and Chia Seeds

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13.1 Introduction

Nowadays, special attention has been given to some bioactive ingredients or nutraceuticals, due to the health-promoting, disease-preventing, or medicinal properties related to its consumption, capturing the interest of consumers. *Nutraceuticals* can be consumed as food supplements or as ingredients of functional foods. Some examples of nutraceuticals are dietary fiber, some lipids, antioxidants, flavonoids, phytochemicals, probiotics, and prebiotics, among others. These can be found in different natural resources such as herbs, fruits, vegetables, cereals, or oil seeds (Milner 2000; Arvanitoyannis and van Houwelingen-Koukaliaroglou 2005).

Both amaranth (*Amaranthus hypochondriacus* L.) and chia (*Salvia hispanica*) are endemic seeds of Mexico and are consumed traditionally since thousands of years ago. Their health benefits are known from ancestral medicine knowledge, but only recent scientific research has demonstrated specific aspects of their chemical composition and relates it to the health benefits which have been attributed to them.

Actually, these seeds have a low commercial value and are not consumed directly by the people of the region where they are produced. The diffusion of the scientific knowledge concerning the health benefits of both seeds, amaranth and chia, could promote their local and global consumption, preventing some illness or as part of a good diet.

This chapter provides information related to bioactive components of amaranth and chia seeds which can be considered as nutraceuticals, remarking the importance of its consumption.

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13.2 Amaranth

The amaranth seed is native of America and has been classified in the Amaranthaceae family. The different species that are cultivated in America are *A. caudatus*, which grows in Peru and Bolivia, *A. cruentus* in Guatemala, and *A. hypochondriacus* in Mexico. This seed is commonly known as "huautli" in Mexico, "kiwicha" in Peru, or "bledos" in Mexico and Guatemala. There are some other species from Asia, *A. blitum*, *A. lividus*, and *A. oleraceus*, and from Africa, *A. viridis*, *A. ascendens*, and *A. gracilis* (Early 1977; Oke 1983).

Amaranth cultivars are widely distributed in tropical and temperate regions all over the world. It can grow in different ecosystems since it tolerates a wide range of temperatures (12–30 °C) and is resistant to droughts; besides, it requires lower water consumption to grow, in comparison to corn or sesame seeds. The production yield of amaranth seeds can be up to 3 t/Ha. Moreover, the leaves of the amaranth plant can also be consumed as vegetable (Matteucci 1998; Saunders and Becker 1984). For these reasons, amaranth cultivars are appreciated worldwide and could be considered a good crop option to the nutritional food market. The amaranth plant and seed are shown in Fig. 13.1.

13.2.1 Origin and General Characteristics

It is known, by archeological excavations, that amaranth was consumed as vegetable since the prehistoric era. And there is no doubt that it is native of Central and South America (Becerra 2000; Morales-Guerrero et al. 2009).

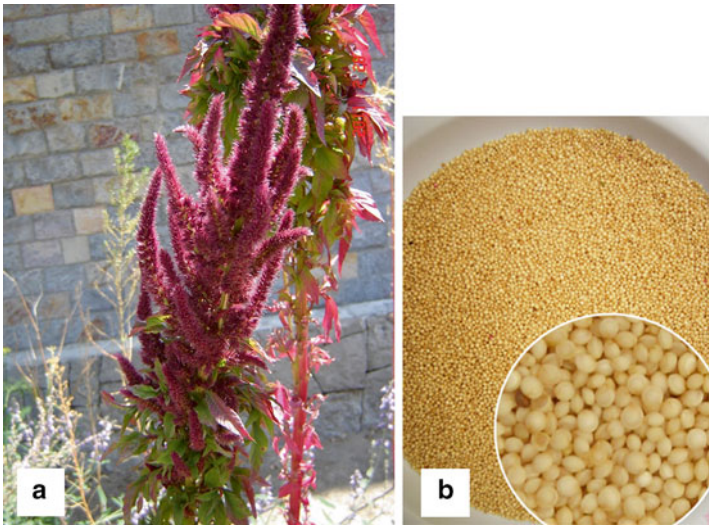

Fig. 13.1 Images of *Amaranthus hypochondriacus*, plant (**a**) and seed (**b**); one seed is 1 mm of diameter, from San Mateo Coatepec, Puebla, Mexico

In Mexico, amaranth seed was very important for pre-Hispanic cultures such as the Mayas and Aztecs. It was used in religious ceremonies as tribute in special commemorations to *Huitzilopochtli* or *Tlaloc*, gods of the war and the rain. During the Spanish conquest, the colonists considered the use of amaranth as a non-Catholic element and prohibited its use and harvest (Sahagún 1970). Even if the production of amaranth was reduced for a long time, traditional food habits and due to scientific researches concerning its high nutritive value, this seed has been reintroduced into the market. Actual economic efforts have been given for amaranth seed production in Mexico; in 2010 it produced 130 t of amaranth (SAGARPA, 2011). Nevertheless, China is the most important producer of amaranth seed in the world, the production yield has raised up to 86 million tons/year (Becerra 2000; Morales-Guerrero et al. 2009).

Amaranth is consumed in different ways; in Mexico, it is commonly used to prepare a traditional candy named "alegría", and it is also used to prepare a hot beverage with water or milk called "atole", and amaranth flour has been also grinded to be used for tortillas and other pastries. In some other American countries like Peru and Bolivia, amaranth is fermented and it is known as "chicha" and the amaranth plant leaves are commonly boiled and used as a colorant for alcoholic beverages (Teutonico and Knorr 1985; Villanueva and Arnao 2007). In the United States amaranth flour is usually used for baking products (Becerra 2000).

In India people have a similar way to use amaranth seed as in Mexico; they also use it to prepare a candy named "laddoos", and in Nepal people produce "sattoo" from amaranth flour (Oke 1983; Early 1977).

The amaranth seed is considered as a pseudo-cereal due to its similar characteristics to real cereal grains, monocotyledons, and for its important content of starch, but is deposited in the perisperm and the germ occupies most of the grain structure; therefore, it is considered a dicotyledon and not a real cereal (Breene 1991).

13.2.2 Nutraceutical Compounds in Amaranth

The most important chemical compounds in amaranth seed are the protein and mineral (expressed as ash) contents. Especially, amaranth content of protein is 18–20 % which reveals to be higher than rice (~7.5 %), corn (~10 %), and wheat (~14 %) (Tovar et al. 2008). The high value of ash content is attributed to the important content of minerals such as potassium, phosphorus, and calcium (800 mg, 530 mg, and 150 mg/100 g of edible seed in dry basis, respectively) (Casillas 1986; Nieto 1990).

An interesting characteristic of the amaranth plant is that leaves are also nutritive, due to a high content of vitamin C (80 mg/100 g) and calcium (267 mg/100 g), this last one almost three times more than calcium content in spinach (Berdanier et al. 2008).

13.2.2.1 Protein

The protein content of amaranth seed differs among the different species, from 11.1 to 20.7 g/100 g (Bressani 1989). Nevertheless, the protein content is remarkably higher than other cereals, as mentioned before. The most important proteins in amaranth seed are albumins and globulins, which represent 60–70 % of the total proteic nitrogen in the grain. The interest to study amaranth proteins relies not only in the nutritional value but to certain characteristics of these, like emulsifier, which provides a wide application for food product development (Villanueva and Arnao 2007).

The amino acid balance of amaranth is quite good and acceptable for the required level for human nutrition, being leucine the limiting amino acid. Amaranth amino acidic ratio (mg of amino acid/g protein) has been reported to be of 70 % for *A. caudatus*, 77 % for *A. cruentus*, and up to 86 % for *A. hypochondriacus* (FAO/OMS/UNU, 1985). These values can be considered of great nutritive value, when comparing them with the ones reported for wheat protein and soy which are of 73 and 74 %, respectively (Sizer and Whitney 2000).

13.2.2.2 Fatty Acids and Squalene

Lipid content of amaranth seeds is among 5–8 % and it is widely recognized to be a natural resource with the highest squalene content which represents 2.4–8.0 % of the total lipid content, depending on the specie (Lyon and Becker 1987; Lehmann 1996; Bruni et al. 2001).

The squalene is an unsaponified lipid and acts as a precursor of the biosynthesis of all the steroids in plants and animals. Among the health benefits attributed to squalene are the decrease of the risk to develop different types of cancer and the reduction of cholesterol levels (Miettinen and Vanhanen 1994; Rao and Newmark 1998; Smith 2000; Hang-Ping and Harold 2003).

The main fatty acids present in amaranth oil are oleic and linolenic acids (omega-3). The oleic acid benefits relate to cardiovascular and hepatic health. It enhances the high-density lipoprotein (HDL) and reduces the low-density lipoprotein (LDL) in the blood, this last considered as the bad cholesterol. Besides, oleic acid prevents the formation of gallstones. According to Mahan and Escott-Stump (2008), oleic acid also participates in the platelet aggregation and plays an important role in regulating lipid metabolism and the corporal weight equilibrium.

Amaranth seed also presents tocotrienols, which are considered potent antioxidants that prevent the peroxidation of lipids and also decrease cholesterol levels in the blood (Villanueva and Arnao 2007).

13.3 Chia

13.3.1 General Aspects

The chia seed belongs to the botanical Lamiaceae family and *Salvia* genus. There are about 900 species but the more widely distributed are *Salvia hispanica* L., *Salvia columbariae* Benth., and *Salvia polystachya. Salvia hispanica* L. is commonly known as "Spanish Salvia," "Mexican chia," or "Chan." Chia is a cereal, native of the southwest of North America and is cultivated from California, Texas, traversing Mexico all the way down in the west and southwest coast down to Chiapas (Alvarez-Chávez et al. 2008; Reyes-Caudillo et al. 2008).

This cereal was cultivated in Mexico by the pre-Colombian civilization (1000 B.C.), by the Mayas and later on, the Aztecs. The chia was introduced to Spain during the colonization and since then it was named "hispanica" (Gentry et al. 1990). In Fig. 13.2 chia plant and seed can be appreciated.

Native people of southern Mexico consumed chia seed to survive during long journeys and wars. They prepared it by adding water but they also used chia seeds to obtain flour and to extract the oil. This oil extract was used for painting their faces and body but also to prepare medicines. As a precious seed, the conquered civilizations of the South of Mexico offered the chia as a tribute to the Aztecs. Since 1991, a group of producers and scientists from different countries of America (Argentina, Bolivia, Colombia, Peru, and the United States) began a collaborative project to diversify chia cultivars and to introduce them into the daily diet (Ayerza and Coates 2005).

Fig. 13.2 Images of *Salvia hispanica*, plant (**a**) and seed (**b**); one seed is 2 mm large, 1.7 mm wide and 0.8 mm width, from San Mateo Coatepec, Puebla, Mexico

13.3.2 Nutraceutical Compounds in Chia

13.3.2.1 Lipids

The lipid content in chia seed is very valuable because it has polyunsaturated fatty acids, which are significantly high among other seed oils, and within it the presence of omega-3, omega-6, and omega-9 fatty acids has been detected. Consuming oils rich in polyunsaturated fatty acids and omega-3 fatty acids prevents hypercholesterolemia and cardiovascular illness (Bushway et al. 1981; Berdanier et al. 2008).

Oil extraction yield from chia seeds depends on the solvent and method applied; oil content varied between 300 and 400 g/kg seed. Chia oil is composed predominantly of unsaturated fatty acids, of which α-linolenic is the major component (about 60 % of the total of fatty acids). The fatty acid profile of chia oil also demonstrates the presence of linoleic, oleic, palmitic, stearic, and eicosanoic acids in decreasing order content, respectively (Ayerza 1995; Ayerza and Coates 2007; Alvarez-Chávez et al. 2008).

Researches from Ayerza (1995) and Ayerza and Coates (2007) demonstrate that chia seed is also an important source of vitamin A (44 μg/100 g), enhancing its nutritional value since this vitamin prevents blindness and cardiovascular illness and reinforces the immunological system. Moreover, when chia is introduced to the diet, the desaturation of α-linolenic acid is retarded, and due to the high content of antioxidants (flavonoids and cinnamic acid), omega-3 acids are more stable during storage.

13.3.2.2 Antioxidants

Phenolic compounds are important antioxidants, naturally and widely present in plants (Shahidi and Naczk 1995). These have been associated to the protective role against cardiovascular illness, cancer, and diabetes, besides retarding DNA oxidation (Berra et al. 1995; Balasundram et al. 2006). Among the phenolic compounds, cinnamic acids (hydroxycinnamic acid, hydroxybenzoic acid, caffeic acid, and chlorogenic acids), coumarins, and isocoumarins have demonstrated a high antioxidant activity. Caffeic acid and chlorogenic acid have been identified in chia seed in a concentration of 66 mg/kg of seed and 71 mg/kg of seed, respectively (Taga et al. 1984; Olthof et al. 2003).

Even if the chlorogenic and caffeic acid have demonstrated an antioxidant activity in in vitro assays, their real effect in vivo is still unknown since both acids could be metabolized by the human body. However, caffeic acid effect on health is controversial since its consumption has been also related to cancer (Olthof et al. 2003).

13.3.2.3 Dietary Fiber

Dietary fiber intake is related to some health benefits including the reduction of cholesterolemia, modification of the glycemic and insulinemic responses, changes in intestinal function, and antioxidant activity (Abdul-Hamid and Luan 2000; Thebaudin et al. 1997).

The high nutritive value of chia is also attributed to its dietary fiber content which is about 27–40 %. Dietary fiber content of other seeds is 50 % lower or more in comparison with chia, for example, corn has 9.2 %, walnuts, 10 %, and almonds, 14 % (Reyes-Caudillo et al. 2008; Ayerza and Coates 2005).

Once the oil is extracted from the chia seed, the residual material presents 50–60 %. When chia seed is immersed in water, mucilage is formed, in which the soluble dietary fiber (SDF) is recovered. In this mucilage, xylose, glucose, and methyl glucuronic acid have been identified (Lin et al. 1994; Ayerza and Coates 2005).

The ratio between insoluble dietary fiber (IDF) and SDF is related to the beneficial physiological effects; the American Dietetic Association recommends a fiber intake of 25–30 g/day with an IDF/SDF ratio of 3:1 (Borderías et al. 2005). Studies in Mexican chia seeds from Chihuahua and Jalisco showed 6.16–6.84 % and 32.87–34.9 % of SDF and IDF content, respectively, which represents approximately 5:1 ratio approximately (Reyes-Caudillo et al. 2008).

13.3.2.4 Proteins

An important aspect of chia, as well as amaranth, is that these seeds do not contain gliadin and therefore are recommended to be included in celiac patient diet (Berdanier et al. 2008).

The chia seed presents a protein content of 19–23 %, which is quite good in comparison to other cereals like corn (8.5 %), wheat (14 %), rice (14 %), oat (15.3 %), and even amaranth (18 %). Nevertheless, chia protein showed a low digestibility either raw, toasted, or in flour (Monroy-Torres et al. 2008).

13.4 Final Remarks

In this chapter, important nutritional components of amaranth and chia seeds have been discussed. These components can be considered as nutraceuticals and their use may improve actual health demands and prevent diseases. The nutritional characteristics of both seeds may be expanded, not only to international consumers but to rural communities in aim of enhancing its consumption and including them in their daily diet.

Amaranth seeds constitute an alternative source of proteins in the human diet, with advantages over other protein sources due to their low content of saturated fats and absence of cholesterol, and can also be used in gluten-free special diets. *A. hypochondriacus* green leaves are also a valuable vegetable that positively prevents anemia (Rangarajan et al. 1998). Another important constituent of amaranth is squalene which can act prophylactically (it has antisclerotic properties and it aids in constipation) (Prokopowicz 2001). On the other hand, chia seeds are not only a nutritious food but have been recommended for their oil content and composition (unsaturated fatty acids); they also are a good source of protein, fiber, and several minerals (Bushway et al. 1981).

Furthermore, the nutraceutical content of different species of amaranth and chia seeds is subject to continuous research, as well as their properties and applications for food product development.

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Chapter 14
Biofunctionality of Chia (*Salvia hispanica* L.) Protein Hydrolysates

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14.1 Introduction

Chia (*Salvia hispanica* L.) is an annual summer herb belonging to the Labiatae family. Native to Mesoamerica, it has been in use by humans for millennia. Along with other basic grains such as corn, beans, and amaranth, chia was a basic foodstuff in several ancient Mesoamerican civilizations (Ayerza 2010). In the Aztec Empire, chia formed part of holy ceremonies as an offering to Nahua gods, and 5000–15,000 t of chia were received annually in the Aztec capital of Tenochtitlan as tribute from conquered nations. After the Spanish conquest, chia was replaced by crops brought from Europe, relegating it to obscurity for the following five centuries (Ayerza 2011). The chia seeds marketed today may descend from seed selected by Nahua botanists centuries ago, although the crop has reached the twenty-first century as a mixed population. This mixed germplasm is still grown by Nahua and Maya people in the mountains of southern Mexico, northern Guatemala, and Nicaragua.

Chia seeds are oval and vary in color from white to dark. White chia seeds represent a small percentage of seed production, are somewhat larger than black seeds, and have slight compositional differences (Moreira et al. 2010). Chia seeds have low moisture content (7.0 %, d.b.) and high oil content (32 %, w.b.), mainly omega-3 (60 %) (Ixtaina et al. 2008). They also contain other compounds with potential health benefits in humans, including proteins, antioxidants, and dietary fiber (Vázquez-Ovando et al. 2009). Its array of beneficial compounds provides chia seed considerable economic potential in the food, pharmaceutical, and chemical industries, but

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specific research is still needed on its biological potential. The present chapter objective was to quantify the antihypertensive and antioxidant potential of chia seed proteins hydrolyzed with an Alcalase®-Flavourzyme® sequential enzymatic system.

14.2 Chia Seeds and Extraction Methods

14.2.1 Chia Seeds

Chia (*S. hispanica* L.) seeds were obtained in Yucatan state, Mexico. Reagents were analytical grade and purchased from J.T. Baker (Phillipsburg, NJ, USA), Sigma (Sigma Chemical Co., St. Louis, MO, USA), Merck (Darmstadt, Germany), and Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The Alcalase® 2.4 L FG and Flavourzyme® 500MG enzymes were purchased from Novo Laboratories (Copenhagen, Denmark).

14.2.2 Protein-Rich Fraction

Chia flour was produced from 6 kg chia seed by first removing all impurities and damaged seeds, crushing the remaining sound seeds (Moulinex DPA139), and then milling them (Krups 203 mill). Oil extraction from the milled seeds was done with hexane in a Soxhlet system for 2 h. The remaining fraction was milled with 0.5 mm screen (Thomas-Wiley®, Model 4). The defatted chia flour was dried in a Labline stove at 60 °C for 24 h. Extraction of the protein-rich fraction (PRF) was done by dry fractionation of the defatted flour according to Vázquez-Ovando et al. (2010). Briefly, 500 g flour was sifted for 20 min using a Tyler 100 mesh (140 μm screen) and a Ro-Tap® agitation system. The PRF yield was calculated as follows: PRF yield = (PRF weight/0.5 particle size flour weight) × 100.

14.2.3 Enzymatic Hydrolysis of Protein-Rich Fraction

The PRF was hydrolyzed in batches by sequential treatment with Alcalase® and Flavourzyme®. A predigestion with Alcalase® for 60 min was followed by incubation with Flavourzyme® at different times; hydrolysis was stopped at 5, 15, 30, 60, 90, 120, and 150 min. A hydrolysis curve was generated with these parameters: substrate concentration, 2 %; enzyme/substrate ratio, 0.3 AU g^{-1} for Alcalase® and 50 LAPU g^{-1} for Flavourzyme®; pH, 7 for Alcalase® and 8 for Flavourzyme®; and temperature, 50 °C. Hydrolysis was done in a reaction vessel equipped with a stirrer, thermometer, and pH electrode. In all treatments, the reaction was stopped by heating to 85 °C for 15 min, followed by centrifuging at 9880 × *g* for 20 min to remove the insoluble portion (Pedroche et al. 2002).

14.2.4 Degree of Hydrolysis

Degree of hydrolysis (DH) was calculated by determining free amino groups with o-phthaldialdehyde following Nielsen et al. (2001): $DH = h/h_{tot} \times 100$, where h_{tot} is the total number of peptide bonds per protein equivalent and h is the number of hydrolyzed bonds. The h_{tot} factor is dependent on raw material amino acid composition.

14.2.4.1 Angiotensin I-Converting Enzyme Inhibitory Activity

Hydrolysate angiotensin I-converting enzyme (ACE) inhibitory activity was analyzed with the method of Hayakari et al. (1978), which is based on the fact that ACE hydrolyzes hippuryl-L-histidyl-L-leucine (HHL) to yield hippuric acid and histidyl-leucine. This method relies on the colorimetric reaction of hippuric acid with 2,4,6-trichloro-S-triazine (TT) in a 0.5 mL incubation mixture containing 40 µmol potassium phosphate buffer (pH 8.3), 300 µmol sodium chloride, 40 µmol 3 % HHL in potassium phosphate buffer (pH 8.3), and 100 mU/mL ACE. This mixture was incubated at 37 °C/45 min and the reaction terminated by addition of TT (3 % v/v) in dioxane and 3 mL 0.2 M potassium phosphate buffer (pH 8.3). After centrifuging the reaction mixture at $10{,}000 \times g$ for 10 min, enzymatic activity was determined in the supernatant by measuring absorbance at 382 nm. All runs were done in triplicate. ACE inhibitory activity was quantified by a regression analysis of ACE inhibitory activity (%) versus peptide concentration, and IC_{50} values (i.e., the peptide concentration in µg protein/mL required to produce 50 % ACE inhibition under the described conditions) were defined and calculated as follows:

$$\text{ACE inhibitory activity}\,(\%) = (A - B)/(A - C) \times 100$$

where A represents absorbance in the presence of ACE and sample, B absorbance of the control, and C absorbance of the reaction blank.

$$IC_{50} = (50 - b)/m$$

where b is the intersection and m is the slope.

14.2.4.2 ABTS Decolorization Assay

The ABTS radical cation was produced by reacting 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) with potassium persulfate according to Pukalskas et al. (2002). To prepare the stock solution, ABTS was dissolved at a 2 mM concentration in 50 mL phosphate-buffered saline. The ABTS radical cation was produced by reacting 10 mL ABTS stock solution with 40 µL K_2S4O_8 solution and allowing the mixture to stand in darkness at room temperature for 16–17 h

before use. Antioxidant compound content in the hydrolysates was analyzed by diluting the $ABTS^{\bullet+}$ solution with PBS to an absorbance of 0.800 ± 0.030 AU at 734 nm. After adding 990 μL diluted $ABTS^{\bullet+}$ solution to 10 μL antioxidant compound or Trolox standard (final concentration 0.5–3.5 mM) in PBS, absorbance was read at ambient temperature exactly 6 min after initial mixing. The percentage decrease in absorbance at 734 nm was calculated and plotted as a function of the Trolox concentration for the standard reference data. The radical scavenging activity of the tested samples, expressed as inhibition percentage, was calculated with the equation:

$$\% \text{ Inhibition} = [(A_B - A_A) / A_B] \times 100$$

where A_B is absorbance of the blank sample ($t = 0$) and A_A is absorbance of the sample.

The Trolox equivalent antioxidant coefficient (TEAC) was quantified by a regression analysis of percent inhibition versus Trolox concentration using the following formula:

$$\text{TEAC} = (\%I_M - b) / m$$

where b is the intersection and m is the slope.

14.2.4.3 Statistical Analysis

All results were analyzed using descriptive statistics with a central tendency and dispersion measures. One-way ANOVAs were run to evaluate protein extract hydrolysis data and in vitro ACE inhibitory and antioxidant activities. A Duncan multiple range test was applied to identify differences between treatments. All analyses were done according to Montgomery (2004) and processed with the Statgraphics Plus ver. 5.1 software.

14.3 Chia Seed Extracts and Biofunctionality

14.3.1 Enzymatic Hydrolysis of Protein-Rich Fraction

Dry fractionation yield of the defatted chia flour was 70.31 % particles >140 μm and 29.68 % particles <140 μm. The PRF (<140 μm) had a higher protein content (44.62 %) than whole chia flour (23.99 %) and defatted chia flour (34.01 %). It was also a good starter material for hydrolysis. Alcalase® exhibited broad specificity and produced hydrolysates with 23 % DH during the 60 min reaction time. The highest DH in the present study (43.8 %) was attained with Flavourzyme® at 150 min (Fig. 14.1). This was at least partially due to predigestion with Alcalase®, which increases the number of N-terminal sites, thus facilitating hydrolysis by Flavourzyme®.

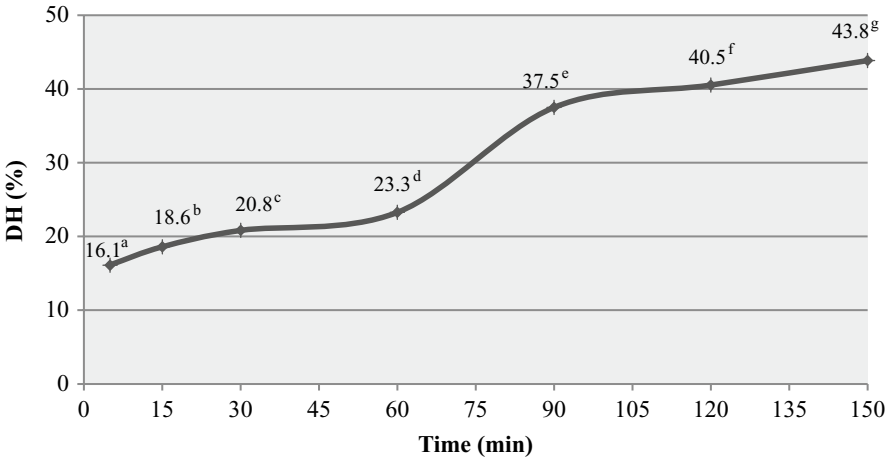

Fig. 14.1 Degree of hydrolysis at different times during enzymatic hydrolysis of a chia (*Salvia hispanica* L.) protein-rich fraction with an Alcalase®-Flavourzyme® sequential system. [a–e] Different superscript letters indicate statistical difference ($P < 0.05$)

Controlled release of bioactive peptides from proteins via enzymatic hydrolysis is one of the most promising techniques for producing hydrolysates with potential applications in the pharmaceutical and food industries. Hydrolysates with >10 % DH have medical applications, while those with <10 % DH can be used to improve functional properties in flours or protein isolates. Several biological properties have been attributed to low-molecular-weight peptides, although producing them commonly requires a combination of commercial enzyme preparations (Gilmartin and Jervis 2000). When hydrolyzed sequentially with Alcalase® and Flavourzyme®, chia seed is an appropriate substrate for producing extensive hydrolysates (DH > 10 %), making it a natural source of potentially bioactive peptides.

14.3.2 ACE Inhibitory Activity

ACE inhibitory activity of the chia protein hydrolysates produced with an Alcalase®-Flavourzyme® sequential system at 90, 120, and 150 min was measured and calculated as IC_{50}. The fact that the alkaline proteases Alcalase® and Flavourzyme® have broad specificity and hydrolyze most peptide bonds, with a preference for those containing aromatic amino acid residues, has led to their use in producing protein hydrolysates with functional and nutritional characteristics better than the original proteins and in generating bioactive peptides with ACE inhibitory activity (Segura-Campos et al. 2010). The chia protein hydrolysates produced with this sequential system exhibited ACE inhibitory activity, suggesting that the peptides released from the proteins are the agents behind inhibition. ACE inhibitory activity in the analyzed

hydrolysates depended significantly on hydrolysis time and therefore on DH. Bioactivity was highest in the hydrolysate produced at 150 min (IC_{50} = 8.86 μg protein/mL), followed by those produced at 120 min (IC_{50} = 20.76 μg/mL) and 90 min (IC_{50} = 44.01 μg/mL).

The in vitro biological potential observed here in the enzymatically hydrolyzed chia proteins was higher than the 140 μg/mL reported by Li et al. (2007) for a rice protein hydrolysate produced with Alcalase®. After a single oral administration in spontaneously hypertensive rats (SHRs), this rice hydrolysate exhibited an antihypertensive effect, suggesting its possible use as a physiologically functional food with potential benefits in the prevention and/or treatment of hypertension. Enzymatic hydrolysates from different protein sources, and with IC_{50} values ranging from 200 to 246,700 μg/mL, have also been shown to have in vitro ACE inhibitory activity as well as antihypertensive activity in SHR. Matsufuji et al. (1994) reported that peptides produced by enzymes such as Alcalase®, and which exhibit ACE inhibitory activity, may resist digestion by gastrointestinal proteases and therefore be absorbed in the small intestine, a property reported in a number of SHR studies. This suggests that the chia protein hydrolysates produced here with Alcalase®-Flavourzyme®, which exhibit ACE inhibitory activity, are capable of resisting gastrointestinal proteases and are therefore appropriate for application in food systems (e.g., functional foods) aimed at those suffering arterial hypertension disorders. Further research will be needed to determine if the peptide mixture exerts an in vivo antihypertensive effect because peptide ACE inhibitory potencies do not always correlate with their antihypertensive activities in SHR.

14.3.3 Antioxidant Activity

Chia protein hydrolysate antioxidant activity, quantified and calculated as TEAC values (mM/mg), decreased as DH increased. The highest TEAC value was for the hydrolysate produced at 90 min (7.31 mM/mg protein), followed by those produced at 120 min (4.66 mM/mg protein) and 150 min (4.49 mM/mg protein); the latter two did not differ ($p < 0.05$). Extensive proteolysis of the chia protein hydrolysates at 120 and 150 min resulted in lower antioxidant activity because it may have generated free amino acids, which are not effective antioxidants. Increased peptide antioxidant activity is related to unique properties provided by peptide chemical composition and physical properties. Peptides are potentially better food antioxidants than amino acids due to their higher free radical scavenging, metal chelation, and aldehyde adduction activities. An increase in the ability of a protein hydrolysate to lower a free radical's reactivity is related to an increase in amino acid exposure. This leads to increased peptide-free radical reactions and an energy decrease in the scavenged free radical, both of which limit a free radical's ability to oxidize lipids (Elias et al. 2008).

Antioxidant activity in the chia protein hydrolysates was measured with an ABTS assay that quantifies an antioxidant's (i.e., hydrogen or electron donor) suppression

of the radical cation $ABTS^{\bullet+}$ based on single-electron reduction of the relatively stable radical cation $ABTS^{\bullet+}$ formed previously by an oxidation reaction. When added to PBS medium (pH 7.2) containing $ABTS^{\bullet+}$, the proteins in the hydrolysates and peptide fractions very probably acted as electron donors, transforming this radical cation (maximum absorbance at 734 nm) into the non-radical ABTS (Segura-Campos et al. 2010). These results demonstrate that chia protein hydrolysates undergo single-electron transfer reactions in the $ABTS^{\bullet+}$ reduction assay, which effectively measures total antioxidant activity of dietary antioxidants and foods. Under the analyzed conditions, the chia protein hydrolysates may have acted as electron donors and free radical sinks, thus providing antioxidant protection. However, this purported antioxidant action needs to be confirmed for each peptide in different oxidant systems and under in vitro and in vivo conditions.

No relationship was observed between antioxidant activity and the hydrolysates with the highest ACE inhibitory activity. This suggests that peptide antioxidant activity may depend on the specific proteases used to produce them, the DH attained, the nature of the peptides released (e.g., molecular weight, composition, and amino acid sequence), and the combined effects of their properties (e.g., capacity for free radical location, metallic ion chelation, and/or electron donation).

14.4 Conclusion

Degree of hydrolysis is the principal factor affecting biological potential levels in the studied chia protein hydrolysates. Increased DH values resulted in higher ACE inhibitory activity but lower antioxidant activity. Hydrolysis of a PRF from *S. hispanica* with the Alcalase®-Flavourzyme® sequential system generated extensive hydrolysates with potential biological activity. This hydrolysis system produces low-molecular-weight hydrolysates, probably peptides, with ACE inhibitory and antioxidant activities and commercial potential as "health-enhancing" ingredients in the production of functional foods.

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Part III
Functional Properties of Fruits and Other Plant Foods

Chapter 15
Forgotten and Less Utilised Plant Species as Functional Food Resources

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15.1 Introduction

In recent years FAO and other leading international organisations' attention is among other global problems focused on less known, forgotten or totally neglected plant species and trying to incorporate them into the pool of generally exploited plants. Bioversity International in Maccarese, Italy (the former International Plant Genetic Resources Institute of FAO), elaborated already in 2000 a Strategic Action Plan for the use of forgotten and unutilised plant species. This document was officially accepted and ratified by many countries worldwide.

Orientation of the global public on less-used and nontraditional plant species, to be applied for economical and nutritional purposes, could not be marked in any case as accidental. This group of plant species is declared by the scientific community as 'plants for the future'. Many of them are spread in different regions of the world. They are often cultivated and used differently, with various degrees of intensity for many purposes—in traditional nutrition, medicine, cosmetics and many other branches. In some countries their mass cultivation started, in others their expansion is secured, and in the rest their introduction is organised. These plants display an elevated tolerance to diseases and pests and often quite modest demands on the cultivation and environmental conditions—therefore they are considered for 'ecological species'.

Fruits and several parts of these species possess often an increased nutritional level, moreover having important phytotherapeutic impacts on human health. Some of such plants could be exploited as decorative trees in the landscape-forming process or as agents in remediation technologies. Therefore, interest for the utilisation

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of these plants is declared not only by the agriculture, but there is a generally growing demand in the pharmacy, cosmetics and biotechnologies as well.

In Slovakia are registered over 50 plant species, which should be included into the group of less utilised species. At the same time their economical value and methodology of their utilisation are well known by the population of all Slovakia regions. Among the most important species should be listed the black mulberry (*Morus nigra* L.), true service tree (*Sorbus domestica* L.), Japanese persimmon (*Diospyros kaki*) and quince (*Cydonia oblonga*), which exert several important functional characteristics proved by both the experimental results of our team and literature data (Brindza et al. 2005b, 2006a, 2007b, 2009; Holecyova et al. 2006). Owing to a limited number of pages, our attention will be focused just on three species—*Morus nigra* L., *Cornus mas* L. and *Sorbus domestica* L.

15.2 Black Mulberry (*Morus nigra* L.)

On one side the black mulberry is counted among the less-used plant species, but on the other side, people of Slovakia are quite fond of this fruit called 'acid strawberry' or 'tree strawberry'. In a traditional folk nutrition, medicine and cosmetics are primarily exploited those fruits, which were collected in full maturity. Anyway, the fruit production is quite low; therefore, it is preferred to use them up for verified phytotherapeutic purposes. Food products prepared from mulberry are different types of compotes, jams, jellies, syrups, musts, non-alcoholic beverages and other original products made according to traditional and/or updated technologies. From dried leaves and fruits are prepared different teas and extracts used for healing of some diseases. Fruits and other mulberry parts and resulting products could be counted among the functional foods, as proved by knowledge gained in research or found in literature data (Holecyova et al. 2006; Brindza et al. 2007a).

15.2.1 Botanical Characterisation

Black mulberry (*Morus nigra* L.) is botanically included into the genus *Morus* and family Moraceae. Genus *Morus* consists of 12 species, occurring prevailingly in the tropical and subtropical climate zones (Rehder 1956). Black mulberry is a highly interesting species, when taking into account the nutritional, phytotherapeutic and/or pharmaceutical standpoint, and therefore this species was selected as the first study object of our experiments.

Minamizawa (1997) and Bendel (1999) declared that the mulberry place of origin is the Caucasus region. Owing to the fact that wildly growing black mulberries are unknown, it is difficult to fix the original descent area (Zeleny and Grünerova 1982). Its transfer to Western Europe is ascribed to Greeks and Romans, and in Slovakia it was introduced later from the West as supposed by Hajnalova (1999).

Fig. 15.1 Black mulberry (*Morus nigra* L.) fruitage shape variability. Photo: J. Holecyova, 2007

To control the mulberry distribution on the Slovakia territory, an inventory of genotypes was made. In 29 village cadastres using GPS system, 964 productive trees (genotypes) of black mulberry were localised. The highest amount of trees was found in the Pukanec cadastre (470) and in the surroundings of nearby village Devičany (115). This locality represents the largest Central European concentration of black mulberry trees occurring on one place. Black mulberry genotypes in Slovakia are growing in an altitude range of 146–517.31 m above the sea level; anyway 37 % of trees may be found in the altitude interval of 300–400 m (Holecyova and Brindza 2006; Holecyova et al. 2006).

15.2.2 Plant Parts Utilised Economically

Fruits of black mulberry are the most important tree part used in practice. In the experimentally evaluated population, fruits with distinct differences in their shape, size and colour (Fig. 15.1) were determined. From the botanical point of view, the mulberry fruit is a tiny key fruit closed in a fleshy corolla (Toman 2003). Fruits are maturing from middle July up to September. When red, the fruits are very sour, but during maturation process, the acid substance amount is lowered, and in a full maturity the fruit is sweet, just with a slight acidity, having a characteristic odour and high amount of blood-red juice. Fruitage could be freely torn from the branches only when fully matured (Kralik and Rosenberger 1993).

Fruits of black mulberry could be consumed directly or used for production of syrup, compote, jam and/or alcoholic drinks as it is generally made not only in Slovakia but in many other countries as well (Bendel 1999).

The fruit juice is an efficient dyeing agent; therefore, it is an excellent source of natural dye often exploited in the food industry. Another possibility is the fermentation of mulberry juice (containing approximately 32 % of tannin, eucalyptol, geraniol and essential oils) into liqueur (Gunes and Cekic 2004). Mulberry fruits are rich sources of anthocyanins and could be a potential source of natural red dye production (Liu et al. 2004).

In the period of 2005–2007 were experimentally evaluated 964 black mulberry genotypes grown in 29 villages of Slovakia (Brindza et al. 2007a). Morphological analyses of fruits resulted in data as follows: fruitage/syncarpy length ranged from

Table 15.1 Fruitage variability of black mulberry (*Morus nigra* L.) population grown in the territory of Slovakia

Trait	*n*	Min	Max	$\bar{x}$	$s_{\bar{x}}$	V (%)
Fruitage length (mm)	462	10.74	27.22	19.92	0.11	11.58
Fruitage width (mm)	462	9.16	16.30	13.18	0.05	7.51
Fruitage weight (g)	450	0.50	4.23	2.43	0.024	21.20
Weight of 1000 seeds (g)	431	1.80	7.60	5.80	0.04	9.37

Fig. 15.2 Black mulberry (*Morus nigra* L.) seed shape variability. Photo: J. Holecyova, 2007

10.74 to 27.22 mm and the width from 9.16 to 16.30 mm. The fruits' weight range was 0.5–4.23 g (Table 15.1).

Interesting differences were found with the black mulberry seeds as well—there are significant variabilities in the shape, size and colour as documented in Fig. 15.2. The seeds are donors of important substances and raw material for production of highly precious oil.

15.2.3 Biochemical Characterisation

To fix the economical value of individual parts of the plant, it was necessary to determine the chemical composition. In Table 15.2 are presented data on protein and amino acid content. Generally, the highest values of the protein concentration were found in male and female inflorescences, the lowest in fruits. Similar results were obtained for the amino acid concentrations as well. In the male and female inflorescences were determined high concentrations of all amino acids including the essential ones. These data are proving that the black mulberry male inflorescences represent another important product, which should be utilised in practice.

On the other hand, in comparison with other plant parts, the highest fructose and glucose content has been found in the mature fruits as shown in Table 15.3. It is understandable—the total amount of saccharides in fruits can distinctly fluctuate in dependence on the genotype, ripeness degree and other factors as indicated by the determined saccharide range of 4.3–19.7 $mg.kg^{-1}$ resulting from the tests provided

Table 15.2 Proteins and amino acid concentration in the different parts of the black mulberry (*Morus nigra* L.) plant (g.kg^{-1})

Component	Male inflorescence	Female inflorescence	Generative buds	Seeds	Mature fruit
Proteins	280.0	308.4	153.4	133.2	15.5
Arginine	18.2	15.8	10.7	16.2	0.7
Glycine	9.9	10.6	8.6	7.8	0.5
Histidine	5.4	6.2	3.5	4.0	0.3
Isoleucine	6.0	7.0	6.5	6.7	0.4
Aspartic acid	53.5	50.5	12.5	10.8	1.0
Glutamic acid	21.5	23.0	11.7	19.2	1.6

Table 15.3 Saccharide concentration in the different parts of the black mulberry (*Morus nigra* L.) plant (mg.kg^{-1})

Component	Male inflorescence	Female inflorescence	Generative buds	Seeds	Mature fruit
Reducing sugars	2.0	2.0	2.0	2.0	16.0
Saccharose	–	–	–	0.12	<1.0
Fructose	1.55	1.07	0.39	0.77	7.59
Glucose	0.86	0.99	1.41	1.5	8.39

Table 15.4 Selected fruitage trait variability in the experimental collection of black mulberry (*Morus nigra* L.) genotypes

Trait	*n*	Min	Max	$\bar{x}$	V (%)
Vitamin C (mg.100 g^{-1})	99	2.26	18.9	7.75	53.56
Total saccharides (mg.kg^{-1})	100	4.3	19.7	10.01	28.16
Organic acids (mg.kg^{-1})	100	0.79	1.73	1.15	15.62
Dyes (mg.kg^{-1})	100	2.1	6.3	3.79	30.84
pH	100	3.21	4.02	3.64	4.63
Fruit dry weight (%)	100	12.66	29.69	19.34	15.29

with 100 genotypes of black mulberry (Table 15.4). Relatively considerable differences were found for tested genotypes in the vitamin C, organic acids and colour substances as well (Table 15.4). The only exception from large variability is represented by pH values of tested genotypes and their products (Table 15.5).

The seeds of black mulberry fruit contain relatively high amount of lipids (16.0 %) and fatty acids, as demonstrated by the analytical results presented in Table 15.5. These data are documenting the suitability of mulberry seeds to be exploited as source of fatty acids with very favourable composition.

Experimentally the chemical composition of ten different foodstuffs prepared from black mulberry was studied, and the results are presented in Table 15.6. The lowest concentrations of saccharides were found in the wine and compotes and the

Table 15.5 Fatty acid concentration in the seeds of black mulberry (*Morus nigra* L.)

Fatty acids	Concentration (mg.100 g^{-1})	Proportion (%)
Lauric acid	15.0	0.11
Myristic acid	12.2	0.09
Pentadecanoic acid	29.9	0.22
Palmitic acid	1256.6	9.24
Palmitoleic acid	20.4	0.15
Heptadecanoic acid	13.6	0.10
Stearic acid	433.8	3.19
Oleic acid	1383.1	10.17
Vaccenic acid	92.5	0.68
Linoleic acid	10099.4	74.26
Linolenic acid	100.6	0.74
Octadecadienoic acid	31.3	0.23

Table 15.6 Chemical composition of products made of black mulberry (*Morus nigra* L.) fruits

Tested products	Total saccharides (mg.kg^{-1})	Organic acids (mg.kg^{-1})	Vitamin C (mg.100 g^{-1})	pH	Dry weight (%)	Dyes (mg.kg^{-1})
Fruit juice	17.24	1.88	0.87	3.47	–	0.63
Compote	11.26	1.91	0.90	3.50	19.04	1.81
Syrup	58.50	1.79	0.36	3.37	–	0.37
Jam	60.01	1.86	0.76	3.31	–	0.93
Jelly	54.50	1.94	0.66	3.28	–	0.92
Liqueur	31.50	0.51	0.73	3.69	–	0.14
Wine	6.50	1.72	0.27	3.68	–	0.09
Freeze-dried fruits	57.00	9.11	–	3.70	85.62	4.99
Fruitage in honey (70 %)	20.00	0.89	1.10	3.44	47.55	0.72
Fruitage in honey (50 %)	98.00	1.08	1.24	3.45	54.74	0.38

highest in concentrated juice, jam, freeze-dried fruits and in fruits tinned in mixture with honey. Organic acids' lowest content has been determined in the liqueur and in fruits preserved with honey (canned), while in other products the concentration of organic acids ranged from 1.72 to 1.94 mg.kg^{-1}, with the highest value occurring in the freeze-dried fruit flesh. Wine and syrup contained very low concentrations of vitamin C (0.27–0.36 mg.kg^{-1}), but substantially higher amount was in fruits preserved with honey. This method of fruit preservation could be counted among old traditional approaches to fruit conservation in several regions of Slovakia. Moreover, these products are often used up to present time as an efficient traditional remedy to heal some diseases. As proved by data in Table 15.6, the fruits are keeping nearly constant values of the pH level in all types of tested products. The highest content

of dyes has been maintained in products with high proportion of fruits or mulberry juice, especially freeze-dried fruit, compote, jam and jelly. Similar data are documented in the literature (Fenner 1888; Alakbarov and Aliyev 2000; Odyova 2001; Wafaa and Attia 2002; Gunes and Cekic 2004; Naderi et al., 2004; Chen et al. 2005).

Bassi (1992) reported 85 g of water, 9.5 g saccharides and 0.39 g proteins in 100 g of mulberry fruits. Mulberry fruit juice could be fermented, as there is contained approx. 32 % of tannin, eucalyptol, geraniol and essential oils (Gunes and Cekic 2004). Koyuncu (2004) quantified using HPLC in black mulberry fruits the concentration ranges of organic acids—malic acid 35.4–198.5 $mg.g^{-1}$, citric acid 5.5–23.4 $mg.g^{-1}$, tartaric acid 4.16 $mg.g^{-1}$, oxalic acid 0.62 $mg.g^{-1}$ and fumaric acid 0.019 $mg.g^{-1}$. Experimental analyses of Elmaci and Altug (2002) for black mulberry fruits showed that the total saccharides ranged from 11.3 to 16.2 %, pH of black mulberry fruits in narrow range of 3.6–3.8 and the total acids from 1.51 to 1.79 %. The sensory traits of black mulberry fruit are quite interesting for the consumers as well, as the fruit is very juicy, sweet and sour, with woody flavour and musk fragrance (Elmaci and Altug 2002).

From the comparison of the white and black mulberry fruits, the resulting data showed in white ones higher amounts of K, Cu, Zn and Mg and lower concentrations of Ca, Fe, vitamin C, pectin and fructose. Other characteristics and physiological properties were similar for both species, e.g. the ratio of glucose/fructose for white and black mulberry is 1:1.3 and 1:1.5, respectively (Wafaa and Attia 2002).

15.2.4 Phytotherapeutic Characterisation

Black mulberry (*Morus nigra* L.) is known not only for its nutritional value and taste but highly appreciated for its use in traditional human medicine as well, based on an elevated content of biologically active substances. For senior people the high concentration of potassium (300 mg K per 100 g^{-1} of fruit) is quite important helping to cover K-deficiency in aged organism; further it is recommended for cardiovascular problems (Darias-Martin et al. 2003).

Mulberry juice exerted the highest inhibition of the cytochrome isoenzyme CYP3A; it was even more efficient than the known inhibitors contained in the pomegranate, orange and grape juices (Hyunmi et al. 2006). Mulberry extract contains high anthocyanin content (200.96 $mg.100\ g^{-1}$); therefore, these fruits are applied in some therapeutic cases (Darias-Martin et al. 2003). Chen et al. (2005) studied anti-hyperlipidaemic and anti-atherosclerotic effects of the mulberry water extract on rats with experimental atherosclerosis.

As documented in the paper of Gunes and Cekic (2004), the black mulberry fruits possess antioxidant and antiseptic activities, and their application by anaemia could bring positive results. Antioxidative effect of black mulberry extracts is exploited by haemoglobin glycosylation, preventing the damage of human erythrocytes and regulating the level of the 'bad' LDL lipoprotein (Naderi et al., 2004).

For healing purposes, fruits of *Mori fructus* are primarily collected. From the pharmaceutical view, the black mulberry fruits could be evaluated as mild laxative, expectorants, tonic and a source of refreshing syrup—S*yrupus mororum* —which is very rich in fruit acids (Fenner 1888).

In Great Britain, the mulberry syrup—*Syrupus mori*—is listed in the medicine register as an official drug (Grieve 1995; Kress 2007).

Due to the sweet taste of mulberry syrup, it is used as remedy for liver, heart and bladder diseases; moreover, in Azerbaijan the so-called Bakmaz (folk medicine based on syrup) is applied for sore throat and cold (Alakbarov and Aliyev 2000).

Some clinical tests showed the possibility to stabilize the blood sugar level control by the black mulberry leaves application (Petlevski et al. 2001; Hallerova 2006). In Canary Islands the mulberry leaves are prescribed as medicine helping to stimulate the insulin production of diabetes (Darias-Martin et al. 2003).

Odyova (2001) included the mulberry leaves among the indication groups as expectorant, diabetic and diaphoretic which have antibacterial effects as well. Concoction prepared from mulberry leaves, fruits or roots is suitable for the healing of diabetes, sore throat and swollen vocal chords.

In China the tea called '*sang ye*' prepared from black mulberry leaves is administered for cold accompanied by fever (Odyova 2001). Leaves are frequently used in homoeopathy in a form of mashed agent '*Teep*', prepared from fresh leaves.

Extract of black mulberry bark was tested on rats, and there was found a positive analgetic effect decreasing the pains more effectively than other drugs (De Souza et al. 2000).

Flowers and fruits of black mulberry are often used in cosmetic branches as well, especially by the production of facial cleanser and body gels or perfumes and conditioning shampoos (Hiteko 2001).

15.3 Cornelian Cherry (*Cornus mas* L.)

Almost on the whole of Slovakia territory are spread freely growing trees and shrubs of Cornelian cherry (*Cornus mas* L.) known under popular name '*drienka*'. For purposes of traditional folk nutrition as well as in medicine andúor cosmetics are preferably exploited fully matured fruits, which after collection are stored in dried or tinned form. Owing to the pleasant and juicy taste of fresh fruit, they could be consumed instantly. The ripe fruits are similar in taste and structure to plums, but the unripe Cornelian cherries are fairly rusty containing some low concentration of pectin. Anyway, these fruits are highly esteemed owing to their verified phytotherapeutic effects. Cornelian cherry fruits are used for preparation of many different products—compote, jam, jelly, syrup, must, non-alcoholic and alcoholic beverages and other original goods made according to traditional and/or updated technologies. Dried leaves and fruits are employed for teas and extracts are suitable for curative purposes. Fruits and other plant parts of Cornelian cherry and products made of them are taken as functional foods, as proved by our experimental studies and reported in literature (Brindza et al. 2006c, 2007b).

15.3.1 Botanical Characterisation

Cornelian cherry (*Cornus mas* L.) is included into the genus *Cornus* and family Cornaceae. *Cornus* genus is composed of 58 species—mostly shrubs or smaller trees occurring in temperate, tropical and subtropical regions (Edye 1988; Murrell 1993; Xiang and Edye 1995).

Cornaceae family is from the developmental view very old—it could be placed into the Mesozoic era. Species of this family originally appeared only in the part of Asia (China, Himalayas). In Europe some of this family species were found in sediments of the Quaternary era. Presently in the temperate European territories, Cornelian cherry is planted as an ornamental wood plant (Klimenko 1990).

In Slovakia the Cornelian cherry as a photophilic and thermophilic species could be found on sunny shrubby hillside, in thin forests and thermophilic limestone localities and often occurs in parks of Slovakia as well (Mercel 1988). Cornelian cherry is a close relative of the *Cornus officinalis* L.

15.3.2 Economically Used Plant Parts

Fruits of Cornelian cherry are in practice the most frequently used plant part of this species. There are many newly bred varieties in Slovakia and abroad having larger fruit and higher production, but our population prefer up to present the fruits of freely growing populations in Slovakia. Therefore, our study was oriented on the evaluation of economic value of these freely growing populations. In the years 2004–2008 were experimentally examined 134 genotypes of Cornelian cherry in several regions of Slovakia (Brindza et al. 2006b, c). From selected genotypes, fruits were withdrawn, and their important morphological traits evaluated (Table 15.7).

In the population of freely growing genotypes, the medium fruit weight in the range of 0.55–3.44 g and fruit length in the range of 12.05–19.55 mm were determined. The flesh of the total fruit weight (the yield) is 65–75 %. Fruits were found out to have different shapes and colours, as well as various degrees of maturity, as shown by their fruit comparison in Fig. 15.3 and stone comparison in Fig. 15.4. Significant differences in the stones colour and shape were detected as well (Table 15.7, Fig. 15.3).

Table 15.7 Variability of fruit and stone traits of freely growing Cornelian cherry (*Cornus mas* L.) genotypes

Evaluated traits	*n*	Min	Max	$\bar{x}$	V %
Fruit weight (g)	134	0.55	3.44	1.41	33.74
Fruit length (mm)	134	12.05	19.55	15.16	9.61
Fruit width (mm)	134	7.43	15.22	10.69	13.54
Stone weight (g)	134	0.11	0.51	0.25	27.65
Stone length (mm)	134	10.16	15.49	12.34	8.79
Stone width (mm)	134	5.14	7.10	5.85	7.36

Fig. 15.3 Variability in shape and colour of Cornelian cherry (*Cornus mas* L.) fruits. Photo: Peter Brindza (2004)

Fig. 15.4 Variability in shape and colour of Cornelian cherry (*Cornus mas* L.) stones. Photo: Peter Brindza (2004)

15.3.3 Biochemical Characterisation

To acknowledge its nutritional and energetic values, several chemical analyses were made. Data in Table 15.8 indicate—the fruit flesh contains around 3.4-g.kg^{-1} proteins—representing very low concentration. Moreover, the amino acid contents are low as well. Differences between the fruits with red and yellow pericarp are relatively few too.

Unripe fruits are fairly sour and bitter, caused by the relatively high concentration of tannic acid. When fully matured, the level of tannic acid is markedly lowered, and at the same time the concentration of saccharides is increasing, particularly

Table 15.8 Protein and amino acid contents in g.kg^{-1} in fresh fruits flesh of Cornelian cherry (*Cornus mas* L.)

Component	Red fruit	Yellow fruit
Proteins	3.4	3.5
Arginine	0.3	0.1
Glycine	0.2	0.1
Histidine	0.1	0.1
Isoleucine	0.2	0.1
Aspartic acid	0.3	0.2
Glutamic acid	0.3	0.3
Leucine	0.2	0.2
Lysine	0.3	0.2
Phenylalanine	0.2	0.2
Proline	0.1	0.1
Serine	0.1	0.1
Threonine	0.1	0.1
Tyrosine	0.1	0.1
Alanine	0.1	0.1
Valine	0.2	0.2

Table 15.9 Saccharides and vitamin contents in Cornelian cherry (*Cornus mas* L.) fresh fruit flesh

Component	Red fruit	Yellow fruit
Reducing saccharides %	86.7	103.7
Saccharose %	<1	<1
Fructose %	33.1	43.1
Glucose %	53.6	60.6
Vitamin C mg.kg^{-1}	167	156
Vitamin E mg.kg^{-1}	5.3	5.6
Vitamin B6 mg.kg^{-1}	<0.5	<0.5
Vitamin B1mg.kg^{-1}	<0.1	<0.1
Vitamin B2 mg.kg^{-1}	<0.1	<0.1

fructose and glucose. Genotype analyses showed the total concentration of reducing sugars in the range of 8.6–10.3 %, fructose 3.3–4.3 % and glucose 5.3–6.0 % (Table 15.9). Demir and Kalyoncu (2003) in studying the group of six types of Cornelian cherry determined the total saccharide content in a range from 6.92 to 8.43 % and the reducing sugars from 6.9 to 8.43 %. Güleryüz et al. (1998) found the total sugars in the range of 4.22–9.96 % and the reducing sugars 2.02–5.66 %. From this comparison follows a considerable consonance between our experimental data and those reported in literature.

Vitamins are an important indicator of fruits' nutritional value. Chemical analyses of 130 samples of fresh flesh showed the range for vitamin C 164.5–385.8 mg.kg^{-1}. Using HPLC method, the vitamin C concentrations ranged from 156 to 167 mg.kg^{-1}. Along with vitamin C, the concentration of vitamin E was measured with a range of 5.3–5.6 mg.kg^{-1} (Table 15.9). These data are drawing attention to another important indicator of Cornelian cherry's nutritional value. Güleryüz et al. (1998) in their paper stated that the Cornelian cherry fruits contain 43.78–76.75 mg

vitamin C. Demir and Kalyoncu (2003) with six Cornelian cherry types determined 54.74–73.11 mg.100 g^{-1} of vitamin C, while Klimenko (1990) with freely growing and bred genotypes in the 5-year interval reported the ascorbic acid concentrations in the range of 70.50–99.80 mg %.

Concentration of lipids has been determined in mutual project with the Faculty of Chemical and Food Technology of the Slovak University of Technology in Bratislava using their HPLC equipment. Total amount of lipids in stones of Cornelian cherry ranged from 3.1 to 5.6 % and in fruit flesh from 0.1 to 0.5 % (Table 15.10).

High amount of lipids (67 % linoleic acid, 18.4 % oleic acid and 7.2 % palmitic acid) in the stones proves the importance of the Cornelian cherry as a source of bioactive substances. Similar results were reported for seeds and oil of Cornelian cherry by two papers (Hohn and Meinschein 1976; Kleiman and Spencer 1982).

In Table 15.11 are demonstrated the relatively high concentrations of mineral substances in the fruit flesh—especially those of potassium, calcium, phosphorus and magnesium. The marked differences in variation coefficient values for individual genotypes could be explained as a consequence of different maturity degrees of tested fruits.

Table 15.10 Content of lipids (%) and some fatty acids (mg.100 g^{-1}) in the stones and freeze-dried fruit flesh of Cornelian cherry (*Cornus mas* L.)

Component	Seeds	Freeze-dried flesh
Total lipids (%)	3.1–5.6	0.1–0.5
Linolenic acid (mg.100 g^{-1})	63.7	27.5
Linoleic acid (mg.100 g^{-1})	2631.8	76.7
Palmitic acid (mg.100 g^{-1})	285.0	39.6
Oleic acid (mg.100 g^{-1})	719.8	18.3
Stearic acid (mg.100 g^{-1})	80.5	4.4

Table 15.11 Variability of mineral and dye substances in fruit flesh of Cornelian cherry (*Cornus mas* L.)

Evaluated traits	*n*	Min	Max	$\bar{x}$	V %
Nitrogen (mg.kg^{-1})	134	3151.0	6443.0	4055.67	17.83
Phosphorus (mg.kg^{-1})	134	425	1363	704.07	25.78
Potassium (mg.kg^{-1})	134	7047	20,327	13674.74	19.07
Calcium (mg.kg^{-1})	134	472	2592	1459.96	27.96
Magnesium (mg.kg^{-1})	134	298	1895	680.96	43.42
Zinc (mg.kg^{-1})	134	3.3	41.0	11.58	42.19
Copper (mg.kg^{-1})	134	1.2	8.1	2.69	39.35
Iron (mg.kg^{-1})	134	6.0	171	29.76	65.07
Sulphur (mg.kg^{-1})	134	396	1645	982.67	24.20
Dye substances (mg.kg^{-1})	134	3.43	37.25	11.98	55.96
Dry weight (%)	134	58.5	83.0	73.93	6.91

Dye content in fruit flesh was found in interval of 3.43–37.25 g.kg^{-1}, and again there was detected a very high variability degree. Similarly as in the case of mineral concentrations, it is interpreted by the following fact: fruit monitoring and their sampling are carried out in the period of August–September, when the fruits are red, but many of them are not in full technological maturity, and therefore the completely ripened fruit comprises higher dye concentration, while the less matured lower one.

In cooperation with the AnalytiCon Discovery GmbH in Potsdam (Germany), the phenolic component occurrence in the fruit flesh of Cornelian cherry was analysed. The chemical analyses using HPLC equipment showed the presence of 28 phenolic substances, among them is up to now 17 components unidentified. Among the identified ones, in the fruit flesh primarily the ursolic and oleanolic acids and nicotine were found. It was reported that the ursolic acid has anti-cancerous, hepatoprotective, anti-inflammatory, anti-ulcerous, antimicrobial, antihyperlipidemic and antiviral effects (Zaletova et al. 1987; Liu et al. 1997). Several authors confirmed the presence of the ellagic acid, gallic acid, ursolic acid, quercetin and rutin in the leaves, flowers and seeds of Cornelian cherry (Delaveau and Paris 1961; Zorina et al. 1966; Grigorescu and Ionescu 1976; Kleiman and Spencer 1982).

Moreover, the Cornelian cherry fruit flesh contains many other components, which had to be omitted owing to limited page range. In the literature are documented high concentrations of tannin ranging from 131.51 to 601.20 mg.L^{-1} (Demir and Kalyoncu 2003), phenolcarboxylic acid, catechol and anthocyanin (Jefremov and Šreter AI 1996), ursolic acid (Liu et al. 1997), furan derivative (Kim and Kwak 1998) and many others.

15.3.4 Phytotherapeutic Characterisation

Healing effects of Cornelian cherry fruits have a long history going back as far as ancient times. Edible fruits were administered against diarrhoea (*enteritis*), what is not ignored even today. Other plant parts as the bark, roots and outshoots were applied against fever (Chevallier 1996).

Traditional medicine in regions of Caucasus and Central Asia used in over 1000 years is the Cornelian cherry (Efendiyev 1964; Damirov et al. 1983;Asadov et al. 1990). Natural remedies made of leaves, flowers and fruits are the traditional medicines prescribed for sore throat, indigestion, measles, anaemia, hepatic and kidney diseases (Asadov et al. 1990). Concentrated fresh fruit juice is prescribed for patients suffering from diabetes (Shukurov 1981; Sokolov and Zamotayev 1985).

Along with the Cornelian cherry fruits, its leaves and bark are quite often used, which contain tonic agents. In traditional medicine in China and Korea, the species *Cornus officinalis* is preferred, having aphrodisiac effects, helping with anaemia and other illnesses as well (Jefremov and Šreter AI 1996).

In Cornelian cherry fruits, the ursolic acid was detected, which is added to many cosmetic agents owing to its anti-ulcerous and antimicrobial properties. It was experimentally confirmed that ursolic acid is inhibiting the growth of some fungi species—*Candida albicans* and *Microsporum lanosum*. In popular medicine, the medicinal plants containing ursolic acid were exploited long ago it was proved which substances are responsible for their therapeutic effectivity. Current scientific research found that this curative power is connected with the isolated and identified substance called ursolic acid (Seeram et al. 2002; Jayaprakasam et al. 2005).

Cornelian cherry allows preparation of many medicaments based on:

1. Leaves and powdered dry fruits helping patients with haemorrhoids and diarrhoea difficulties (Damirov et al. 1983; Asadov et al. 1990).
2. Substances extracted from the bark and concentrated juice are in traditional medicine applied to cure skin injury and furunculosis—ulcerous skin (Gubanov et al. 1976; Lewis and Elvin-Lewis 1977; Damirov et al. 1983; Asadov et al. 1990; Muhammed 1993).
3. Scientists in the former Soviet Union found out that the fruit flesh and oil pressed for stones are suitable to heal and regenerate inner and outer damaged epidermic tissues (Tzitzin et al. 1963; Lewis and Elvin-Lewis 1977; Damirov et al. 1983; Asadov et al. 1990).
4. Fruits, bark and leaves possess antimicrobial activity against several species—*Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* (Roja and Smith 1977; Asadov et al. 1990).

Last but not least, presently increasing attention to foodstuffs is given constantly which could help with preventive measures against body fat accumulation, especially when induced by erroneous nutrition, and/or with a possible lowering of diabetes and cardiovascular diseases. To solve these important problems of the current population, it would be advisable purposefully and effectively to utilise the means given to us by nature—the fruits and different products made of Cornelian cherry (*Cornus mas* L.) plant parts.

15.4 True Service Tree (*Sorbus domestica* L.)

Presently the true service tree is ranked among less known fruit trees with a low degree of domestication. Despite this poor position of *Sorbus domestica* L., its fruits or even whole trees are used for preparation of traditional food, for medicine/cosmetics production and for landscape forming. The fruit has astringent, anti-inflammatory and pain-relieving properties.

The first forms of this genus could be dated into the limestone era as it is supposed by Gabrijeljan (1978). Theophrastus in antique Greece described this species in the years 371–285 BC documenting that it is cultivated in the Mediterranean area over 2000 years as a fruit tree.

The origin of the *Sorbus domestica* L. is in Asia Minor from where it was gradually introduced to Balkans and later to the rest of Europe. In Central Europe it is occurring in southern regions up to altitude of 650 m above the sea level. The appropriate climatic conditions for this plant are limited to the South and Central Europe with the highest occurrence in the Balkan Peninsula, Italy and south of France (Rotach 2003). In the majority of Central European countries, this species is considered as a valuable biological resource, which should be preserved and used in practice for different purposes (Rotach 2003).

There are available data showing that in 1992 in Switzerland approximately 170 trees of *Sorbus domestica* L. were registered, in Austria around 100–150 prevailingly old trees, Germany 3500–4500, countries of the former Yugoslavia and Greece around 10,000, Czech Republic 200–300, Luxembourg 33 and Slovakia 350 trees (Pagan and Paganova 2000).

15.4.1 Genus Sorbus *Botanic Characterisation*

The true service tree is part of the genus *Sorbus* in the Rosaceae family, where over 80 species are listed. It is quite difficult to distinguish individual species. The name *Sorbus domestica* L. is older than 2000 years. The name '*domestica*' has been introduced by Matthiolus in 1953 in the book on herbs edited in Prague and it is the Latin root for homely and domestic (von Schmeling and W 1992). The word '*sorbus*' is derived from the Arabic '*sorben*' or from Celtic '*sor*', both defining sour, constrictive character (Kytka 1987). It has been documented that people utilised these fruits for a very long time.

The true service tree usually grows in the tree form with height of 10–16 m with a crown wide up to 20 m. Crowns are mostly pyramidal, but occur rounded or sometimes semirounded forms as well. The bark of a young tree is smooth of grey-brownish colour, later transforming into more rough with lengthwise furrowed scratches. Under suitable conditions, these trees could survive up to 500–600 years.

The true service tree has oddly assembled leaves usually with 6–10 yokes. Their colour is sharp green with several shades, from the bottom side olive green and in autumn dyed to dark red.

Inflorescence of the true service tree are formed by rich multiarmed semiglobular corymb. In Slovakia, the flowering begins in May lasting up to June. The flower is white or white rose having five corolla petals with length of 50–70 mm and medium diameter of 16–18 mm (Fig. 15.5). The flowers are regular and hermaphroditical.

The fruit of the true service tree is a pome. Based on the study of 120 genotypes grown in Slovakia (Brindza et al. 2009), the variability of a medium fruit weight in a range of 4.91–18.64 g was determined (Table 15.12). The average fruit weight has been determined in a range of 2.9–22.6 g (Naništova 2005) von Schmeling, W (1992).

In this evaluated population the mean fruit height was in the range of 19.84–36.29 mm. Pagan and Paganova (2000) determined the same trait range as 15–40 mm

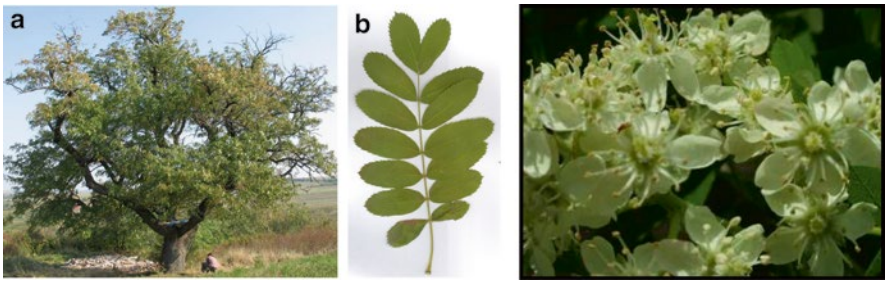

Fig. 15.5 Crown habitus (**a**), leaf (**b**) and inflorescence of the true service tree (*Sorbus domestica* L.). Photo: J. Červenakova, 2005

Table 15.12 Trait variability of true service tree (*Sorbus domestica* L.) fruits

Traits	*n*	Min	Max	x	V %
Fruit height [mm]	102	19.84	36.29	25.30	13.07
Fruit width [mm]	102	18.91	32.58	24.94	11.67
Fruit shape index	102	0.86	1.40	1.02	9.93
Stem length [mm]	95	0.50	14.12	4.68	54.97
Fruit weight [g]	120	4.91	18.64	10.14	30.45

and Naništova (2005) 14.3–37.2 mm. The mean fruit width measured with our genotype collection was found in the range of 18.91–32.58 mm (Table 15.12). The mean fruit diameter has been fixed in ranges of 15–20 mm by Karpati (1960), 20–30 mm by Pagan and Paganova (2000), 13–34 mm by Bignami (2000) and 13.1–33.8 mm by Naništova (2005).

Significant variability was found with the fruit shape as well when studying the genotype collection. The fruits are mostly pear-like, apple-like and of rounded shapes. Miko and Gažo (2002) in their experiments made on Slovakia territory identified six basic morphological groups of true service tree genotypes according to their fruit shapes—those being flatly round, round, pear-like, pear conical, oviform and cylindrical—fully corresponding with the results of Bignami (2000).

Significant differences between genotypes were detected in the case of fruit colour as well. During the maturation period, yellow, yellow-green or orange fruits with so-called red spot of various intensity on the sunny site were found. Moreover, the fruit surface is covered by a number of rusty sclerenchymatous idioblasts. Matured fruit is soft, coloured to cinnamon shade with white lenticel dimension dots. They are fairly aromatic with sweet and sour taste.

The fruit yield of freely growing true service trees in Slovakia ranged from 300 to 500 kg. Similar data were reported by Cvopa (1985). This species is known for irregular fruit-bearing capacity—there is a typical principle that in 3 years one will get good yield just two times. Owing to crown dimensions, fruit picking is quite difficult; therefore, it is usual to shake the fruits down off the tree. Another possibility is to comb, but then the fruits should be stored for a few days to allow their maturation. In one fruitage, 4–15 fruits are formed as documented by Bignami (2000).

Fig. 15.6 Flower (**a**) and seeds (**b**) of true service tree (*Sorbus domestica* L.). Photo: J. Červenakova, 2005

Table 15.13 Trait variability of true service tree (*Sorbus domestica* L.) seeds

Traits	*n*	Min	Max	$\bar{x}$	V %
No. of seeds in fruit [pcs]	63	1.11	4.31	2.15	34.28
Seed weight in fruit [g]	63	0.03	0.18	0.09	35.48
Seed length [mm]	65	5.90	10.61	6.76	10.35
Seed width [mm]	65	4.23	5.98	5.07	5.31
Seed thickness [mm]	65	1.30	3.87	1.72	19.29
Seed shape index	65	1.21	1.94	1.34	7.67
Weight of ten seeds [mm]	65	0.28	0.33	0.30	4.54

Seeds are economically esteemed as well. In our experiments in one fruit, we found 1–5 seeds (Table 15.12). Pagan and Paganova (2000) found 2–3 seeds, Bignami (2000) 2–5 (rarely even up to ten) Miko and Gažo (2002) 1–5 seeds.

Seeds are shining, shaped widely oviform with tapered base, coloured light brown, cinnamon or even dark brown (Fig. 15.6). Length of seeds ranged from 5.9 to 10.6 mm, width 4.2–5.9 mm and thickness 13–3.87 mm (Table 15.13), while in the literature reported data for seed length are 5–8 mm and width 3–6 mm. Pagan and Paganova (2000) and Cvopa (1985) presented similarly 6 mm for the length and 1.5–2 mm for the width.

From the fully matured fruits could be gained 80–89 % of pure flesh by rubbing (Fig. 15.7). Even the garbage produced by fruit rubbing consisting of stems, skin, core and seeds has some economic value.

15.4.2 Biochemical Characterisation

Chemical analyses of true service tree fruits and other plant parts showed a significant variability in composition. Dry weight of fresh fruit ranged from 18.1 to 23.8 %, while the frozen sample range was 31.7–32.2 % and frozen flesh 23.1–27.8 %. In the freeze-dried samples of flesh and exocarp, the dry weight values of 94 % and 95 %, respectively, were determined. Dry weight of seeds represents 93.2 % (Table 15.14).

Fig. 15.7 Fruits (**a**), rubbing process of matured fruits, and (**b**) paste/flesh of true service tree (*Sorbus domestica* L.) fruits. Photo: J. Červenakova, 2005

Table 15.14 Chemical composition of selected fruit parts of true service tree (*Sorbus domestica* L.)

Samples	Dry weight (%)	pH	Total saccharides mg.kg^{-1}	Organic acids mg.kg^{-1}	Dyes
Fresh fruits	18.1–23.8	3.4–3.7	–	–	–
Frozen fruits	31.7–32.2	3.6–3.8	25.5–26.0	0.6–0.7	0.40
Frozen flesh	23.1–27.8	3.6–3.7	11.4–19.0	2.0	0.35–0.42
Freeze-dried matured flesh	94.0–94.4	3.9–4.0	59.0–71.0	1.8–2.5	6.55–7.33
Freeze-dried exocarp	95.0	3.7–4.1	56.8–65.0	2.0	7.75
Seeds	93.2	–	8.0	2.0	–

Table 15.15 Saccharide content in different fruit parts of the true service tree (*Sorbus domestica* L.)

Fruit part	Fructose (%)	Glucose (%)	Reducing sugars (%)
Flesh	8.6–12.0	4.2–7.7	13.9–18.0
Exocarp	7.9–11.1	3.6–5.5	12.0–16.1
Seeds	1.6–2.0	0.47–0.76	1.6–2.5

Results in Table 15.14 are complemented by the pH values as well. True service tree fruits contain considerable amount of digestible saccharides, namely, glucose and fructose, as shown in Tables 15.14 and 15.15. Further, there are para-ascorbine acid and sorbit (Kresanek and Krejča 1977), and glycide occurring in some plant species included into Rosaceae family. Exocarp and mesocarp contain many times higher content of fructose in comparison to other plant and animal species (Gunišova 2006).

In the fresh fruit flesh, the protein concentration was 0.44–0.65 g.kg^{-1}, in exocarp 0.38–0.56 g.kg^{-1} and in seeds 29.81–34.20 g.kg$^{-1.}$ Seed proteins contain an interesting amount of essential amino acids (see Table 15.16), which is even higher than in the eggs, with exception of lysine (Gunišova 2006). Vitamins found in fruit play again an important role as documented in Table 15.17 and fatty acids in Table 15.18. Several authors reported an elevated amount (1.61 %) of pectins (Cvopa 1985), organic acids and dyes (Albrecht 1993).

Table 15.16 Amino acid content in the seeds of the true service tree (*Sorbus domestica* L.)

Amino acid	Arg	Gly	His	Iso	Asp	Glu	Leu	Lys	Phe	Pro	Ser	Thr	Tyr	Ala	Val
Seeds	2.8	1.5	0.6	1.4	2.4	7.0	1.6	0.7	1.2	1.4	0.7	0.6	0.7	1.2	1.1

Table 15.17 Vitamin content in different fruit parts of the true service tree (*Sorbus domestica* L.)

Fruit part	B1 $mg.kg^{-1}$	B2 $mg.kg^{-1}$	B6 $mg.kg^{-1}$	C $mg.kg^{-1}$	E $mg.kg^{-1}$
Flesh	0.80–1.30	0.78–2.10	0.98–2.60	0.89–0.98	1.0–2.30
Exocarp	1.50	<1	<1	0.85	<1
Seeds	4.30	–	–	<1	18.05

Table 15.18 Fatty acid content in seeds of the true service tree (*Sorbus domestica* L.)

Indicator	Pentadecanoic	Palmitic	Stearic	Oleic	Linoleic	Linoleic isomer
Formula	C 15:1-10c	C 16:0	C 18:0	C 18:1-c	C 18:2-9c, 12c	C 18:2-c10, c12
RT (min)	10.18	11.04	14.47	14.94	15.89	17.98
Content (%)	25.56	11.46	2.26	20.80	24.31	9.33

Termentzi et al. (2009) determined in the true service tree fruits novel compounds belonging to the hydroxybenzoic acid derivatives, polyphenolic phenylpropanoid derivatives, quercetin glycosides, flavonol glycoside, quercetin dimmer and biphenyls.

15.4.3 Economically Exploited Plant Parts

Already in the Roman times, people highly appreciated the true service tree qualities. We should thank everybody who was engaged in the preservation activity to keep this species for us and for the future generations. Anyway, the tree numbers or better said the species participation on the total fruit production is nowadays distinctly diminished. Actually, this species is strongly endangered and occurs very scarcely. Service tree was fairly popular in the past; now it is dwindling in our villages. Therefore, this species should be taken as highly endangered and we should not lose such economically perspective tree.

It is regrettable that the service tree is presently ranked among the so-called forgotten or less utilised fruit species. This species is producing fruit directly consumable, when fully matured. When it has a browned exocarp and yellow flesh, the fruit is sweet or slightly sour and aromatic. In some localities, it is returning in the former position enlivening the folk food. Fruit is in the Slovak kitchen traditionally used in many forms—dried, candied, canned or processed to mixed marmalades and jams (Cvopa 1985). In several countries the fruits are used for wine conservation, e.g. in France, Germany and Switzerland, and already in the eighteenth century, the must

made of service tree fruits was added to apple must, what resulted in natural cleaning resulting in improved taste, better quality and longer lifetime without addition of other preservation substances. The mixing ratio of apple wine with the service tree fruit must is advised from 3:1 to 3:2. Moreover, there is a bonus effect—the adjusted apple wine is more friendly to the consumer stomach and intestines due to better digestion. Apple juice producers in some regions of Germany use the juice of the proanthocyanidin-rich service tree fruits ('Speierling', *Sorbus domestica* L.) as a taste-improving additive (Ölschläger et al. 2004). Fruits are suitable for preparation of high-quality liqueurs and distillates, which are on the market sold for interesting prices (Kytka 1987; Pagan and Paganova 2000).

Dried fruits are suitable for the production of marmalades, jelly and fruit paste. Dried and grated fruits are used for dusting of sweet meals and cakes. In Bulgaria, the fruits are sterilised in sweet and sour brine, in Ukraine they are added to confection and cookies, and in many countries, it is produced as a fine liqueur named 'sorbet'.

The wood of true service tree contains high amount of tannin, and the bark is a good resource for production of tannin (Cvopa 1985; Rotach 2003). Seeds are not consumable owing to amygdaline which could be converted to highly toxic hydrogen cyanide (Velgosova and Velgos Š 1998).

15.4.4 Phytotherapeutic Characterisation

From the medicinal point of view, the service tree fruits have mildly diuretic, laxative, antirheumatic and antipyretic effects. In the past, they were very requested, and in folk medicine they are used as remedy for digestive disorders of both, people and utility animals. Indeed, their consumption regenerates the normal regime of intestines, as documented in many literature sources. In the case of stomach difficulties or diarrhoea, processed fruits were recommended, which should be after picking cut in halves and dried in the sun. Low concentration of phytoncides occurring in the fruits has antibacterial and antimycotic effects; moreover there was a registered improvement in the blood circulation in veins, inhibition of blood coagulation, hypertension and blood vessel elasticity.

Labuda et al. (2005) studied the matured fruits of service tree growing in Slovakia contaminated by microscopic fungi. In the tested fruits was detected an endogenous contamination by microscopic fungi, although previously the surface of all fruits was sterilised. *Cladosporium cladosporioides* (Fresen.) de Vries, *Alternaria alternata* (Fr.) Keissl and *Penicillium expansum* Link with the occurrence frequency of 88, 63 and 54 %, respectively, were the dominant species contaminating the fruits. Another 24 species were found in the matured fruits, although their incidence was fairly lower. What is important, all tested isolates of *Penicillium expansum*, *Penicillium carneum* Frisvad, *Penicillium paneum* Frisvad and *Penicillium griseofulvum* Dierckx produced mycotoxin—patulin. In a few cases were detected producers of citrinin, griseofulvin and zearalenone. It seems that the maturation process of service tree fruits is connected with the occurrence of microscopic fungi, which

are known as species responsible for the fruit rotting. At the same time, it should be noted that the service tree fruits could be taken as potential donors of fungal secondary metabolites including some mycotoxins.

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Chapter 16
Antioxidant Properties and Health Benefits of Date Seeds

Moran Brouk and Ayelet Fishman

16.1 Introduction

The date palm (*Phoenix dactylifera* L.) is considered a symbol of life in the desert, because it tolerates high temperatures, drought, and salinity more than many other fruit crop plant species. It is one of the oldest trees from which man has derived benefit, and it has been cultivated since ancient times. Remains of dates have been found on a number of Neolithic sites, particularly in Syria and Egypt suggesting that they were being eaten by man as much as 7000–8000 years ago (Morton 1987; El-Juhany 2010). The world production of dates is 7.9 million tons per year with Egypt, Iran, and Saudi Arabia being the largest producers (FAOSTAT 2010).

For thousands of years, the fruits (dates) of the date palm were consumed as staple food and believed to have remarkable nutritional, health, and economic value (Al-Shahib and Marshall 2003; Mansouri et al. 2005; Al-Farsi and Lee 2008a; Rock et al. 2009). Dates provide a good source of energy (213 and 314 kcal/100 g—fresh and dried respectively) mostly due to the high carbohydrate content, averaging 54.9 and 80.6 g/100 g for fresh and dried fruit, respectively (Al-Farsi and Lee 2008a).

In addition to numerous evidences regarding the valuable antioxidant and anti-mutagenic properties of the date palm fruit (Vayalil 2002; Mansouri et al. 2005; Hong et al. 2006; Rock et al. 2009), recent studies indicate that the seeds of the dates also possess high levels of phenolics, antioxidants, and dietary fiber, even higher than those present in the date flesh (Hamada et al. 2002; Al-Farsi et al. 2007). This review focuses on the antioxidant properties and health benefits of date seeds and describes a new coffee-substitute drink based on date seeds.

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16.1.1 Composition of Date Seeds

It is estimated that the date seeds constitute approximately 10 % w/w of the total weight of the ripe date fruit (Hamada et al. 2002; Habib and Ibrahim 2011). The seed composition depends on the variety but contains approximately 8–10 % moisture, 4.0–5.6 % protein, 6.0–8.5 % oil, 1 % ash, and 75–81 % carbohydrates including soluble sugars and mainly dietary fibers (Devshony et al. 1992; Besbes et al. 2004b; Al-Farsi et al. 2007; Nehdi et al. 2010; Al Juhaimi et al. 2012). The soluble sugars comprise glucose, fructose, raffinose, stachyose, sucrose, and galactose in average concentrations of 3.5, 3.8, 3.2, 3.7, 3.5, and 2.2 g/kg respectively (Al Juhaimi et al. 2012). Date seeds are rich in potassium 350–400 mg/100 g, phosphorus 200 mg/100 g, magnesium 70 mg/100 g, and calcium 40 mg/100 g and to a smaller extent iron—10–20 mg/100 g (Al-Shahib and Marshall 2003; Nehdi et al. 2010; Al Juhaimi et al. 2012).

At room temperature the seed oil is a yellow liquid having a refractive index of 1.456 (Nehdi et al. 2010). Carotenoids present in substantial amounts (5.5 mg/100 g oil) are responsible for the visible color measured at 420–470 nm (Nehdi et al. 2010). The fatty acid composition of date seed oil comprises nearly 50 % of monounsaturated fatty acids primarily due to abundance of oleic acid. Saturated fatty acids account for 40 % with lauric (C12:0, 18 %), myristic (C14:0, 10 %), and palmitic (C16:0, 12 %) being the most plentiful, and linoleic acid (C18:2) accounts for the remaining 10 % (Besbes et al. 2004b; Saafi et al. 2008; Al Juhaimi et al. 2012). These values can slightly change depending on the cultivar, with the Allig brand for example, containing only 27 % saturated fatty acids and 23 % polyunsaturated fatty acids (Besbes et al. 2004b).

Sterols comprise the main unsaponifiable fraction in date seed oil with a level of 300–350 mg/100 g oil (Nehdi et al. 2010). Differences in sterol composition make them suitable for determining the botanical origin of oils and hence detecting adulteration. The major sterol in date seed oil is β-sitosterol (75 %) followed by campesterol and Δ5-avenasterol (10 % each) similarly to few other vegetable oils such as olive oil and grape seed oil (Besbes et al. 2004a). Date seed oil contains 50 mg of tocopherols and tocotrienols per 100 mg of oil (Nehdi et al. 2010) with a profile similar to palm oil (Besbes et al. 2004a). The major tocols are α-tocotrienol, γ-tocopherol, and γ-tocotrienol at 34.01, 10.30, and 4.63 mg/100 g, respectively. These compounds possess antioxidant properties and they are active as vitamin E, albeit to different extents, making them particularly important for human health.

In recent years phenolic antioxidants have received increasing attention due to their beneficial effects on human health and their high potential in preventing a variety of pathologies such as cardiovascular diseases, diabetes, and cancer (Crozier et al. 2009). Their antioxidant activity is attributed to their ability to donate a hydrogen or electron and delocalize the unpaired electron within the aromatic structure (Fernandez-Panchon et al. 2008). Phenolic antioxidants are widely distributed in the plant kingdom and thus in a wide variety of food products including dates (Fernandez-Panchon et al. 2008; Saafi et al. 2009). Indication of the total phenolic

content in a sample can be provided by the Folin-Ciocalteu method. This method is an electron transfer-based assay which measures the reducing capacity of a solution, and has been correlated with phenolic content (Prior et al. 2005). Al Farsi and Lee (Al-Farsi and Lee 2008b) examined the phenol content of Mabseeli date seeds grown in Oman and a level as high as 184 mg ferulic acid equivalents/100 g wet weight was obtained depending on the extraction solvent (water vs. 50 % acetone) and on experimental parameters such as temperature, solvent/seed ratio, and number of extraction cycles. In a different study, levels of 3102–4430 mg gallic acid equivalents/100 g fresh weight were reported for three varieties (Al-Farsi et al. 2007). Various factors such as variety, growing conditions, maturity, season, geographic origin, fertilizer, soil type, storage conditions, and amount of sunlight received, among others, might be responsible for the observed differences, as well as experimental protocols (Al-Farsi et al. 2007). The specific phenolic compounds identified using high performance liquid chromatography (HPLC) are hydroxylated derivatives of benzoic acid (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, and vanillic acid) and cinnamic acid derivatives (caffeic acid, *p*-coumaric acid, ferulic acid, *m*-coumaric, and *o*-coumaric acid) (Al-Farsi and Lee 2008b). Compounds typical of olives such as tyrosol, hydroxytyrosol, and oleuropein were also reported (Besbes et al. 2004a). The oxygen radical scavenging capacity (ORAC) values give an indication of the ability of different compounds to scavenge oxygen radicals in vitro which is a true measure of antioxidant performance, through a hydrogen atom transfer reaction mechanism (Prior et al. 2005). While the exact relationship between the ORAC value of a food and its health benefit has not been established, it is believed that foods higher on the ORAC scale will more effectively neutralize free radicals in biological systems as well. Al-Farsi and co-workers reported values of 580–929 and 146–162 Trolox equivalents per gram fresh weight for date seeds and date fruits, respectively (Al-Farsi et al. 2007).

16.2 Health Attributes of Date Seeds

According to the free-radical theory of aging, foods containing antioxidants such as vitamins or polyphenolic compounds will slow the oxidative processes and free-radical damage that can contribute to age-related degeneration and disease (Manach et al. 2004; Fernandez-Panchon et al. 2008). Recent studies show that date fruit consumption (and mainly the Hallawi variety) by healthy subjects, confers beneficial effects on serum triacylglycerol and oxidative stress and does not worsen serum glucose and lipid/lipoprotein patterns, and thus can be considered an anti-atherogenic nutrient (Rock et al. 2009). Owing to their high level of antioxidants (Al-Farsi et al. 2007), date seeds were also examined recently for their health benefits. Male rats were fed a basal diet containing 0, 70 or 140 g/kg date seeds for 30 days (Habib and Ibrahim 2011). Indication of oxidative damage was assessed in the liver and serum, and antioxidant status was assessed in the liver. The results showed that date seeds

significantly reduced liver and serum malondialdehyde (a lipid peroxidative damage product) and serum lactate dehydrogenase and creatine kinase. The levels of α-tocopherol, ascorbic acid, and glutathione and the activities of catalase, glutathione peroxidase, and superoxide dismutase in the liver were not altered by dietary treatments. Blood counts and serum biochemical parameters were not altered as well. Therefore, Habib and Ibrahim concluded that the results obtained suggest a protective effect of date seeds against in vivo oxidative damage, possibly through the action of their bioactive antioxidants (Habib and Ibrahim 2011).

Ben Abdallah et al. investigated the effects of date seed oil on epididymal sperm characteristics, testicular antioxidant enzyme activities, and testicular lipid peroxidation in male mice treated with different doses by intraperitoneal route for 4 weeks (Ben Abdallah et al. 2009). They found a significant increase in sperm count, motility, and viability in all treated animal groups in comparison with the control group. In addition, the percentage of abnormal sperm was significantly lower in the treated groups. In a subsequent study, the researchers evaluated the effect of date seed oil on human male spermatozoa under induced hydrogen peroxide stress conditions (Ben Abdullah et al. 2009). Results showed that incubation with H_2O_2 alone led to a significant increase in lipid peroxidation (57.83 %) associated with a significant decrease in sperm motility, sperm viability, and percentage of reacted acrosome. Date seed oil improved sperm motility and protected spermatozoa against the deleterious effects of H_2O_2 on motility, viability, acrosome reaction, and lipid peroxidation. Human epidermal keratinocytes were also shown to be protected against H_2O_2-induced stress by date seed oil (Dammak et al. 2010).

16.3 Uses of Date Seeds in Food

16.3.1 Bread Enrichment

Derived from the growing world production and consumption of dates, the amount of date seeds is also growing rapidly. This waste product of the date industry is typically discarded or used as fodder for animals (Hamada et al. 2002; Besbes et al. 2004a; Al-Farsi and Lee 2008b). Potential uses of date seed oil are the cosmetics and pharmaceutical industries especially in light of the recent promising in vivo and in vitro results in animals and human (Ben Abdallah et al. 2009; Ben Abdullah et al. 2009; Dammak et al. 2010). With respect to usage in food, date seed milled into coarse powder was incorporated into Saudi Mafrood flat bread at 0, 5, 10, and 15 % replacement levels in comparison to wheat bran (Almana and Mahmoud 1994). Breads containing milled date seed fractions were slightly lower in protein and slightly higher in fat but substantially higher in total and soluble dietary fiber than the control bread. Coarse fraction at 10 % replacement level was found to increase the total dietary fiber contents in bread by fourfold without a significant adverse effect on bread quality. Wheat bran at the same level of replacement increased

the total dietary fiber content in bread by only threefold. Furthermore, breads containing 10 % milled coarse fraction were better or similar to the corresponding wheat bran control in sensory evaluation (Almana and Mahmoud 1994).

16.3.2 Coffee-Substitute Hot Beverages

By roasting and grinding date seeds, they can be exploited for the preparation of hot beverages as coffee-like brew. Such a drink, made of roasted date seeds, has been traditionally consumed in the Arabic world (Rahman et al. 2007). With the growing awareness regarding health foods and functional foods, coffee-substitute drinks which can provide a high antioxidant content accompanied by low caffeine intake are of public interest. Recently, a commercial date seed-based hot beverage marketed by the brand name Espressodate® was launched in Israel. This date-based coffee is prepared from roasted and grinded date seeds, blended with 5 % coffee beans and sugar, and commercialized either as instant or espresso coffee. We analyzed this drink on a serving cup basis in order to evaluate the actual consumption of antioxidants by consumers in comparison to coffee (Table 16.1).

Table 16.1 Total phenolic content and oxygen radical scavenging capacity (ORAC) of hot beverages

Method of preparation	Sample	Total phenolics[a]	ORAC values[b]
Suspension in hot water	Espressodate[c]	4.5 ± 0.16	17 ± 2
	Black grounded coffee	9.1 ± 0.8	24 ± 0.4
	Espressodate with 10 % unroasted Majul seeds	7.72 ± 0.07	23 ± 2
	Unroasted Majul seeds	16 ± 3	38 ± 4
Espresso machine	Espressodate	10.5 ± 0.02	35 ± 3
	Espresso coffee	39 ± 4	75 ± 5
	Espressodate with 10 % unroasted Majul seeds	20 ± 2	44 ± 1
Filter preparation	Filter drink from Espressodate	2.28 ± 0.04	11 ± 0.6
	Filter drink from Espressodate with 40 % unroasted Majul seeds	7.4 ± 0.1	19 ± 2
Soluble instant coffee	Taster's choice	5.3 ± 0.4	21 ± 0.4
Instant coffee substitutes	Chicory based	1.31 ± 0.03	2.1 ± 0.1
	Organic barley	3.3 ± 0.1	10 ± 0.8
	Decaffeinated instant coffee	5.4 ± 0.05	20 ± 1

Standard deviations are presented for experiments which were performed in duplicates or triplicates

[a]Measured by the Folin-Ciocalteu assay and expressed as gallic acid equivalents (mM)

[b]Oxygen radical scavenging capacity expressed as mM Trolox equivalents

[c]Espressodate is a commercial drink made from roasted date seeds with 5 % coffee beans

The Folin-Ciocalteu values of the date seed-based hot beverages ranged from 2.28 ± 0.04 to 20 ± 2 mM gallic acid equivalents, depending on the sample preparation procedure (Table 16.1). The results indicated that date seed beverage made with an espresso machine contains more phenols than a hot water suspension and more than filter brew. The content decreases from 10.5 > 4.5 > 2.28 mM gallic acid equivalents. This is despite the fact that the amount of coffee powder used for preparation is in the opposite order (14 g for espresso, 20 g for hot water-based date coffee, 16 g for filter brew). Apparently, the espresso machine extracts more antioxidants than just hot water or a filter process. In addition, from the results it is evident that date seed coffee substitute contains less phenolics than coffee. The amount is only 50 % when the coffee is prepared using hot water (4.5 ± 0.16 vs. 9.1 ± 0.8), and 27 % for espresso preparation. However, comparing to soluble instant coffee (Taster's Choice®) the values are similar. Moreover, other coffee substitutes, as chicory and organic instant barley, contain only 29 % and 73 % phenolics, respectively, relative to that of date seed beverage prepared by suspending in hot water.

It was suggested that the phenolic content declines during the roasting process of the seeds. This was confirmed by extracting unroasted seeds with hot water in a similar way to the date drink (Table 16.1). The unroasted Majul seeds contained 16 ± 3 mM gallic acid equivalents compared to 4.5 ± 0.16 for date seed coffee substitute in hot water. Therefore, unroasted seeds were added to the date seed drink in order to evaluate if there is an overall improvement in phenolic content. In most cases, especially for the espresso machine-derived drink and filter preparation, the phenolic content indeed improved.

The total phenolic compounds of ready-to-drink orange juice and nectar was evaluated and reported to range from 18.7 to 54.2 mg of gallic acid per 100 mL beverage (Stella et al. 2011). Orange juice is considered by the consumers as a healthy beverage, providing a good source of antioxidants. Nevertheless, the phenolic contents of the juice drinks tested were lower than those obtained for the Espressodate beverages (the orange juice Folin-Ciocalteu values correspond to 1.1–3.2 mM gallic acid equivalents).

The ORAC values presented in Table 16.1 correspond well with the total phenol content. Espressodate beverage prepared with an espresso machine exhibited higher activity than hot water extraction or using a filter method. This is the same order of activity obtained for the Folin-Ciocalteu method: espresso > hot water > filter. Likewise, black grounded coffee has higher ORAC values than date seed coffee substitute (1.4-fold), similarly to the Folin-Ciocalteu values (twofold). Moreover, the unroasted seeds showed a significant level of ORAC values. Adding unroasted seeds to date seed beverages prepared in different methods resulted in a moderate increase in ORAC values.

Identification of specific antioxidants in the date seed-based hot beverages using HPLC-mass spectra analysis revealed the presence of several polyphenols similar to those found in the date fruits by Mansouri et al. (Mansouri et al. 2005). Among these polyphenols are the sinapic acid and hydrocaffeic acid. In addition, the presence of cinammic and caffeic acid derivatives was detected together with small amounts of protocatechuic acid, which was previously reported in oils extracted from date seeds (Besbes et al. 2004a).

16.4 Conclusions

Date seeds contain significant amounts of beneficial food ingredients such as oleic acid, dietary fibers, and polyphenols. These compounds have been associated with reduced incidence of cardiovascular diseases and improved overall well-being. Date seeds have been recently exploited for production of staple foods such as bread and hot beverages without impairment to sensory characteristics. Using an undesired by-product of the date industry as a raw material for functional foods has great economic potential and is thus expected to grow in the coming years.

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Chapter 17
Antioxidant Capacity of *Capsicum chinense* Genotypes

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17.1 Introduction

Antioxidants are effective lipid peroxidation inhibitors. They play a vital function in food preservation and in the cell defense mechanism against oxidative damage and are an important tool in the prevention of degenerative diseases such as Alzheimer's and cancer (Sun et al. 2007). Fruits and vegetables contain a wide variety of antioxidant components which protect against harmful free radicals and provide a number of other health benefits, including lower incidence and mortality rates of cancer and heart diseases (Velioglu et al. 1998). Their combination of color, flavor, and nutritional value has made pe ppers widely popular as a food ingredient. Many of these benefits originate in peppers' wide array of phytochemicals and high contents of vitamin C and carotenoids, which are vital nutritional antioxidants in the human

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diet. Vitamin C is the most abundant water-soluble antioxidant in the human body and is believed to aid in cancer prevention by inhibiting formation of N-nitrous compounds in the stomach and stimulating the immune system. Carotenoids are fat-soluble antioxidants found in many fruits and vegetables and are required for human epithelial cellular differentiation. Peppers are also known to contain high levels of the phytochemicals known as neutral phenolics or flavonoids, important antioxidant components which may reduce risk of degenerative diseases (Zhang and Hamauzu 2003).

Capsicum genus fruits owe their intense orange and red colors to carotenoid pigments synthesized during fruit ripening. The carotenoids responsible for final fruit color are capsanthin, capsorubin, and capsanthin 5,6-epoxide. These can only be found in the *Capsicum* genus (Guil-Guerrero et al. 2006). The aim of this chapter was to identify and quantify the characteristic compounds (e.g., polyphenols, carotenoids, ascorbic acid) responsible for antioxidant capacity in habanero pepper *Capsicum chinense* Jacq. genotypes grown in Yucatan state, Mexico.

17.2 Extraction and Analysis of *Capsicum chinense*

17.2.1 Capsicum chinense *Jacq. Genotypes*

Capsicum chinense Jacq. genotypes were donated by the germplasm bank of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, of Mexico. Material was selected according to mature fruit color 82 days after transplantation. Selected colors and materials were orange (Orange and L-184), red (Red and L-149), yellow (L-110 and L-36), and brown (L-37). Pepper fruits from the selected genotypes were ground in a blender at a 1:2 (fruit/distilled water) ratio. Samples were frozen and dried at −45 °C and 133×10^{-3} mbar in a lyophilizer.

17.2.2 Capsicum chinense *Jacq. Extracts*

Extracts were produced by first suspending 1 g (d.b.) lyophilized sample in 20 mL methanol aqueous solution at 80 % (v/v) and stirring for 3 h at room temperature. The suspension was centrifuged at $2500 \times g$ for 15 min, the supernatant separated and a second extraction done. The two supernatants were mixed and filtered through No. 41 Whatman paper.

17.2.2.1 Total Polyphenols

Total polyphenol concentration in all samples was quantified by the Folin-Ciocalteu method, according to Singleton et al. (1999). The reaction mixture consisted of 500 μL sample in 4.5 mL water to which 200 μL Folin-Ciocalteu and 500 μL saturated

Na_2CO_3 solutions were added. This mixture was stirred and 4.3 mL distilled water added. Absorbance was read at 765 nm after 1 h. Total polyphenol content was calculated as Trolox equivalents.

17.2.2.2 Total Carotenoids

Total carotenoid concentration was quantified according to Rodriguez-Amaya and Kimura (2004). Briefly, 1 g fresh sample was homogenized in 20 mL acetone and the supernatant decanted. This process was repeated until attaining complete removal of all pigments. The sample was filtered and washed with 30 mL acetone, the acetone evaporated, and the dry sample dissolved in 60 mL petroleum ether. The resulting solution was filtered, transferred quantitatively to a 100 mL volumetric flask, and volume completed with petroleum ether. Of this solution, 2 mL was placed in a test tube with 8 mL petroleum ether. Absorbance was read at 475 nm and concentration calculated with a β-carotene curve.

17.2.2.3 Ascorbic Acid

Ascorbic acid concentration in all samples was quantified using the official AOAC titrimetric method (AOAC 1990). Briefly, 2 mL of 3 % metaphosphoric acid, 8 % acetic acid sample extract was titrated with indophenol solution (25 % 2,6-dichlorophenol and 2 % $NaHCO_3$ in water) until a light but distinct rose pink color appeared and persisted for more than 5 s.

17.2.2.4 ABTS Decolorization Assay

Following Pukalskas et al. (2002), the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation was produced by reacting 10 mL ABTS stock solution with 40 μL $K_2S_4O_8$ solution and allowing the mixture to stand in darkness at room temperature for 16–17 h before use. The percentage decrease in absorbance at 734 nm was calculated and plotted as a function of Trolox antioxidant concentration for standard reference data.

17.2.2.5 ß-Carotene Decolorization Assay

Antioxidant activity was quantified according to Miller (1971). The assay is based on determining the decolorization exhibited by ß-carotene in response to the action of linoleic acid oxidation products. In a test tube, 1 mL of 0.2 mg/mL ß-carotene in chloroform was added to a mixture of 20 mg linoleic acid and 200 mg Tween 20. The chloroform was evaporated and 50 mL bidistilled water added. Aliquots (2.5 mL) of this mixture were added to tubes containing 100 μL sample. Water was used

as a reference blank and 2,6-di-tert-butyl-4-methyl phenol (BHT) dissolved in 96 % ethanol was used as a positive control. Samples were incubated at 50 °C; aliquots taken at 30, 60, 90, and 120 min; and absorbance measured at 470 nm.

17.2.2.6 Statistical Analysis

All results were analyzed using descriptive statistics with a central tendency and dispersion measures. One-way ANOVAs were run to evaluate polyphenols, carotenoids, and ascorbic acid contents, as well as ABTS and β-carotene decolorization. A LSD multiple range test was applied to determine differences between treatments. All analyses were done according to Montgomery (2004) and processed with the Statgraphics Plus version 5.1 software.

17.3 Antioxidants in *Capsicum chinense* Extracts

17.3.1 Total Polyphenols

Total polyphenol content in the different genotypes ranged from 20.54 to 20.75 mg/100 g sample (Fig. 17.1). Content was higher ($p<0.05$) in red (L-149 and Red) and brown (L-37) genotypes and did not differ ($p>0.05$) among yellow and orange genotypes. Polyphenols are secondary metabolites widely distributed in plants. They play a variety of functions in plants, including defense against pests and stresses, and add flavor, aroma, and color to fruits and vegetables (Espín de Gea and Tomás-Barberán 2006). The more intensely colored genotypes studied here had the highest polyphenol content, which agrees with previous reports of high phenolic content in red genotypes (Zhang and Hamauzu 2003).

17.3.2 Total Carotenoids

Carotenoid content ranged from 1.00 to 1.26 mg/100 g sample (Fig. 17.2). The highest levels were observed in L-36, Orange, L-184, and L-37, which did not differ ($P<0.05$). Carotenoids are terpenoid compounds formed by the condensation of eight isoprene units. The presence of specific chemical groups at the chain terminus determines carotenoid chromophore properties and allows their classification into two families based on color: red and yellow/orange (Hornero-Méndez et al. 2000). *Capsicum* fruits owe their intense coloring to carotenoid pigments (Guil-Guerrero et al. 2006), which coincides with the high carotenoid content observed here in all seven studied genotypes.

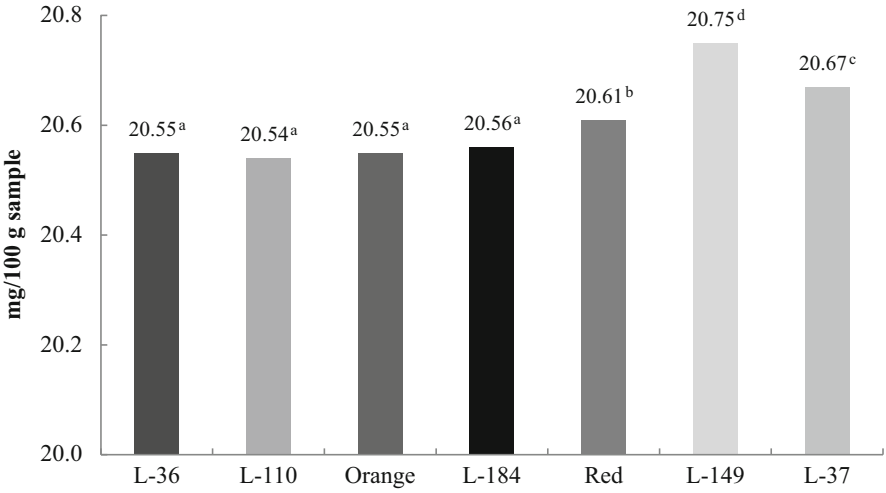

Fig. 17.1 Polyphenol content in *C. chinense* Jacq. genotypes

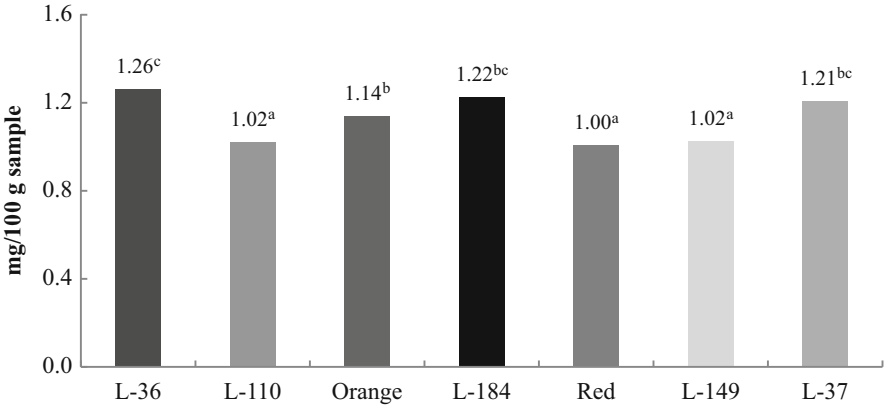

Fig. 17.2 Carotenoid content in *C. chinense* Jacq. genotypes

17.3.3 Ascorbic Acid

Ascorbic acid content varied between the studied genotypes from 187.24 to 281.73 mg/100 g sample (Fig. 17.3). The orange genotype had the highest level and L-149 the lowest. The high ascorbic acid content of peppers is one of their primary nutritional qualities. Factors such as genotype, environment, and fruit maturity affect levels of ascorbic acid and other nutritional compounds. Ascorbic acid levels were high in all the studied genotypes, a result which agrees with reported increases in ascorbic acid content as fruit matures, the highest content being reached at full maturity (Bosland and Votava 2000).

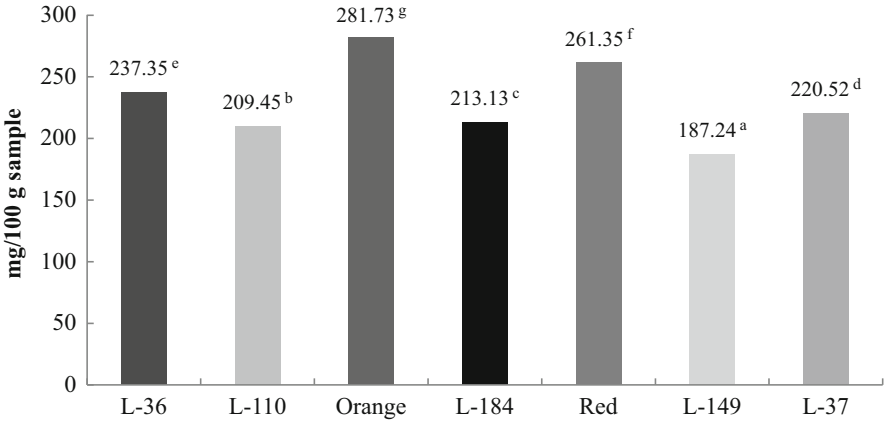

Fig. 17.3 Ascorbic acid content in *C. chinense* Jacq. genotypes

17.3.4 Antioxidant Assay with ABTS Decolorization

Trolox equivalent antioxidant capacity (TEAC) (mM/mg sample) differed ($P<0.05$) between the genotypes and ranged from 1.55 to 3.23 mM/mg sample (Fig. 17.4). The TEAC assay is based on scavenging of the $ABTS^{\bullet+}$ radical cation by the antioxidants present in a sample. The $ABTS^{\bullet+}$ radical has a bluish-green color with maximum absorbance at 734 nm (Re et al. 1999). When antioxidant compounds are present in the reaction medium, they capture the free radical, causing a loss of color and a consequent reduction in absorbance which corresponds quantitatively to antioxidant concentration.

Scavenging ability (%) also varied significantly ($P<0.05$) between the genotypes, with a high of 94.98 % and a low of 44.46 % (Fig. 17.5). Scavenging ability values exhibited the same behavior as the TEAC values: the highest values in the L-36 and red genotypes and the lowest in L-37. Values for the L-36 and red genotypes were higher than those of the other genotypes. This higher antioxidant capacity in the yellow, orange, and red genotypes agrees with observations made by Guil-Guerrero et al. (2006) that antioxidant capacity in *C. annuum* genotypes is comparable to that of commercial antioxidants, with the highest activity in orange, red, and yellow genotypes.

17.3.5 Antioxidant Assay with β-Carotene Decolorization

Beta-carotene decolorization is an effective antioxidant activity assay. In the absence of an antioxidant, β-carotene undergoes rapid discoloration since the free linoleic acid radical attacks the β-carotene molecule, which loses its double bonds and consequently its characteristic orange color. During the 120-min decolorization trial,

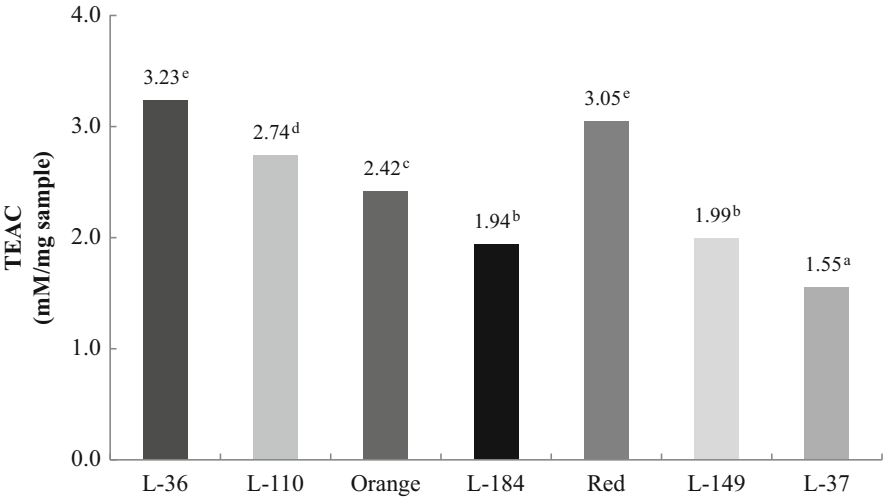

Fig. 17.4 Trolox equivalent antioxidant capacity (TEAC) in *C. chinense* Jacq. genotypes

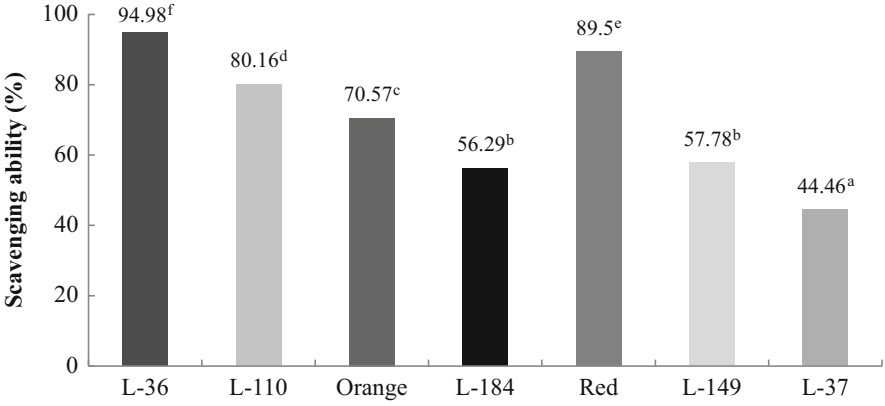

Fig. 17.5 Scavenging ability in *Capsicum chinense* Jacq. genotypes

antioxidant capacity decreased over time (Fig. 17.6). Values ranged from 36 to 57 % β-carotene bleaching during the first 30 min, with L-36 having the highest percentage and L-149 the lowest percentage. After 120 min, values ranged from 11 to 32 %. Overall, L-149 was the most stable antioxidant of the studied genotypes since its capacity decreased only 33 % after 120 min.

Phenolic compounds, carotenoids, and ascorbic acid can contribute to antioxidant activity in fruits, vegetables, and grain products (Velioglu et al. 1998). Free radical scavenging is one of the known mechanisms by which antioxidants inhibit the lipid oxidation caused by free radicals. The high polyphenols, carotenoids, and ascorbic acid contents observed here in all the genotypes suggest that their observed antioxidant activities are largely due to their antioxidant content.

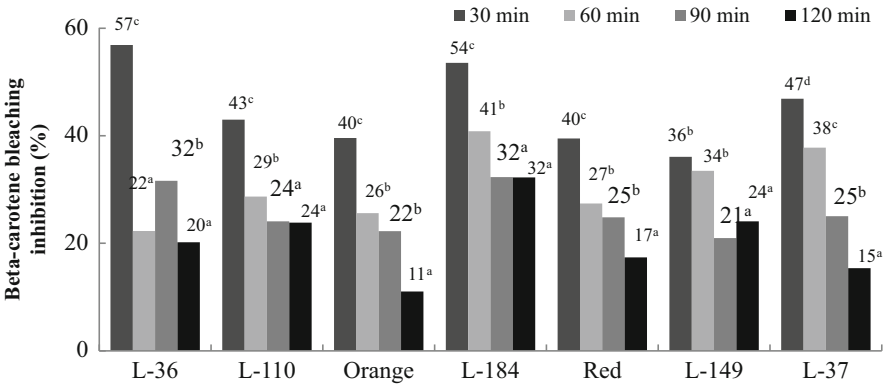

Fig. 17.6 β-carotene bleaching inhibition in *Capsicum chinense* Jacq. genotypes

17.4 Conclusion

Fruit of the habanero pepper *C. chinense* Jacq. genotypes analyzed here can be considered a good source of some antioxidant compounds, especially polyphenols, carotenoids, and ascorbic acid.

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Chapter 18
Functional Components and Medicinal Properties of Cactus Products

Laura Iturriaga and Mónica Nazareno

18.1 Introduction

The Cactaceae is a family constituted by more than 1600 species (Gibson and Nobel 1986; Barhlott and Hunt 1993). Cacti grow up in arid and semiarid environments where the lands generally are water limited and traditional fruits and vegetables have scarce possibilities to survive. Thus, these plants have evolved by developing special physiological traits and distinctive appearances, such as stem morphology, spine properties, nocturnal stomata opening (CAM plants), attractive flowers, etc.

Cacti are native to Mesoamerica, although nowadays are widely spread not only along America, from Canada (Spiers 1982) to Patagonia in Chile and Argentina (Kiesling 1988), but also all over the world. Cacti are now ubiquitous in Europe, mainly in Italy and other places of the Mediterranean Basin, in Africa especially in Northern countries as Tunisia and Morocco, in Asia, and in Oceania as well (Fig. 18.1).

Ancient Mesoamerican civilizations have taken advantages of cacti as good sources of food, feed, and medicine. Pictures belonging to the Codice Florentino which present images of the everyday life in the Aztec culture in Mexico dating from the sixteenth century report such uses of cacti by this pre-Columbian culture (Fig. 18.2).

The anatomy and morphology of cacti allow their development in harsh environments and strongly hard stress conditions; therefore, they are alternative crops for

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Integrating Food Science and Engineering Knowledge Into the Food Chain 12,
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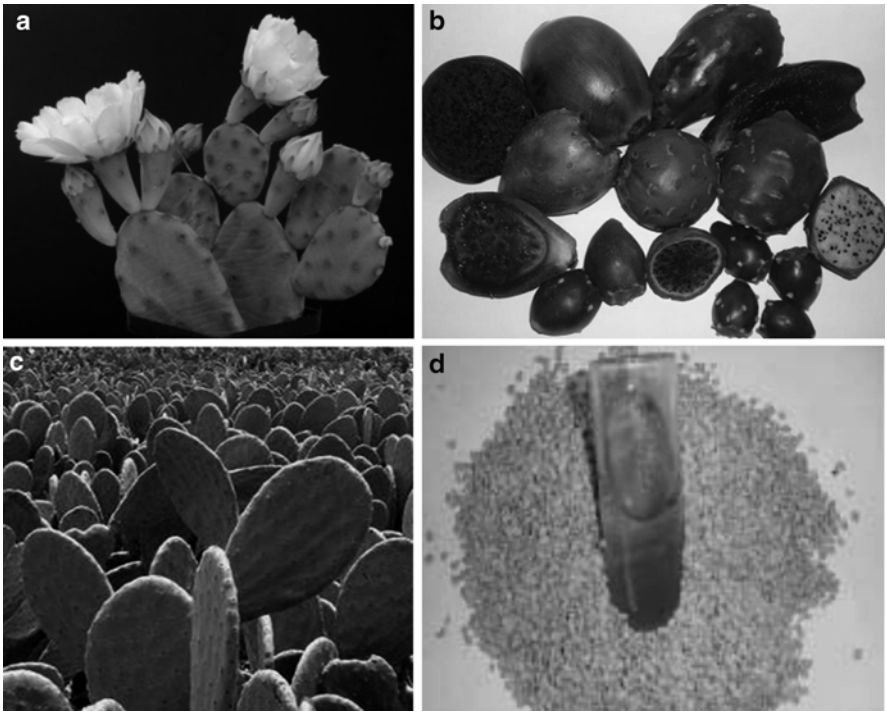

Fig. 18.1 (**a**) *Opuntia* plant with its typical colorful flowers; (**b**) fruits from different cactus species; (**c**) *Opuntia* spp. crop; (**d**) seeds and oil

Fig. 18.2 Picture taken from Codice Florentino (1575)

areas where other species hardly grow. Cacti have adapted to diverse environmental conditions from arid and semiarid areas at sea level to high altitude lands such as the Andes of Peru, as well as tropical regions of Mexico. For this reason, these species may be a promising food resource to many different ecological zones.

There are about one hundred cactus species, mainly of the genus *Opuntia*, which yield edible fruits. Their successful cultivation may be achieved in arid lands, where only few plants can survive (Pimienta-Barrios 1994). Currently, most of the cactus fruits offered in the global market belongs to the *Opuntia ficus-indica* species whose fruits are known as "cactus pear."

However, the main benefits of cacti have to do with the entire utilization of the different parts of these plants and not only their fruits as Table 18.1 shows.

In recent years there is a global trend toward the use of phytochemicals present in natural sources as antioxidants and functional foods. Natural bioactive substances can be used in the food industry to replace synthetic additives, antioxidant, and colorants (Nazareno et al. 2011). Besides, there is increasing evidence that the use of these substances may have beneficial effects on consumers' health beyond their nutritive action. The growing demand for nutraceuticals parallels by an increased effort in developing natural products for the prevention or treatment of human diseases.

Cacti can be considered an important source of bioactive substances and excellent candidates for nutraceutical and functional food preparation. Recent data has revealed high content of some chemical constituents in fruits, cladodes, seeds, and flowers, which can add value to cactus products. Additionally, some of their constituents show promising characteristics in terms of functionality.

Table 18.1 Functional constituents of the different parts of a cactus plant and their uses for food product preparation

Plant parts	Main constituents	Uses as food products
Fruit	Sugars	Fresh fruits
	Vitamin C	Marmalades
	Betalains	Juices
	Polyphenols	Candies
	Fiber	Liquors
		Syrup
Cladodes	Fiber	Salad vegetable
	Mucilage	Pickles
	Minerals	Flour and additives
	Chlorophyll derivatives	
Flowers	Flavonoids	Infusions
	Betalains	Salad vegetable
Seed	PUFA, MUFA, and sterols	Cosmetic products
	Polysaccharides	
Root	Flavonoids	Infusions

18.2 Plant Parts and Their Functional Constituents

18.2.1 Cladodes

Cladodes are specialized stems from cacti, which generally show spines. The sizes of the spines may vary within different species, but spineless plants can also be found (for instance, *O. ficus-indica* cv. Burbank's Spineless is a spineless cultivar). Young tender stems, called "nopalitos," are consumed as fresh vegetable in México and some places in the United States (Cantwell 1995). Besides, they are used as an ingredient in a diversity of dishes including sauces, salads, soups, snacks, pickles, beverages, candies, and desserts (Saenz-Hernandez et al. 2002). Nopalitos are generally obtained from *Opuntia ficus-indica* L., although they can proceed from *O. robusta* or *Nopalea* spp.

Chemical composition of fresh cladodes has been reported by Canceco et al. (2001) indicating a 92 % (w/w) for moisture content, 3.8 % (w/w; d.b.) for proteins, 1.2 % (w/w, d.b.) for fat content, and 25 % (w/w, d.b.) for ashes, for two-year-old cladodes. Mucilage content was considered as remaining solid content reaching a 71 % (w/w, d.b). On the other hand, reports of fresh "nopalitos" show that the composition is mostly water (91 %) and proteins (1.5 %), lipids (0.2 %), total carbohydrates (4.5 %), and ashes (1.3 %) with 90 % being calcium. Besides they contain 11 mg/100 of vitamin C and 30 μg/100 g of carotenoids; their fiber content (1.1) is comparable to that of spinach (Saenz-Hernandez et al. 2002).

The most important functional component present in cladodes is the mucilage and in a minor importance (mainly due to its content) the proteins.

18.2.1.1 Mucilage

The mucilage is a polysaccharide that may occur in specialized storage cells or free within cells or intracellular spaces of the chlorenchymatic and parenchymatic tissue of the cladodes (Ting 1997; Terrazas-Salgado and Mausseth 2002). It is a high molecular weight (MW) polymer (3.4×10^6) (Cardenas et al. 1997) having two fractions: the one of high MW (14.2×10^6) and the other one of low MW (~4000), with the latter containing mainly protein (80 % w/w) in the case of mucilage from *Opuntia ficus-indica* fruits (Majdoub et al. 2001).

The dried mucilage had in average 5.6 % moisture, 7.3 % protein, 37.3 % ash, 1.14 % nitrogen, 9.86 % calcium, and 1.55 % of potassium, as was informed by Sepúlveda et al. (2007).

As to its chemical composition, the mucilage has been considered a polymer similar to pectin composed of L-arabinose, D-galactose, D-xylose, and L-rhamnose as neutral sugars in higher quantity and D-galacturonic acid in smaller (Trachtemberg and Mayer 1981; Medina-Torres et al. 2000; Majdoub et al. 2001). In contrast, earlier studies about its chemical composition reported the mucilage as being a neutral polysaccharide made up of arabinose, galactose, rhamnose, and xylose (Amin et al. 1970).

Hydrocolloids are biopolymers (polysaccharides or proteins) that, due to their water affinity, can modify the properties of aqueous media by forming colloidal solutions. As such, they can exhibit thickening, gelling, stabilizing, and emulsifying properties, which make their ingredients much employed in formulated food products. These properties, called "functional properties," can vary thoroughly depending on the chemical structure, conformation, and hydrodynamic volume of the biopolymer. The physical stability of aqueous dispersions is favored when the viscosity of the continuous phase is enhanced with the addition of the hydrocolloids, especially if the inner phase shows no affinity to the outer phase.

Rheological studies carried out by Medina-Torres et al. (2000), Cardenas et al. (1997), and Iturriaga (2006) indicate that the mucilage is a thickener hydrocolloid that shows a shear-thinning behavior (pseudoplastic). These materials may exhibit during flow a decrease of the apparent viscosity (η_{app}) when the shear rate increases. Figure 18.3 shows the flow properties of mucilage solutions at three different concentrations. Viscosity curves were adjusted using power law model obtaining K and n parameter. The former defined as "viscosity coefficient" (in Pa.s^n) and the latter referring to "flow behavior index" (dimensionless). Figure 18.3 shows that the higher the concentration, the higher the K value, which indicates the thickening properties of this polysaccharide, and the higher the concentration, the lesser the n value, which indicates a more pseudoplastic system.

Shear-thinning behavior is very common in fruit and vegetable products as well as in polymer melts (Steffe 1996).

Some hydrocolloids can develop gel-type structures, formed by a tridimensional network of intermolecular junction zones and a continuous aqueous phase entrapped

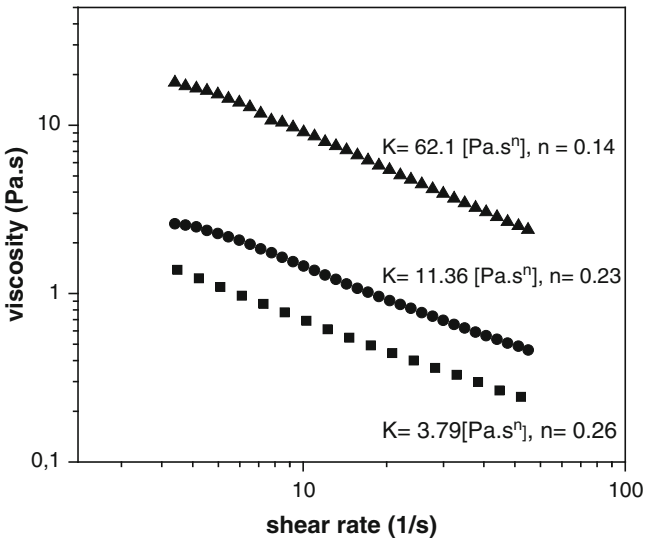

Fig. 18.3 Flow curves of mucilage aqueous solutions at (**a**) [*filled square*] 3 % w/w, (**b**) [*filled circle*] 5 % w/w, and (**c**) [*filled diamond*] 10 % w/w

into this matrix. Gel strength depends on the polymer concentration as well as on the type and number of the intermolecular interactions. Typical rheograms can be found when dynamical rheological studies are performed on these systems. In fact, frequency sweep plots, obtained within the LVR (linear viscoelasticity range), show a G′ value that is independent of (storage modulus) and far greater than the G″ (loss modulus) over the whole range of frequency (Giboreau et al. 1994), giving solid viscoelastic properties. Figure 18.4 shows dynamical rheograms from mucilage solutions at three concentrations. These results correspond to samples presenting some structured matrix or organized solution called "weak gel" (Clark and Ross-Murphy 1987). In these cases, viscous component dampens the response to an imposed stress and the system appears as a fluid, even at higher concentrations. In fact, Saag et al. (1975) have described mucilages as biopolymers that form molecular networks able to retain large amounts of water. Other studies have reported mucilage solutions behaving as a viscoelastic fluid (Cardenas et al. 1997; Medina-Torres et al. 2000).

Effects on the viscosity due to medium pH and ionic strength changes were reported by Medina-Torres et al. (2000); pH and of ionic strength increases produced an increase in the viscosity meaning that the mucilage behaves as a polyelectrolyte.

One of the main functional properties of hydrocolloids is that of being an O/W emulsions stabilizer. Depending on their amphiphilic characters, hydrocolloids can also act as emulsifiers, like gum arabic (Dickinson 2003). According to the

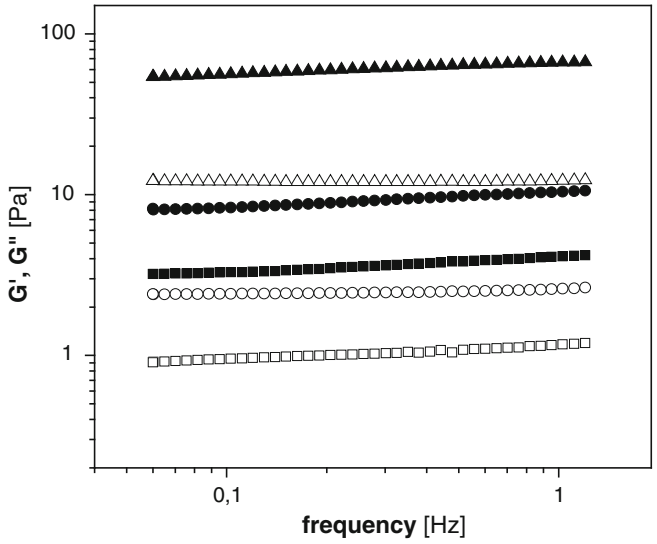

Fig. 18.4 Frequency sweeps of mucilage solutions at (**a**) [*filled square*] 3 % w/w, (**b**) [*filled circle*] 5 % w/w, and (**c**) [*filled diamond*] 10 % w/w. *Solid symbols* (G′) and *open symbols* (G″)

thickening properties of the mucilage, studies of stabilizing properties against coalescence on O/W food emulsions have been carried out by Quinzio et al. (2009). Promising results showed high stabilities against coalescence for emulsions formulated with mucilage and guar gum reaching about 95 % of oil retained in the inner phase after centrifuging, with respect to emulsions stabilized with carboxymethyl cellulose, which presented a stability of 12%.

18.2.1.2 Nopal Flour as Cladode Product

Besides the use of their soft shoots, cladodes are grinded to flour and other products. Nopal flour is a rich source of dietary fiber reaching up to 43 % on dry basis (Saenz-Hernandez et al. 2002). These flours are used as fortifiers in foods prepared using flours from another sources. Figure 18.5 includes several products available in the market made out of nopal flour (tablets, tortillas, puddings).

Fig. 18.5 Food and nutraceutical products prepared using nopal flour

18.2.2 Fruits

Many cactus species produce edible fruits. The typical cactus pear fruit is an oval-shaped berry with an average weight of 100–200 g. The juicy pulp contributes 60–70 % to the total fruit weight and contains numerous small hard seeds (varying from 100 to more than 400 per fruit). Cactus fruits are good sources of nutrients like sugars as well as bioactive constituents as vitamins, pigments, and other secondary compounds with antioxidant properties (Moßhammer et al. 2006; Kuti 2004).

Nutrient contents increase with fruit maturity reaching up to 15 % of sugars. Fruit pulp is rich in calcium and magnesium, being its levels up to 59 mg/100 g and 98.4 mg/ 100 g, respectively (Stintzing et al. 2001). Cactus pear shows a high ascorbic acid level that can reach figures up to 30 mg/100 g (Piga et al. 2003). Such content is higher than that found in apples, pears, grapes, and bananas. Total free amino acid content (257.24 % mg/100 g) is higher than the average of other fruits.

Cactus pear is a non-climacteric fruit and presents a low respiration rate (Cantwell 1995) and low ethylene production (Lakshminarayana and Estrella 1978); hence, its nutrient concentration remains almost the same after harvest. Postharvest variation in fruit properties such as pH, acidity, total soluble solids, and acetaldehyde and ethanol contents is not significant; however, marked changes in vitamin C content has been reported due to storage conditions (Inglese et al. 2002; Coria-Cayupán et al. 2009).

Fruit pigment composition depends strongly on the species, variety, or cultivar. Besides, the concentrations of chlorophylls, betalains, and carotenoids in the fruit vary according to several factors. One of the most significant is the fruit ripening status (Coria-Cayupán et al. 2011). Betalains are the main pigments responsible for the ripe fruit colors, and, therefore, they have a strong influence in the consumer acceptance. These pigments are water-soluble nitrogenous compounds and they are also found in other plants as beetroot. According to their chemical structures, betalains are classified into two different groups, the betaxanthins, yellow- and orange-colored substances, and the betacyanins, red- to purple-colored ones (Hendry and Houghton 1996). They differ only in the chemical group linked to the betalamic acid moiety. Betacyanins are ammonium conjugates of betalamic acid with cyclo-DOPA, while betaxanthins are conjugates with amino acids or amines. Betalain composition has been characterized in cactus pear fruit (Stintzing et al. 2003). In addition to color, the same pigments have antioxidant properties higher than that of ascorbic acid (Stintzing et al. 2005; Butera et al. 2002). In contrast to anthocyanins, another group of natural pigments, betalains are stable in a wider pH range (Stintzing and Carle 2004). This property makes the latter ideal for use as food colorants in low-acid products (Stintzing et al. 2001). Due to the wide structural variety and, hence, color diversity, betalains constitute a very promising source of natural colorants to be used as functional colorants. Compared with red beets, cactus pear offers a wider range of colors; besides, due to its natural character, it can be used free of certification (Stintzing et al. 2002).

The presence of phenolics has also been detected in cactus pulp fruit. Kuti (1992) has reported an antioxidative effect due to the major flavonoids found in cactus fruits like quercetin, kaempferol, and isorhamnetin (Kuti 1992). Flavonol derivatives found in *Opuntia* ssp. have been compiled (Stintzing and Carle 2005). Concerning the use of cactus fruits for functional food preparation, one aspect to take into account is that higher phenolic contents are expected in the peel, rather than the pulp (Stintzing et al. 2005). Consequently from a functional point of view, processing both peel and pulp appears to be advantageous.

Other bioactive constituents have been found in the skin of *Opuntia ficus-indica* fruit; a series of studies about the polysaccharide fraction have been reported by Habibi et al. (2003, 2004a, 2004b, 2005).

Opuntia ficus-indica var. saboten is a widely spread cactus in Southwestern Korea, where it is used as a functional food. Antioxidant activity of this cactus pear variety has been reported by Lee et al. (2002b) as corresponding to well-known antioxidants, such as catalase, α-tocopherol, and ascorbic acid.

The interest on natural antioxidants is remarkably increasing these days. These substances present the ability to scavenge free radicals or other non-radical oxidant species delaying or retarding the oxidative damage produced by them. Numerous investigations indicate that free radicals are involved in oxidation processes resulting in cell aging and the origin of serious pathologies such as cancer, heart diseases, degenerative illness of the nervous system, and malaria (Halliwell and Gutteridge 1989; Trush and Kensler 1991). Many biochemical and clinical studies suggest that natural and synthetic antioxidant compounds are helpful in treating diseases mediated by oxidative stresses.

Cactus pears are usually consumed as fresh fruits, juice, dessert, as well as processed products like jams, jelly, marmalades, ice creams, syrups (locally named "arrope"), and candies. Dehydrated fruit sheets are also offered in several countries. One of the better-known and accepted products for massive consumption worldwide is jam. It is easy to make by cooking the fruit pulp and adding sugar. Alcohol and vinegar are also elaborated out of *Opuntia ficus-indica* fruit through different processes.

18.2.3 Seeds and Seed Oil

After fruit processing in juice and jam preparation, great amounts of seeds are usually discarded. The cactus pear fruit contains many hard-coated seeds that represent 10–15 % of the pulp weight. Seed endosperm constituents are arabinan-rich polysaccharides (Meyer et al. 1980), being D-xylan the major seed coat component (Habibi et al. 2003). In addition to lipids, seeds have been reported to accumulate proanthocyanidins (Nieto 1987). The fruits contain a large number of seeds although their oil content is relatively low. Indeed, oil constitutes 7–15 % of the whole seed weight. Seeds from 11 commercial cactus pear cultivars were analyzed for oil content and fatty acid composition by South African researchers. They reported

palmitic acid content ranging between 11.4 and 15.9 %, considerably lower than that of cotton oil. Linoleic acid content varied between 61.4 and 68.9 %. The α-linolenic acid of all the cultivars was less than 1 %. The oleic acid content varied between 12.4 and 16.5 % (lower than that of cotton seed). Unsaturated fatty acids made up about 80 % of all fatty acids (Ennouri et al. 2005). Therefore, although the seed oil content is relatively low, the fatty acid composition indicates that it has potential as oil for the health product market (Labuschagne and Hugo 2010). The seeds which are ground or pressed to produce oil are the most lucrative part of the plant. The oil is part of more than 40 cosmetic products and sells at a very high price as pure skin oil. It takes approximately 1 t of these tiny seeds to make 1 L of oil. The *Opuntia* seed oil is obtained by cool pressed seeds and some of its main applications are being developed by the cosmetic industry.

18.2.4 Flowers

Cactus flowers are also rich in active compounds such as flavonoids. Flavonoids were identified in *Opuntia* flowers by chromatographic methods as quercetin 3-glucoside, quercetin 3-rutinoside, and kaempferol 3-glucoside (Clark et al. 1980). Quercetin 3-galactoside (hyperin) and the isorhamnetin 3-rutinoside (narcissin), 3-galactoside, and 3-rhamnoglucoside were found in the flowers of *O. lindheimeri* (Rösler et al. 1966). A varied composition of betalains has been also found in colored cactus flowers (Kobayashi et al. 2000).

Flowers, as cladodes or nopalitos, can be eaten as vegetable. Besides, to take advantages of their recognized health beneficial properties, infusions of dried flowers are consumed as medicinal teas.

18.3 Medicinal Properties

Numerous plants were used by ancient civilizations to cure diseases and heal wounds for thousands of years. Cactus cladodes, fruits, and flowers have been traditionally used as medicines in several countries. Cladodes are still used in folk medicine for the treatment of gastric ulcer and for its healing activity as therapeutic agents. The prostate cancer-preventing properties of cactus dried flower infusions are also well known.

Recent scientific research confirmed that cactus plants may be efficiently used as a source of bioactive phytochemicals, such as mucilage, fibers, pigments, and antioxidants.

Numerous medicinal properties of cactus products have extensively been assessed by scientific research groups using in vitro and in vivo systems. Table 18.2 summarizes the most remarkable results and the plant sources analyzed for the performed studies.

Table 18.2 Review of the reported medicinal properties of cactus products

Medicinal properties	Studied species and active part of the cactus plant	Studied system and reference
Cancer-preventive properties	Aqueous extracts of cactus pear	Ovarian and cervical epithelial cells, as well as ovarian, cervical, and bladder cancer cells, Zou et al. (2005)
Antiviral action	*O. streptacantha* cladode extract	Intracellular virus replication inhibition and extracellular virus inactivation, Ahmad et al. (1996)
Antihyperlipidemic effect and cholesterol-reducing action	*Opuntia* sp. cladodes	Guinea pigs, Fernandez et al. (1992); guinea pigs, Fernandez et al. (1994)
	Opuntia robusta fruits	Nondiabetic hyperlipidemic humans, Wolfram et al. (2002)
	Opuntia ficus-indica cladode	Rats, Galati et al. (2003a, b)
	Opuntia ficus-indica seed oil	Rats, Ennouri et al. (2006a)
	Opuntia ficus-indica seeds	Rats, Ennouri et al. (2006b)
	Cactus pear seeds and oil	Rats, Ennouri et al. (2007)
	Opuntia ficus-indica var. saboten	Mice, Oh and Lim (2006)
Liver protection	*Opuntia ficus-indica* fruit juice	Liver, Galati et al. (2005)
Antiobesity factor	*Opuntia sp*. cladode	Humans, Frati Munari et al. (2004)
Hypoglycemic and antidiabetic effects	*Opuntia megacantha*	Diabetic rats, Bwititi et al. (2000)
	Opuntia lindheimeri	Diabetic pigs, Laurenz et al. (2003)
	Opuntia ficus-indica, *Opuntia lindheimeri*, and *Opuntia robusta*	Diabetic rats, Enigbokan et al. (1996)
	O. streptacantha	Humans, Meckes-Lozyoa and Roman-Rams (1986)
	O. monacantha cladode extract	Diabetic rats, Yang et al. (2008)
	Opuntia ficus-indica seeds	Rats, Ennouri et al. (2006a)
	Opuntia ficus-indica seed oil	Rats, Ennouri et al. (2006b)
	Opuntia streptacantha	Humans, Frati Munari et al. (1991, 1992)
	Opuntia fuliginosa fruit extract	Rats, Trejo-Gonzalez et al. (1996)

(continued)

Table 18.2 (continued)

Medicinal properties	Studied species and active part of the cactus plant	Studied system and reference
Anti-inflammatory actions	*Opuntia humifusa* leaf extracts	Nitric oxide-producing macrophage cells, Cho et al. (2006)
Antiulcerogenic and antigastritis effects	*Opuntia ficus-indica* cladodes	Rats, Galati et al. (2001)
	Opuntia ficus-indica fruit juice	Rats, Galati et al. (2003a, b)
	Opuntia ficus-indica cladodes	Rats, Galati et al. (2002a, b)
	Opuntia ficus-indica var. saboten stems	Rats, Lee et al. (2002a)
	Opuntia ficus-indica var. saboten fruit	Rats, Lee et al. (2001)
Healing properties	*Opuntia ficus-indica* cladodes	Human, Hegwood (1990)
Neuroprotective action against neuronal oxidative injuries	*Opuntia ficus-indica* var. saboten flavonoid extract	Primary cultured rat cortical cells, Dok-Go et al. (2003)
Neuroprotective action against cerebral ischemia	*Opuntia ficus-indica extracts*	In vitro studies in cultured mouse cortical cells and in vivo studies in gerbils, Kim et al. (2006)
Decreasing effect on the oxidative stress in humans	*Opuntia ficus-indica* fruit	Humans, Tesoriere et al. (2004); in vitro human LDL, Tesoriere et al. (2003)
	Cactus pear fruits	Ex vivo human cells, Tesoriere et al. (2005)
	Opuntia robusta fruits	Humans, Budinsky et al. (2001)
Protection upon nickel-induced toxicity	*Opuntia ficus-indica* cladode extract	Rats, Hfaiedh et al. (2008)
Oxidative damage induced by zearalenone	*Opuntia ficus-indica* cladode	Mice, Zourgui et al. (2008)
Diuretic effect	*Opuntia ficus-indica* cladodes, flowers, and non-commerciable fruits	Rats, Galati et al. (2002a, b)
DNA damage reduction	*Opuntia ficus-indica* fruit extract	Human peripheral lymphocytes, Siriwardhana et al. (2006)

Antioxidant	*Opuntia ficus-indica* var. saboten leaves	DPPH radical, Saleem et al. (2006)
	Opuntia ficus-indica fruit extract	DPPH radical, HO• radical by ESR; Siriwardhana et al. (2006)
	Opuntia ficus-indica fruit juice	DPPH radical, Galati et al. (2003a, b)
	Opuntia spp. fruit	ORAC and TEAC in vitro assays, Stintzing et al. (2005)
	Opuntia ficus-indica fruit extract	Lipid oxidation in red blood cells and in LDL, ABTS radical cation assay, in vitro reducing assay; Butera et al. (2002)
	Opuntia monacantha cladode extract	DPPH radical, Valente et al. (2010)
	O. ficus-indica, *O. lindheimeri*, *O. streptacantha*, *O. stricta* var. stricta fruits	ORAC, Kuti (2004)
	Opuntia ficus-indica fruit extract	Oils and emulsion model systems, Siriwardhana and Jeon (2004)
	Opuntia humifusa leaf extracts	DPPH and xanthine oxidase assays, Cho et al. (2006)
	Opuntia ficus-indica var. saboten flavonoid extract	Inhibition of xanthine oxidase activity, DPPH radical scavenging, lipid peroxidation inhibition; Dok-Go et al. (2003)
Platelet function	Prickly pear fruits	Humans, Wolfram et al. (2003)
Other studies	Fruit betalain extract	Endothelial ICAM-1 expression inhibition, Gentile et al. (2004); human myeloperoxidase, Allegra et al. (2005); beneficial effects in benign prostatic hyperplasia, Jonas et al. (1998)
	Fruit betalain extract	
	Flower extracts	
Alcohol hangover symptoms alleviation	*Opuntia ficus-indica* plant extract	Humans, Wiese et al. (2004)

Scientific studies in experimental models have confirmed that lyophilized cladodes have significant antiulcer effect, protect from gastric lesions, and possess anti-inflammatory activity. Diet supplementation with cactus pear fruits in healthy humans has shown to decrease the oxidative stress and, therefore, improves their overall antioxidant status. Cactus pear fruits have also been studied for ovarian cancer prevention. Their ability in suppressing carcinogenesis of in vitro and in vivo models has been already assessed. *Opuntia ficus-indica* cladodes were supplied to hypercholesterolemic rats, and a marked decrease in cholesterol and triglycerides levels was found in plasma samples. Experiments in diabetes mellitus non-insulin-dependent patients have confirmed the hypoglycemic effects of *Opuntia streptacantha* cladodes. Moreover, consumption of cactus young cladodes has shown to reduce obesity and blood glucose level. Experiments concerning the antiviral action of cactus cladode extracts have been conducted against viruses such as herpes, HIV-1 virus, and influenza A.

A great variety of functional foods, nutraceuticals, and cosmetic products from cactus plants are currently available in the global market. High value-added products as healthy foods can be prepared from cacti as juices, marmalades, candies, liquors, and syrups. However, the functional properties of cactus products should be more efficiently exploited by food, cosmetic, and pharmaceutical industries.

18.4 Conclusions

Cactus as a multifunctional plant offers the possibility of taking advantages or gaining benefits from the whole plant: fruits, cladodes, flowers, and seeds. In fact this plant can provide not only fresh food but also processed products maintaining its functional and medicinal properties.

However, further studies about cactus functional products are needed to improve their transfer to a technological scale in order to gain competitiveness and market positioning against other traditional foods already settled in the consumer preferences.

Cacti are especially significant plant resources in arid and semiarid lands where population deals with subsistence economies. This crop is a promising factor to promote local development through ventures devoted to increase the added value of cactus products as food, nutraceuticals, as well as fodder industries.

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Chapter 19
Chios Mastic Gum and Its Food Applications

Adamantini Paraskevopoulou and Vassilios Kiosseoglou

19.1 Introduction

Chios Mastic Gum (CMG) or Mastiha is the dried exudate of the shrub-like tree *Pistacia lentiscus* L. var. *chia* of the Anacardiaceae family, cultivated exclusively in the south of the Greek island Chios (Fig. 19.1). Attempts to grow the mastic tree in other Greek regions and countries have been reported, but they met very limited success. This is probably related to the temperate climate of the island and the underwater volcanic zone of the area and Chios' ground. Cultivation of mastic tree originates in ancient years, during the Hellenistic period. Despite competition from other species of mastic at that time, Chios mastic's superior quality established the gum as a unique natural product with significant commercial value and exclusivity of supply. CMG has been accepted and established as a Greek product, and so Greece is the only country that has the right to produce it (European Union Law (123/1997)). Production and commercial exploitation of the gum is organized in the island of Chios by a cooperative of mastic growers. Due to systematic research, development, and promotion, the sales of CMG and its reputation have seen an increase in the last decades, leading to a higher income for the mastic producers.

From early July until late September, vertical slits, 4–5 mm deep and 10–15 mm long, are made in the trunks and branches of the mastic tree. A tree can receive from between 20 and 100 slits, depending on age. The initially liquid exudate that oozes out from the bark incisions is a semitransparent colorless resinous material which is allowed to remain under the tree and coagulate while being exposed to the local environmental conditions for a period of several days. The dried resin drops are then collected by hand, subjected to sieving to remove foreign materials, washed with soap

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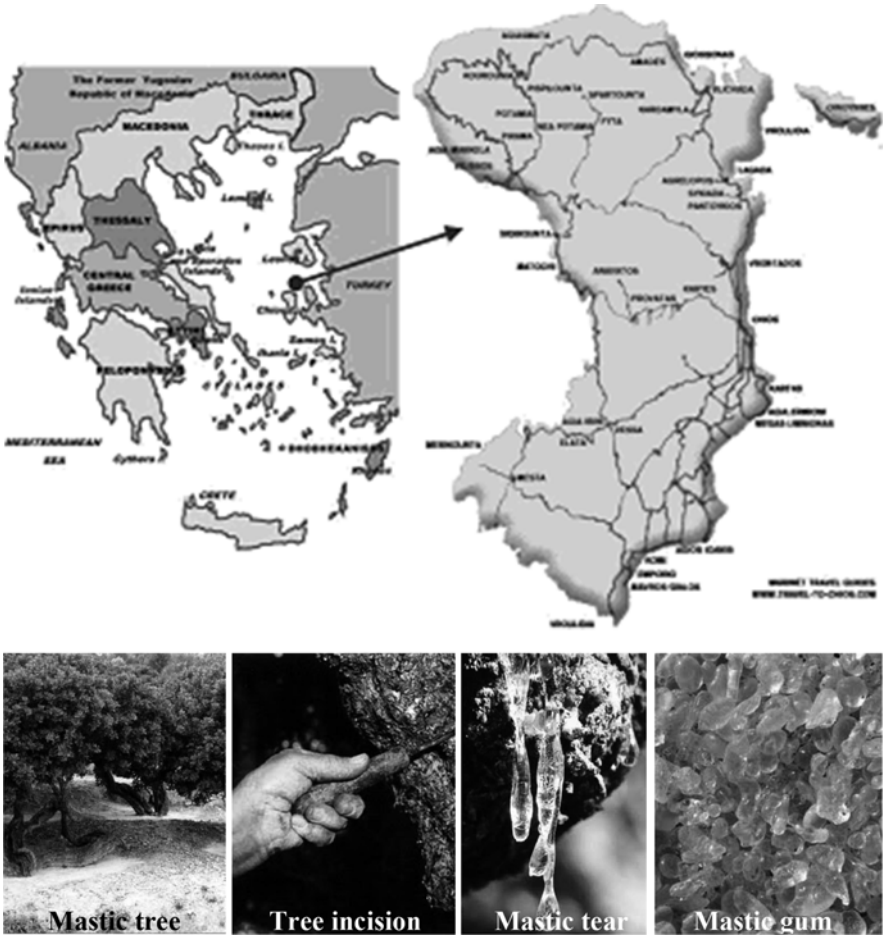

Fig. 19.1 Map of the island of Chios and steps of Mastiha collection

water, and then several times with cold water, allowed to dry in air and handed over to the local factory. In order to become commercially available, the raw mastic particles are subjected in the factory to multiple washing steps and then carefully treated with special knifes to remove any tightly adhering on the drop surface debris. Following drying in air, the particles are classified according to size and stored in cold environment to avoid extensive color and flavor deterioration (Perikos 1988) (Fig. 19.1).

The distinctive mastic gum flavor is connected with the presence of essential oil constituents, representing about 1–3 % of the final product, while the nonvolatile fraction consists mainly of triterpene acids and alcohols. In the nonvolatile fraction is also included a polymeric material representing between 3 and 10 % of the resin, which, according to van den Berg et al. (1998), is polymyrcene and was found to act as a plasticizer of the monomeric mastic gum fraction (Kehayoglou et al. 1994).

The annual production yield of dried mastic gum ranges between 100 and 150 t. The gum finds use either as a natural chewing gum on its own but mainly as a natural chewing gum base in admixture with additives such as sugar and lecithin for the preparation of the well-known Chios chewing gum or Chios chicle. In addition, it often finds culinary uses in the traditional Greek, Turkish, or Arabic kitchen, mainly as a flavoring and/or texturizing ingredient. Alternatively, the gum is subjected to steam distillation to recover the volatile essential mastic gum oil which is exploited as flavoring material in food and drink products as well as in cosmetics and perfumery. Apart from their flavoring and texture-modifying properties, CMG constituents also exhibit food preservation and antioxidative activity. On the other hand, the treatment by mastic gum consumption of various gastric malfunctions and ulcer therapy possibly by causing structural changes within the *Helicobacter pylori* bacteria cell structure have also been reported. The gum also finds many non-food uses, the main ones being in the preparation of pharmaceutical products, like medical creams and dental tooth paste, paint varnish, and artist color oil.

19.2 Chemistry and Physicochemical Properties

CMG is mainly composed of triterpenes/triterpenoids and essential oil constituents (mainly terpenes). It also contains a series of phenolic compounds such as tyrosol, and p-hydroxy-benzoic, p-hydroxy-phenylacetic, vanillic, gallic, and trans-cinnamic acids. Its chemical composition has been evaluated by a number of researchers (Papageorgiou et al. 1991; van den Berg et al. 1998; Magiatis et al. 1999; Daferera et al. 2002; Assimopoulou and Papageorgiou 2005; Dietemann et al. 2005; Koutsoudaki et al. 2005; Kaliora et al. 2004). All have showed the predominant presence of α-pinene and β-myrcene, with the β-myrcene percentage being the determinant of the marketability of mastic gums.

Regarding the CMG essential oil fraction, there is a proportion between the α-pinene and β-myrcene contents, which characterizes its authenticity. Percentages of ~60–80 % for α-pinene and 7–20 % for β-myrcene represent an acceptable oil quality, while increases of β-myrcene in the oil mixture devalue its quality (Daferera et al. 2002). Moreover, the (+)/(−)α-pinene ratio, which should not be less than 99:1, is considered as an important discriminating factor of product's possible adulteration. The mastic gum oil is characterized by the presence of α-pinene, β-pinene, β-myrcene, limonene, linalool, and β-caryophyllene. Other compounds are also present at concentrations less than 0.5 % (Table 19.1).

The mastic gum essential oil chemical composition varies and depends on gum quality. Consecutively, the gum quality is influenced by its purity, the collection time, and the duration between exudation from the trunk and the collection. Daferera et al. (2002), who studied the chemical composition of mastic gum oils from resins collected over different durations, found a large range of α-pinene and β-myrcene percentages (33.7–72.8 and 3.8–63.5, respectively) probably due to the collection time of resin and the duration between its exudation from the trunk and the collection.

Table 19.1 Chemical composition of the essential oil from gum *P. lentiscus* var. *chia*

Compound	1[a]	2[a]	3[a]	4[a]
α-Pinene	77.10	66.48	72.1	63.3
Camphene	1.04	0.83	0.7	0.6
β-Pinene	2.46	3.29	2.9	3.3
β-Myrcene	12.27	8.34	16.5	25.0
Limonene	0.95	1.26	1.0	1.5
Methyl-o-cresol	0.44	1.17	0.7	0.6
Linalool	0.48	2.84	1.0	0.5
β-Caryophyllene	1.47	2.04	1.1	0.9

[a]Data obtained from: (1) Papageorgiou et al. (1991), (2) Magiatis et al. (1999), (3) Daferera et al. (2002), (4) Koutsoudaki et al. (2005)

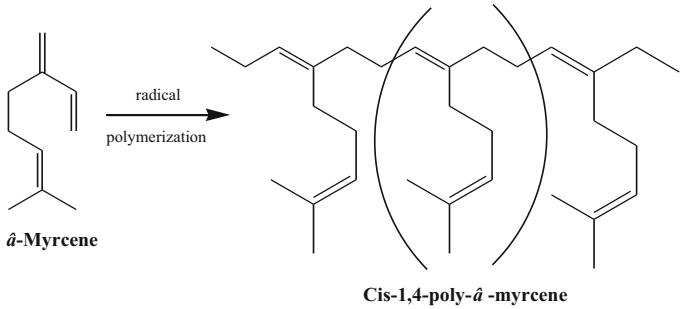

Fig. 19.2 Formation of mastic polymer from myrcene by radical polymerization (as suggested by van den Berg et al. 1998 and Dietemann et al. 2005)

The concentration of *β*-myrcene was increased and exceeded *α*-pinene in resins collected immediately, while it decreased to less than 20 % in resins left to mature physiologically over a maximum time of 2 months.

The polymeric fraction was found to have a broad molecular weight distribution up to about 100,000 Da (van den Berg et al. 1998). Its structure has been characterized as 1,4-poly-[*β*-myrcene], predominantly present in the *cis*-conformation for about 75 % (Fig. 19.2). *β*-Myrcene, the second most abundant compound in the essential oil fraction, is the only compound with conjugated double bonds which are relatively prone to polymerization. According to van den Berg et al. (1998), the tree produces relatively large amounts of *β*-myrcene which is polymerized once the resin exudes from the tree by radical chain reactions. Dietemann et al. (2005) revealed the absence of a polymer fraction in dark harvested mastic gum as well as a smaller amount of triterpenoid dimerization products compared to conventionally harvested mastic, which could be related to the reduced radical formation in light protected samples.

The biological properties of CMG are mainly attributed to its triterpene/triterpenoids constituents. The chemical composition of triterpenes of both neutral and acidic fraction has been reported by several authors (Barton and Seoane 1956;

Table 19.2 Triterpenes (*presented as methyl esters*) identified in the acidic fraction of *P. lentiscus* var. *chia* resin. The most abundant compounds are in bold print

	Acidic fraction compounds[a]	% (w/w) of triterpenic fraction	% (w/w) of acidic fraction	% (w/w) of resin
1	Methyl 11-oxo-3*β*-hydroxy-28-norolean-17-en-6-oate	0.6	1.6	0.2
2	Methyl moronate	0.5	1.3	0.2
3	**Methyl oleanonate**	**3.8**	**9.7**	**1.4**
4	Methyl 3*β*-acetoxy-6*β*-hydroxy-olean-18-en-28-olate	0.2	0.4	0.06
5	Methyl 3*β*-acetoxy-6*β*-hydroxy-dihydro--isomasticadienolate	0.1	0.4	0.05
6	**Methyl isomasticadienonate**	**24.4**	**63.3**	**8.9**
7	Methyl 3-epi-isomasticadienolate	*tr*[b]	*tr*	*tr*
8	**Methyl masticadienonate**	**9.3**	**24.1**	**3.4**
9	Methyl 3*β*-acetoxy-20,30-dehydro-12- lupen-28-oate	0.1	0.3	0.04
10	Methyl olean-12,18-dien-3-olate	0.2	0.5	0.06

[a]As reported by Assimopoulou and Papageorgiou (2005)
[b]*tr*: found in traces

Seoane 1956; Monaco et al. 1973; Mills and White 1989; Marner et al. 1991; Stern et al. 2003; Papageorgiou et al. 1997; Colombini et al. 2000; Dietemann et al. 2001; Assimopoulou and Papageorgiou 2005). The major constituents are isomasticadienonic acid, masticadienonic acid, and 28-norolean-17-en-3-one. All compounds identified in *P. lentiscus* var. *chia* resin are presented in Tables 19.2 and 19.3.

In the acidic fraction of *P. lentiscus* var. *chia* resin 10 triterpenes were identified (Table 19.2.): methyl 11-oxo-3*β*-hydroxy-28-norolean-17-en-6-oate, methyl moronate (18-oleanene), methyl oleanonate (12-oleanene), methyl 3*β*-acetoxy-6*β*-hydroxy-olean-18-en-28-olate, methyl 3*β*-acetoxy-6*β*-hydroxy-dihydro-isomasticadienolate, methyl isomasticadienonate (8-tirucallene), methyl 3-epi-isomasticadienolate, methyl masticadienonate (7-tirucallene), methyl 3*β*-acetoxy-20,30-dehydro-12-lupen-28-oate, and methyl olean-12,18-dien-3-olate. As established, isomasticadienonic acid seems to be the major constituent in resin (24.4 % of total triterpenic fraction), followed by masticadienonic acid (9.3 %) and oleanonic acid (3.8 %). These three triterpenes comprise 37 % of the total triterpenic fraction (neutral and acidic) of resin, while the proportion of isomasticadienonic/masticadienonic acid is 2.6.

In the neutral fraction of the resin 26 triterpenes were identified (Table 19.3): lupenone, 3*β*-hydroxy-6*β*-hydroxymethyl-28-norolean-17-ene, 3*β*-hydroxy-28-norolean-17-en-6-al, tirucallol, 3-methoxy-28-norolean-12-ene, dammaradienone,

Table 19.3 Triterpenes identified in the neutral fraction of *P. lentiscus* var. *chia* resin. The most abundant compounds are in bold print

	Neutral fraction compounds[a]	% (w/w) of triterpenic fraction	% (w/w) of neutral fraction	% (w/w) of resin
11	Lupenone	0.2	0.7	0.1
12	3*β*-Hydroxy-6*β*-hydroxymethyl-28--norolean-17-ene	0.6	1.7	0.2
13	3*β*-Hydroxy-28-norolean-17-en-6-al	0.6	1.6	0.2
14	Tirucallol	0.2	0.5	0.06
15	3-Methoxy-28-norolean-12-ene	0.7	1.9	0.2
16	Dammaradienone	0.9	2.6	0.3
17	28-Norolean-12-en-3-one	1.1	3.1	0.4
18	*β*-Amyrone	1.3	3.6	0.5
19	Olean-18-en-3-one	1.0	2.7	0.3
20	**28-Norolean-17-en-3-one**	**19**	**53.4**	**6.9**
21	28-Norolean-12,17-dien-3-one	*tr*[b]	*tr*	*tr*
22	6-Methyl-28-norolean-17-en-3-one	0.6	1.6	0.2
23	3-Methoxy-28-norolean-17-ene	0.7	1.9	0.2
24	3*β*-Acetoxy-28-norolean-17-ene	*tr*	*tr*	*tr*
25	3-Oxo-28-norolean-17-en-6-al	0.5	1.3	0.2
26	3*β*-Hydroxy-6-methyl-28-norolean-17-ene	*tr*	*tr*	*tr*
27	Olean-18-en-3-ol	0.2	0.5	0.07
28	3*β*-Hydroxy-dammarane-derivative	*tr*	*tr*	*tr*
29	20,24-Epoxy-25-hydroxy-dammaren-3-one	0.1	0.4	0.04
30	3*β*-Hydroxy-epoxy-dammarane derivative	0.3	0.8	0.1
31	Hydroxydammarenone	0.8	2.2	0.3
32	28-Nor-17-oleanen-3-ol	0.1	0.4	0.05
33	**Oleanonic aldehyde**	**5.4**	**15.2**	**2.0**
34	Isomasticadienolic aldehyde	0.4	1.1	0.1
35	11-Oxo-*β*-amyrin acetate	0.3	0.8	0.1
36	Norlupenone	0.7	2.0	0.2

[a]As reported by Assimopoulou and Papageorgiou (2005)

[b]*tr*: found in traces

28-norolean-12-en-3-one, *β*-amyrone, olean-18-en-3-one, 28-norolean-17-en-3-one, 28-norolean-12,17-dien-3-one, 6-methyl-28-norolean-17-en-3-one, 3-methoxy-28-norolean-17-ene, 3*β*-acetoxy-28-norolean-17-ene, 3-oxo-28-norolean-17-en-6-al, 3*β*-hydroxy-6-methyl-28-norolean-17-ene, olean-18-en- 3-ol, 3*β*-hydroxy-dammarane derivative, 20,24-epoxy-25-hydroxy-dammaren-3-one, 3*β*-hydroxy-epoxy-dammarane derivative, hydroxy-dammarenone, 28-nor-17-oleanen-3-ol, oleanonic aldehyde, isomasticadienolic aldehyde, 11-oxo-*β*-amyrin acetate, and norlupenone. 28-Nor-17-oleanenes were the main components in the neutral fraction of *P. lentiscus* var. *chia* resin. More specifically, the most abundant compound

was found to be 28-norolean-17-en-3-one (53 % of neutral fraction or 19 % of total triterpenic fraction).

The chemical composition of gum is influenced by the resin collection technique. Liquid collection, which is a newly applied technique that increases resin productivity, seems to affect the qualitative as well as the quantitative composition of triterpenes in CMG in comparison to resin collected traditionally, in the form of tears or droplets, by longitudinal incisions in the tree. This is of great importance, since the biological activity of resin may be influenced by different triterpene composition. In liquid collection method, where a stimulating agent (e.g., ethrel) is used for resin excretion after incision of the tree, mastic gum is produced in a fluid form with a characteristic odor. In a recent study, conducted by Assimopoulou and Papageorgiou (2005), it was reported that resins collected by applying either the traditional or the liquid method were composed of several different minor triterpenes. In the liquid collection resin, eight compounds were identified in the acidic and 11 in the neutral fraction, while seven compounds were not present in resin traditionally collected. More specifically, in the acidic fraction of liquid collection resin, eight triterpenes were identified, four of which were not identified in traditionally collected mastic gum, namely methyl 11-oxo-masticadienonate, methyl isomastica-8,12-dienolate, methyl 3-acetoxy-3-epi-isomasticadienolate, and methyl 3-acetoxy-3-epi-masticadienolate. In the neutral fraction of liquid collection resin, 11 compounds were identified, from which 3-β-acetoxy-12-oleanene, 3-β-acetoxy-isomasticadienolic aldehyde, and 28-norolean-12-en-3-ol were not contained in traditionally collected *P. lentiscus* resin. The main triterpenes in resin sample collected by use of stimulating agents were isomasticadienonic acid, masticadienonic acid, and 28-norolean-17-en-3-one (22.5, 14.7, and 36 % w/w of triterpenic fraction, respectively). As it was demonstrated from the quantitative analysis of the resin samples, the one sample collected using ethrel contained about the same percentage of isomasticadienonic acid as the sample traditionally collected, increased percentage of masticadienonic acid and significantly increased percentage of 28-norolean-17-en-3-one (19–36 %), something that was attributed to the use of the stimulant agent, which, probably, stimulates the biosynthesis of 28-norolean-17-ene derivatives.

Freshly harvested mastic is colorless. The discoloration of commercial mastic becomes notable within 1 year, when a light yellow color is visible. Within a number of years, the yellow tint turns into a strong yellow-orange color. The yellowing of freshly harvested mastic tears could be due to oxidation initiated by sunlight irradiation during harvest, as explained by Dietemann et al. (2001). In another study, the same group of researchers revealed that the harvesting and cleaning methods influence the mastic's aging behavior (Dietemann et al. 2005), as its radical content was found to depend on the extent of light exposure during harvest, size of mastic tears, mastic quality, and recent storage conditions. According to their observations, the radical formation was mainly caused by light exposure, but also by impurities in the resin, while it was generally dependent on surface-to-volume ratio and aging conditions.

The composition of mastic resin may also vary with the season, environmental conditions (weather, soil etc.), age of the tree, or depend on the individual tree's secretion, which might well influence the resin's aging behavior.

19.3 Health Effects, Antioxidant, and Antimicrobial Properties

Chios mastic was known for its medicinal properties since ancient times, while it is still used as a traditional folk remedy throughout the eastern Mediterranean. Ancient Greeks used it to cure snakebites, while Indians and Persians used it to fill dental cavities. Its usefulness in prevention of digestion problems and colds is first mentioned by a number of ancient Greek authors, e.g., Hippocrates, Dioscorides, and Theophrastus. In medieval times, mastic was greatly appreciated by sultan's harems as a breath freshener and teeth whitener. In some parts of Africa or Asia, especially Hong Kong, mastic has been esteemed for its aphrodisiac properties. In Saudi Arabia the mastic is offered as a nuptial present from the groom to the bride in order to keep away evil spirits and presumptive rivals.

In recent times, mastic has been reported to possess antioxidant, radical scavenging activity, anti-inflammatory, aphrodisiac, anticancer, antifungal, and antibacterial activity, being particularly effective against gastrointestinal disorders like dyspepsia and peptic ulcer. In addition, regular consumption of mastic has been proven to absorb cholesterol, thus easing high blood pressure and reduce the risk of heart attacks. Because of this wide spectrum of biological activities, which are mainly attributed to its triterpene/triterpenoid and essential oil constituents, mastic has gained respect and attracted much attention among the scientific and medical community as a natural substance in medicine and healthy food preparation.

Several studies have been published on CMG with regard to its effectiveness against *Helicobacter pylori* and peptic ulcers. *H. pylori* is a Gram-negative spiral bacterium that colonizes the stomach. Its incidence in Europe is in the range of 10–25 % and has been falling during the last decades while in the developing world it is estimated that its incidence is much higher (Magalhaes-Queiroz and Luzza 2006). Infection with *H. pylori* is etiologically linked to gastritis, peptic ulcer disease, primary B cell gastric lymphoma, and adenocarcinoma of the stomach. *Helicobacter pylori* can be eradicated but this is difficult to achieve and at least two antibiotics and an acid suppressant are required to achieve eradication (Malfetrheiner et al. 2007). Side effects for these regimes are common and a major concern is the development of antimicrobial resistance.

Earlier clinical studies conducted by Al-Habbal et al. (1984) and Al-Said et al. (1986) during 1980s indicated that CMG was effective against gastric and duodenal ulcers. More specifically, the administration of mastic (1 g daily) relieved the pain and healed the stomach and duodenal ulceration in the majority of the patients suffering from peptic ulcers within 2 weeks (Al-Habbal et al. 1984). Al-Said et al. (1986), who studied the effect of mastic on experimentally induced gastric and duodenal ulcers in rats, observed that mastic at an oral dose of 500 mg/kg reduced significantly gastric secretions, protected cells, and reduced the intensity of gastric mucosal damage. In another study, Huwez et al. (1998) showed that mastic can cure peptic ulcers by killing *Helicobacter pylori* bacteria at concentrations as low as 0.06 mg/mL, while Paraschos et al. (2007) indicated that mastic has only a modest ability

to eliminate *H. pylori*, suggesting that refining mastic by removing the polymer poly-*β*-myrcene may make the active components, in particular isomasticadienolic acid, more available and effective. In their study, Paraschos et al. (2007) revealed that the acidic fraction of mastic gum led to an approximately 30-fold reduction in *H. pylori* colonization of infected mice. In addition, Kottakis et al. (2008), who investigated the existence of arabinogalactan proteins in CMG extracts, stated that they exhibit antibacterial activity in vitro against *H. pylori* suggesting that these molecules could provoke morphological alterations in *H. pylori* and therefore inhibit its growth in vitro. The same researchers, in another study, explored the effect of CMG-extracted arabinogalactan proteins (AGPs/CMG) both in vitro and in vivo, under the presence of *H. pylori* neutrophil-activating protein (*HP*-NAP), and showed that 1 g of mastic gum daily reduced the protein that stimulates neutrophils to release cytokines (Kottakis et al. 2009). Cytokines increase inflammation specifically in relation to gastric ulcers in the presence of *H. pylori*.

On the other hand, the activity of mastic gum against *H. pylori* in vivo was the subject of studies with conflicting results. Two in vivo studies, one in mice and the other in humans, showed no eradication of *H. pylori* and only a modest antibacterial activity (Bebb et al. 2003; Loughlin et al. 2003). However, in a recent study, Dabos et al. (2010a, b) showed that mastic gum possesses antibacterial activity against *H. pylori* in vivo and is able to eradicate it from patients. These researchers conducted a randomized controlled trial in order to assess the efficacy of mastic gum monotherapy or in combination with a proton pump inhibitor on *H. pylori* eradication and to compare this efficacy with the standard treatment regime. According to their results, 38.5 % of the patients were eradicated while in most of them the urea breath test (UBT) value decreased compared to the pretreatment reading. Although mastic gum monotherapy did not achieve eradication rates as high as the standard (76.92 %) it could be used as a rational alternative regime in patients either unwilling to undergo eradication with the usual triple therapy regime or being unable to tolerate it due to side effects. The therapeutic effects of mastic gum were attributed to constituents belonging to the class of mono- and sesquiterpenoids (essential oil) and triterpenoids (e.g., masticadienonic acid).

Chios Mastiha appears to be efficacious also in the treatment of functional dyspepsia. Dyspepsia is a very common problem that affects a lot of people worldwide. It is a medical condition characterized by chronic or recurrent pain in the upper abdomen, upper abdominal fullness, and feeling full earlier than expected when eating. Dyspepsia in the absence of an identifiable structural lesion in the upper gastrointestinal system is referred to as functional dyspepsia and is estimated to affect about 15 % of the general population in western countries (Talley et al. 1999). According to Dabos et al. (2010a, b) the gum significantly improves symptoms in patients with functional dyspepsia. Their results demonstrated that there was a marked improvement of symptoms in 77 % of patients receiving CMG, while individual symptoms that showed significant improvement were stomach pain in general, stomach pain when anxious, dull ache in the upper abdomen, and heartburn.

Mastic gum has also been evaluated for possible clinical effectiveness in patients with Crohn's disease. Kaliora et al. (2007), based on the information that a major

constituent of mastic, namely oleanolic acid, is among the best-known triterpenes with biological properties against chemically induced liver injury in laboratory animals exerting anti-inflammatory and antitumor-promotion effects (Liu 2005), examined the effects of supplementation with mastic in patients with mildly to moderately active Crohn's disease. Their results demonstrated that mastic gum treatment clearly improved the clinical features of the disease and regulated inflammation and antioxidant status. Taking into account the harmful side effects of the long-term use of corticosteroids, further research in larger cohorts is required in order the beneficial role of this natural product to be established.

Taking into consideration its antibacterial and antimicrobial properties, Mastiha is also a promising agent to prevent oral diseases, especially plaque-related diseases such as dental carries. Earlier studies have demonstrated that chewing mastic gum prevents plaque formation or reduces it when it has already been formed on those teeth surfaces that can be reached by the mass of Mastiha during its methodical chewing (Topitsoglou-Themeli et al. 1984). In agreement with these observations, the results of Takahashi et al. (2003) and Aksoy et al. (2006) also suggest that regular use of mastic gum may be useful to control dental caries via its inhibitory action against overall bacterial growth and antiplaque formation activity. Mastic gum has an antibacterial activity against one of the most important cariogenic oral bacteria (*S. mutans*), which leads to decalcification of enamel and surface caries, and it may be useful for maintaining oral hygiene by reducing the bacterial growth (*mutans streptococci*) in saliva. Moreover, from a convenience perspective, the usage of mastic gum may be useful in cases where other means (e.g., mouth rinses) would be unfeasible but a chewable antiplaque agent would be desirable. It is also worth noting that chewing mastic gum, with a unique pleasant flavor, can help people relax and recover from both physical and mental work. Furthermore, the mastic acts as breath freshener and improves the flow of saliva, which is required for the appreciation of taste and in general for improvement of stomatal hygiene.

Moreover, mastic gum is considered as a natural supplement for zinc to enhance male sexuality and prostate function. For a long time, mastic has been esteemed for its aphrodisiac properties. Sawidis et al. (2010) compared the trace element zinc content of mastic from *P. lentiscus* var. *chia* to that of other natural resins as well as the quantity released after a certain time of chewing. Among all gums studied only the Chios mastic released an amount of zinc in the mouth and gastrointestinal system (~50 %), whereas commercial gums studied provoked deficiency of this element for the human organism. These results indicate that the gum could be a natural source of zinc and could be used to provide it to humans in the case of minor deficiency of this trace element. The slow release of the trace element (during the long chewing time) follows the biological rates of intake and metabolism by the human organism. This product has advantages over others commercially available, e.g., pills, because CMG is a natural product with long history of usage, while no undesirable side effects have ever been attributed to it.

P. lentiscus has also been traditionally regarded as an anticancer agent, especially on tumors of breast, liver, stomach, spleen, and uterus. These traditional beliefs are in line with recent studies demonstrating that CMG induces apoptosis (Balan et al. 2005)

and possesses antiproliferative activity (Balan et al., 2007) in colon cancer cells. As it was suggested by these researchers, CMG might be developed into a chemotherapeutic agent for the treatment of human colon and other cancers. Loutrari et al. (2006), based on reported observations concerning the monoterpene perillyl alcohol, examined whether mastic oil could also suppress tumor cell growth and angiogenesis. Perillyl alcohol, which is found in traces in mastic oil, is of great clinical interest due to its established chemopreventive and chemotherapeutic potential demonstrated in a variety of rodent tumor models (Belanger 1998; Crowell 1999), while it acts as an angiogenesis inhibitor as well (Loutrari et al. 2004). These researchers found that mastic oil concentration and time exerted an antiproliferative and proapoptotic effect on human leukemia cells and inhibited the release of vascular endothelial growth factor from mouse melanoma cells, emphasizing that mastic oil through its multiple effects on malignant cells and endothelial cells might be a useful natural dietary supplement for cancer prevention (Loutrari et al. 2006).

P. lentiscus has also been associated with cardiovascular protection and hepatoprotection. It inhibits human LDL oxidation in vitro (Andrikopoulos et al. 2003) and, due to the triterpenes, it acts on peripheral blood mononuclear cells to elicit an antioxidant/antiatherogenic effect (Dedoussis et al. 2004). It has been shown that the gum exerts its effect mainly through increasing the antioxidant defense system, and effectively lowering the levels of serum cholesterol in human subjects. This action has also been reported by Triantafyllou et al. (2007) who demonstrated that CMG powder could have a hepatoprotective/cardioprotective role in vivo in humans by primarily acting on the hepatocyte, and therein modifying the lipoprotein metabolism, either at the level of apolipoprotein biosynthesis or at the level of lipoprotein receptor expression.

Besides, CMG has been proved to have strong antioxidant activity in a variety of oil substrates (lard, olive oil, corn oil, and sunflower oil) (Assimopoulou et al. 2005). The best concentration of the resin presenting the highest activity depended on the substrate. In olive oil, at concentrations of 0.1 and 0.15 % w/w, showed high antioxidant activity and its essential oil, even at low concentrations (0.02 %), strongly retarded its oxidation. The combination of gum with citric acid presented a synergistic effect in both sunflower oil and corn oil.

The essential oil of *P. lentiscus* var. *chia*, traditionally used as a food additive, is also a natural antimicrobial agent that has found extensive uses in medicine in recent years. In a recent investigation, correlating the antimicrobial activity with individual components or the mastic oil itself (Koutsoudaki et al. 2005), several trace components that appear to contribute significantly to the antibacterial activity of mastic oil were identified: verbenone, *R*-terpineol, and linalool, while the sensitivity to these compounds was different for different bacteria tested (*Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*). Its activity has been attributed to the combination of several bioactive compounds, including some of the trace elements, working synergistically.

Due to its antimicrobial properties, mastic oil could also be used as a more "natural" alternative for the maintenance or extension of a product's shelf life. As there is considerable interest in the possible use of unconventional food additives, either to

prevent the growth of food-borne pathogens or to delay the onset of food spoilage, the naturally occurring essential oil from CMG inhibitory action on *Staphylococcus aureus*, *Salmonella enteritidis*, *Lactobacillus plantarum*, and *Pseudomonas* spp. has been exploited (Tassou and Nychas 1995). The addition of mastic gum in broths as well as in a model food system (skim milk) inoculated with these organisms inhibited their growth.

19.4 Traditional Food Products Based on Mastic

19.4.1 Chewing Gum

The unique physicochemical characteristics of CMG and its composition in terms of flavor constituents, combined with its well-recognized by the scientific community health-promoting potential, have been exploited over for centuries by habitants of the eastern Mediterranean and the Middle East area in the preparation of a number of traditional foods and beverages. A large proportion of the annual CMG production is exploited as a natural gum base in the preparation of the famous Chios chewing gum, as an alternative to chewing gum products prepared with synthetic gum base. From ancient times and over the years, the dried mastic gum particles were masticated, as their mixing with saliva in the mouth at body temperature provide a very durable and pliable bit. The product was very popular as it had the particular smell and also it was well known by changing the smell of the mouth to be better and, moreover, by being effective for the health of the stomach.

As was suggested by Kehayoglou et al. (1994), the property of Mastiha particles to turn into chewing gum when subjected to mastication is connected with the presence in the gum of an insoluble in alcohol polymeric fraction which acts as a plasticizing agent of the monomeric mastic gum fraction. The polymeric fraction content may depend on environmental conditions prevailing during the time of mastic gum collection as well as on the method of collection and the length of product storage. In addition the treatment of mastic with steam, a process applied for the recovery of essential oil, results in an increase of hardness due to the removal of the oil constituents which probably also have a plasticizing action on the resin. When this polymeric fraction was isolated and incorporated in natural mastic resin at 10 and 20 % levels, a significant reduction in the product hardness was observed by these investigators. Even more effective as plasticizers appeared to be food additives such as wax and lecithin. Their incorporation in mastic gum produced a drastic reduction in the products resistance to compression which depended on the level of addition.

There are still people who prefer to chew the natural Mastiha, to take full advantage of its flavor and health-promoting properties. This raw product, however, rates low in certain textural characteristics especially in hardness during chewing and stickiness to the teeth. These poor textural characteristics of the natural gum and

changing consumer trends over the last decades, as well as the competition from chewing gum products based on synthetic gum, have led to mixing of the gum with a number of food additives to develop the Chios chewing gum or Chios chicle. The product is prepared by mixing natural Mastiha and mastic oil with sweeteners, stabilizers, gums, and plasticizers, aiming at improving the flavor and textural characteristics of the final product. Chios chicle is also available in a sugar-free form incorporating xylitol as the sweetener.

19.4.2 Confectionery, Desserts, and Bakery Products

CMG and its oil have been used over the years for the preparation of an appreciable number of traditional confections, desserts, and bakery products of the cuisines of the East Mediterranean countries, mainly for flavoring purposes. Many of these products have now been commercialized and are found in the market although their production and marketing is still performed by local small-scale production units, patisserie and bakeries. The range of such products may include sweets and candy such as Turkish delight (Lukumia in Greek), Khalva, Vanilia (a mixture of mastic gum, sugar, and vanilla), ice cream, yogurt, and bakery products such as cakes, cookies, and tsoreki, a very well-known specialty bakery product exhibiting a very elastic structure and containing eggs, butter, and milk, mainly consumed in Easter but also all the year round.

In addition to flavoring effect, CMG incorporation in these products may also bring about a substantial modification of their textural characteristics. Most of the food products are considered multiphase systems exhibiting diverse compositional and structural characteristics. The incorporated mastic gum particles may become involved in various interactions with the food components and as a result, their presence is expected to influence to a different extent the food textural characteristics, depending on the type of food product and its composition. The building up of structure of most of the semisolid or solid foods is based on the presence in the system of biopolymers (polysaccharides and proteins) which are involved in molecular interactions and the development of a matrix that entraps and immobilizes the solvent (water) and all other food constituents. Experience has shown that mastic gum incorporation in certain confectionery or bakery products (ice cream, cakes, etc.), which owe their structure to biopolymer interaction and matrix formation, has a profound effect on their texture. This leads to the conclusion that some kind of interaction should take place between the mastic particles and the food matrix. As Mavrakis and Kiosseoglou (2008) have suggested, the mastic particles in biopolymer gel matrices may act either as active or negative fillers of the resulting composite structure depending on the polymer involved in gel matrix development. More specifically, active filler effects were observed in the case of matrices based on egg white albumen and were attributed to the development of interactions between the matrix protein molecules and those adsorbed to the mastic particle surface. On the other hand, negative filler effects and deterioration of mechanical properties were

observed in the case of polysaccharide-based matrices attributed to lack of interaction between the mastic gum particles and the polysaccharide matrix, leading to particle flocculation due to depletion.

19.4.3 Alcoholic and Nonalcoholic Beverages

CMG and mastic oil are used as flavoring ingredients in the production of a number of Greek alcoholic drinks, especially liqueurs and ouzo. The liqueur Chios Mastiha is a very well-known alcoholic drink prepared by mixing in water potable alcohol, Mastiha powder, and sugar. Ouzo is a widely consumed throughout Greece anise-flavored alcoholic drink, which is sometimes mixed with CMG to obtain the characteristic flavor of Mastiha.

A nonalcoholic drink, sometimes flavored with CMG, is soumatha. The drink is based on a water mixture of comminuted almonds and sugar and is consumed in certain areas of Greece during the summer period. This product is in effect an oil-in-water emulsion and suspension at the same time, as its basis is extracted from almond oil bodies in combination with almond particles both suspended in the water phase.

The distribution of mastic oil constituents between the oil and the water phase in soumatha may affect flavor release from this mixed dispersion system and hence perception by the consumer. The same should hold for any other Mastiha-containing liquid product appearing in the form of an oil-in-water emulsion (e.g., cream liqueur) or solid and semisolid food product having a protein or polysaccharide-based structure with oil droplets embedded in the biopolymeric network (e.g., ice cream and yogurt). As Paraskevopoulou et al. (2009) have reported, the partition of mastic gum volatiles between an air–liquid interface in a hydroalcoholic model system depends on the type of emulsifier, and in some cases on oil droplet size and the nature of the dispersed oil phase. These workers suggested that the product composition and structural characteristics may strongly influence the sensory properties of a mastic-flavored food or drink, something that should be taken into consideration when developing such products.

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Chapter 20
Capsicum chinense: Composition and Functional Properties

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20.1 Introduction

Habanero pepper (*Capsicum chinense* Jacq.) was introduced to the Yucatan Peninsula, Mexico, via the Antilles. The Yucatan Peninsula is currently the largest habanero pepper-producing region worldwide, with 732 ha under cultivation and 3700 t harvested annually (Cázares-Sánchez et al. 2005). Habanero pepper fruit has a limited number of applications. It is primarily marketed as a fresh vegetable in the food industry, but is also processed for its oleoresins and capsaicin for inclusion in products such as cosmetics, tear gas, and organic insecticide. Habanero pepper fruits are hollow berries with two, three, or four cavities. Unripe fruits are green and become orange, red, or brown during maturation. Ripe habanero fruits are typically 2–6 cm long (Fig. 20.1).

20.2 Composition and Functional Properties

20.2.1 Nutrient Composition

Peppers are an important source of nutrients in the human diet, particularly antioxidants such as vitamins A and C and neutral and acidic phenolic compounds. In habanero pepper, moisture content is within ranges reported for other commercial

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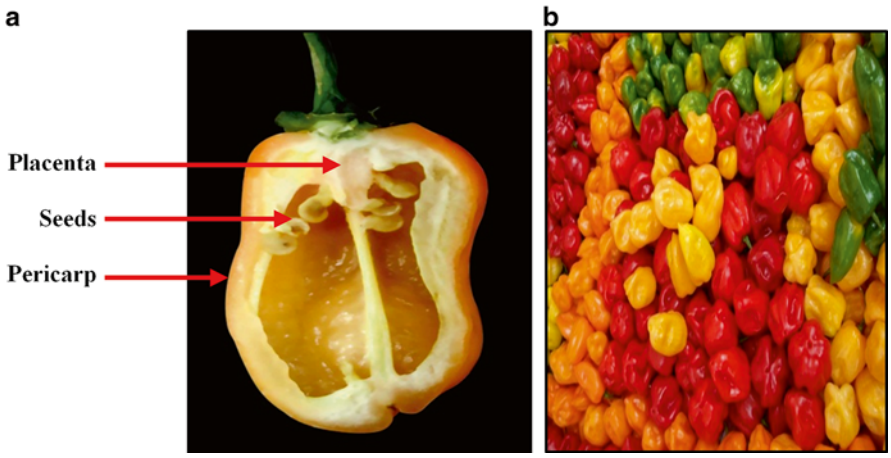

Fig. 20.1 (**a**) Habanero pepper fruit structure, (**b**) color characteristics of the maturation process

Table 20.1 Proximate composition and vitamin C content of habanero pepper

Parameter	Content (g/100g)
Moisture	89.8 ± 0.4
Crude protein	1.52 ± 0.10
Lipids	1.52 ± 0.09
Ash	0.72 ± 0.2
Available carbohydrates	9.20 ± 0.26
Vitamin C	0.122 ± 2.52

pepper varieties. This parameter has a direct effect on other nutrient values in fresh weight. It is to be expected therefore that crude protein, lipids, ash, and available carbohydrates (Table 20.1) were also in agreement with values reported for other commonly consumed peppers (Guil-Guerrero et al. 2006).

Habanero pepper has particularly high levels of carbohydrates (9.20 g) and crude protein (1.52 g) and very adequate vitamin C content (0.122 g) for 100g of fruit. Overall, this pepper appears to be an excellent contribution to human nutrition.

20.2.2 Capsaicinoids and Pungency

Capsaicin is the main capsaicinoid in habanero pepper, followed by dihydrocapsaicin. These two compounds are about twice as potent to the taste and pain nerves as the minor capsaicinoids nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin. Considered by many to be the hottest pepper in the world, habanero pepper has a pungency range of 150,000–300,000 Scoville units. Capsaicin is believed

to be synthesized in the interlocular septa of chili peppers by addition of a branched-chain fatty acid to vanillylamine; specifically, capsaicin is produced from vanillylamine and 8-methyl-6-nonenoyl CoA (Guil-Guerrero et al. 2006). Most of total fruit capsaicin is found in the placenta (62 %), followed by the seeds (37 %) and the pericarp (1 %) (Fig. 20.1).

20.2.3 Antimicrobial Properties

A survey of the Mayan pharmacopoeia revealed that *Capsicum* species tissues were included in a number of herbal remedies for a variety of ailments of probable microbial origin. Aqueous extracts from fresh *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens* varieties have been tested for their antimicrobial effect against bacteria and yeast. Antimicrobial activity in *Capsicum* species is at least partially attributable to two pungent compounds found in capsaicin and dihydrocapsaicin. Cichewicz and Thorpe (1996) reported that aqueous extracts of *C. chinense* exhibit varying degrees of inhibition against *B. cereus*, *B. subtilis*, *C. sporogenes*, *C. tetani*, and *S. pyogenes*. The antimicrobial properties of peppers can also be attributed to peptides in their seeds (Brito-Argaez et al. 2009). These peptides are small molecules and apparently function as constitutive elements of pepper species' pre-existing innate defense system. Once isolated, those compounds with antimicrobial properties can be included in the formulation of natural defensive compounds for topical use in humans and/or animals or applied directly or in dilute solutions in agriculture.

20.2.4 Antioxidant Capacity

Antioxidants are important inhibitors of lipid peroxidation. They not only protect food from degrading but also function as a cell defense mechanism against oxidative damage and are vital in the prevention of degenerative diseases such as Alzheimer's or cancer (Sun et al. 2007). *Capsicum* genus fruits owe their intense orange and red color to carotenoid pigments synthesized during ripening. Three carotenoids found only in the *Capsicum* genus are responsible for final fruit color: capsanthin, capsorubin, and capsanthin 5,6-epoxide (Guil-Guerrero et al. 2006). A comparative study of carotenoid composition in the fruit of three Mexican pepper varieties showed the major carotenoids to be β-carotene and β-cryptoxanthin as a provitamin A precursor (Collera-Zúñiga et al. 2005). Overall, the concentration of carotenoids, ascorbic acid, flavonoids, phenolic acids, and other antioxidants increased as pepper fruit reached maturity. Their high carotenoid content in mature stages makes colored pepper varieties good antioxidant sources (Chu et al. 2002).

20.3 Conclusion

Habanero pepper (*Capsicum chinense* Jacq.) is a promising source of some nutrients and functional compounds.

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Chapter 21
Nuts and Dried Fruits Potential as Functional Foods

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21.1 Introduction

21.1.1 Functional Food

Nowadays the society presents a growing concern about the food impact on human health. The functional food concept was created in the last century and has been presenting a constant increase of importance on the consumer's life.

Modern society life style, life expectancy increasement and medication high costs are factors that lead food industry to study food properties that might help to the prevention and treatment of several illnesses.

Functional food concept arose during the 1980s in Japan, being regulated as *Foods for Specific Health Use* (*FOSHU*) in 1991 (Mark-Hebert 2004). Although there are several definitions, according to *International Food Information Council* (*IFIC*) "Functional foods are foods or dietary components that may provide a health benefit beyond basic nutrition" (Functional Foods 2009). It is important to enhance that these products must be consumed regularly, included in a balanced diet and regular physical exercise, in order to benefit its properties.

Consumer's interest on food health benefits has been increasing, as shown by this food market segment growth. According to a TNS study, an international market study company, Portuguese people spent about 200€ million in 2007, an increasement of 12.8 % when compared to the previous year (Marques 2008). Moreover, global functional food market is expected to worth about 175€ billion by 2012, a 25 % rise concerning 2007 (Starling 2009).

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21.1.2 Mediterranean Diet

Dietary styles are very important when considering health benefits. Mediterranean diet concept arose in the 1950s, when some Mediterranean countries dietary pattern (including Portugal) were associated to low mortality and high life expectancy (Lorgeril and Salen 2006). Its protective properties against cardiovascular diseases, cancer, diabetes, and neurological diseases are attributable to its main component properties (Ortega 2006), as summarized in Table 21.1.

21.1.3 Nuts and Dried Fruits

Influenced by dietary pattern, nuts and dried fruits are consumed regularly in Portugal. These foods are consumed as a whole or incorporated in traditional confectionary, especially during Christmas season.

According to DAFNE—DAta Food NETworking (2005), consumption of nuts and dried fruits had risen between 1990 and 2000 (see Fig. 21.1), being chestnut and dried fig the most consumed fruit in each category.

Despite this growing tendency, Portuguese production is still insufficient. According to INE data (Instituto Nacional de Estatística 2005), in 2005 nuts cultivation occupied circa 70,000 ha, expressing this sector's importance in national

Table 21.1 Food sources that contribute to Mediterranean protective character

Component	Food source
Fiber	Fruit, legumes, and cereals
Monounsaturated fat	Olive oil, omega-3 rich fish
Antioxidants	Vegetables, legumes, nuts

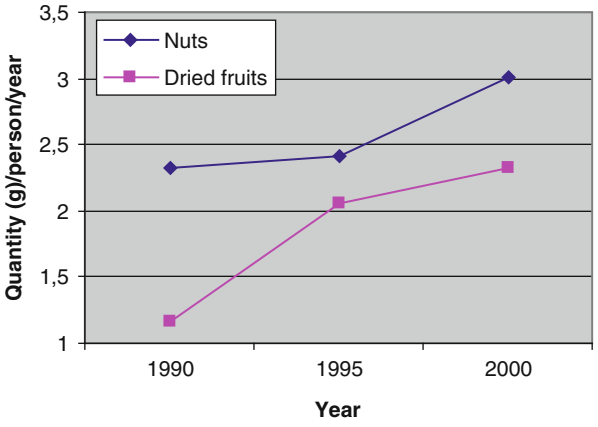

Fig. 21.1 Portuguese nuts and dried fruits consumption (1990–2000)

agriculture. Notwithstanding, there was a decreasing of 11 % when compared to 2001 data of Agricultural Ministry (Gabinete de Planeamento e Politica Agro-Alimentar 2001), which reflects the debilities of this sector. Production has also registered a reduction from 64.430 to 50.844 ton between 1998–2000 and 2003–2005 (GPPAA 2007). Trás-os-Montes is the region with highest nut production, representing 86 % of total cultivation area. Chestnut is the leader fruit, followed by almond and carob. Walnut and other nuts have low representativeness.

Regarding dried fruits, there is little information about this sector. Since Portugal has great climate conditions for fruit drying, it is important to study this activity concerning production, consumption, and commercial transactions. This evaluation will allow analyzing its strength and weakness, allowing quality and competitive improvements. Dried fruits production is distributed along the country, which was not expected since Algarve and Alentejo are regions with higher temperatures during the year.

21.2 Nuts and Dried Fruits Potential as Functional Food

Functional food protective properties are related to ingredients which have a positive effect on treatment/prevention of certain diseases. Therefore, it is important to analyze the nutritional composition in order to evaluate food functional properties. In this chapter, for each category, a nutritional characterization is performed followed by a functional properties analysis.

21.2.1 Nuts

21.2.1.1 Nutritional Properties

Nuts are food with a hard shell with fruit inside. Only its interior is consumed.

Nuts are characterized as the best protein vegetable source, being an excellent alternative for vegetarians to reach RDI, between 50 and 60 g protein/day (Gebhardt and Thomas 2002). They are also rich in fiber and have good amounts of monounsaturated and polyunsaturated fatty acids, recognizably known by its positive effects on organism. Like other fruits, nuts are good sources of vitamins, minerals, and antioxidants (Irish Nutrition and Dietetic Institute 2007).

Almond (*Prunus amygdalus* Batsch) is particularly rich in α-tocopherol and manganese, being an important source of other minerals such as copper, magnesium, and phosphorous as well. It has also moderate percentage of fiber and protein (mainly arginine). Some studies evaluated almond antioxidant potential, concluding that its skin is very rich in antioxidants (Takeoka and Dao 2003; Wijeratne et al. 2006; Chen et al. 2006). Thus almonds should be consumed with its skin, rather than peeled fruit, in order to benefit from its antioxidant properties.

Hazelnut (*Corylus avellana* L.) lipid composition (high oleic acid content and lower presence of saturated acid fat) makes its oil a very good alternative to olive oil, main fat used in Mediterranean diet. Phytosterols (β-sitosterol), polyphenols, and α-tocopherol are also present in large quantities in hazelnut oil (Alasalvar et al. 2006). Similarly to almonds, hazelnut skin is rich is phenolic compounds which confers hazelnut good antioxidant properties (Shahidi et al. 2007). This fruit is also a good source of minerals as well some vitamins, being especially rich in α-tocopherol (Alasalvar et al. 2003). According to available literature, there is little information about minerals and vitamin content of Portuguese varieties. It is suggested a physico-chemical study of national species in order to evaluate its potential.

In 2000, Sze-Tao and Sathe found that glutamic acid and arginine are the dominant amino acids in *walnuts* (*Juglan regia* L.). In the same study, it was concluded that this fruit has also good content of vitamins and minerals (Sze-Tao and Sathe 2000). Çaglarirmak developed a research about Turkish walnuts, founding that linoleic acid is present in higher quantity (above 40 %). Polyunsaturated fatty acids profile makes this fruit oil suitable to cook and temper salads, being also a good alternative to olive oil. Walnuts consumption is advisable for sportsman due to its lipid richness. The same study allows affirming that this fruit is a good source of proteins. Phenolic compounds are present mostly in shell (Labuckas et al. 2008). It would be instructive to perform a physico-chemical study on Portuguese cultivars.

In a Portuguese study on *chestnut* (*Castanea sativa* Mill.) species, cultivated in the North and Center of Portugal, it was reported that this fruit has low amounts of protein and fat, being however a good source of fiber. Authors also concluded that starch is presented in high content, representing almost 30–45 % of its weight (Costa et al. 2008). Chestnut sub-products are richer in phenolic compounds, particularly polyphenols and flavonoids, when compared to fruit (Barreira et al. 2008).

Pine (*Pinus pinea* L.) planted in Portugal has high content of fat, protein, magnesium, and phosphorous. Linoleic and oleic acid are the most representative fatty acids (Costa and Evaristo 2008). It would be beneficial to study national varieties amino acids profile, since these components are known to have an important role on human health.

There are very few studies on *Carob* (*Ceratonia siliqua* L.) chemical composition. It is known that aspartic acid and alanin are the dominant amino acids in pod (Ayaz et al. 2007) and carob germen contains high levels of glutamic acid and arginine (Bengoecha et al. 2008). Flower is richer in protein (about 4.7 %), followed by fruit and syrup (Ozcan et al. 2007). Based on these results it is important to evaluate Portuguese Carob composition, in order to maximize its potential.

21.2.1.2 Functional Properties

Numerous studies have been performed on nuts functional food properties. Obtained results show that its consumption might help preventing cardiovascular, cancerigenous, and neurodegenerative diseases, as well as diabetes.

Formerly nuts consumption was not advised due to its high energetic value, which might lead to weight gain. However, energy absorption is not complete due to its high content of fiber, protein, and unsaturated fatty acids. Based on cardiovascular protective properties, FDA suggests that 42 g/day consumption of almonds, hazelnuts, pines and walnuts, among others, may reduce coronary diseases incidence, if incorporated in a low saturated fat and cholesterol diet, characteristic of Mediterranean diet (Taylor 2003).

Functional properties of almonds, hazelnuts, walnuts, carobs, pines, and chestnuts are discussed below:

1. Almond
   Several studies have been performed in order to study almond functional properties, highlighting its contribution for protection against:
   - *Cardiovascular disease*: High content in monounsaturated fatty acids, as well as α-tocopherol are major factors for this protective effect, according to a study performed in 2002 by Jenkins and other researcher (Jenkins et al. 2002). In 1998, a study was conducted with 48 hypercholesterolemic individuals to evaluate almond effects on low density (LDC) and high density cholesterol (HDC). Consumption of 100 g/day resulted in a significant reduction of total cholesterol (TC) and LDC, when compared with other fat sources (with olive oil and with cheese and butter). The obtained results encourage investigation on almond oil effect on cardiovascular diseases prevention, followed by comparison with olive oil protective properties. The same study indicated that fiber category, high arginine:lisin lysine ratio and vegetable sterols have positive effects on lipoproteins (Spiller et al. 1998). A study performed in rats (Chen et al. 2005) shows that flavonoids present in almond skin act synergistically with E and C vitamins, improving low density lipoproteins resistance to oxidation. It would be instructive to continue similar research in human subjects.
   - *Diabetes*: High unsaturated fat content (García-Lorda et al. 2003), antioxidant potential (Jenkins et al. 2006; García-Lorda et al. 2003) and high ω-6:ω-3 ratio are factors that may contribute to almond potential on diabetes prevention. It is also known that copper insufficiency lowers glucose tolerance. Consequently, studies should be conducted in order to comprehend this mineral contribution to avoid this disease.
   - *Cancer*: In 2001, Davis and Iwahashi concluded that almond consumption may have an anti-carcinogenic effect, though it is not clear which components are associated to this property (Davis and Iwahashi 2001). Thus, further experiments should be conducted in order to confirm this ability and understand its action mechanism. The combination between α-tocopherol, calcium, and fiber may contribute to cancer protection.
   - *Osteoporosis*: Calcium, an extremely important component to bone formation, is associated to osteoporosis prevention. Since almonds have high content of this mineral, it would be interesting to study if this fruit consumption might help avoiding this illness.

- *Hepatic diseases*: Oxidative stress and high levels of triglycerides are associated to liver diseases (Morisco et al. 2008). Since almond has a good antioxidant potential, as well unsaturated fatty acids high content, it would be beneficial to study almond effect on hepatic diseases.
- *Neurological diseases*: Parkinson and Alzheimer diseases may be related to oxidative stress. As mentioned previously, almond antioxidant potential may also have a positive effect on the prevention of these disorders.

A laboratory study performed by Institute of Food Research (IFR) scientists indicates that almond has a prebiotic effect, due to its lipid fraction (Mandalari et al. 2008). It is necessary to test this property in humans.

2. Hazelnuts
   Fatty acids profile as well as high content in antioxidants components give hazelnuts a protective role on:

   - *Cardiovascular diseases*: Fatty acids profile, antioxidants content (Durak et al. 1999), and phytosterols (Amaral et al. 2006) play important roles in heart disease protection. Selenium concentration is inversely linked to cardiovascular diseases risk. Since this component is relevant in hazelnut mineral composition, it might also contribute to prevent heart illness.
   - *Cancer*: There are no evaluations about hazelnut anti-carcinogenic potential. High content in Selenium, known by its protective properties against this illness (Zeng and Combs 2008), might give this fruit consumption capacity to reduce risk of cancerous diseases. Additionally, antioxidants and phytosterols may also influence hazelnuts properties, thus it is recommended to study hazelnut effect in tumors development.
   - *Diabetes*: Likewise almonds, high unsaturated fatty acids and antioxidants content might provide protective characteristics against diabetes 2, though it is necessary for epidemiological experiments to confirm.
   - *Hepatic diseases*: Hazelnut consumption may contribute for liver pathologies reduction, due to its richness in antioxidants and unsaturated fatty acids.
   - *Neurological diseases*: As mentioned previously, oxidative stress reduction may contribute to Parkinson and Alzheimer avoidance. Antioxidants presence in hazelnuts may play an important role in protective ability of this fruit consumption regarding this illness.
   - *Depression and anxiety*: B complex vitamins and folate appears to be inversely associated to depression and high anxiety levels (Hintikka et al 2003). Therefore, further investigation is needed to study hazelnuts consumption effect on reduction of the above-mentioned symptoms.

3. Walnuts
   Walnuts are a very energetic fruit, making it suitable for sportsman with a regular physic effort. It is also appropriate to people with high brain drain due to its phosphorous and magnesium high levels.

   Equally to other nuts, its components allow protection against some syndromes like:

- *Cardiovascular diseases*: Several studies have been performed in order to evaluate walnuts ability to promote heart health (Chisholm et al. 1998; Zambón et al. 2000; Almario et al. 2001; Ros et al. 2004). Although consumed amounts and experimental period vary between evaluations, results allow concluding walnut consumption lowers TC and LDC, thus reducing risk factor associated to this sickness. High content in ω-3 fatty acids and antioxidants (highlighting melatonin) are the main components associated to this effect (California Walnuts Health Research 2006). Arginine, as previously referred, may also contribute to these positive properties.
- *Diabetes*: Individuals with diabetes type 2 have higher probability of having high levels of HDC, raising cardiovascular diseases risk. As expected, investigation performed in 2004 and 2005 by Tapsell and other researchers confirmed that walnuts consumption can reduce diabetes risk, as well as contribute to its control, due to unsaturated fatty acids action (Tapsell et al. 2004; Tapsell et al. 2005).
- *Bone diseases*: ω-3 fatty acids as well as antioxidants play a significant role on bone formation in addition to osteoporosis and rheumatoid arthritis prevention (Seifert and Watkins 1997). Since these components are part of walnuts composition, further experiments are needed to test if this fruit consumption might contribute to this pathologies avoidance.
- *Cancer*: Similarly to bone diseases, antioxidants and ω-3 fatty acids are important factors that may contribute to anti-carcinogenic walnut capability, though it is necessary to confirm this fruit consumption effect on tumor development.
- *Neurological diseases*: High antioxidant potential may confer this fruit protective ability against Alzheimer and Parkinson, likewise other nuts referred earlier.

4. Carob
   Carob functional properties have been associated to fiber and antioxidants content. Although, there are other components that provide this fruit's beneficial health characteristics, such as:

   - *Cardiovascular diseases*: In 1999, a study was performed in order to study fiber from carob effects in rat TC and triglycerides, when compared to psyllium husk. Carob fiber was significantly more effective (Pérez-Olleros et al. 1999). It is then important to evaluate if carob consumption can help preventing heart pathologies in humans. Arginine, an important amino acid in this fruit composition, might also contribute to this positive effect.
   - *Cancer*: In 2008 Klenow and other researchers discovered that carob might contribute to reduce intestinal colon cancer (Klenow et al. 2008). It would be advantageous to perform similar investigations concerning prostate and breast cancer.
   - *Anemia*: Iron plays an important role in some anemia's prevention, being present in high levels in carob. Consequently, it would be beneficial to study if there is an inversely proportional relation between this fruit consumption

and this sickness development, as well as if fiber may eliminate/prejudice its action since it reduces minerals absorption.

Additionally, it is known that iron insufficiency in children may delay growing and create learning difficulties. It is therefore advisable to incorporate carob in children food, since it contributes to attain DRI, 5–28 mg/day (Gebhardt and Thomas 2002).

5. Pine
   So far there are no evaluations on pine functional properties. Its lipid fraction may confer protective properties against cardiovascular and cancerous diseases, as well as contribute to diabetes type 2 control. Pine consumption may also prevent anemia and learning/growing troubles in children, due to iron content. It may also avoid inflammatory diseases (through zinc action) and reduce anxiety and depression, due to B complex vitamins high content.
6. Chestnut
   Unlike other nuts, chestnut is poor in unsaturated fatty acids. Even though, it has other favorable characteristics to cardiovascular disease prevention, such as antioxidant potential and fiber. Additionally, fiber may play an important role on intestinal colon cancer reduction and diabetes control/prevention.

21.2.2 Dried Fruits

21.2.2.1 Nutritional Properties

Dried fruits are fresh fruits submitted to a drying process in order to extend its shelf-life, as a result of microbial growth inhibition. Studied dried fruits are: dried plum, dried pear, dried fig, and raisin. Traditionally, they are sun-dried, where sun is the heat source. This technique has disadvantages and limitations and, therefore, new methods were developed using air to dry fruits. Convective air-drying is the most common process in food industry.

Drying affects fruit's nutritional quality: fiber and minerals are concentrated; vitamins A, B, and C content are severely decreased, and there is also a reduction on phenolic compounds level. High antioxidant potential compounds are formed as a result of *Maillard*[1] reactions. Sweetness and high fiber levels make dried fruits suitable to be incorporated in cereal bars or other snacks, being an excellent alternative to chocolates.

Generally dried fruits are very good sources of fiber and minerals. Alike other fruits, they have low content of protein and fat. High sugar concentration is the reason why these foods are not advisable to diabetic people. Except "Pêra passa de Viseu" and "Ameixa D'Elvas," there are few investigations on nutritional properties of fruits dried in Portugal. Since they present some important characteristics that

[1] Chemical reaction between an amino acid or protein and a reduced sugar.

may valorize national species, it is advisable an higher effort from scientific community to study national products nutritional properties.

Nutritional properties of fig (*Ficus carica*), pear (*Pyrus communis*), grape (*Vitis vinifera* L.), and plum (*Prunus domestica*) are briefly discussed below.

Fig (*Ficus carica*) is an excellent source of complex B and A vitamins, being also rich in iron, magnesium, calcium, and potassium. Among usually consumed fruits, fig has the highest calcium content (235 mg/100 g fruit), just below orange, being a good alternative for people intolerant to lactose for attaining RDI (~800 mg/day). Dried figs' fiber content is relatively high (about 20 % RDI), with 28 % soluble fiber (Vinson 1999). It would be important to study national dried fig nutritional properties in order to maximize its potential.

"*Pêra passa de Viseu*" (*Pyrus communis*) is one of the best characterized fruits. It has a high fiber content (12.6 % humid basis), mainly insoluble (Barroca et al. 2006). Since air-drying was the method used, it would be instructive to evaluate if sun-drying would produce similar results. In 2002, a study on phenolic compounds content allowed to conclude that sun-drying caused a reduction of 32 % (25 to 7.9 g/kg humid basis). Generally, there was a reduction on phenolic compounds level, except for arbutin (Ferreira et al. 2002). According to "Tabela de Composição dos Alimentos" (Martins et al. 2006) dried pear is an excellent source of potassium, sodium, calcium, magnesium, and iron, containing also good levels of A, C, and complex B vitamins.

After dried, *grape* (*Vitis vinifera* L.) fiber and B2 and B6 vitamins content significantly increase, as well as protein level. A vitamin percentage drastically reduces and minerals generically rise. Its high energetic level makes dried grape consumption suitable for sportsman (Martins et al. 2006). It is desirable to chemically characterize Portuguese-dried grape, especially antioxidants and carotenoids evaluation in pulp, pips, skin, and vine leaves.

High fiber content (almost 3 g fiber/100 g fruit) is one of the most recognized characteristic of *plum* (*Prunus domestica*), superior to dried beans and any other fruit. Additionally it has good levels of minerals and A and B complex vitamins (Doymaz 2004). β-caroten is the most abundant carotenoid (Stacewicz-Sapuntzakis et al. 2001). Plum has also good antioxidant content, particularly neoclorogenic and clorogenic acids (Kayano et al. 2002; Piga et al. 2003).

21.2.2.2 Functional Properties

Dried fruits are very appreciated for their intense flavor. Drying process affects nutritional quality, producing food rich in fiber and minerals, therefore having beneficial properties to organism. Functional properties of dried fig, dried pear, raisins, and dried plum will be discussed below.

1. Dried fig
   There is no knowledge about this fruit's functional properties evaluation. However, nutritional composition allows affirming that dried fig consumption may help preventing:

- *Cardiovascular diseases*: fiber and complex B vitamins content, components acting on cholesterol absorption, may help preventing this illness. Additionally, magnesium contributes to arterial tension control (Saris et al. 2000), a risk factor associated to coronary diseases.
- *Osteoporosis*: Calcium, potassium, and magnesium play an important role on bone formation (Tucker et al. 1999). Consequently, dried fig possibly will have positive effects on osteoporosis prevention, as well as other bone pathologies, since minerals mentioned above are present in considerable amounts.
- *Anemia*: In 2009, Kolsteren and other researchers affirmed that anemic patient's supplementation with A vitamin, iron, and zinc is more effective than just with iron (Kolsteren et al. 2009). Since these components are present in high levels, it is believed that dried fig consumption will have positive effects in anemia's prevention/treatment.
- *Diabetes*: Magnesium and antioxidants, particularly A vitamin, are nutrients that complement diabetes treatment (Guerrero-Romero and Rodríguez-Morán 2005). Fig richness in these compounds can provide positive properties on this disease treatment.
- *Cancer*: A, B6, and B12 vitamins, fiber, and calcium are known as having anti-carcinogenic properties. Therefore, dried fig, which has high content in these nutrients, indicates that this fruit consumption may possibly prevent tumors formation.

2. Dried pear
   There are unknown studies about this fruit benefic properties. However, minerals composition allows affirming that "Pêra passa de Viseu" consumption may contribute to anemia and osteoporosis prevention. Additionally, this fruit consumption possibly protects against diabetes, cancer, and cardiovascular diseases, due to its fiber content.
3. Raisins
   - *Cardiovascular diseases*: Raisins consumption possibly helps on heart syndromes prevention, since it is rich in fiber and antioxidants.
   - *Diabetes*: Combined action between fiber and antioxidants help controlling glucose blood levels, reducing diabetes risk.
   - *Cancer*: Resveratrol, a polyphenol present in grapes, has protective properties against cancer (Latruffe 2001). It would be instructive to study drying effect on this compound, in order to verify if protection against cancer is still present in dried grape. Additionally, fiber can contribute to intestinal colon cancer prevention.
   - *Neurological diseases*: B6 and B12 action over homocystein, present in high levels in individuals affected by this disease (Dantas et al. 2008), reduces neurological sickness incidence, possibly conferring raisins a protective effect against Alzheimer and Parkinson pathologies.
4. Dried plum
   Dried Plum is one of the dried fruits whose functional potential is better studied. Phenolic compounds, fiber, and minerals are components that help explaining the following protective characteristics against:

- *Cardiovascular diseases*: Previous studies performed in rats revealed that fiber-dried plum lowers TC and LDC (Tinker et al. 1994; Lucas et al. 2000), reducing cardiovascular diseases risk. It would be interesting to evaluate if this fruit consumption has the same effects in human beings. β-carotene can also contribute to good heart health.
- *Diabetes*: Fructose is the dominant sugar in plum. This sugar is associated to a moderate insulin absorption, thus controlling sugar levels increasement. Clorogenic acid (one of the main phenolic compounds in plum), fiber, and copper also contribute to this biological benefit. Therefore, dried plum can play an important role on diabetes control (Stacewicz-Sapuntzakis et al. 2001).
- *Cancer*: Plum ingestion is commonly associated to its laxative ability. This is mainly due to sorbitol, fiber, and more recently, to chlorogenic and caffeic acids (Stacewicz-Sapuntzakis et al. 2001). Consequently, plum may reduce intestinal colon cancer risk. In 2002, a study was performed on dried plum consumption effect in estrogens levels, concluding this fruit helps reducing breast cancer-associated metabolites, through fiber action (Kasim-Karakas et al. 2002).
- *Osteoporosis*: Combined action between calcium, copper, and bore causes plum ingestion ability to raise bone density and osteoporosis prevention. Organic acids allow increased absorption of minerals that contribute to bone formation (Stacewicz-Sapuntzakis et al. 2001).
- *Neurological diseases*: Plum can possibly reduce neurological diseases as a result of antioxidant content.

21.3 Conclusions

Nuts, particularly walnuts and carob, are very energetic thus being suitable to be incorporated in energetic supplements for sportsman or cereals bar. Additionally, fatty acids, fiber, and antioxidants high content contribute to protective properties against cancer, cardiovascular diseases, diabetes type 2, as well as neurological pathologies. Satiety sensation, caused by fiber and vegetable protein action, when consuming nuts, may help on obesity prevention/control. Almonds and hazelnuts antioxidant richness may contribute to reduce hepatic diseases risk. Arginine's high content associated to calcium levels may cause almond consumption an additional protection against osteoporosis, particularly for lactose intolerant individuals. Nuts sub-products (shell, skin, leafs, among others) are an excellent source of natural antioxidants. Industrial extraction represents an opportunity to take advantage of these sources commonly wasted. Nuts lipid fraction is more favorable when compared to olive oil, being a good alternative to the most used fat in Mediterranean diet. Since nuts are good sources of vegetable protein, it is suggested to hydrolyze its proteins to be incorporated in fortified food formula.

Regarding dried fruits, although Portugal has very good climate conditions for its cultivation, there is few information about this sector, hindering its potential

analysis. Sun-drying is the most common method used to dry fruits. When compared to industrial drying, it is less effective and produces a lower quality final product. There are few investigations about dried fruit nutritional composition as well as functional properties. As nuts, dried fruits may contribute to diabetes type 2, cancer, cardiovascular diseases, and osteoporosis prevention. Additionally, dried pear and dried fig consumption possibly reduces anemia risk, through iron and other minerals that potentiate its action. These fruits can be a chocolate substitute, since they are sweet, do not cause caries, are a good source of fiber, and contain better lipid fraction.

Concluding, nuts and dried fruits produced in Portugal have several functional potentialities that should be exploited to not only valorize national varieties but also stimulate these products consumption.

Further investigations are recommended in order to a full nutritional characterization of Portuguese nuts and dried fruits. Regarding nuts, it would be instructive to study if almond and carob can contribute to osteoporosis prevention/treatment, as well as almond and hazelnut protective effects against hepatic diseases. Concerning dried fruits, it would be beneficial to characterize Portuguese market as well as confirmation of functional properties mentioned in this work.

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Chapter 22
Functional Food and Sustainable Development Once Met in the Argan Forest: The Tale of the Argan Oil

Dom Guillaume and Zoubida Charrouf

22.1 Introduction

The High Atlas mountain range covers most of the Northern part of Africa. It extends from Morocco to Tunisia for almost 1240 miles. In its central and eastern parts, i.e., its Tunisian and Algerian parts, respectively, its Southern slopes fall almost directly to the Sahara Desert, the largest hot desert in the world. On its western side, that is to say its Moroccan side, the High Atlas mountain range plunges into the Atlantic Ocean. Interestingly, South of the High Atlas, and exclusively in Morocco, an additional mountain range named the Anti-Atlas extends from the Atlantic Ocean toward the Northeast. Such situation is peculiar since the Anti-Atlas has led to isolate at about latitude 30° North the Souss valley whose size is 1600 square miles, an area slightly smaller than the size of Delaware. The Anti-Atlas protects the Souss valley from the direct and deleterious effects of the Sahara desert. Open onto the Atlantic Ocean on its western end, the climate of the Souss valley is therefore tempered by the maritime influence of the current of the Canaries. Consequently, the Souss valley acts as a buffer ahead of the Sahara desert and constitutes a unique biotope where annual average temperatures are 14 °C (57 °F) in the Northern part and 20 °C (68 °F) in the Southern region. However, the temperature amplitude is quite large and daily temperatures possibly go from −3 °C (26 °F) to 49 °C (120 °F). The annual rainfall also presents a large geographic variability. The

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average rainfall is 11 in./year but it can be as low as 2 in./year in the most arid areas and up to 20 in./year on some steep and mountainous slopes. Wind-associated evaporation is generally high. Combined, wind- and water-induced soil degradation varies from 870 to 1700 tons/square mile/year. Exclusively endemic in this area is the argan tree (Benzyane et al. 1999). This region that presents an exceptional climate and biotope is frequently referred to as "the argan forest." It was declared a Biosphere Reserve by UNESCO in 1998.

The argan tree [*Argania spinosa* (L.) Skeels; Sapotaceae] is a fascinating tree. Expectedly, it has been the topic of scientific investigations for years (Grieve 1917). The argan tree was previously named *Argania sideroxylon* (Roem. & Schult.). The name *Argania spinosa* means "spiny argan tree" whereas *Argania sideroxylon* means "iron-wood argan tree." Both names depict two of the major tree specificities. The argan tree is an 80-million-year-old relic tree species. It anciently covered the major part of Northern Africa from where it totally disappeared during Quaternary glaciations, with the exception of the trees growing in the Souss valley since they found there optimum conditions for their survival (Kenny and De Zborowski 2007). Nowadays, the argan tree is the only species of the family Sapotaceae that grows in the subtropical zone; every other species is tropical.

The argan forest covers about 3166 square miles. Argan tree mostly grows in the Souss valley dry lowlands and on the South-exposed mountain slopes of the Anti-Atlas. Indeed, argan tree can grow at the sea level (along the Atlantic Ocean) as well as in mountainous locations, up to 5000 ft. However, it does not support freezing periods. Argan tree growth is very slow and trees take 15 years to mature. Depending on its surrounding, soil fertility, and past damage, argan tree is either shrubby or up to 7–10 m. It is an evergreen tree whose trunk becomes gnarly when old. Its leaves are 1 in. long and a quarter of an inch large. Adult argan tree is extremely resistant; it can live for up to 200 years, and can survive long periods of drought. In case of such events, its leaves fall down but new leaves are rapidly formed when water returns (Morton and Voss 1987). The argan treetop is dense and wide. Underground, argan tree is equipped with a deep root system that can reach water in the moist layers well below the soil crust. Symbiotic association between fungus and argan tree root would be necessary for a harmonious tree development (Nouaïm and Chaussod 1994). Argan tree exists under various phenotypes. Resulting differences impact argan leaves that can be sometimes quite short, but are more easily observable on argan fruit that can be round, oval, or spindle shaped. Some argan nuts are also much easier to break than others.

Altogether, the argan tree is remarkably well adapted to its subtropical habitat. It has even become an essential ecological actor in the Souss valley and its surrounding rocky slopes since it stabilizes the soil, reduces wind- and water-induced erosion, maintains through leaf-perspiration moisture in the air, acts as a water pump through evapo-perspiration, shelters small and wild animals, and shades young seedlings and domestic cultures. Desertification becomes irreversible as soon as top soil gets lost due to erosion and dune settlement. Therefore, it is easy to understand that the argan tree is an important actor in the Souss valley incessant fight against desertification. Winning this fight is essential since desertification is a frightening

phenomenon that slowly corrupts the agrarian system in all the Sub-Saharan part of Africa. Alarmingly, negative signs have already been frequently noticed in the argan forest for several years. Particularly, its area has been divided by two during the twentieth century and, in some places, tree density is almost 2/3 what it was 50 years ago, getting as low as 78 trees/mi^2 (Kenny and De Zborowski 2007). If no measures are rapidly taken, anticipated climate change will certainly irremediably accelerate this dramatic trend, and the argan tree will become a severely endangered species. Simultaneously, the major part of the Souss valley will become a desert (Fassi 2003). Recognizing the urgency of acting before it is too late, a vast Moroccan government-supported program aimed at safeguarding the argan forest, has been going on in Morocco for 20 years. This program also rapidly got the support of some active members of University Mohammed V-Agdal. Several private or non-governmental organizations have joined the movement more recently, as Association Ibn Al-Baytar being one of the most active. Rapidly, the sustainable development of the argan forest has emerged as the only realistic solution capable of rescuing the argan forest, the Souss valley, and its dwellers as well.

22.2 The Argan Forest

Argan forest dwellers are often named *Berbers*. Because these dwellers have established their own cultural identity for centuries, they consider the name *Amazigh* (plural *Imazighen*) as more representative and more appropriate for their culture even though this name is scarcely used, even in Morocco. Most rural Amazigh families are very poor. They get the major part of their meagre income from agriculture-related activities. Amazigh live is dependent on the argan tree. So, access to the argan forest and farming activity in the argan forest are rigorously regulated (Kenny and De Zborowski 2007). Imazighen directly use argan wood in most of their daily activities: heating, cooking, carpentry, house and fencing construction, charcoal making, or tool building for crafting. Argan leaves are used to feed cattle and represent sometimes the only source of vegetarian nutriments available. The canopy of grouped argan trees provides shadow to humans, animals, and culture during summer hot hours. Imazighen also indirectly use the argan tree. The oil prepared from its fruit kernels is used for dietary, medicinal purposes or to prepare beauty products. Finally, argan tree is the central theme of several traditional amazigh songs.

The dramatic shrinking of the argan forest area results from the combined effect of unfortunate natural events, and human long-term mismanagement, as well. Indeed, it is indisputable that several consecutive extremely arid years have fragilized the argan forest between years 1970 and 2000, but the overuse of argan wood as fuel, the destruction of young trees by several hundreds of unattended goats freely grazing in the forest, and an uncontrolled tourism-supporting policy are also responsible for the argan forest decrepitude. As a proof, the high charcoal demand that has undeniably weakened the argan forest during the first half of the twentieth century does not exist anymore. However, deforestation in order to permit

the culture of various types of citrus fruits and the implantation of greenhouses where fresh vegetable is grown all over the year to satisfy the tourism industry is a major problem in most fertile places of the Souss Valley. In remote mountainous regions, the argan forest is frequently overgrazed. Sheeps, camels, and goats freely circulate in the forest where they devastate young tree shoots and eat young leaves and fruit. Argan trees are also sometimes replaced by olive trees used for the production of olive oil. Olive trees grow more rapidly than argan trees, so the time necessary to get the first harvest is reduced. Such an argument is of prime importance when survival is a daily challenge. However, olive tree culture is not as well adapted to the Souss Valley biotope as argan tree endemic growing can be. On the long term, olive trees contribute to accelerate the erosion of the argan forest. Regretfully, argan forest dwellers themselves share the responsibility of having mismanaged the forest for a very long time. They have too often and for too long considered the argan forest as a "free garden" or an "eternal gift of God" and they have totally ignored the simplest rudiments of forest care. Therefore, safeguarding the argan forest became vital. Nevertheless, it could neither go against the Amazigh interest nor be achieved without their active participation (Charrouf and Guillaume 2009). With those bases established, the sustainable development of the argan forest could now begin.

Sustainable development is aimed at preserving biodiversity and respecting the livelihoods of rural communities; it requires the simultaneous achievement of environmental, economic, and social sustainability (Rosen 2008). However, and generally speaking, management of a sustainable development program in developing countries presents its own specificities. Indeed, survival is often the major problem faced by the poorest and, in that case, environmental issues become a non-priority. Such environmental issues will be considered only if they are linked to basic economic matters. In the specific case of the argan forest, the efforts necessary for the long-term rescue of the argan tree obviously necessitated the support of a large majority of its dwellers that would likely have to modify their way life. Hence, it could be anticipated that the participation of the argan forest dwellers would only be sequential and would be gradually reinforced if changes had a rapid and positive impact on the dweller daily income. Only tangible proofs could get argan forest dweller confidence.

Therefore, the potential of the argan tree was considered and different possibilities were rapidly evaluated. Boosting the value of argan oil emerged as the best mean to ensure the sustainable development of the argan forest (Charrouf et al. 2008). Indeed, due to its chemical composition, argan oil possesses cardioprotective properties (El Monfalouti et al. 2010). It is also an efficient free-radical scavenger activity, a property particularly valued in dermocosmetology (Guillaume and Charrouf 2011). Therefore, argan oil could easily represent the archetype of functional food generally looked for by consumers of developed countries, the kind combining antiaging and hypo-lipidemiant properties. Argan oil unique hazelnut taste was thought to be the final touch to convince the mass market. The program resulting from this idea anticipated that, in case of success, one part of the profits generated by argan oil marketing should return to the native population while another part would be used for the preservation of the argan forest, ascertaining its

sustainable development. The genius idea of this program was that the modernization of argan oil production methods would be the key to allow its swift implementation as a must as functional food (nutraceutical) on the international market. Nowadays, 20 years after the beginning of this program, argan oil is sold on the European, Asian, and Northern American markets, and argan oil is nicknamed "the most expensive oil in the world."

22.3 The Argan Oil

At the present time, three different types of argan oil coexist: edible argan oil, beauty argan oil, and cosmetic argan oil. All three types are prepared from the kernels contained in the argan fruit stone. Argan oil can also be categorized into three types based on its preparative process: traditional, semi-mechanized, and industrial. Edible and beauty argan oils are prepared either traditionally or using the semi-mechanized method. Cosmetic argan oil is exclusively industrially prepared.

22.3.1 Traditional Extraction Method

For centuries, Imazighen have prepared a single type of argan oil and used it indifferently as edible or beauty oil. In the Amazigh society, for cultural and religious reasons, argan oil preparation is exclusively a woman task that follows an ancestral process (Fig. 22.1).

Peel of fresh fruit contains a milky latex that makes the peel so sticky that it cannot be removed efficiently. So, once the ripe fruits have been collected, between May and September, they are sun-dried for a few days in an open space. Optimum length of this drying period has been evaluated to be between 10 and 15 days (Harhar et al. 2010a). Because rainfall is scarce in the argan forest during the summer months, rain protection is unnecessary. When argan fruit peel is dry, brown, and wrinkled, it becomes crisp and can be manually removed, affording argan nuts. Argan nuts are then manually broken. Women hold the nuts between their thumb and index along the longest seed diagonal and violently hit them with a stone. Between one and three white kernels are released. Then, kernels are gently roasted for a few minutes. Over-roasting affords an oil presenting an unpleasant burned taste so this step has got to be carefully handled. Argan shells are not wasted, they are used to produce the gentle fire necessary to achieve a good roasting temperature. Roasted kernels are then manually crushed using a homemade millstone composed of a bedstone and a center-pierced cone-shaped rotating piece, into which the kernels are frequently introduced. The brownish viscous liquid obtained is mixed with water and the resulting dough is kneaded by hand for several minutes. Slowly, the dough becomes solid and releases an emulsion from which argan oil is decanted. The solid residue remaining after maximum oil has been collected is traditionally

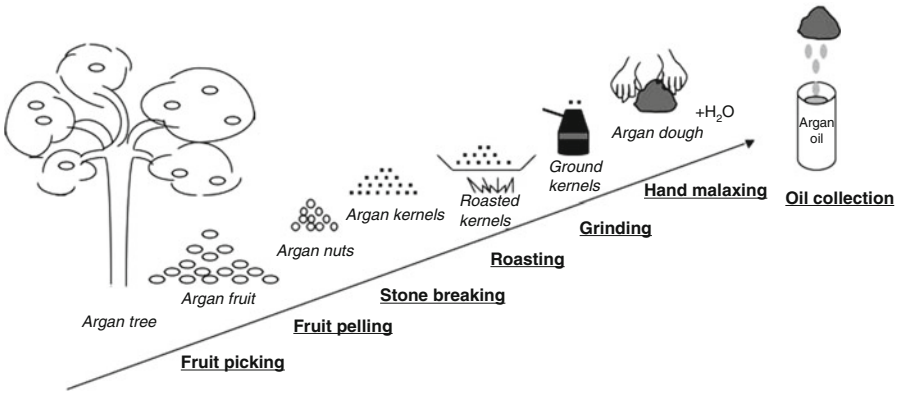

Fig. 22.1 Processing line for argan oil preparation following the traditional method

used to feed goats and cows. The traditional process is long and painful. From 100 kg of dried fruits, only 2–2.5 L of oil is obtained after 58 h of work, for a single person. In villages, groups of women often from the same family gather to prepare argan oil and then share their production, making the full process less painful. Fruit peeling is particularly laborious and time-consuming. To avoid this step, argan fruits are sometimes given to goats. Indeed, goats are very fond of the pulp and they naturally regurgitate peeled-argan nuts after a few hours. Peeled argan nuts can be collected in the middle of the goat stools. However, such a method obviously raises microbiological. It also raises quality concerns as the taste of the oil and its chemical composition are altered. The addition of water to the ground kernels is also a frequent source of chemical and bacteriological contamination since running water is still scarce in most locations of the argan forest and unsafe water sources and often used. Such steps are inconsistent with the production of high quality argan oil. Furthermore, preservation of traditionally prepared argan oil is often limited to a few weeks even though some amount of salt is sometimes added to prolong the preservation period. Therefore in the amazigh society, moderate amounts of argan oil are prepared at one time or larger amounts are shared between several households. In both cases, it is necessary to frequently repeat the extraction process. When not used in the family circle, traditionally prepared argan oil is sold, in reused plastic bottles, to tourists along the roads of Morocco. In this later case, partial adulteration by cheap oils is frequent and sometime artificially colored vegetable oils are sold as argan oil.

22.3.2 Semi-Mechanized Extraction Method

The success of the sustainable development of the argan forest is based on the implantation at different locations of the argan forest of a new type of cooperatives that have developed stringent quality standards to prepare argan oil. To respect the

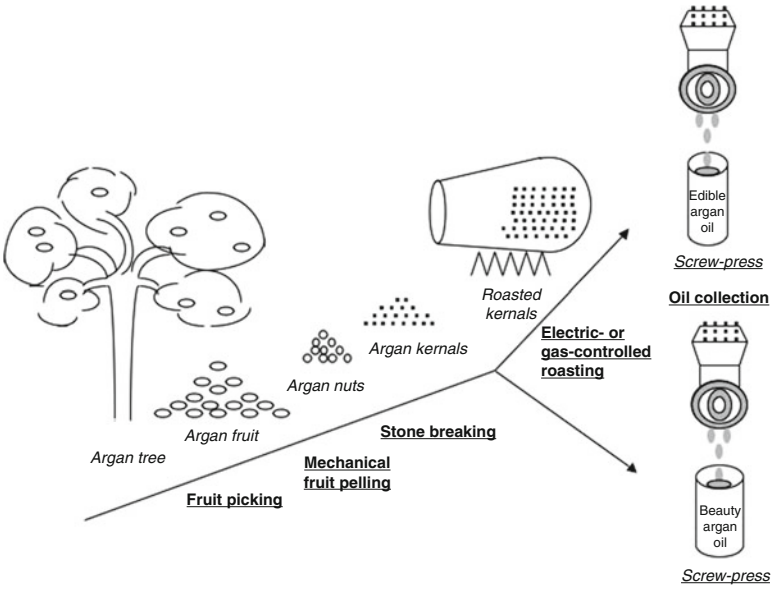

Fig. 22.2 Processing line for edible and beauty argan oil preparation following the semi-mechanized method

tradition, these cooperatives are woman-only cooperatives. The consequences of the cooperative implantation on the local economy, the population, the social equilibrium, as well as their evolution, are periodically and extensively analyzed by Lybbert (Lybbert et al. 2002, 2003, 2009; Lybbert 2007). So this aspect will not be further detailed here. Cooperatives buy argan fruits from argan forest dwellers. Before the implantation of cooperatives, argan nuts were only sold to argan wholesale buyers and price varied according to supply. Therefore, dwellers could not count on predictable price, nor did they guarantee any particular quality. Cooperatives buy argan fruit at a determined price but they do not buy goat-depulped fruits. To make sure that fruits have not been improperly peeled, only pulped fruits are purchased by the cooperatives. Also highly importantly, in these cooperatives argan oil is extracted using a totally new method that requires the use of mechanical presses (Fig. 22.2). This makes the hand-mixing of the dough and water addition unnecessary and consequently eliminates possible water-induced bacterial contamination. Simultaneously, the use of mechanical presses has dramatically increased the yield in oil and the press cake now contains only minute traces of oil. However, it still remains a good cattle food since its protein content is unchanged.

When argan oil is prepared in the family circle, each family is able to evaluate the necessary amount of fruit, often gathered by kids, required to prepare enough oil for a given period of time. In the woman cooperatives, huge quantities of fruits are purchased between May and September. Fruit peeling is then achieved using scratching machines. Therefore the time necessary to peel the fruits is strongly reduced. The preparation of an essential oil endowed of insect repellent properties

from these large amounts of peel gathered by the cooperatives to prepare is presently being investigated. Then nuts are broken and kernels stored until they are pressed. Storage at 4 °C (39 °F) allows the perfect preservation of kernel quality for almost 1 year (Harhar et al. 2010b). For the roasting step, gas- or electric-roasters are used. Standardization of the roasting step is also essential since it allows the production of an oil of reproducible flavor on a large scale. Kernel collection from argan nuts has not been modified and is still performed manually. In the cooperatives, the overall time necessary to prepare 1 L of oil has been reduced by one-third compared to the traditional method, the most painful tasks being the most shortened. Protected from sunlight, shelf life of semi-mechanically prepared argan oil is up to 2 years and the taste between each batch is reproducible. Depending on argan oil physicochemical properties (see Chap. 10.3.4), edible argan oil can be extra virgin, virgin, or regular virgin (SNIMA 2003). Lampant argan oil cannot be used as human food (SNIMA 2003). Extra virgin argan oil is the type of argan oil found in the gourmet stores of developed countries.

In cooperatives, beauty argan oil is also prepared. To produce this type of oil, the roasting step is simply skipped, the other steps remaining unchanged. However, filtering is often necessary to avoid a sometimes-milky appearance resulting from the presence of gums.

Beauty oil is gold colored whereas edible argan oil is copper colored. Beauty oil shelf life is slightly shorter than that of edible argan oil likely due to the formation following a Maillard-like process of not fully identified preservative compounds in edible argan oil during the roasting step.

22.3.3 Industrial Extraction Method

Several major cosmetic Laboratories introduce some amount of argan oil into their skin care products or shampoos. Cosmetic Laboratories extract argan oil from unroasted pulverized kernels with chemical solvents as hexane, cyclohexane, or dichloromethane. After solvent removal by evaporation, argan oil is obtained (Fig. 22.3). Polyphenols and other minor components found in beauty oil are not necessarily extracted by the industrial process and natural or nonnatural antioxidants are often added to industrial argan oil to increase its preservation and cosmetic properties. Also to satisfy the cosmetic industry standards, argan oil is often deodorized and decolored before being introduced in cosmetic preparations.

22.3.4 Chemical Composition and Some Physical Properties of Argan Oil

Argan oil contains 99 % of glycerides and 1 % of unsaponifiable matters. The glyceride fraction is composed at 95 % of triacylglycerides. Oleic and linoleic acid are the two main fatty acids (46–48 %, 31–35 %, respectively) found in argan oil. Both

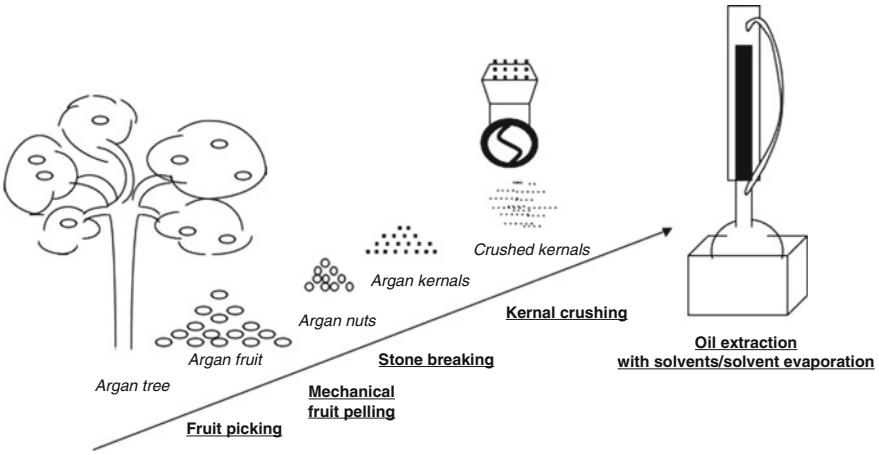

Fig. 22.3 Processing line for cosmetic argan oil preparation following the industrial method

are unsaturated fatty acids, the type presenting the highest interest in terms of human health. Palmitic acid is the third main fatty acid found in argan oil (11–14 %). It is a saturated fatty acid (Charrouf and Guillaume 1999). Argan oil can be characterized by its acid value expressed in percent of oleic acid. The extra virgin label means that argan oil acid value is below 0.8; the virgin label indicates an acid value below 1.5 while regular virgin argan oil has an acid value below 2.5. An oil whose acid value is above 2.5 is depicted as lampant (SNIMA 2003).

Unsaponifiable matters include carotenes (37 %), triterpene alcohols (20 %), tocopherols (8 %), and xantophylles (5 %). Among tocopherols, γ-tocopherol is majoritary (69 %). It is a strong anti-oxidative agent. Several other phenols have also been identified in argan oil (Charrouf and Guillaume 2002). Sterols belong to the triterpene alcohol group. Their physiological potential is also high. Furthermore, the low level in campesterol in argan oil, a sterol found in most vegetable oils, has been advantageously used to design an easy implement method to detect argan oil adulteration by other vegetable oils (Hilali et al. 2007). The combined presence of all these (poly)phenols, sterols, and carotenes even in very small amount, is likely to be responsible for some of argan oil pharmacological properties (Charrouf and Guillaume 2008).

22.3.5 Pharmacological Properties of Argan Oil

Imazighen are well aware of the health benefits of argan oil. They use it to treat liver diseases and they claim that argan oil has choleretic and hepatoprotective properties. Argan oil is also traditionally indicated to cure hypercholesterolemia and atherosclerosis. Applied on the skin, beauty argan oil is said to be useful to eliminate

skin pimples, juvenile acne as well as chicken pox pustules. Application of beauty argan oil is welcome in case of dry skin, dry hair, and to reduce the appearance rate of wrinkles.

Argan oil composition has led to suspect that it could possess even more favorable pharmacological properties and the "amazigh diet" is now recognized for its numerous benefits on human health (Charrouf and Guillaume 2010). However, not every claim has been necessarily scientifically demonstrated. Hence, because both argan and olive oils present high levels of squalene and of γ-tocopherol, a potent antioxidant, the presumed chemoprotective effect attributed to olive oil has also been attributed to argan oil (Khallouki et al. 2003). To confirm those claimed virtues, the benefits of argan oil consumption on human health have been evaluated by several epidemiologic or clinical studies. The chemoprotective effect of argan oil has been confirmed by specific investigations on prostatic cells. Those studies showed that argan oil tocopherols possess a cytotoxic activity and exert an inhibitory effect on the proliferation of hormone-independent as well as hormone-dependent prostatic cell lines. γ-Tocopherol and argan oil polyphenols are also efficient for prostate cancer prevention, whereas squalene and argan oil polyphenols exert an antiproliferative effect on specific cell lines. Additional studies have shown that argan oil polyphenols specifically interrupt the insulin-signaling cascades at the MEK1/2-ERK1/2 interface.

However, argan oil is mostly known for its preventing effects on obesity and adverse cardiovascular outcomes. The hypocholesterolemic properties of phenols, phytosterols, and tocopherols are now well known. Not surprisingly, argan oil phenolic fraction has been shown to prevent LDL oxidation, they are also responsible of the antiatherogenic property of argan oil. A paraoxonase-related mechanism could also explain the hypolipidemic and hypocholesterolemic properties of argan oil.

Finally, concerning the antidiabetic activity of argan oil, it has only been evidenced on rats. It should also be noted that the first case of allergy to argan oil has been recently reported. It would be due to the presence of a 10 kDa protein belonging to the family of oleosins that is also encountered in peanut and sesame (Astier et al. 2010).

22.4 Conclusion

The sustainable development of the argan forest, which was considered as a utopia 20 years ago, appears to be on a favorable track for the moment. This success results from the positioning of argan oil on the highly competitive but highly lucrative international nutraceutical market. Of course, without argan oil unique organoleptic and pharmacological properties nothing would have been possible. Nevertheless, it should be kept in mind that a lot of work and a lot of sacrifices have been necessary to achieve this task. Convincing people to take part to the project, persuading them to continue in case of difficulties, making sure that all commitments are respected,

and overcoming adversity requires a lot of time, energy, and devotion to the project. For interested readers, more details can be found in Charrouf et al. (2011). Even now, after 20 years nothing is really totally ascertained. Indeed, the still ongoing preservation program has delivered very positive signs for the preservation of the argan tree and of the argan forest biotope, at witnessed by reforestation of large areas in the argan forest. But should the argan forest be planted with selected seeds as often suggested? Should the argan forest be changed in a vast argan orchard simply because the world felt in love with argan oil? Should not a sustainable preservation looking as close as possible like a natural process be encouraged? Who should give the answers to these questions? Dwellers, forest managers, argan oil retailers, politics, scientists…?

Furthermore, the success of the entire program is based on a single produce, and therefore remains highly fragile. As it is, a decline in argan oil popularity or the production of large quantities of argan elsewhere than in the argan forest would undoubtedly also mean the end of the sustainable development program of the argan forest as it is designed. This is why the geographical indication granted to argan oil in 2009 was an important step for all the partners of the argan tree/forest project. Geographical indication can be used for selected products only coming from a specific geographical location. The geographical origin has to be recognized as responsible for the major part of the product qualities and fame. Similarly to trademarks, geographical indications give a monopoly right. The rules implemented in the woman cooperatives are so stringent that they certify the quality of argan oil and the environmental specificity of the argan forest certifies the uniqueness of the biotope. Such protection puts on additional asset in argan forest dweller hands (Charrouf et al. 2002). Now they have got to not only watch the argan tree fructify every year but also to learn how to make the income of their precious oil fructify as well.

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Part IV
Honey and Beverages with Functional Properties

Chapter 23
Functional and Nutritional Properties of Different Types of Slovenian Honey

Mojca Korošec, Urška Kropf, Terezija Golob, and Jasna Bertoncelj

23.1 Introduction

Honey is the natural substance that honey bees (*Apis mellifera*) produce from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of coniferous and latifolius plants, which the bees collect, bring into the hive, transform by combining with specific substances of their own, deposit, dehydrate, and leave in honeycombs to ripen and mature. Honey has been used as a sweetener and a home remedy since ancient times (Allsop and Miller 1996; Crane 1975). In fact it has been the only available sweetener for humans until the rise of industrial sugar production at the beginning of nineteenth century. Honey, intended for human consumption, may not be altered by man as it is set also in the Council Directive 2001/110/EC relating to honey (EC 2002).

The annual world honey production totals about 1.4 million tons, which represents less than 1 % of world's sugar production. China is the largest honey producer, followed by Turkey, Argentina, Ukraine, and the USA, not necessary in this order. According to available statistics, Spain produces the most honey among EU members. Slovenia, with average annual honey production of 2000 tons, ranks about 70th place on world's ranking (FAOSTAT 2011). However, the production per capita, 0.9 kg of honey, is in Slovenia significantly higher compared to EU (0.4 kg/per capita) or world average (0.2 kg/capita). The consumed amounts of honey are slightly higher than those produced; therefore, it is not surprising that mainly Slovenian honey is consumed, while the imported honey takes only 14 % share (Statistical Office of Republic of Slovenia 2011). Generally, the consumption of

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honey may vary considerably among countries, and ranges from very low in China and Argentina (annually 0.1–0.2 kg/capita) up to high (1–1.6 kg/capita/year) in Germany, Austria, and Greece (CBI Market Information Database 2011).

23.2 Types of Honey

Bees forage on diverse plant species with differently available sources, i.e., nectar and/or honeydew. The great diversity of plants foraged is reflected in the honeys produced. Although no honey is completely identical to another it may be classified regarding its principal characteristics. In general, honey types are divided into two big groups regarding the main source:

- Nectar honey, produced from nectar of different plants
- Honeydew honey, produced from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants

According to Directive relating to honey (EC 2002) the floral origin may be stated on the label when honey derives mainly or in whole from the indicated botanical source and possesses typical physicochemical, sensory, and microscopic characteristics. In several European countries honey with botanical denomination represents almost half of the marketed honey, is often regarded as more valuable and therefore gains higher price than blended nectar or honeydew honey (Persano Oddo and Bogdanov 2004).

Natural geographic conditions and climatic diversities in Slovenia provide a wide spectrum of honey bee pastures. Appropriate technology, consistent beekeeping, and careful handling during honey production and postproduction may enable a high quality honey of different types regarding the botanical origin. In Slovenia different types of honey are traditionally produced and Slovenian consumers are accustomed to choose among them (Table 23.1).

Traditionally acacia and multifloral honey as nectar honey types, linden and chestnut honey as blended nectar and/or honeydew honey, and spruce, fir, and forest honey as honeydew honey types are produced in Slovenia. Individual types of honey differ in physicochemical and sensory characteristics as well as in the proportion and composition of pollen grains. Consumers' choice usually depends on typical sensory characteristics, where honeys with lighter colors are preferred over darker ones.

Table 23.1 Types of Slovenian honey

Pasture source	Type of honey
Nectar	Multifloral, acacia (*Robinia pseudoacacia*), canola (*Brassica napus*), buckwheat (*Fagopyrum esculentum*), *Prunus mahaleb* honey, and dandelion (*Taraxacum officinale*) honey
Nectar and/or honeydew	Linden (*Tilia* sp.), chestnut (*Castanea sativa* Mill.), and maple (*Acer pseudoplatanus* L., *A. platanoides* L.) honey
Honeydew	Forest, spruce (*Picea abies* (L.) Karst.), fir (*Abies alba* Mill.), and *Metcalfa pruinosa* honey

Besides being a natural sweetener honey has been used also for healing. Considering it in this aspect it is necessary to highlight the very significant differences in antioxidant and antimicrobial activity that give certain honey types a specific value, e.g., Australian manuka honey, and chestnut honey from Slovenia.

23.3 Composition of Honey

Honey is a remarkably complex food that, according to existing scientific data, contains more than 200 phytochemicals—biologically active compounds, which presence and content in honey depend on its botanical and also geographical origin. The main components of honey are carbohydrates, predominantly glucose and fructose, and water. Some minor components present in honey are minerals, phenolic acids and flavonoids, ascorbic acid, proteins, certain enzymes, amino acids, organic acids, α-tocopherol, carotenoids, and Maillard reaction products.

The quality of honey and its functional properties depend on the content of abovementioned components as well as on the content of pollen, activity of the enzymes, and sensory characteristics, i.e., typicality and intensity of color, odor, taste, and aroma. The pollen type and content as well as physicochemical and sensory characteristics vary among different types of honey and may be also influenced by different factors such as climate conditions, rock and soil conditions, and vegetation conditions as well as beekeeping practice. The value ranges of the principal physicochemical parameters in different Slovenian honey types, obtained from analyzing over 1000 samples during long-term monitoring, are presented in Table 23.2. The requirements of the honey quality on Slovenian market are set by Rules relating to honey (2011), which is in line with Council Directive relating to honey (EC 2002). The document provides the highest or lowest admissible values, respectively, for principal parameters of honey quality: the sum of fructose and glucose content, the content of sucrose, water, water-insoluble matter and hydroxymethylfurfural, the electrical conductivity, free acids and diastase activity.

23.3.1 Content of Water

The water content in honey is one of the most important criteria of honey quality. The Council directive (EC 2002) states that honey may contain up to 20 % of water. The lower the water content, the more viscous, thick, and unchanged honey is, since such conditions in honey are unsuitable for growth of osmophilic yeasts and thus prevent fermentation of honey. The honey with less than 15 % of water is more viscous and crystallizes more quickly. The water content depends on the type and amount of pasture, climatic conditions, especially in the period of plants' flowering and nectar secretion, the type of the beehives and the beekeeping practice. Over 90 % of Slovenian beekeepers use AŽ (Alberti-Žnideršič) beehives, which enable production of honey with lower water content. Slovenian honey contains usually between 14 and 17 % of water (Table 23.2).

Table 23.2 The principal physicochemical parameters in Slovenian honey types

Honey type	Water (g/l00 g)	EC (mS/cm)	pH	Free acids (mEq/kg)	Diastase number	Protein (g/l00 g)	Proline (mg/kg)	Fructose (g/l00 g)	Glucose (g/l00 g)	Sucrose (g/l00 g)	F/G
Acacia	13.5–17.5	0.11–0.27	3.6–4.4	6.5–23.1	5.9–13.7	0.13–0.21	197–447	33.6–45.1	21.9–31.3	2.12–8.28	1.31–1.67
Linden	14.5–17.8	0.55–1.07	4.1–6.1	6.1–21.4	9.6–21.2	0.13–0.24	225–398	33.0–43.0	29.5–39.3	0.09–3.51	0.91–1.32
Chestnut	13.7–18.2	0.96–2.25	4.8–6.2	7.3–26.0	16.3–28.4	0.31–0.40	457–776	17.7–24.9	17.4–32.7	2.02–3.29	1.40–1.64
Fir	13.8–17.7	0.89–1.57	4.7–5.8	14.1–27.5	11.3–24.4	0.18–0.36	323–506	28.1–35.0	23.6–29.6	1.00–4.89	1.19–1.31
Spruce	14.3–18.5	0.92–1.63	4.3–5.5	17.7–45.1	12.7–24.2	0.18–0.38	231–495	28.1–42.8	23.1–30.9	1.23–3.75	1.04–1.41
Multifloral	14.4–18.0	0.24–0.84	3.9–5.2	8.7–42.7	10.8–24.7	0.18–0.42	309–534	33.2–39.2	28.5–35.5	1.32–4.35	0.95–1.32
Forest	13.5–17.0	0.81–1.68	4.4–5.2	14.7–43.1	13.3–30.0	0.20–0.49	322–461	24.9–36.4	22.9–31.6	1.72–4.61	1.01–1.36

EC electrical conductivity

23.3.2 Electrical Conductivity

Electrical conductivity is a parameter that provides useful information on the quality and possible adulteration of honey, and is also useful in identifying different types of honey, especially in distinguishing between the honey from the nectar and the honey from honeydew. It depends on the content of mineral salts, organic acids, proteins, and other complex substances such as sugars and polyols.

The measurement of electrical conductivity is in Slovenia one of the basic analysis, carried out also on the field by beekeeping advisors, to check the accuracy of the labeling of honey. According to the Directive relating to honey (EC 2002) the electrical conductivity of honey from the nectar—except for the chestnut and linden—is not more than 0.8 mS/cm, while the electrical conductivity of honey from honeydew and chestnut honey is not less than 0.8 mS/cm. Linden honey is an exception, which may have electrical conductivity below or above the value set. Acacia honey has the lowest electrical conductivity among Slovenian honey types, but the highest values that may exceed even 2 mS/cm in the individual seasons are typical for chestnut honey.

23.3.3 Carbohydrates in Honey

Carbohydrates are the most abundant component of honey and from the nutritional aspect undoubtedly the most important. They affect the physicochemical properties of honey, i.e., viscosity, crystallization, and hygroscopicity. The content and distribution of different carbohydrates in honey is related to the botanical origin, composition and secretion of nectar, presence of enzymes, climatic conditions, type, and physiological state of bees and strength of bee colonies.

Invert sugar, i.e., mixture of fructose and glucose represents with 65–95 % the major part of carbohydrates in honey. The content of invert sugar is higher in the honey from nectar (≥60 %) than in the honey from honeydew (≥45 %). Fructose or D(−) fructose is very hygroscopic, quickly soluble in water and crystallizes very slowly. Glucose or D(+) glucose is less soluble in water and forms stable crystalline form of α-D glucose monohydrate at temperatures lower than 50 °C. The ratio of fructose and glucose (F/G) is strongly dependent on the botanical origin and is characteristic for each type of honey. Most honey types contain slightly more fructose (approximately 40 %) than glucose (approximately 34 %); therefore, the F/G value is greater than 1.0. The rate of crystallization of such a honey is smaller and honey remains liquid for a longer time. Among Slovenian types of honey acacia and chestnut honey are the most fructose-rich types, with the F/G ratio 1.5 and 1.4, respectively.

Disaccharides are present in smaller extent, following by some other oligosaccharides. Although they are present in honey in minority, their distribution may be used as an index of honey authenticity and floral origin (Nozal et al. 2005). Nectar and honeydew honeys usually do not significantly differ in the amount of disaccharides, e.g., sucrose, palatinose, turanose, and maltose, but there are discrepancies in the content of trisaccharides (Swallow and Low 1994; Golob and Plestenjak 1999;

Table 23.3 Average content of different di- and trisaccharides in nectar and honeydew honey types from Slovenia

Compound	Average ± SD (g/100 g)	
	Nectar honey	Honeydew honey
Glucose	29.38 ± 3.97	26.97 ± 2.43
Fructose	37.27 ± 2.73	33.31 ± 3.64
Sucrose	3.47 ± 1.50	3.03 ± 0.88
Palatinose	0.89 ± 0.08	0.97 ± 0.23
Turanose	1.62 ± 0.25	1.83 ± 0.41
Maltose	2.11 ± 0.44	2.07 ± 0.67
Isomaltose w. Gentiobiose[a]	1.86 ± 0.67	1.83 ± 0.54
Panose	0.59 ± 0.04	0.61 ± 0.06
Erlose	1.60 ± 0.50	2.19 ± 1.03
Raffinose	<LOQ	2.21 ± 1.45
Melezitose	<LOQ	2.53 ± 1.60
Maltotriose	0.70 ± 0.14	0.92 ± 0.36

[a]Chromatographic condition did not enable separation of the two carbohydrates; *<LOQ* carbohydrate was detected, but the amount was too little to be quantified; *SD* standard deviation

Cotte et al. 2004; Nozal et al. 2005; Korošec et al. 2009). While erlose, maltotriose, and panose proved to be present in both, nectar and honeydew types of honey, are raffinose and melezitose trisaccharides, typical for honeydew honeys (Table 23.3). The presence of melezitose in nectar honey indicates that nectar was mixed with honeydew.

23.3.4 pH Value and Acidity of Honey

Honey contains a variety of organic acids (acetic, butyric, citric, formic, lactic, malic, oxalic, fumaric, amber, glycolic, and tartaric) and inorganic acids, which impart a distinctive taste and aroma of honey, contribute to its preservation and have impact on the antibacterial and antioxidant activity.

Total content of acids in honey is not related to its botanical origin, while on the contrary pH value is. Honeydew honey contains more minerals, which act as a buffer; therefore, such honey has higher pH value and less acidic taste. The content of free acids is one of the most important parameters when checking the quality of honey, namely the increased values indicate the ongoing of a fermentation process. In this process osmophilic yeasts convert sugar firstly into alcohol then into the acids and carbon dioxide. pH values of Slovenian honeys are generally ranging between 3.5 and 5.5, with the lowest pH in the acacia, and the highest in chestnut honey. According to legislation (EC 2002) up to 50 mEq of free acids per kg honey are allowed.

23.3.5 Proteins and Amino Acids

Proteins in the honey are not attributed with greater nutritional importance, since their content in honey is low. Already in 1978 White reported an average content of nitrogen (0.04 %) and protein (0.2 %) in honey and highlighted also the large standard deviations. Also Anklam (1998) in her review article states that the amount of protein in honey is usually lower than 0.5 %. Researches of Slovenian honey types have shown that the average protein content ranges from 0.16 g in 100 g of acacia honey up to 0.35 g in 100 g of chestnut honey (Kropf et al. 2010).

Amino acids in honey originate from the nectar or honeydew, pollen and honey bees (González Paramás et al. 2006). Their content is understandably low and varies from 712 mg/kg in acacia to 994 mg/kg in forest honey from Slovenia (Slovenian unpublished data). Among 27 amino acids determined in honey proline is the only noteworthy due to its highest content (Hermosin et al. 2003). Its amount in honey depends on the botanical source and the degree of raw material processing by the bees, and ranges from 301 mg/kg in acacia honey to 617 mg/kg in chestnut honey (ranges for different Slovenian honey types are given in Table 23.2). The proline content is related to the honey ripeness, quality, authenticity as well as botanical origin and may serve as an indicator of honey adulteration. As set by International Honey Commission (2002), the lowest admissible value for proline content in ripe, unadulterated honey is 180 mg/kg. The research of naturally occurring antioxidant substances in honey broadened the role of proline in honey. Namely, Meda and coworkers (2005) demonstrated a positive correlation between proline content and the radical scavenging activity.

23.3.6 Hydroxymethylfurfural

Hydroxymethylfurfural (5-hydroxymethylfuraldehyde, HMF) is a cyclic aldehyde formed at the degradation of carbohydrates, especially fructose and glucose in the acidic environment of honey (Molan 1996). The speed of HMF formation depends on the temperature. Knowing the content of HMF is an important criterion in determining the quality of honey. Fresh honey contains low amounts of HMF, from 0 to 0.2 mg/kg. Increased content is an indicator of inappropriate heating or improper storage of honey. The legislation (EC 2002) set the limit at 40 mg of HMF per kilogram of honey, and for honey from countries with tropical climates not more than 80 mg/kg. Crystallization is a natural process that occurs in several honey types during storage and consequently liquefaction of honey is needed prior further processing. Appropriate heating conditions of honey (short-term higher temperature or tempering at up to 40 °C) do not significantly affect the formation of HMF.

More than half of the honey in Slovenia is sold privately, at beekeepers'; therefore, it is important that they are informed about appropriate heating technique of honey. The knowledge is disseminated via seminars and field work of beekeeping advisors'

network organized by Slovenian Beekeepers Association. The implementation of the education into beekeepers' practice was proved by results of a 5-year monitoring of HMF content in artisanal honey, when over 600 samples were collected and analyzed. None of the samples contained HMF above the legal value (40 mg/kg) and on average in 75 % of the samples the HMF amount was 5 mg/kg or lower (Kandolf et al. 2010).

23.4 Elements

Honey is not considered an important source of elements because the total content of elements or ash in nectar honeys is usually lower than 0.6 %, and in honeydew honeys lower than 1.0 %. The elemental composition of a particular honey sample greatly depends on the composition of the nectar or honeydew and pollen from which honey originates. Among macroelements honey contains only K, ranging from 0.17 g/kg to more than 5 g/kg. Among the microelements, which occur at levels above 1 mg/kg in honeys are: Al, B, Ba, Br, Ca, Cl, Fe, Mg, Mn, Na, P, Rb, S, Sr, and Zn. The trace elements, which are found in honey, are: Ag, As, Cd, Co, Cr, Cu, Li, Mo, Ni, Se, Pb, etc.

Some primary studies on Slovenian honeys demonstrated that acacia, linden, and chestnut honeys greatly differ in elemental content (Golob et al. 2005; Nečemer et al. 2009). These honey types also differ in their physicochemical parameters (Kropf 2009; Kropf et al. 2010). This statement was also confirmed by Bogdanov and coworkers (2007), who ascertained that botanical factors have the greatest influence on the trace element content of honey when studying environmental, geographical, and botanical aspects of the mineral content in honey.

An extensive research on elemental composition of a large number of honey samples from Slovenia was done in years 2004–2006 (Kropf 2009; Nečemer et al. 2009; Kropf et al. 2010). With the total reflection X-ray spectroscopy (TXRF) 271 samples from 7 types of honey were analyzed and 14 elements were determined. According to the average values in all analyzed samples seven elements were arranged from the most to the least frequent in the next order: K > Cl > Ca > S > Rb > Mn > Br. It was observed that this order was not the same for all honey types: acacia and linden honey contained more manganese than rubidium, honeydew types of honey contained more sulfur than calcium (Table 23.4). Obtained data enable the classification of honey according to botanical and geographical origin with the elemental content values, stable isotope ratios, and basic physicochemical parameters.

Since the content of individual elements in honey is low, its contribution to recommended daily intakes (RDI—Recommended daily intake) of the various macro- and microelements is negligible. Bogdanov et al. (2008) mentioned that from the nutritional point of view, especially for children aged 1–15 years, three elements (chromium, manganese, and selenium) are mentionable. In the same contribution they pointed out the elements sulfur, boron, cobalt, fluorine, iodine, molybdenum, and silicon, which can be important in human nutrition, although there are no RDI

Table 23.4 Elemental content, determined with the TXRF method, in different honey types of Slovenian origin

	Content of elements (average ± SD) (mg/kg)						
Honey type	K	Cl	Ca	S	Rb	Mn	Br
Acacia	278 ± 78	95 ± 52	17.3 ± 7.7	47 ± 19	0.72 ± 0.32	1.68 ± 1.27	0.60 ± 0.26
Linden	1800 ± 349	379 ± 139	69 ± 23	50 ± 27	5.5 ± 2.9	3.55 ± 1.56	1.02 ± 0.43
Chestnut	3590 ± 657	240 ± 217	148 ± 33	42 ± 24	17.0 ± 7.7	23.2 ± 9.0	0.55 ± 0.23
Fir	3170 ± 555	333 ± 134	35 ± 18	71 ± 26	22.0 ± 7.0	5.03 ± 1.93	0.59 ± 0.12
Spruce	2950 ± 494	322 ± 74	47 ± 17	70 ± 26	13.9 ± 6.1	7.07 ± 2.3	0.58 ± 0.22
Multifloral	1120 ± 352	264 ± 85	61 ± 25	56 ± 25	2.97 ± 1.63	3.12 ± 1.59	0.65 ± 0.25
Forest	2940 ± 561	310 ± 79	59 ± 19	57 ± 21	13.7 ± 7.8	6.74 ± 2.51	0.59 ± 0.25

Table 23.5 Honey phenolic and non-phenolic compounds with antioxidant activity (Gheldof et al. 2002; Al-Mamary et al. 2002; Küçük et al. 2007)

Phenolic compounds		
Phenolic acids	Flavonoids	Non-phenolic compounds
Benzoic acid	Pinobanksin	Enzymes
Cinnamic acid	Pinocembrin	Catalase
Caffeic acid	Chrysin	Glucose oxidase
p-Coumaric acid	Galangin	Organic acids
Ferulic acid	Apigenin	Ascorbic acid
Gallic acid	Quercetin	Maillard reaction products
Ellagic acid	Kaempferol	Amino acids
Vanillic acid	Luteolin	Proteins
Chlorogenic acid	Tectochrysin	Proline
	Hesperetin	
	Pinostrobin	
	Myricetin	

values proposed for these elements. While selenium was identified in all samples of Slovenian honey, but its content was less than the detection limit (0.53 mg/kg), the defined amount of chromium may not represent only its natural content in honey, but also the unknown proportion of this element due to contamination during honey harvesting and processing. The average contents of manganese ranged from 1.68 mg/kg in acacia to 23.2 mg/kg in chestnut honey (Kropf 2009).

23.5 Antioxidants

Honey can serve as a source of natural antioxidants. The components in honey responsible for its antioxidative effect are flavonoids, phenolic acids, ascorbic acid, catalase, peroxidase, carotenoids, and Maillard reaction products (Table 23.5). The quantity of these components varies widely according to the botanical and

geographical origin of honey. In addition, processing, handling, and storage of honey may influence its composition (Gheldof and Engeseth 2002; Turkmen et al. 2005; Küçük et al. 2007).

The phenolic profiles of honeys are determined by their botanical and geographical origin(s), and by the climatic conditions of the area. Therefore, identification and quantification of the phenolics in honey is of great interest (Gómez-Caravaca et al. 2006). The flavonoids constitute one of the largest groups of naturally occurring phenolic compounds that have health-related properties. They are derived from plants, and when the plants are used by bees to collect nectar or honeydew, these bioactive components are transferred into the bee honey. Their presence and concentration in honey is attributed to the plants visited by the bees, health of the plant, season, and environmental factors. Three subgroups of flavonoids with similar structures are present in honey, namely the flavones, the flavonols, and the flavanones (Viuda-Martos et al. 2008).

The total phenolic contents of Slovenian honeys were determined by the modified Folin–Ciocalteu method, and they varied widely among the honey types, as can be seen from Table 23.6. The lowest values of total phenolic content were determined in the acacia honeys, rising further in linden, multifloral, and chestnut honeys. The highest values of total phenolic content were obtained for the honeydew types of honey, namely fir, spruce, and forest honeys. The average total phenolic content was in close agreement with data reported in other studies of European honeys (Beretta et al. 2005; Lachman et al. 2010).

Contents of individual phenolic compounds in Slovenian honey were determined by means of liquid chromatography with mass spectrometer as detector (Bertoncelj 2008; Bertoncelj et al. 2011). Carbohydrates and other disturbing substances were removed from honey by solid-phase extraction before LC/MS analyses. The phenolic acids and flavonoids determined in the analyzed honey samples were *p*-coumaric acid, caffeic acid, ellagic acid, chlorogenic acid, myricetin, luteolin, quercetin, naringenin, apigenin, kaempferol, pinocembrin, chrysin, and galangin. Cinnamic acid, β-phenyllactic acid, pinobanksin, isorhamnetin, pinostrobin, fisetin, and eriodictyol were only tentatively identified on the basis of their molecular weights and

Table 23.6 Total phenolic content (mg GAE/kg) and antioxidant activity in different types of honey (Bertoncelj et al. 2007)

Honey type	Total phenolic content (mg GAE/kg)	Antioxidant activity (FRAP method) (μM Fe(II))
Acacia	25.7–67.9	56.8–86.0
Linden	63.4–109.0	94.6–155.1
Chestnut	146.8–272.3	238.3–469.5
Fir	163.4–285.7	320.8–582.2
Spruce	185.7–239.0	277.5–495.4
Multifloral	126.8–194.6	181.1–262.9
Forest	192.3–270.1	371.6–494.1

GAE gallic acid equivalents

available literature data. The results obtained indicate that most honeys had similar but quantitatively different phenolic profile. All honey types contained variable amounts of propolis-derived flavonoids, namely pinocembrin, pinobanksin, chrysin, and galangin. Their quantity in honey depends on the presence and content of propolis. Kaempferol and apigenin were present in all honey types analyzed, quercetin in most types, while the content of flavonoids myricetin, luteolin, and naringenin were very low. Flavonoid myricetin was not detected in acacia and linden honey, while chestnut honey did not contain naringenin. All analyzed honey types contained *p*-coumaric, affeic, cinnamic, and β-phenyllactic acid. Chlorogenic and ellagic acid were determined in some samples of Slovenian honey types except in acacia honey. Direct comparisons of the phenolic profiles of Slovenian honeys with the literature data are difficult, since other studies have involved different extraction and detection techniques; the number of components determined is also very variable.

For determination of the antioxidant capacity of Slovenian honeys FRAP assay (ferric reducing/antioxidant power) was used, which is a simple direct test that is widely used for antioxidant activity determination in many different samples, including honey (Benzie and Strain 1996; Beretta et al. 2005; Blasa et al. 2006; Küçük et al. 2007; Taormina et al. 2001). As shown in Table 23.6, the antioxidant activity for different types increased in the following order of the honeys: acacia < linden < multifloral < chestnut < spruce < forest < fir honey. These results are similar to those obtained by Beretta et al. (2005) and Lachman et al. (2010). A significant correlation between the total antioxidant activity, determined by the FRAP method, and phenolic content was observed, which indicates that phenolics are one of the main components responsible for the antioxidant behavior of honey. Gheldof et al. (2002) stated that phenolic compounds significantly contribute to the antioxidant activity of honey, but in spite of this, it seems that antioxidant activity appears to be a result of the combined activity of honey phenolics, peptides, organic acids, enzymes, and Maillard reaction products.

All types of Slovenian honey contain phenolic compounds and possess antioxidant activity. The total phenolic content and antioxidant activity vary greatly among different types of honey and was found to be the highest in darker types, namely chestnut, fir, spruce, and forest, while the lightest honey types, acacia, linden, and multifloral, show low total phenolic content and consequently lower antioxidant capacity. Regarding this statement, honey, especially darker types, may provide a significant additional source of dietary antioxidants.

23.6 Antibacterial Activity

Although honey is most widely used as a sweetener it forms part of traditional medicine in many cultures (Gómez-Caravaca et al. 2006). The first evidence of the contribution of the antibacterial capacity of honey dated in 1979 (Dustman). The substances with antimicrobial activity, so-called inhibins were classified in two systems by Bogdanov (1997): (1) the system due to the action of the hydrogen

peroxide in honey that is produced by glucose oxidase in the presence of heat and light and (2) stable non-peroxide activity independent of light and heat. Some authors have found that non-peroxide antibacterial activity is more important than the peroxide in terms of antibacterial effects. However, for optimum antibacterial activity, honey should be stored in a cool, dark place and be consumed when fresh (Alvarez-Suarez et al. 2009).

Hydrogen peroxide is generally poorly stable and prone to decay, but since it is continuously produced by the enzyme, some quantity is always present in honey. Research on the content of peroxide in Slovenian honey (Slovenian unpublished data) has shown a great diversity among the honey types as well as within a type of honey. Conclusions were in line with findings of other authors, i.e., the honey with naturally low enzyme activity (low diastase and invertase activity) has also a minimum content of peroxide. The highest content of peroxide in samples of Slovenian honey was found in forest (77.8 μg H_2O_2/g honey/h) and fir (76.6 μg H_2O_2/g honey/h) honeys, and the lowest in acacia honey (19.7 μg H_2O_2/g honey/h).

Typical antibacterial properties of honey are reflected in its stability during storage. In a study of Taormina et al. (2001) the inhibitory effectiveness of honey on the microorganisms *Escherichia coli*, *Salmonella typhimurium*, *Shigella sonnei*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*, responsible for food spoilage, was demonstrated. The results pointed out that the darker honeys have greater inhibitory effect than lighter ones. Darker honeys also showed higher antioxidant activity. Since antimicrobial activity of the darker colored honeys was not eliminated by catalase inhibition, non-peroxide components such as antioxidants also contributed to controlling the growth of some foodborne pathogens. By Bogdanov (2010) the following antibacterial factors in honey are responsible for antibacterial action: osmotic effect of sugars, pH and honey acids, hydrogen peroxide and others: phenolics, carbohydrates, proteins, and non-determined ones.

The study, carried out on six types of Slovenian honey (Kralj Kunčič et al. 2011) showed highly significant and positive antimicrobial effect of chestnut honey. Resistance of bacterial species (*Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) and fungal species (*Aspergillus niger*, *Aureobasidium pullulans*, *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Cladosporium cladosporioides*, *Penicillium chrysogenum*, and *Rhodotorula mucilaginosa*) was tested on chestnut, forest, fir, linden, acacia, and multifloral honey. Chestnut honey had the best antibacterial and antifungal activities, which appear to be mainly due to its high peroxide property, while the other types of Slovenian honey varied considerably. Undiluted Slovenian chestnut honey therefore represents a complex, natural substance with potential for medicinal purposes, such as for the topical treatment of skin and wound infections.

Unlike the manuka honey (*Leptospermum scoparium*), in which the highest antimicrobial activity is resulting from non-peroxide components, the major part of the antimicrobial effectiveness of Slovenian chestnut honey is a consequence of hydrogen peroxide.

Weston et al. (1999) found that manuka honey contains several phenolic compounds, among which methyl syringate was found to possess significant

antimicrobial activity against *Staphylococcus aureus* (Alvarez-Suarez et al. 2009). While manuka honey has an international recognition for use in medical purposes, the application for Slovenian chestnut honey has been filed and is currently being processed.

It was proved that honey has a preventive effect of honey on tooth caries (decay) and diseases of the mucous membrane in the mouth due to the components contained. Sucrose, the primary sugar that causes plaque to adhere to teeth, is present in honey in smaller amounts; moreover, honey contains different phytochemicals with antimicrobial activity to prevent the growth of dental pathogens. Molan (2001) and English with coworkers (2004) reported inhibitory effect of manuka honey on growth of the dental plaque bacteria and potential therapeutic role of the honey in the treatment of gingivitis and periodontal disease. Currently researches on effectiveness of other honey types are in progress. Slovenian periodontologists have found that among Slovenian honeys the canola and linden honey most effectively inhibits the growth of oral bacteria *Aggregatibacter*, followed by multifloral, acacia, forest, chestnut, and fir honey. Additionally, research efforts are focused on determination of the honey component with principal antimicrobial or bacteriostatic effect like methylglyoxal is in *Leptospermum* spp. manuka honey (Podržaj et al. 2010).

23.7 Prebiotic Properties

Prebiotic is defined as "a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota" (FAO Technical Meeting 2007). Detailed characterization of such component is required (source, origin, purity, chemical composition and structure, vehicle, concentration and amount in which it is to be delivered to the host).

Honey contains several oligosaccharides and some low molecular weight polysaccharides and thus presumably possesses prebiotic properties. Namely, the mentioned carbohydrates are likely to resist human enzymes and therefore be available as a nutrient of the microflora in the large bowel.

To date only reports of in vitro test on honey effect on growth and activity of intestinal microflora, and no data on human clinical studies are available. Furthermore, Slovenian honey has not been undergone any laboratory study of this kind. However because of the comparable amounts of oligosaccharides to those reported for different European honeys, the topic must not be neglected.

Kajiwara and coworkers (2002) reported that growth promoting effect of honey on five strains of bifidobacteria is similar to that of commercial fructo- and galactooligosaccharides and inulin. It was found out that honey oligosaccharides exert the prebiotic effect mainly in a synergistic way, although panose proved to enhance the growth of bifidobacteria the most (Ustunol 2000). Different bifidobacteria strains responded differently regarding the type of honey applied, which is attributed to the source specific carbohydrates contained. According to results of Croatian study of milk sweetened with honey (Lucan et al. 2009) chestnut honey has greater

prebiotic activity as acacia honey. From the available data it may be presumed that honeydew honeys, which contain higher amount and larger spectra of oligosaccharides, possess also greater prebiotic activity; however, more research is needed to confirm this.

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Chapter 24
Functional Properties of Honey and Some Traditional Honey Products from Portugal

Teresa Cavaco and Ana Cristina Figueira

24.1 Introduction

Honey and some traditional honey products (mead and abbamele or água-mel) are some attractive sources of nutraceuticals and medical ingredients for healthy foods (Kücük et al. 2007). These products are rich in secondary metabolites as natural antioxidants (phenolic acids and flavonoids) and contribute to human health.

The research on bioactive compounds in honey shows that the regular intake of phenolic compounds is associated with a reduced risk of heart diseases. In these diseases, phenolic compounds had protective effects as antithrombotic, anti-ischemic, antioxidant, and vasorelaxant (Idris et al. 2011; Khalil 2010). Work from this research groups led to the suggestion that flavonoids decrease the risk of coronary heart disease by three major actions: improving coronary vasodilatation, decreasing the ability of platelets in the blood to clot, and preventing low-density lipoproteins (LDLs) from oxidizing. In this chapter we will describe the presence of some nutraceutical products in honey and the effect of processing on their presence in some traditional honey products.

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24.2 Honey

Honey is one of the most complex foodstuffs produced by nature, and certainly the only sweetening agent that can be used by humans without processing. Honey is produced by honey bees (*Apis mellifera*) from carbohydrate-containing exudates produced by plants (Terrab et al. 2004), Fig. 24.1.

Honey has a wide range of applications in the food industry. It can be processed for direct consumption or be used as an ingredient of various processed food products. Due to its superior nutritional value and unique flavor, natural bee honey is preferred by consumers (Qiu et al. 1999).

The composition of honey (see Table 24.1) is variable, owing to the differences in plant types, climate, environmental conditions, and contributions of the beekeeper (Anklam 1998; Azeredo et al. 2003). Several types of honey are produced in Portugal (Mendes et al. 1998). Different types of honey depend highly on the types of flowers used by the bees, as well as regional and climatic conditions (Anklam 1998; Mendes et al. 1998). Honey commercialized throughout the world varies greatly in quality, which is assessed largely on the basis of color, flavor, and density (Mendes et al. 1998; Bogdanov 2002).

Honey is a remarkably complex natural liquid that is reported to contain at least 200 substances and considered to be part of traditional medicine (Bogdanov et al. 2004). It has been used in ethno medicine since the early humans, and in more recent times, its role in the treatment of burns, gastrointestinal disorders, asthma, infected wounds, and skin ulcers has been "rediscovered" (Al-Mamary et al. 2002; Kücük et al. 2007).

Honey is a sweet and flavorful natural product which has been consumed for its high nutritive value and its contribution to human health. It has been demonstrated that honey, on a fresh weight basis, is similar to many fruits and vegetables in its antioxidant capacity. The antioxidant activity of honey, however, varies greatly depending on its floral source. There is little knowledge about the profiles of antioxidant substances in honey from various floral sources. The variation in these profiles might be responsible for the widely varying abilities of honey to protect against oxidative reactions. In addition, these constituents present in honey have antioxidant properties. These include phenolic acids and flavonoids, certain enzymes

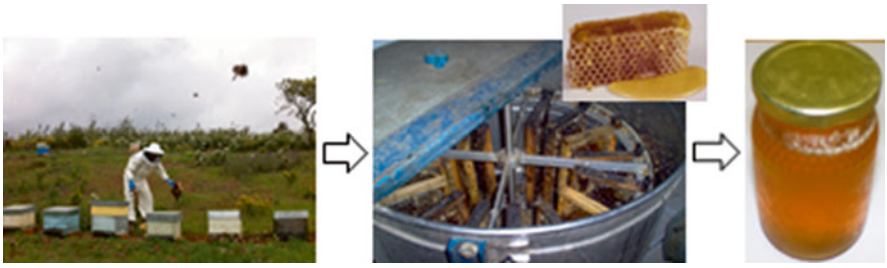

Fig. 24.1 Production and extraction of honey

Table 24.1 Physicochemical parameters and antioxidant activities of different honeys (Al et al., 2009; Akbulut and Musa 2009; Cavaco and Figueira 2006; Idris et al. 2011; Kücük et al. 2007; Meda et al. 2005; Silici et al. 2010)

Parameters	Produced in the Algarve, Portugal	Produced in other countries
Moisture (%)	14.80–17.27	15.40–20.00
Conductivity (μs/cm)	130–540	200–800
Ash (%)	0.06–0.18	0.03–1.23
pH	3.27–4.28	3.70–5.21
Total acidity (meq/kg)	13.41–20.00	16.00–32.00
HMF (mg/100 g)	0.02–28.29	18.00–94.00
Water activity	0.51–0.54	0.47–0.56
Proline (mg proline/100 g)	4.35–48.67	48.67–216.00
Reducing sugars (mg/100 g)	34.06–63.63	67.70–73.70
Sucrose (g/100 g)	0.00–1.00	0.00–3.34
Fructose (g/100 g)	19.00–45.09	20.84–45.70
Glucose (g/100 g)	21.65–46.00	28.58–45.40
Total flavonoids (mg quercetin equivalents/100 g)	1.40–25.80	0.91–28.25
Total phenolic compounds (mg gallic acid/100 g)	24.72–48.67	2.00–201.08
DPPH (% inhibition)	15.88–50.22	35.80–90.73

(glucose oxidase and catalase), and others, namely ascorbic acid, protein, and carotenoid (Alvarez-Suarez et al. 2010; Meda et al. 2005; Molan and Betts 2004). Other authors have studied the phenolic and flavonoid contents of honey to determine if a correlation with floral origin and its antioxidant capacity exists (Meda et al. 2005; Tomás-Barberán et al. 2001).

Honey has therefore a great potential to serve as a natural food antioxidant, since its antioxidant concentration, although variable, is high (Bogdanov et al. 2004). Research indicates that honey has functional properties in human health promotion that depend largely on the floral source. These properties could be associated to honey's high osmolarity, antibacterial properties, and antioxidant capacity (Alvarez-Suarez et al. 2010; Effem 1988). The therapeutic potential of honey is gradually growing and scientific evidences for the effectiveness of honey in several experimental and clinical conditions are beginning to emerge. Honey has been reported to be effective in gastrointestinal disorders, in healing of wounds and burns, as an antimicrobial agent and to provide gastric protection against acute and chronic gastric lesions (Meda et al. 2005).

The antioxidant activity of phenolic compounds might significantly contribute to the human health benefits of plant foods and beverages such as red wine and tea. Honey contains a great number of phenolic compounds, which are generally acknowledged to be of considerable importance because of its chemoprotective effect in human beings (Arráez-Román et al. 2006).

Since antiquity, mankind has been keeping bees, mainly as a source of both honey (nature's natural sweetener) and beeswax (mainly for candlelight). In those times, the only way to separate them was by removing the honeycombs from the beehives, crumbling and squeezing them. Honey was further removed by rinsing the beeswax with warm water (Montereau 2011). During this process, the honey water was considered as a by-product, which was left over. This was the beginning of a large variety of honey by-products, some of which will be covered in the next sections.

24.3 Mead

Mead is an alcoholic beverage produced from an aqueous solution of honey, under the effect of osmotolerant yeasts, containing 7–22 % (v/v) of ethanol (Gupta and Sharma 2009; Mendes-Ferreira 2010). Mead has been made from about 7000 BC, and is rather important both for the Africans and the Europeans. Its production is believed to have started in Africa, from where it was brought through the Mediterranean into Europe (Gupta and Sharma 2009; Montereau 2011; Teramoto 2000).

There is a large variety of meads, depending on the source of honey, concentration of the initial dilution, additives, type(s) of yeast(s) used in fermentation, and the ageing process, Table 24.2. Further to these, fruit juices or pieces, spices, herbs, and vegetables are amongst the most frequent additions mentioned in the literature (Anon 1997; Gupta and Sharma 2009; Andrews 1997).

For mead elaboration, honey mashes are usually diluted to 18–37 % (w/v) (Fig. 24.2.), at pH 3.6–5.5 and temperatures 20–35 °C (Chagas et al., 2008; Gupta and Sharma 2009; Koguchi et al. 2009; Mendes-Ferreira 2010; Navrátil et al. 2001; Qureshi and Tamhane 1985, 1986,1987; Roldán et al. 2011; Sroka and Tuszynski 2007).

Additives such as citric acid, malic acid, tartaric acid, diammonium monohydrate, diammonium phosphate, diammonium hydrogen phosphate, ammonium dihydrogen phosphate, potassium sodium tartrate, magnesium sulfate, calcium sulfate, bentonite sodium form, potassium bitartrate, magnesium chloride, calcium chloride, SO_2, potassium chloride, potassium metabisulphite, ammonium chloride, potassium dihydrogen phosphate, are so incorporated (Chagas et al., 2008; Gupta and Sharma 2009; Koguchi et al. 2009; Mendes-Ferreira 2010; Navrátil et al. 2001; Pereira et al. 2009; Qureshi and Tamhane 1985, 1986,1987; Roldán et al. 2011; Sroka and Tuszynski 2007).

The honey musts are then usually inoculated with strains of *Saccharomyces cerevisiae* (Chagas et al., 2008; Gupta and Sharma 2009; Koguchi et al. 2009; Mendes-Ferreira 2010; Navrátil et al. 2001; Pereira et al. 2009; Qureshi and Tamhane 1985, 1986,1987; Roldán et al. 2011; Sroka and Tuszynski 2007), *Hansenula anomala* (Qureshi and Tamhane 1987) or a mixture of *Saccharomyces cerevisiae* and *Hansenula anomala* (Qureshi and Tamhane 1986). The fermentation process proceeds until the amount of total soluble sugars stabilizes, usually for a concentration of residual sugars lower than 5 g/L. At this stage, mead is siphoned, matured, and bottled (Gupta and Sharma 2009; Roldán et al. 2011).

Table 24.2 Different types of mead (Andrews 1997; Anon 1997)

Types of mead	
Traditional mead	A fermented honey beverage made from approximately two and one-half pounds of honey diluted with one gallon of water only
	Mead made primarily from honey, water, and yeast. Honey should be expressed in aroma and flavor. Additives of any type are allowed at subthreshold levels. The mead should have a neutral acidity-sweetness-tannin balance. It can be made using generic or blend of honeys (Standard traditional mead), or honey from a particular flower source, with clover and wildflower honeys being the exceptions (varietal honey traditional meads)
Hydromel	Weak, or watered mead
Sack mead	Mead that is made sweeter by the addition of 20–25 % more honey; a sauterne-like beverage
Metheglin	Spiced mead; originally spiced with a combination of herbs (gruit) but later hops became more popular
	A mead made with spices or herbs. The spices should be expressed in the aroma and flavor of the mead, but usually won't appear in the color. There should be a pleasing but not necessarily equal honey-spice balance in the mead. Metheglins containing more than one spice should also have a good balance between the different spices, though stronger spices tend to dominate. Often a blend of spices may give a character greater than the sum of its parts
Sack metheglin	Sweet spiced mead; traditionally similar to vermouth. Mixed Category Mead (A mead that combines ingredients from two of the three previous categories. The mead should exhibit the character of all of the ingredients, and should show a good blending or balance between the various flavor elements)
Braggot	A mead made with malted barley or wheat (also spelled Bracket)
Melomel, or mulsum	Mead made with fruit juice
Spiced melomel or fruited metheglin	A mead made with fruit and spices
Cyser	A melomel made with apple juice or cider; similar to a sherry wine
Apple pie mead (cyser with mulling spices)	A mead made with apples and allspice, cinnamon, cloves, ginger, nutmeg, citrus rind, mace or other mulling spices
Pyment, or clarre	A melomel made with grape juice; sometimes referred to as honey-sweetened grape wine
Hyppocras	Spiced pyment. A mead made with grapes and spices. Grassy white wine character or buttery chardonnay character is appropriate in hippocras (or pyment) only
Maltomel	Mead made with malt and fruit
Malteglin	Mead made with malt and spices

As expected, the physicochemical composition of mead (a fermented drink) is considerably different from the one for honey (Tables 24.1. and 24.3.). This is particularity noticeable for ethanol, °Brix and both reducing and residual sugars.

Phenolic compounds are a widespread group of antioxidants present in mead, these compounds are taken from plants by the honey bees (*Apis mellifera*). Regarding

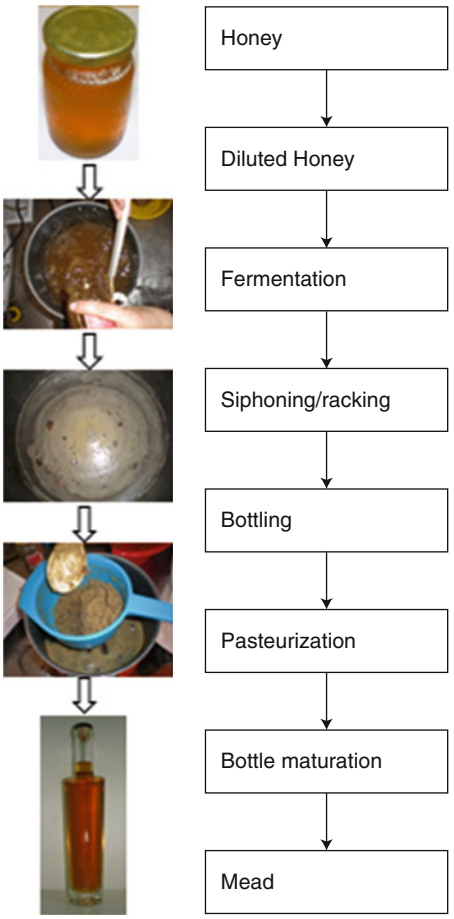

Fig. 24.2 Procedure for production of mead

the biological compounds present in meads, they depend not only on the honey floral source, but also on the other various ingredients, namely fruit juices, plants, black rice, spices (as mentioned in Table 24.2.) used in mead preparation (Gupta and Sharma 2009; Kahoun et al. 2008; Koguchi et al. 2009;). Mead demonstrates a high antioxidant activity, which is usually associated to protective effects as antithrombotic, anti-ischemic, antioxidant, and vasorelaxant (Idris et al. 2011; Khalil 2010).

24.4 Abbamele or Águamel

Abbamelle or água-mel is a dark, viscous, honey-like product traditionally produced in the Southern parts of Europe, namely in Sardinia (Italy) and Alentejo and Algarve (Portugal) (Figueira and Cavaco 2012; Jerković et al. 2011; Spano et al. 2008). These are subproducts originating from the honey industry. Briefly, it is obtained by scalding previously crumbled honeycombs (wax from the bee hives)

Table 24.3 Antioxidative activity of different types of mead (adapted from Gupta and Sharma 2009; Koguchi et al. 2009)

Type of mead	Free SO_2 (ppm)	Bound SO_2 (ppm)	Titrable acidity (g/L tartaric)	Volatile acidity (g/L acetic)	% Ethanol	°Brix	pH	Residual sugars (%)	Reducing sugars (mg/mL)	CO_2 output (g)	Final nitrogen (mg/L YAN)
Commercial mead from soya honey	43.2	56.8	5.9	0.6	11.8	8.2	3.64	1.0			
Home-brewed mead from soya honey	3.2	16.0	2.1	1.3	4.6	13.0	3.3	20.0			
Buckwheat experimental mead	9.5	14.1	2.97	0.48	11.5	7.9	3.23	18.0			
Soy experimental mead	7.4	20.0	3.89	0.91	6.4	14.0	2.74	20.6			
Chinese milk vetch honey experimental mead			2.6		4.8		3.1		91.3	4.6	
Chinese milk vetch honey and black rice experimental mead			2.7		10.4		3.3		2.5	10.3	
Chinese milk vetch honey and polished rice experimental mead			2.6		8.1		3.2		32.3	8.1	

(continued)

Table 24.3 (continued)

Type of mead	Free SO_2 (ppm)	Bound SO_2 (ppm)	Titrable acidity (g/L tartaric)	Volatile acidity (g/L acetic)	% Ethanol	°Brix	pH	Residual sugars (%)	Reducing sugars (mg/mL)	CO_2 output (g)	Final nitrogen (mg/L YAN)
Buckwheat honey experimental mead			2.8		9.5		3.1		4.9	9.2	
Buckwheat honey and black rice experimental mead			2.5		10.3		3.4		1.8	10.2	
Buckwheat honey and polished rice experimental mead			2.8		10.4		3.3		4.3	9.8	
Experimental mead from Portuguese heather honey	0.0±0.0	30.7±0.4	3.0±0.1	0.63±0.04	10.8±0.7		3.67±0.13				24.5±4.9
Experimental mead from commercial multifloral honey			4.87±0.21	1.370±0.179	9.04±0.02		2.95±0.15	<0.6			

Table 24.4 Antioxidative activity of different types of mead (adapted from Gupta and Sharma 2009; Koguchi et al. 2009)

Type of mead	Total phenolic content (mg/mL gallic acid eq.)	DPPH radical scavenging activity (mM Trolox eq.)
Commercial mead from soya honey	3102.93	16.06
Home-brewed mead from soya honey	163.63	7.12
Buckwheat experimental mead	300.6	3.79
Soy experimental mead	167.16	3.47
Chinese milk vetch honey experimental mead	100	0.071
Chinese milk vetch honey and black rice experimental mead	200	0.329
Chinese milk vetch honey and polished rice experimental mead	100	0.096
Buckwheat honey experimental mead	300	0.398
Buckwheat honey and black rice experimental mead	400	0.454
Buckwheat honey and polished rice experimental mead	300	0.406

Table 24.5 Phenolic compounds and hydroxymethylfurfural concentration in mead samples (adapted from Kahoun et al. 2008)

Type of compound	Compound	Concentration ranges (mg/L)
Toxic	Hydroxymethylfurfural	2.74–157
Benzoic acid hydroderivatives	Gallic acid	0.04–6.63
	Protocatechuic acid	0.06–3.08
	Gentisic acid	0.06–0.77
	Vanillic acid	0.08–2.61
	Syringic acid	0.10–1.80
Cinnamic acid derivatives	Caffeic acid	0.15–6.38
	Chlorogenic acid	0.16–14.1
	p-Coumaric acid	0.08–10.6
	Ferulic acid	0.04–3.74
	Isoferulic acid	0.05–1.41
	Sinapic acid	0.08–0.51
Phenylacetic acid	4-Hydroxyphenylacetic acid	0.07–1.53
	3-Hydroxyphenylacetic acid	0.11–0.12
	Homoprotocatechuic acid	0.06–0.32
Sensorically significant compounds	Vanillin	0.12–54.8
	Ethylvanillin	0.050–31.0
Other phenolic compounds	Protocatechuicaldehyde	0.03–0.12
	Esculetin	0.12
	(+)-Catechin	0.44–4.04

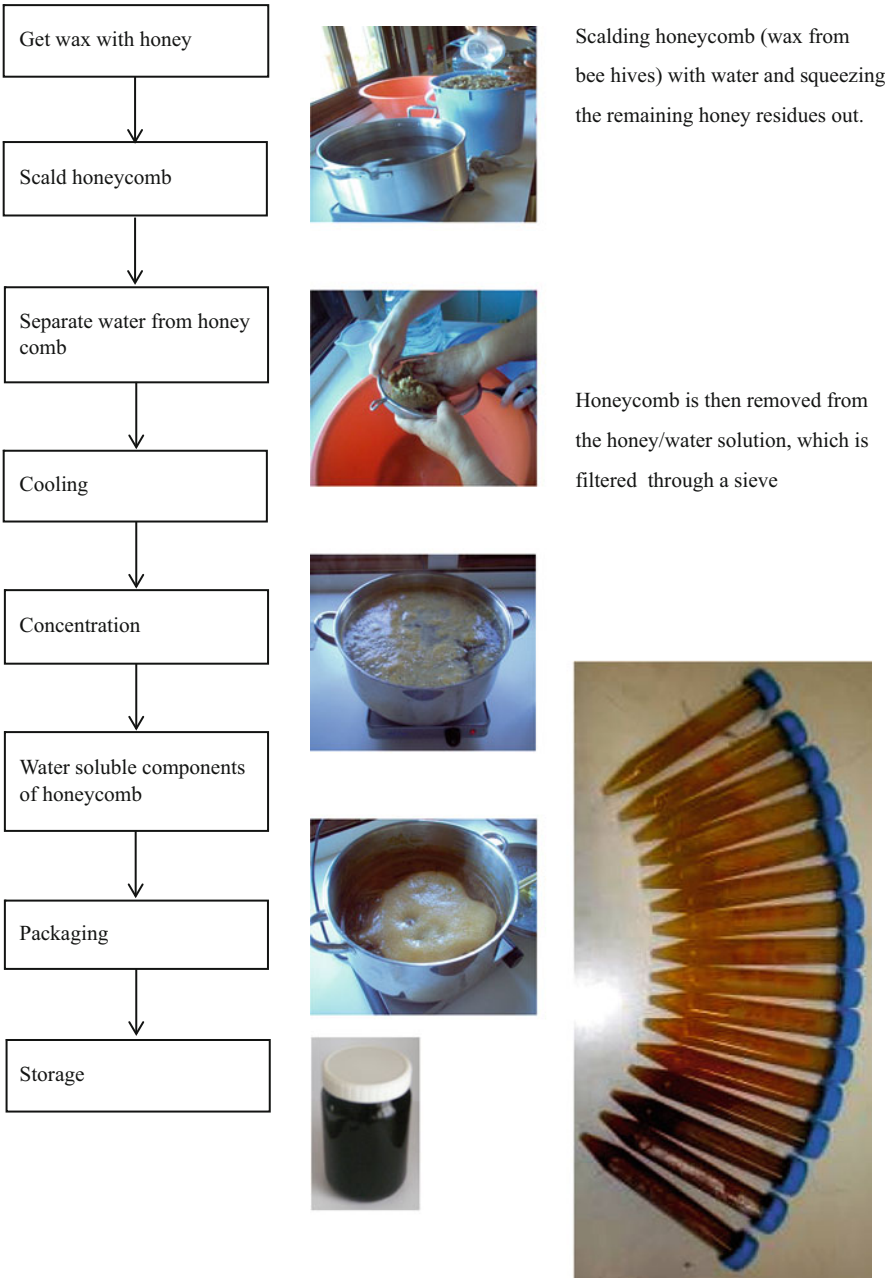

Fig. 24.3 Procedure for production of água-mel

Table 24.6 Physicochemical parameters and antioxidant activity from different água-mel (Figueira and Cavaco 2012; Spano et al. 2008)

Parameters	Industrial	Traditional	Produced in Algarve, Portugal
pH	3.36–3.92	3.21–3.84	3.21–3.70
Free acidity (meq kg^{-1})	26.10–75.70	26.20–87.6	24.00–70.20
Water content (%)	17.40–27.70	19.40–25.4	17.00–25.00
Electrical conductivity (mS cm^{-1})	0.41–0.81	0.19–0.60	0.15–0.80
Invertase activity (U kg^{-1})	0.00–1.02	0.02–0.08	
HMF (mg/kg)	111.20–1823.60	1007.00–4405.80	40.00–60.41
Total polyphenols (mg GAE/kg)	1297.80–1959.80	1491.20–4469.50	1000.15–1523.20
FRAP (mmol Fe^{2+}/kg)	13.30–19.40	19.9–71.2	
DPPH (mmol TEAC/kg)	3.80–5.70	5.10–23.3	0.91–28.25

Table 24.7 Biological activity of honey and traditional honey products (Al-Mamary et al. 2002; Alvarez-Suarez et al. 2010; Giorgi, 2011; Khalil 2010; Jerković et al. 2011)

Products	Biological activities
Honey	Treatment of burns, gastrointestinal disorders, asthma, infected wounds and skin ulcers has been "rediscovered", antioxidant effects, antimicrobial effects, reduced risk of heart diseases, antithrombotic, anti-ischemic, antioxidant, vasorelaxant, decreasing the ability of platelets in the blood to clot, preventing low-density lipoproteins (LDLs) from oxidizing, antiparasitic activity, antimutagenic, infant nutrition
Abbamele or águamel	Anti-inflammatory, antimicrobial effects
Mead	Antioxidant effects

with water and squeezing the remaining honey residues and also pollen out. The wax is then removed by filtration, and the liquid, to which it is common to add peels or pieces of citrus fruits, is simmered until the appropriate amount of soluble solids (°Brix) is attained (Figueira and Cavaco 2012; Jerković et al. 2011; Spano et al. 2008), Fig. 24.3.

Água-mel is traditionally consumed as a sweetener, on soft cheese, as a honey-like spread, on bread or sandwiches, or as filler for sweets (Figueira and Cavaco 2012; Spano et al. 2008).

The physicochemical composition of água-mel is generally similar to the common levels found for honey (Tables 24.1 and 24.6). This is particularly so for electrical conductivity, pH, and free acidity (Spano et al. 2008). Exceptions are, as expected, the so-called "thermolabile" analytes. Therefore, invertase activities are substantially lower, whilst 5-hydroxymethyl-2-furaldehyde (HMF) is very high in all samples (Figueira and Cavaco 2012; Jerković et al. 2011; Spano et al. 2008). Heating does, unfortunately, also affect the amino acids initially present in honey, and even proline (a relatively abundant amino acid found in honey) is absent from

água-mel (Spano et al. 2008). This is suggested to be due to an increased rate of the Maillard reaction (a chemical reaction which takes place between the amino acids and the reducing sugars initially present in the honey) during heating (Spano et al. 2008). Moisture contents vary substantially, and depend mainly on the moisture content of the raw materials (honey and honeycombs), the amount of water added during processing, and the temperature and length of the heating process (Spano et al. 2008).

The total antioxidant activities (determined using the FRAP test), the antiradical activities (determined using the DPPH test) and the total phenolic contents show values that are much higher than the observed for honeys (Tables 24.1 and 24.6). It's also worth to mention a linear correlation between the total phenolic content and both the antioxidant activity and the antiradical activity. In addition, total phenolic contents show to be similar or even higher than the found for red wine, fruits, and vegetables (Figueira and Cavaco 2012; Jerković et al. 2011). These findings lead to the suggestion that água-mel may be considered as an additional source of bioactive compounds in the human diet.

24.5 Conclusions

Honey and some traditional honey products (mead and abbamele or água-mel) do have different physicochemical and biological characteristics, due to variations in the honey botanical origins, appearance, and sensory perception. Their main components are always the carbohydrates fructose and glucose, but in addition to these, honey contains a large number of minor constituents with antioxidant activities, which result in numerous nutritional and biological effects, namely antimicrobial, antioxidant, antiviral, antiparasitic, antiinflamatory, antimutagenic, anticancer, and immunosuppressive activities (Table 24.7.).

The high biological activities observed for honey are, however, not exactly the same shown by mead and água-mel. This is due to the fact that in the case of these traditional honey products, the physicochemical and biological characteristics are affected by the processing methodology. Both mead and água-mel production involve some heating steps, which results in increases in the concentration of some compounds with a deleterious effect in human health, as in the case of HMF. In addition, heating also decreases the amino acids' concentration in the final honey products. These support the need for the research of alternative production methods, using milder heating treatments.

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Chapter 25
Functional Aspects of Tea *Camellia sinensis* as Traditional Beverage

Anna Gramza-Michałowska

25.1 Introduction

Tea is the commonly consumed refreshing and thirst-quenching beverage made from the leaves of *Camellia sinensis* and is widely known for its health benefits. People used tea as a social and medicinal beverage for centuries. It is a plant cultivated in many countries of the world and consumed in probably every corner. Nowadays tea and its constituents are the subject matter of many research due to their possible beneficial properties in the human body. It was stated that main tea constituents, polyphenols, scavenge free radicals, helping the human body in fighting with major degenerative diseases like cancer or simple aging. Chinese legend claims that around 2737 B.C. Emperor Sheng Nung, called the Divine Healer, discovered tea and taught people how to prepare and drink it. Since then tea became a daily consumed beverage in China and later in other countries.

It is easy to notice the increased awareness in functional food, possessing positive human health benefits or bioregulatory functions. Research confirmed that tea originated substances possess antioxidant properties as trapping agents and free radical quenchers, presenting a wide range of health benefits for human health and wellness.

Numerous research showed desirable food, consuming habits including tea beverage drinking. As it was known for centuries and latest research had confirmed tea beverage is a drink that could contribute to overall health and prevention of many diseases. It must be also underlined that tea is new source of natural substances presenting antioxidant activity that are acceptable by the consumers, and which allow to increase quality and shelf life of food products.

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25.2 Tea Cultivation and Manufacture

Botanically tea plant is an evergreen shrub or tree from the *Theaceae* family, species *Camellia sinensis*. Presently cultivated tea plants belong to botanical varieties: Chinese tea shrub (*Camellia sinensis*) as well as the Indian tea tree (*Camellia assamica*) (Sanderson 1972; Annonymus 2000). Leaves of var. *assamica* are large and trunk is tall, var. *sinensis* however is smaller leaf and its trunk is bush type (Chu 1997). Generally leaf of tea is dark green and shiny, growing round and opposite, flowers are white and pink and fruits are small and brown color (Chu and Juneja 1997).

Mukhtar and Ahmad (1999) have found that the average daily consumption of tea brew reaches 120 mL/person, but it is also important that tea consumption preferences are different in various regions of the world. The tradition of tea drinking is specific in Japan and Northern China inhabitants prefer pure and regarded as healthier—green tea; oolong tea is mainly consumed in Taiwan and Southern China, most Europeans and Americans however prefer fermented black tea.

One of the most popular tea classifications is based on different fermentation degree: non-fermented (white and green), semi-fermented (oolong and yellow) as well as totally fermented (black) (Harbowy and Balentine 1997; Chen et al. 2006). Recently very popular became Pu-erh tea, which is also *Camellia sinensis* but its processing involve microorganisms in the fermentation not the enzyme oxidation. As a result of leaves special treatment with the fungus *Aspergillus niger* Pu-erh tea is well known for a unique flavor and suppressing fatty acid synthase expression (Mo et al. 2008). Pu-erh tea is commonly called as "fat burner."

Tea plantations are very popular in Japan, China, India, and Taiwan (Weisburger 1997; Fernandez et al. 2002). Years ago the only mountainous areas had been regarded as the best for tea cultivation, nowadays mainly hilly and flat terrain are occupied because of large scale of mechanical farming, which makes tea production easier and cheaper than the high mountain growing expensive leaves.

The production of teas is conducted in many different ways, but mainly it consists of few stable stages. The first picking of tea leaves runs in late April to May (first flush), than on the turn of June, followed by early August. Picking is mostly done mechanically, while manual picking is only for limited kinds of tea (Sen-cha or Gyokuro). During the harvesting the mostly destructive factor is frost, which could devastate tea shoots. To avoid such influence electric fans have been set up on poles in places where the frost is mostly to occur (Fig. 25.1). Main role of those fans is to send the warmer air down to the surface to elevate the temperature. Other common practice is covering the shoots with polythene or sprinkling tea plants with water (Hara 2001a, b).

Tea leaves of tea undergo several stages of processing, varying with the temperature and time of processing. Figure 25.2 presents typical process flowchart for tea production. Fresh leaves after the plucking are withered overnight, which allows receiving special fragrance, rendering leaves more pliable to rolling. During the rolling process oxidative enzymes and catechins start to interact. In fresh leaves they are separated, but during rolling catechins from vacuole in the palisade layer and

Fig. 25.1 Tea *Camellia sinensis* cultivation and warming fans

enzyme from the epidermal layer bounds for interactions. This process of enzymatic oxidation, often called tea leaves fermentation, leads to formation of dimmers and other highly complexed compounds, and in effect the appearance of the dark-brown or black color and suitable aroma (Chu and Juneja 1997; Lin et al. 1998). Partially fermented yellow and oolong tea undergoes a considerably shorter fermentation time than black tea. Production of gentle and constringent green tea involves partial withering of tea shrub or leaves, afterwards roasting to inactivate oxidative enzymes (polyphenol oxidase and glycosidase), rolling up, drying, and sorting (Balentine et al. 1997).

Basic machines in the manufacture of green tea are as following: tea leaf feeder, steaming machine, steamed leaves cooling machine, primary tea roller, dryer, tea roller, secondary drying tea roller, final drying tea roller, drier, sorting, and packing machine. For quality assurance and longer storage tea should have the moisture reduced to 2–3 % and stored in a dry, cool, and dark place. Certain tea brands are packed with the nitrogen gas to protect tea from oxidative deterioration.

25.2.1 Chemical Composition

Tea leaves contain various chemical compounds. The tissue of tea leaves is mainly carbohydrates, including cellulosic fiber, protein, and lipids, other substances are polyphenols, vitamins A, B1, B2, C, niacin, amino acid—theanine, minerals Ca, P, Mg, Zn, Fe, Na, K, and caffeine (Tsushida and Taeko 1977; Chu and Juneja 1997;

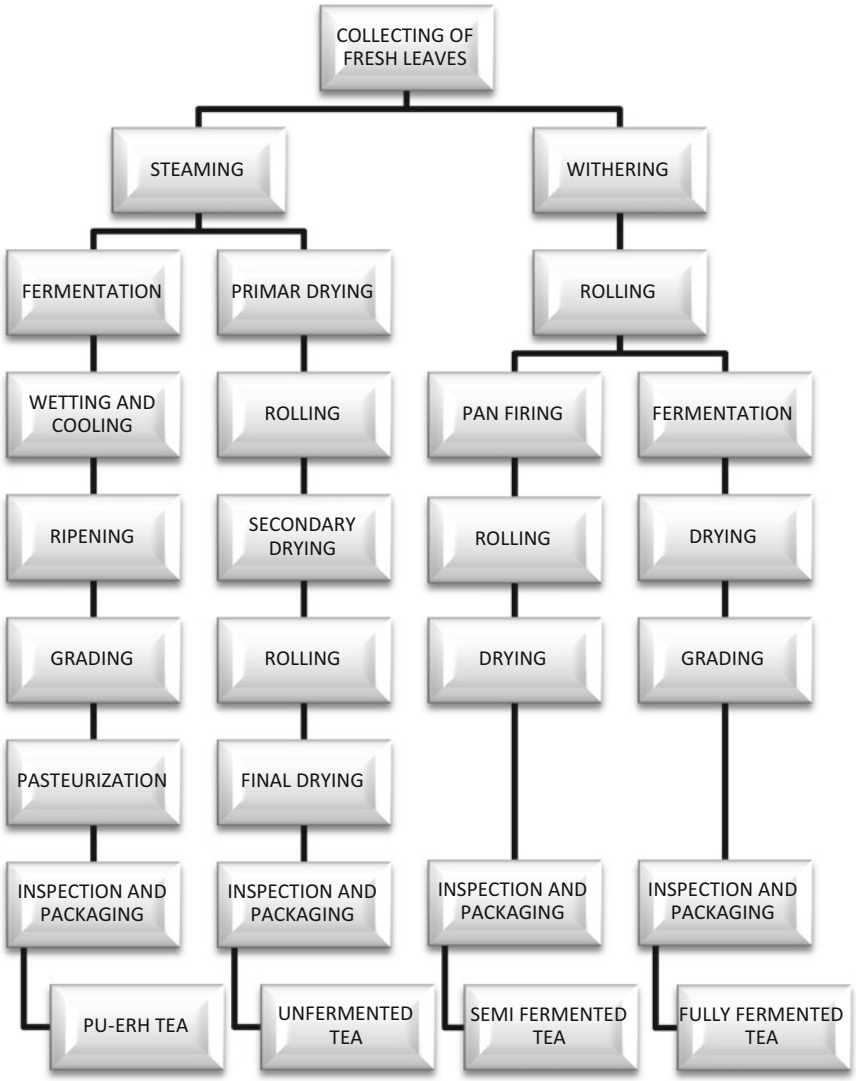

Fig. 25.2 Tea manufacture (Hara 2001a, b; Mo et al. 2008)

Fernandez-Caceres et al. 2001; Ferrara et al. 2001). Another important tea constituents are pigments, chlorophyll in green tea and orange theaflavins and brown thearubigins in fermented teas (Bailey and Nursten 1993; Balentine 1997; Higashi-Okai et al. 2001; Sava et al. 2001; Gramza and Korczak 2004). Among all substances in tea probably the most active are polyphenols, which are plant secondary metabolites, not synthesized in *fauna* world. In plants polyphenols role is protection from UV irradiation, pests, pathogens, and also giving the attractive for insects color (Parr and Bolwell 2000). Tea polyphenols are basically from flavonoids and

flavanols group, making up to 30 % of its dry weight but only 10 % of the dry weight of black tea (Wang et al. 2000a). However mostly noticeable among polyphenols are catechins, extracted within water, and responsible for brew astringency and bitter taste. Tea catechins mainly consist of (+)-catechin C, (−)-epicatechin EC, (+)-gallocatechin GC, (−)-epigallocatechin EGC, (−)-epicatechin gallate ECG, and (−)-epigallocatechin gallate EGCG, which are synthesized through the malonic and shikimic acid metabolic pathways (Graham 1992; Balentine et al. 1997; Chu 1997; Hollman 2001; Gramza et al. 2006).

Many research showed that the differences in levels of substances strictly depend from the plant species, tea kind, and leaf structure, which influence further leaching kinetics, harvesting practices, and manufacturing practices (Wang et al. 2000a, b; Khokhar and Magnusdottir 2002). Green tea composition is dominated by the presence of simple flavonoids, which are transformed to complexed forms of theaflavins and thearubigins during the fermentation process (Graham 1992; Balentine et al. 1997; Hagerman and Carlson 1998; Yanishlieva-Maslarowa and Heinonen 2001). Tannins are the other compounds responsible for the specific astringent brew's aroma and taste (Chung et al. 1998; Hagerman et al. 1998; Kallithraka et al. 2000; Riedl and Hagerman 2001). It was stated that tea leaves catechin content correlates with infusion quality (Thanaraj and Seshardi 1990).

Comparison of green and black teas resulted in lower catechins amount in black tea, but larger quantity of gallic acid than in green tea (Khokhar and Magnusdottir 2002). In order to remain the possibly highest biological effect consumers must be aware of tea leaf quality and brewing methods influence on bioactive components content. Graham (1992) and Mukhtar and Ahmad (1999) found that a cup of green tea contains high level of catechins and could be 90–400 mg of polyphenolic antioxidants, of which EGCG is 200 mg.

25.2.2 Factors Influencing Tea Leaves Polyphenol Content during Processing

During the fermentation (or enzymation) tea leaves are subject to the polyphenol oxidase activity, resulting in oxidation of catechins to quinones, further polymerization to bisflavans and complexed structures like theaflavins, thearubigins, and higher molecular mass compounds (Stagg 1974; Bailey and Nursten 1993; Lin et al. 1998; Tanaka et al. 2002). According to results of Chen et al. (1998a, b), pH increase causes an increase in catechin degradation, while acidic pH resulted in its large stability (Zhu et al. 1997; Friedman and Jurgens 2000). Practically consumers should put, e.g., lemon slice into a cup to stabilize catechins. There are many ways for tea consumption; it could be as pure infusion, with additives like lemon, sugar or milk and cream, which is very popular in Ireland, England, and Canada (Hollman et al. 2001). Results showed possible negative effect of milk addition on biological potential of tea brewing (Lorenz et al. 2007; Ryan and Petit 2010).

25.3 Biological Action and Health Effects of Tea Compounds

Before drinking tea leaf was one of the "ancient chewing gum." According to Chu (1997) during centuries, the merit of tea as a physiologically functional agent or the habit of eating tea leaves has gone, and today's consumer use tea leaves as for brewing and as supplements in order to lead a "healthy lifestyle" (Scalbert and Williamson 2000; Gramza et al. 2005a, Gramza and Korczak 2005; Gramza-Michalowska and Bajerska-Jarzebowska 2007). Tea constituents possess wide range of biological activity presented in Table 25.1.

Beneficial impact of tea compounds on human organism is widely known for many centuries and confirmed by recent studies (Ramarathnam et al. 1995; Sato and Miyata 2000; Yang and Landau 2000; McKay and Blumberg 2002; Wu and Wei 2002). Research showed that the undeniable benefits of tea drinking have been proved for consumers of the traditionally brewed tea by method called Chan-you, where powdered green tea leaves, are flooded with small portions of hot water, beaten to consistency of cream and consumed (Sadakata et al. 1992). Long time ago tea was prescribed for upset stomach, cold, or flu. Recent years have supported those potentials by the results of many different trials.

Tea therapeutic value is also a result of tea polyphenols activity in scavenging superoxide radicals. In effect of mentioned activity tea could be a helpful tool in preventing oxidative stress-related and other degenerative diseases (Halliwell et al. 1995; Unno et al. 2000). Oxygen is an indispensable component for living organism which undergoes through many transformations leading to reactive oxygen species ROS (superoxide anion radical ($O2^{\bullet-}$), hydroxylic radical ($^{\bullet}OH$), and hydroperoxide (H_2O_2) (Squadriato and Peyor 1998). Since human body possesses a very effective defensive system against oxidative stress induced by ROS there is no problem, but this system diminish with aging, leading to disturbances in red-ox balance (Osawa et al. 1995; Sato and Miyata 2000; Wu and Wei 2002). Results showed that antioxi-

Table 25.1 Tea *Camellia sinensis* constituents biological activity

Tea biological activity
Cancer chemoprevention (antioxidative activity, carcinogen trapping, inhibition of nitrosation reaction, inhibition of tumor biochemical signals)
Prevention of dental caries (inhibition of bacterial cells adherence and glucan synthesis)
Antibacterial and antiviral (e.g., *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*)
Anti-inflammatory and immunostimulatory agent
Antidiabetic (beneficial effect against lipid and glucose metabolism disorders)
Lowering the incidence of cardiovascular disease (hypocholesterolemic and antioxidant)
Deodorizing effect (food and human)
Improvement of neurological and psychological functions
Weight and obesity management
Remedy for diarrhea
Longevity

Combined from: Kim and Masuda (1997), Sato and Miyata (2000a, b), Wu and Wei (2002), Khan and Mukhtar (2007)

dative efficiency of a polyphenol depends on the degree of absorption and metabolism in living cells (Lonchampt et al. 1989).

Tea *Camellia sinensis* is very potent antibacterial and antiviral agent, fighting bacterial infections in humans and preventing food products from pathogenic bacteria contaminations. Most foodborne pathogenic bacteria consist of *Clostridium perfringens*, *Bacillus cereus*, *Escherichia coli*, or *Salmonella* could be destroyed by tea polyphenols (Okubo and Juneja 1997). Another potent attribute of tea polyphenols is possible anticarcinogenic action by inhibiting of mutagenicity and tumorigenicity (Kinlen et al. 1988; Oguni et al. 1988; Ahmad et al. 1997; Katiyar and Mukhtar 1997; Mukhtar and Ahmad 1999; Swiercz et al. 1999; Inoue et al. 2001; Smith and Dou 2001). Since nearly 30 % of cancer could be the result of incorrect nutritional habits, it is possible that suitable diet manipulation may be potent tool in cancer prevention (Weisburger 1996; Jankun et al. 1997; Fujiki et al. 1998); however, no clear anticarcinogenic mechanism had been found yet. Well proved are tea germicidal properties, allowing to decrease pathogens population (*Escherichia coli*) without influencing the *Lactobacillus* and *Bifidobacterium* existing in large intestine (Sakanaka et al. 1989; Okubo and Juneja 1997). Tea catechins inhibit RNA reverse transcriptase, playing an essential part in HIV virus replication (Nakane and Ono 1989; Gupta et al. 2002). Results of Middelton and coworkers (1998) showed that tea flavonoids inhibit release of some allergic reactions factors, such as leukotrienes and prostaglandins, by modifying enzymes activity. Tea polyphenols are also responsible for plasma cholesterol reduction and lipid lowering effect, hypoglycemic action, and hypotensive action. Results suggested that daily consumption of more than ten cups of green tea results in a decrease in blood cholesterol level (Imai and Nakachi 1995), and protection of low density lipoprotein (LDL) fraction against oxidation, through antiplatelet properties and by activation of prostaglandin synthesis (Acker et al. 1998; Hodgson et al. 1999; Sung et al. 2000).

Caffeine (trimethyl derivative of purine 2,6-diol), an alkaloid which in tea is called theine is proven to be cardiac stimulant, diuretic, stimulating the cerebral cortex and further inducing excitation in the central nervous system, accelerating toxic substances removal from organisms, irritating to the gastrointestinal tract and sleepless for certain people (Passmore et al. 1987; Chu and Juneja 1997; Woodward and Tunstall-Pedoe 1999; Gramza-Michałowska 2014). Research showed however that a reasonable caffeine consumption (four cups of tea leaves brewing daily) doesn't cause an increase in the incidence of heart disease (Myers 1991).

Research showed also the possible negative effect of drinking tea infusions. It was found that tea consumption resulted in the slowing down of iron absorption (Disler et al. 1975a, b) and increase of anemia (Merhav et al. 1985). Also the presence of condensed tannins inhibits trypsin and blocks proteins lowering total nutritious value of food (Los and Podsetek 2004). Looking through the results of negative impact of tea drinking on human health no data on its daily intake was found (Takahashi and Ninomiya 1997). Nevertheless in the era of supplements it is very important to measure possible toxic effect of consumed polyphenols. Summarizing it must be written that health effect of polyphenols is a result of many factors (Yang 1999), but polyphenols bioavailability and metabolism has not yet been explained.

25.3.1 Tea Polyphenols in Body Weight Regulation

Results of research showed possible effect of tea on overweight and obesity, which further leads to hypertension, coronary heart disease, non-insulin-dependent diabetes mellitus and certain forms of cancer (Stunkard 1996). It was shown that regular tea consumption (± four cups per day) helps weight controlling and loosing for obese people (Chen et al. 1998a, b; Han et al. 1999; Sato and Miyata 2000; Chantre and Lairon 2002; Wu et al. 2003; Nagao et al. 2005; Chan et al. 2006). Results of Shimotoyodome et al. (2005) showed that combined dietary green tea extracts consumption and regular exercise could stimulate fat reduction, and attenuate obesity induced by a high fat diet in mice. Research showed that tea components may promote body weight and fat loss by stimulating thermogenesis (Dulloo et al. 1999, 2000; Komatsu et al. 2003; Berube-Parent et al. 2005). The thermogenic effect of green tea was attributed to its caffeine and catechin content (Astrup et al. 1990; Dulloo et al. 2000). Research of Kao et al. (2000) showed that EGCG may interact specifically with a component of a leptin-independent appetite control pathway. Although tea components are regarded as active substances for body weight reduction, more well-designed and controlled clinical studies are still needed.

25.3.2 Tea Catechins Toxicity

Tea catechins as a part of tea beverage are consumed abundantly nowadays with no toxic influence. There is no problem when catechins are consumed naturally with the beverage, but its administration in pure form (capsules or tablets) as supplement is rather questionable. From experimental data it became certain that even huge amount of catechins should not harm human beings (Hara 2001a, b)

25.3.3 Bioavailability and Absorption of Tea Leaves' Components

After consumption tea catechins undergo many changes. Catechin is absorbed, methylated, and conjugated in the liver and excreted in the bile and urine (Hara 2001a, b). EGCG however, which is the most potent antioxidant in tea, after oral administration reaches stomach, small intestine, large intestine, and then it is removed with feces. This proves that the EGCG is not degraded in the stomach or small intestine.

It was proven that absorption and metabolism of polyphenols depends highly on its chemical structure (Price and Spitzer 1994; Hollman 2001). Polyphenols are absorbed from the digestive tract, penetrating the blood and binding with albumins, possibly masking their antioxidant activity. Further in stomach flavonoids undergo

hydrolysis to simpler constituents, than absorption and work as antioxidants (Arts et al. 2001, 2002). Flavonols and flavanols are metabolized mainly in the liver and large intestine (Takahashi and Ninomiya 1997; Manach et al. 1999; Rechner et al. 2002). Polyphenols however are not absorbed when bile is secreted into a small intestine and disintegrated by colon bacteria (Griffiths and Smith 1972). Research showed that some quantity of phenols remain in the body after consumption, suggesting that regular consumption of tea permits maintenance of high catechin levels (Nakagawa et al. 1997; Saganuma et al. 1998; Yang et al. 1998, Yang 1999).

25.4 Antioxidative Potential of Tea Compounds

Antioxidative activity of tea polyphenols is based on many mechanisms beginning from the radical scavenging, hydrogen donating, metal chelating, inhibiting enzymes activity, absorbing UV radiation, decomposing peroxides and nonradical products, or partial regenerating of primary antioxidants (Salah et al. 1995; Rice-Evans et al. 1997; Yanishlieva-Maslarowa 2001; Gramza et al. 2005a, b). Since oxidative reactions are very complexed, the potential of tea polyphenols depends on kind and number of substituents in the ring. Results showed that the hydroxylation degree and position is of basic meaning for polyphenols antioxidant activity (Fig. 25.3) (Dreosti 1996, 2000). One of the most active antioxidant compounds is EGCG, consisting of eight active free groups (OH). Very important for antiradical potential is presence of the o-dihydroxy structure in the B ring, which presence results in the higher stability of radical, that is participating in electron delocalization (Salah et al. 1995; Burda and Oleszek 2001; Gramza and Korczak 2005).

Recent developments in the area of tea bioactive compounds allowed to incorporate them into different food products. Probably the best protective efficiency was measured in lipids and lipid-containing products. Since the oxidation reactions are normal pathway in living organism it also happens in other components, crucial for human life and well-being. Best antioxidative potential was characterizing tea polyphenols, especially catechins.

Oxidative reactions are typical reactions not only for human body, but also for food components. Food industry manage that processes by using synthetic antioxidants like BHT, or BHA, but it became questionable to use it since possibly carcinogenic properties were found. New trends in food production assumed usage of natural, plant derived sources of such compounds. It was proved that tea posses many compounds with antioxidative potential, the only debatable is the direction of use since tea antioxidative compound act in dependence of the reaction environment. Many research showed antioxidant activity in delaying the oxidative changes in lard, linoleic acid, edible oils, and meat products (Hara 2001a, b).

Another very promising tea polyphenols potentials is the radical scavenging activity. Free radicals, molecules with one unpaired electrons are highly reactive and are inevitably generated in our body during the metabolism. Oxygen radicals like superoxide anion radical ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), or hydroxyl radical ($^{\bullet}OH$)

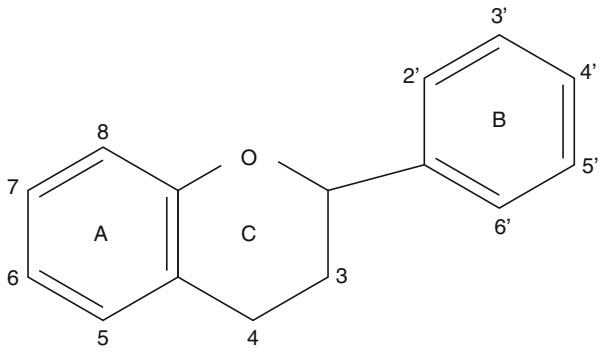

Fig. 25.3 Basic structure of flavonoids

are commonly produced both in human body, resulting in various diseases and aging, and in food products, resulting in deterioration and rancidity. According to antiradical potential of tea components its high potential was proven. Commonly used radicals scavenging activity methods like $DPPH^{\bullet}$, $ABTS^{+\bullet}$, ORAC, and PCL showed promising trends for further applications. It was also found that pure tea catechins were not as active as its crude or purified extracts (Gramza-Michalowska et al. 2007; Gramza-Michalowska 2008). The reason was probable synergistic or antagonistic interactions between extracts components.

25.4.1 Properties of Tea Polyphenols in Food

Addition of tea polyphenols to food products is mainly directed for inhibition of lipid oxidation processes that occurs. Lipids and lipid-containing products unfortunately for the consumers undergo oxidation, resulting in a sequence of unfavorable changes, like rancidity, color or texture changes, and nutritional value decrease (Gray 1978; Frankel 1998).

Many research showed that there is no antioxidant that is active in all food products, mainly because of activity in different food systems, affinity to different phases, processing stability, and agreement with legal standards (Houlihan and Ho 1985; Giese 1996; Gramza et al. 2007). Tea components do not show similar activity in different conditions. It was stated that tea constituents had been promising antioxidants in bulk oils than lipid emulsions (Salah et al. 1995; Huang and Frankel 1997; Roedig-Penman and Gordon 1997; Gordon and Roedig-Penman 1998; Amarowicz and Shahidi 1995; Samotyja et al. 2004; Gramza et al. 2007; Gramza-Michalowska et al. 2007). Its antioxidative activity was often comparable with activity of commercial antioxidants like BHT, TBHQ, or α-tocopherol (Wanasundara and Shahidi 1994, 1996; Chen et al. 1996; Koketsu and Satoh 1997; Gramza et al. 2006)

Also very high antioxidative potential was examined in food products. Tea polyphenols administration allowed to inhibit oxidation processes in chicken muscle (Tang et al., 2001, 2002), however no protection was found in frozen meat balls (Korczak et al. 2004). Other research showed inhibition of mutagenic compounds formation on tea extracts coated meat and afterwards cooked on the griddle (Weisburger et al. 2002).

25.5 Tea Commercial Products

Today's consumers no longer need to brew fresh tea; the market offers many different products, comparable with tea value (Gramza and Regula 2007). The most popular is Ice tea, produced widely from different kinds of tea, like green, black, or oolong. However the best potential market for that kind of products is in Japan, were tea addition is as common as citric acid in European drinks. Most popular is canned tea, which basic production scheme consists of tea leaves extraction and filtration, additives introduction (sugar, citric acid, flavors, milk, etc.), pasteurization, filling the container, seaming, cooling, the weight and seaming inspection, and package (Hara 2001a, b).

According to all tea activities in vitro and in vivo there is still further investigation needed, just to understand benefits and contributions of tea polyphenols to human life. However food products enrichment with tea leaf constituents could profitably influence its oxidative stability and improve life and health by additional incorporation into the human body.

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Chapter 26
Horchata de Chufa: A Traditional Spanish Beverage with Exceptional Organoleptic, Nutritive, and Functional Attributes

Eugenia Martín-Esparza and Chelo González-Martínez

26.1 Cultural and Economic Values

"Horchata de chufa" is a milky-like product obtained from dried tiger nuts (chufa) tubers (*Cyperus esculentus* L.). This nonalcoholic beverage is commonly produced in the east region of Spain, where a well-developed industry has emerged, which sold not only in Spain but also in Mexico, Panama, Dominican Republic, USA, and South France. It is of a great economic importance in Spain. According to the industrial production survey, 47,685 thousand liters of horchata was manufactured in 2005, representing a retail market value of some 27.3 million euros (INE 2006). Horchata consumption in Spain is about 50 million liters per year. This product presents a relevant consumer acceptance, who considers horchata as a refreshing and very pleasant beverage, mainly consumed during the summer months, with excellent sensorial characteristics, a characteristic flavor and a high energetic level (Varo-Galván et al. 1998). In addition, the high nutritional quality of horchata makes it even more attractive to consumers, which are nowadays very sensitive to the need of healthy foods intake.

Chufa cultivation and horchata production also presents economic and social values assimilated by its population through several centuries, being part of its culture. In fact, horchata de chufa is considered to be originated in Valencia (Espert et al. 1990). Actually most of tiger nuts and horchata produced in Valencia are typified, produced, and commercialized under the Designation of Origin "*chufa de Valencia*" rules (Regulating Council, RC), ensuring the homogeneity and optimum quality of such products. Because of the importance of this nonalcoholic beverage, the Spanish Government (B.O.E 1988, 1990, 1991) has defined the different kinds

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of horchata and has regulations on ingredients, additives, microbial tolerances, sanitary procedures, storage temperatures, and packaging (Mosquera et al. 1996). Nowadays (RC 2002), this product is commercialized as:

- Natural: fresh product non-heat treated with a minimum of 12°Brix at 20 °C, 2.2 % starch content, and 2.5 % fat content; minimum pH of 6.3; total sugar content (expressed as sucrose) higher than 10 %; starch and fats exclusively proceed from dry tubers.
- Natural pasteurized: it is the natural horchata thermally treated below 72 °C, without additive or processing aid addition. Composition and physicochemical and organoleptic characteristics must be the same as those obtained in the natural horchata.
- Pasteurized and sterilized: it is the horchata technologically processed in order to totally or partially eliminate or transform the starch content, and thermally treated to extend its shelf life.
- Concentrated (up to a minimum of 42°Brix at 20 °C) and with a minimum pH of 6; when reconstituted, organoleptic and microbiological characteristics must be those of the natural horchata.
- Condensed (up to a minimum of 60°Brix at 20 °C) and a minimum of 4.2 % starch content and 5.4 % fat content.
- Powdered (water content below 5 %): it is the horchata technologically processed in order to totally or partially eliminate or transform the starch content into particles or solid granules, and dried.

26.2 Nutritional and Functional Characteristics

Horchata is rich in carbohydrates (12.2 g/100 mL), being 9–10 g/100 mL sucrose and 2–3 g/100 mL starch. Fat content is around 2.4–3.1 % (w/v), mainly oleic acid (77 % of total fat, RC 2002) and linoleic acid (9 % of total fat), being this fatty acid profile similar to that of olive oil (Martínez-Valls 2003; Mosquera et al. 1996). Protein content oscillates between 0.6 and 1.4 % (w/v), it is highly digestible and arginine is the major amino acid, followed by glutamic acid and aspartic acid (RC 2002). With the exception of histidine, the essential amino acid contents in horchata are higher than the amount in the model protein proposed for adults by the FAO/OMS (Cortés et al. 2005). It is also remarkable the content on thiamine B1, niacin, and folic acid (CESNID 2002). Mineral content of horchata includes major elements as K, P, Mg, and Ca and minor elements as Fe and Zn.

As commented before, horchata has a great potential in the food market due to its compositional content, as it is considered as a high nutritional quality (Cortés et al. 2005). In addition to its nutritive value (as a source of carbohydrates, lipids, proteins, and several minerals and vitamins, as already described), some functional properties are attributed to this nonalcoholic beverage. The high content of oleic acid and vitamin E has a positive effect on cholesterol level, preventing

hypercholesterolemia, hypertriglyceridemia, and arteriosclerosis (Bixquert-Jiménez 2003). It has been also found to have digestible properties (it is antidiarrhea, antiflatulent, and diuretic) and to assist in reducing the risk of colon cancer as a consequence of its amino acid and starch contents (Bixquert-Jiménez 2003). The presence of arginine makes it suitable for diabetic and overweight people. It appears to be an ideal beverage for children, older people, and sportsman as it provides a high easily available energy supply (carbohydrates are mainly starch and sucrose).

The nutritional and sensory characteristics of horchata depend on many factors, including the chemical composition of the tuber (which is somehow influenced by harvest region) and the manufacturing process (Cortés et al. 2005).

26.3 The Manufacture Process

Traditionally, horchata was homemade and the producer directly sold the product to the consumer. During the last decades, small industries have emerged, in which natural horchata is daily produced and distributed under refrigerated conditions. The technological process is rather simple and consists basically on the washing, rehydration, grinding, water extraction, and sugar addition of the dried tiger nuts. A scheme is shown in Fig. 26.1.

After washing with potable water under stirring conditions to remove rests of sand and other impurities, damaged tiger nuts by insects or microorganisms are eliminated by flotation in a saline solution (salt concentration between 15 and 17°Baumé). Selected tiger nuts are again washed with potable water to remove the rests of brine from its surface before rehydration.

Rehydration of tiger nuts is industrially carried out by immersion on water at the temperature given by water local network (between 5 and 25 °C, depending on season) for a certain time (between 8 and 24 h) depending on tiger nut and water characteristics. It is an important step along horchata's line production as it reduces the surface rugosity as a result of swelling. This leads to a more effective disinfection (next step) by immersion in an active chlorine solution (1 % minimum concentration) with mechanical stirring during at least 30 min. Once again it is necessary to wash the tiger nuts to eliminate rests of germicide. Rehydrated nuts are then ground in a mill, where water is added to avoid caking and product retention. The obtained pulp is introduced in a press consisting of a cylindrical and perforated sieve where the walls are continuously swept by blades while the residue (pulp) remains in the internal surface. Water is at the same time added through showers. For industrial purposes, extraction process is optimal when a maximum fat content is attained as further reformulation (water and sugar addition) after extraction is required to achieve a certain fat concentration on the final product (sometimes up to 3.6 %). The extracted liquid is sweetened (sucrose addition) and finally cooled (0–2 °C). This temperature must remain during distribution and selling of the final product. The obtained product, named natural horchata, is manufactured daily for its consumption due to its high microbial load (above the million cell counts per mL)

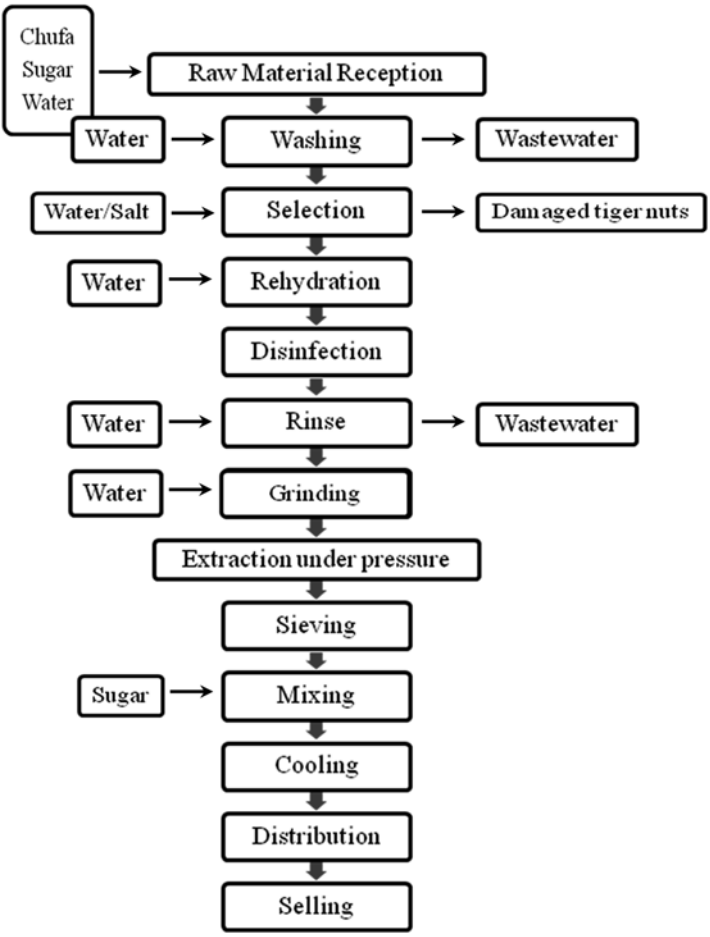

Fig. 26.1 Natural horchata (nonthermally treated) manufacturing steps

(Espert et al. 1990). Thermal treatments (pasteurization, UHT, sterilization) are normally used in the industry to extend its shelf life and these products are presented in the markets as pasteurized or sterilized horchata.

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