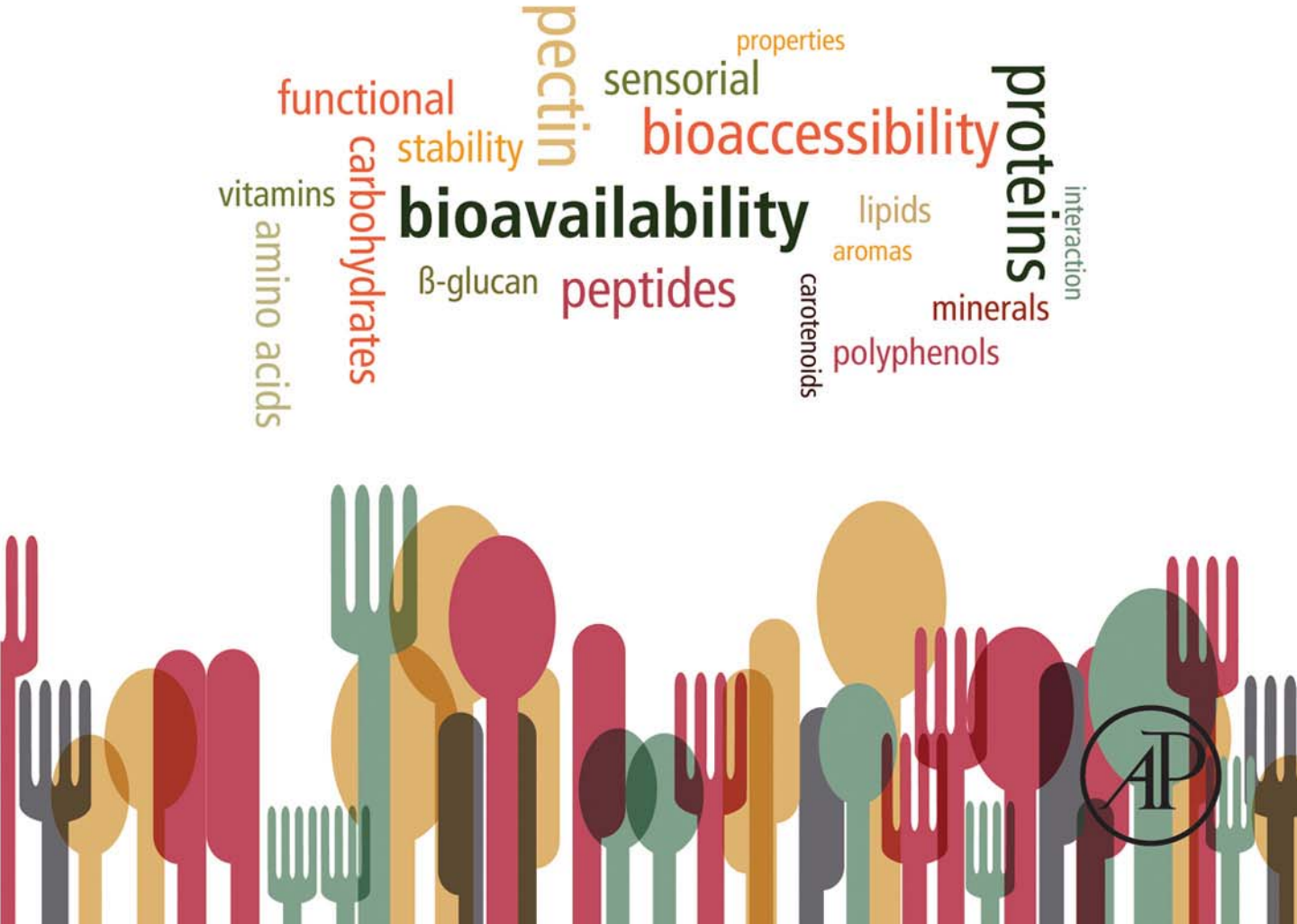
Nutraceutical and Functional Food Components

Effects of Innovative Processing Techniques

Edited by
Charis M. Galanakis



Nutraceutical and Functional Food Components

This page intentionally left blank

Nutraceutical and Functional Food Components

Effects of Innovative Processing Techniques

Edited by

Charis M. Galanakis

Galanakis Laboratories, Chania, Greece

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1800, San Diego, CA 92101-4495, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-805257-0

Publisher: Nikki Levy
Acquisition Editor: Megan Ball
Editorial Project Manager: Jaclyn Truesdell
Production Project Manager: Nicky Carter
Designer: Mark Rogers

Typeset by MPS Limited, Chennai, India

Contents

This page intentionally left blank

List of Contributors

Milica Atanacković Krstonošić
University of Novi Sad, Novi Sad, Serbia

Francisco J. Barba
Universitat de València, Burjassot, Valencia, Spain

Mira Bursać
University of Novi Sad, Novi Sad, Serbia

Silvina Rosa Drago
UNL, Santa Fe, Ciudad Autónoma de Buenos Aires, Argentina; Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

Eliana Noemi Fissore
Buenos Aires University (UBA), Buenos Aires, Argentina; National Research Council of Argentina (CONICET), Buenos Aires, Argentina

Charis M. Galanakis
Galanakis Laboratories, Chania, Greece

Cristina García-Viguera
CEBAS-CSIC, Murcia, Spain

Lia Noemi Gerschenson
Buenos Aires University (UBA), Buenos Aires, Argentina; National Research Council of Argentina (CONICET), Buenos Aires, Argentina

Amadeo Gironés-Vilaplana
University Miguel Hernández, Orihuela, Alicante, Spain

Jelena Hogervorst Cvejić
University of Novi Sad, Novi Sad, Serbia

Seyed Vali Hosseini
University of Tehran, Karaj, Iran

Raffaella Inchingolo
Alma Mater Studiorum-Università di Bologna, Bologna, Italy

Anet Režek Jambrak
University of Zagreb, Zagreb, Croatia

Nagendra Prasad Krishnamurthy
Monash University Malaysia, Selangor, Malaysia

Javier Marhuenda
Universidad Católica San Antonio de Murcia (UCAM), Murcia, Spain

Uroš Miljić
University of Novi Sad, Novi Sad, Serbia

Silvia A. Moreira
Universidade de Aveiro, Aveiro, Portugal

Diego A. Moreno
CEBAS-CSIC, Murcia, Spain

Jayesree Nagarajan
Monash University Malaysia, Selangor, Malaysia

Rui P. Queirós
Universidade de Aveiro, Aveiro, Portugal

Mavinakere Eshwaraiah Raghunandan
Monash University Malaysia, Selangor, Malaysia

Ramakrishnan Nagasundara Ramanan
Monash University Malaysia, Selangor, Malaysia

Maria Teresa Rodriguez-Estrada
Alma Mater Studiorum-Università di Bologna, Cesena, Italy; Alma Mater Studiorum-Università di Bologna, Bologna, Italy

Ana Maria Rojas
Buenos Aires University (UBA), Buenos Aires, Argentina; National Research Council of Argentina (CONICET), Buenos Aires, Argentina

Mauro D. Santos
Universidade de Aveiro, Aveiro, Portugal

Jorge A. Saraiva
Universidade de Aveiro, Aveiro, Portugal

Dubravka Škevin
University of Zagreb, Zagreb, Croatia

Reza Tahergorabi
North Carolina Agricultural & Technical State University, Greensboro, NC, United States

Urszula Tylewicz
Alma Mater Studiorum-Università di Bologna, Cesena, Italy

Débora Villaño
Universidad Católica San Antonio de Murcia (UCAM), Murcia, Spain

Zhenzhou Zhu
Wuhan Polytechnic University, Wuhan, China

Preface

Foods contain major and minor components as well as bioactive compounds that are of primary importance for human nutrition. The importance of these compounds accelerated the development of innovations in the food industry, generating the so-called *functional foods* and *nutraceuticals*. Whole foods like fruits and vegetables represent the simplest example of functional foods, as they are rich in bioactive compounds and have a well-established protective role against the development of diseases. Nutraceuticals represent any substance that provides medical or health benefits, including the prevention and treatment of diseases. Contrarily to functional foods, nutraceuticals are commodities derived from foods used in the medicinal form of pills or capsules. The preparation of foods fortified with functional components requires integration of diverse aspects under evaluation. These include, for instance, separation techniques, toxicological assessments, and stability and activity tests. On the other hand, processing has an impact on the final food products. Applied technologies may influence the content and effectiveness of nutrients, e.g., loss of bioactive compounds or diminution of their functionality typically increases more and more as foods are processed, stored, and transported.

Novel, nonthermal technologies (e.g., ultrasounds, high-hydrostatic pressure, pulsed electric field, high voltage electrical discharge, cold plasma) promise to treat foods without destroying their nutritional components and sensorial characteristics, which are normally affected during heat treatment. The latest techniques are today applied in both research institutes and food industries, promising to shorten processing times, control Maillard reactions, improve product quality, and enhance functionality. The implementation of these technologies together with other trends and practices of the food industry (e.g., nanoencapsulation, food waste recovery, emerging need for innovations, etc.) have brought new developments, data, and state-of-the-art in the field. Indeed, this renaissance has changed the way food components are incorporated inside foods and thus consumed. As a result, food technologists that deal with the development of functional foods and nutraceuticals must consider:

1. the effect of thermal and nonthermal processing technologies on food components in spite of their functional properties and preservation ability;
2. the available and optimized extraction and formulation processes; and
3. the innovative and sustainable applications in foods.

Thus there is a need for a resource that covers the latest developments in the food industry and their trends and practices therein. This text hopes to fill in this gap by highlighting the impact of recent food industry advances on different parameters of food components (e.g., nutritional value, physical and chemical properties, bioavailability and bioaccessibility characteristics) and the final products (e.g., applications, shelf-life during storage, sensory characteristics).

The book consists of 10 chapters. Chapter 1, Introduction, provides a state-of-the-art in nutrition, prior to discussing the current trends of the food industry in relation to functional foods and nutraceuticals development. Detailed definitions of *bioavailability*, *bioaccessibility*, and *bioactivity*, and the factors affecting them are provided in order to understand better these key functions. Chapter 2, Proteins, Peptides, and Amino Acids, discusses respective food compounds as well as their modifications during processing with emerging technologies. Chapter 3, Carbohydrates,

discusses the respective effect of innovative technologies on carbohydrate properties, giving special attention to compounds with a chain length up to nine carbon atoms, inulin, starch, and dietary fibers such as pectin and β-glucan. Chapter 4, Lipids, deals with the impact of nonthermal technologies on the bioaccessibility of lipids and their stability against oxidation. Similarly, Chapter 5, Minerals, focuses on particularly iron, zinc, and calcium which typically show low bioavailability in consumption. Methodologies to estimate in vivo and in vitro bioaccessibility of minerals as well as to measure bioavailability in humans are also discussed. The implementation of emerging technologies to improve the stability and bioaccesibility of vitamins, polyphenols, and carotenoids in foods through their metabolism and health-promoting activities are described in Chapter 6, Vitamins, Chapter 7, Polyphenols, and Chapter 8, Carotenoids, respectively. Innovative extraction techniques for the recovery of these bioactive compounds from food sources and by-products as well as their effects on functional properties are also highlighted. Chapter 9, Food Aroma Compounds, provides an overview of the main natural and technology-derived food aroma compounds, with a critical focus on the novel extraction methods, delivery strategies, and the effects of innovative processing technologies on the acceleration of Maillard reactions. Finally, Chapter 10, Interaction of Compounds, deals with the interactions of food compounds (as discussed in previous chapters) induced by the application of nonthermal technologies.

This book is intended to support food scientists, technologists, engineers, chemists, and professionals working in the food science field as well as researchers and product developers dealing with food processing and innovative applications. It could be used as textbook and/or ancillary reading in graduate- and postgraduate-level courses on food science and related technologies.

I would like to take this opportunity to thank all the authors and contributors of this book for their high-quality work in bringing together theoretical and technical issues in an integral and comprehensive text. I consider myself fortunate to have had the opportunity to collaborate fruitfully with so many knowledgeable colleagues from Argentina, China, Croatia, India, Iran, Italy, Malaysia, Portugal, Serbia, Spain, and the United States. Their acceptance of the editorial guidelines and their dedication to the book's concept is highly appreciated. I would also like to thank the acquisition editor Megan Ball for our collaboration on this project and all of the Elsevier team, particularly Jacklyn Truesdell and Karen Miller for their assistance during editing and Nicky Carter during the production process.

Finally yet importantly, a message for you, the reader. In a collaborative project of this size, it is impossible for it not to contain errors. Thus if you find errors or have any objections to its content, I would really appreciate it if you would contact me.

Charis M. Galanakis
Galanakis Laboratories, Chania, Greece
ISEKI Food Association, Vienna, Austria

CHAPTER

INTRODUCTION 1

Charis M. Galanakis
Galanakis Laboratories, Chania, Greece

1.1 STATE-OF-THE-ART IN NUTRITION

Food manufacture is currently attracting significant scientific and public interest due to extensive media coverage of diet-related diseases and their influence on the health and well-being of communities (Day, Seymour, Pitts, Konczak, & Lundin, 2009). As a result, more and more consumers believe that foods contribute directly to their health (Mollet & Rowland, 2002). According to the World Health Organization and the Food and Agriculture Organization, certain dietary patterns along with lifestyle habits constitute major risk factors in relation to the development of chronic diseases (WHO, 2003). A major problem facing affluent societies and the rest of the world is reduced activity and lack of exercise, which can lead to obesity and the so-called "metabolic syndrome" (Moebus & Stang, 2007). Changes in eating habits, consumption of fast foods, and environmental factors can also adversely affect the health of humans around the world (Shahidi, 2009).

This approach has led to increased consumer demand for healthy and nutritious foods with not only balanced calorific content, but also with additional health-promoting functions (Bech-Larsen & Scholderer, 2007; Hasler, 2002). To date, the primary concern of the food industry has been to provide consumers with safe food. However, while safety is still of paramount importance the nutritional and caloric composition of foods is becoming equally concerning (Day et al., 2009). Foods are now intended to prevent nutrition-related diseases as well as to improve physical and mental well-being (Betoret, Betoret, Vidal, & Fito, 2011). Governments and consumers worldwide endorse this trend by accepting that high-quality healthy products that are also convenient need to be developed through innovative multidisciplinary research programs (Day et al., 2009).

1.2 FUNCTIONAL FOODS AND NUTRACEUTICALS

Foods contain major and minor components as well as bioactive compounds (e.g., antioxidants, peptides, carbohydrates, lipids, and glucosinolates) that are important for human nutrition. Consequently, their importance has initiated a surge of research and product development in the food industry. In order to adapt to these consumer drivers and enhance the physiological functionality of inherent nutrients, the food industry is developing so-called "functional foods" (Day et al., 2009),

Nutraceutical and Functional Food Components. DOI: http://dx.doi.org/10.1016/B978-0-12-805257-0.00001-6

a term that was first used in Japan. Indeed, the Japanese were the first to observe that food could play a role beyond gastronomic pleasure and nutrient supply to the human organism. Japan was also the first country to legislate these products in the FOSHU (Foods of Specified Health Use) legislation, and it has the highest number of functional foods on the market. Europe and the Americas later incorporated this concept (López-Varela, González-Gross, & Marcos, 2002). The American Dietetic Association (ADA) has classified all food as functional at some physiological level, pointing out that "the term functional food should not be used to imply that there are good and bad foods." In addition, it states that "all food can be incorporated into a healthful eating plan—the key being moderation and variety" (ADA, 2004). Whole foods like fruits and vegetables represent the simplest example of functional foods since they are rich in bioactive compounds, which protect the body's cells against oxidative damage and reduce the risk of developing certain cancers (Day et al., 2009). It is important to note that functional food must be a food (not a drug), and beneficial effects should be obtained by consuming it in normal amounts within the regular diet.

The lack of consensus between Europe and the United States for concrete definitions has led to the use of different terms, increasing confusion among professionals and consumers. In general, the United States prefers the term "nutraceutical" (López-Varela et al., 2002), which refers to any substance, food, or part of a food that provides medical or health benefits, including the prevention and treatment of diseases (Kaur & Das, 2011). However, in contrast to functional foods, nutraceuticals are commodities derived from foods used in the medicinal form of pills, capsules, potions, and liquids and again render demonstrated physiological benefits. The term nutraceutical has now been grouped together with herbal and other natural health products (Shahidi, 2009).

The average consumer prefers natural products over chemical versions since people want to eat food with the desired health benefits rather than take medicine separately (Betoret et al., 2011). The increasing demand for functional foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for improved quality of life (Roberfroid, 2007). In many cases, it is believed that certain unprocessed or minimally processed foods have better health benefits than their processed counterparts. However, this assumption may not hold when considering particular phytochemicals, e.g., lycopene in tomato (Shahidi, 2009).

Food components with functional properties are extracted and used as additives in foodstuff due to their ability to provide both advanced technological properties and health claims to the final product (Galanakis, Markouli, & Gekas, 2013). Epidemiological studies have shown that health benefits (e.g., reduced risk of coronary heart disease and stroke, diabetes, obesity, and cancer) may be attributed to the consumption of both macro- and micronutrients. For instance, macromolecules like soluble dietary fiber is known for its ability to lower blood lipid level and at the same time shows advanced gelling properties. Therefore it can be used to replace fat in foods, stabilize emulsions, and improve the shelf-life of food products (Elleuch et al., 2011; Galanakis, 2011, 2015; Galanakis, Tornberg, & Gekas, 2010c; Patsioura, Galanakis, & Gekas, 2011; Rodríguez, Jiménez, Fernández-Bolaños, Guillén, & Heredia, 2006). Proteins have also been used as fat replacements in milk products, flavor enhancers in confectionaries, and as food and beverage stabilizers (Galanakis, Chasiotis, Botsaris, & Gekas, 2014; Kristinsson & Rasco, 2000; Pogaku, Seng, Boonbeng, & Kallu, et al., 2007; Prakash, 1996).

Natural antioxidants typically include smaller compounds (e.g., polyphenols, carotenoids, tocopherols, and ascorbic acid) that have been connected to both nutritional (reduction of oxidative stress, prevention of cancer, arteriosclerosis, aging processes) and functional (preservative of

vegetable oils and emulsions) properties (Galanakis, 2015; Galanakis, Kotanidis, Dianellou, & Gekas, 2015; Moure et al., 2001). Other compounds of interest include glucosinolates and its derived forms (isothiocyanates), which are potent antimicrobials and have been associated with important health benefits (e.g., the reduction of degenerative diseases like cancers of the lungs and alimentary tract). Additionally, some of them can also be used as unique flavorings (e.g., in mustards) (Deng et al., 2015).

In the past few years, new products based on fruit or vegetable and milk have been appeared in Europe and North American markets. These products have wider consumer acceptance and higher nutritional value, largely due to their higher bioactive compound content and antioxidant capacity (Andlauer & Furst, 2002; Heckman, Sherry, & de Mejia, 2010). However, the design and development of functional foods should not only be carried out based on the desired nutritional function. The appearance and sensory properties of foods are also important attributes to the consumer, thus the color, texture, taste, and mouth feel should also be taken into account (Day et al., 2009). From a manufacturing point of view, the most popular functional food product format is beverages since they are relatively easy to formulate. In the case of soft solid foods, the structure-derived quality aspects (e.g., stability, texture, and taste) are of high importance for consumer acceptance of foods as well as for the bioavailability of micronutrients (Parada & Aguilera, 2007). Food manufacturers face a series of technical challenges during fortification of foods with bioactive compounds. For instance, processes should be selected carefully to maintain both functionality of bioactive compounds as well as the quality and sensory attributes of the food (Day et al., 2009).

1.3 BIOAVAILABILITY, BIOACCESSIBILITY, AND BIOACTIVITY OF FOOD COMPONENTS

The preparation of foods fortified with functional components requires integration of diverse aspects under evaluation. These include selecting the appropriate source, detecting the bioactive compounds, applying separation and recovery techniques, performing toxicological assessments, and finally taking stability, activity, and bioaccessibility measurements (Korhonen, 2002). At this point, it is important to carefully define the terms "bioavailability," "bioaccessibility," and "bioactivity" (Fig. 1.1), which are often used indistinctly to express similar functions.

1.3.1 BIOAVAILABILITY

Overall, bioavailability includes gastrointestinal (GI) digestion, absorption, metabolism, tissue distribution, and bioactivity. However, the term has several meanings depending on the research area it is used in. For instance, from a pharmacological point of view, bioavailability is the rate and extent to which the therapeutic moiety is absorbed and becomes available at the drug action site (Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009). From a nutritional point of view (which is of particular interest in the current book), bioavailability refers to the fraction of the nutrient that is stored or is available in physiological functions (Fairweather-Tait, 1993). It is a key term for nutritional effectiveness, as not all the amounts of bioactive compounds are used effectively by the organism (Blenford, 1995). For example, when different foods come into contact with the mouth or digestive tract, various interactions may take place affecting phytochemical bioavailability

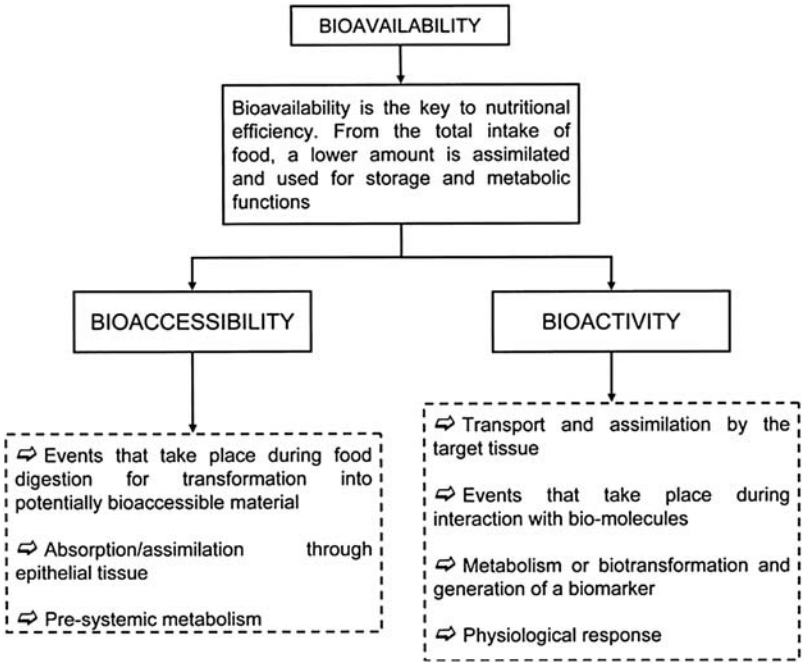


FIGURE 1.1

Definitions of bioavailability, bioaccessibility, and bioactivity. Physiochemical events involved at each stage.

Adapted from Fernández-García, E., Carvajal-Lérida, I., & Pérez-Gálvez, A. (2009). In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. Nutrition Research, 29, 751–760.

(e.g., fat enhances quercetine bioavailability in meals) (Lesser, Cermak, & Wolffram, 2006). Therefore bioavailability expresses the fraction of ingested nutrient or bioactive compound that reaches the systemic circulation and is ultimately utilized (Wood, 2005).

Bioavailability is important in all different definitions of functional foods, e.g., when a claim for nutritional and/or health properties is made. First, the nutrient or component that provides this benefit should be efficiently digested and assimilated. Thereafter, once absorbed, it must perform a positive function in the body (Fernández-García et al., 2009). However, there are practical and ethical difficulties when measuring the bioactivity of food components on specific organ sites. In these cases, the term "bioactivity" is not used and bioavailability is defined as the fraction of an oral dose of an active metabolite that reaches the systemic circulation (Schumann et al., 1997). Subsequently, bioavailability is determined using in vivo experiments as the area under the plasma concentration of the compound obtained after administration of an acute or chronic dose (Rein et al., 2013).

1.3.2 BIOACCESSIBILITY

Before becoming bioavailable, bioactive compounds must be released from the food matrix and modified in the GI tract. Thus bioavailability includes the term bioaccessibility (Fernández-García

et al., 2009). Indeed, it is important to analyze whether the digestion process affects bioactive compounds and their stability before concluding on any potential health effect (Carbonell-Capella, Buniowska, Barba, Esteve, & Frigola, 2014).

Bioaccessibility is defined as the quantity of a compound that is released from its matrix into the GI tract, becoming available for absorption (e.g., enters the bloodstream) (Benito & Miller, 1998; Heaney, 2001). This term includes digestive transformations of foods into material ready for assimilation, the absorption/assimilation into intestinal epithelium cells as well as the presystemic, intestinal, and hepatic metabolism. However, beneficial effects of unabsorbed nutrients such as calcium binding of bile salts in the tract are missed by definitions based on absorption (Carbonell-Capella et al., 2014). Bioaccessibility is usually evaluated by in vitro digestion procedures, generally simulating gastric and small intestinal digestion, sometimes followed by Caco-2 cell uptake (Courraud, Berger, Cristol, & Avallone, 2013).

Bioaccessibility has not been a priority goal thus far during initial development of functional foods for two reasons. First, the current experimental models do not allow us to distinguish bioavailability effectiveness from bioaccessibility and assimilation. Secondly, there is no consensus among US and European legislations in spite of the requirement to integrate this parameter.

Bioaccessibility is directly influenced by the composition of the food matrix and by the synergies and antagonisms that may be established between the different components, permitting a potentially digested material to be available to the body (Fernández-García et al., 2009).

1.3.3 BIOACTIVITY

Bioactivity is the specific effect upon exposure to a substance. It includes tissue uptake and the consequent physiological response (e.g., antioxidant, antiinflammatory). It also includes information on how the bioactive compounds are transported and reach the target tissue, how they interact with biomolecules, metabolism, and biotransformation characteristics, as well as the biomarker generation and consequent physiological responses (Fig. 1.1) (Fernández-García et al., 2009). Digestibility applies specifically to the fraction of food components that is transformed into potentially accessible matter through all physical and chemical processes that take place in the lumen (Carbonell-Capella et al., 2014). On the other hand, assimilation refers to the uptake of bioaccessible material through the epithelium by some mechanism of transepithelial absorption (Etcheverry, Grusak, & Fleige, 2012). One example where only bioactivity applies concerns nondigestible polysaccharides, oligosaccharides, and dietary fiber. These compounds produce several health benefits, although they are not absorbed (Roberfroid, 2002).

Bioactivity measurements (in vivo, ex vivo, and in vitro) are based on the events that take place during the time the bioactive component interacts with biomolecules. This interaction generates a metabolite, a signal, or a response that will continue to modulate and amplify until the systemic physiologic response is produced (health benefit). The experimental procedures used to measure bioactivity need to be adjusted to every health benefit claim separately (Vaisberg, Lenzi, Hansen, Keon, & Finer, 2006). The scientific support of claims of what a food can do (healthy properties or reduced risk of disease) is based on bioactivity data. Claims of a food's nutritional content are provided by bioaccessibility without the need for performing bioactivity studies (Fernández-García et al., 2009).

1.3.4 BIOACTIVE COMPOUNDS

Bioactive compounds are phytochemicals found in foods that are capable of modulating metabolic processes and resulting in the promotion of better health. They exhibit beneficial effects such as antioxidant activity, inhibition or induction of enzymes, inhibition of receptor activities, and induction and inhibition of gene expression (Correia, Borges, Medeiros, & Genovese, 2012). The bioaccessibility and bioavailability of each bioactive compound differs greatly, and the most abundant compounds in ingested fruit are not necessarily those leading to the highest concentrations of active metabolites in target tissues (Manach, Williamson, Morand, Scalbert, & Remesy, 2005). Indeed, when studying the role of bioactive compounds in human health, bioavailability is not always well known (Carbonell-Capella et al., 2014).

Bioactive compounds are found in fruits, vegetables, and whole grains (Carbonell-Capella, Barba, Esteve, & Frigola, 2013; Gil-Chávez et al., 2013). They include an extremely heterogeneous class of compounds (polyphenolic compounds, carotenoids, tocopherols, phytosterols, and organosulfur compounds) with different chemical structures (hydrophilic or lipophilic), distribution in nature (specific to vegetable species or ubiquitous), range of concentrations both in foods and in the human body, possible site of action, effectiveness against oxidative species, and specificity and biological action (Carbonell-Capella et al., 2014; Porrini & Riso, 2008). Several factors interfere with the bioavailability of antioxidants, e.g., the food source or the chemical interactions among the phytochemicals and biomolecules present (Parada & Aguilera, 2007). For instance, fruit antioxidants are commonly mixed with different macromolecules such as carbohydrates, lipids, and proteins to form the food matrix. In plant tissue, carbohydrates are the major compounds found, mainly in free and conjugated forms (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004).

1.3.5 FACTORS AFFECTING THE BIOACCESSIBILITY AND BIOAVAILABILITY OF BIOACTIVE COMPOUNDS

After consumption, the nutrients that are present in a food or drink are released, absorbed into the bloodstream, and transported to their target tissues. Different nutrients differ in their bioavailability, which means that they are not utilized to the same extent. Release of the nutrient from the food matrix, effects of digestive enzymes in the intestine, binding and uptake by the intestinal mucosa, transfer across the gut wall to the blood or lymphatic circulation, systemic distribution and deposition, metabolic and functional use, and excretion can affect nutrient bioavailability. The latter is mediated by external (e.g., characteristics of the food matrix, chemical form of the nutrient) and consumer internal (e.g., gender, age, nutrient status, and life stage) factors. The bioavailability of macronutrients (carbohydrates, proteins, and fats) is usually very high, e.g., more than 90% of the amount ingested.

Bioaccessibility is the first step in making a nutrient bioavailable. In this step, the nutrient is liberated from the food matrix and turned into a chemical form that can bind to and enter the gut cells or pass between them. Chewing, enzymatic digestion of the food in the mouth, mixing with acid and enzymes in the gastric juice, and release into the small intestine are the unit operations of the process by which the nutrients are rendered bioaccessible. The small intestine is the major site of nutrient absorption. Enzymes of the pancreatic juice continue breaking down the food matrix. Certain procedures involved in food preparation like cooking, chopping, or pureeing collaborate

with mastication and enzymes to the digestibility of food matrices (EUFIC-The European Food Information Council, 2010).

The oral bioavailability of bioactive compounds is drastically affected by the restricted release of compounds from plant matrix, the solubility in GI fluid, the permeability across intestinal epithelial cells, as well as the enzymatic and chemical reactions occurring within the GI tract (McClements & Xiao, 2014). Four essential steps are necessary for the effective absorption of bioactive compounds:

1. release from food matrix,
2. incorporation into bile-salt micelles,
3. absorption by epithelial cells, and finally
4. incorporation into the chyclomicrons with secretion into lymphatic system.

1.4 THE TREND OF EMERGING TECHNOLOGIES IN FOOD PROCESSING

Food processing has an impact on the chemical constituents as well as on the physical and sensory properties of the final product. Applied technologies may influence the content of bioactive compounds leading to changes in their functional properties (e.g., bioavailability, bioaccessibility, and bioactivity) and potential health benefits. Indeed, loss of phytonutrient foods becomes more and more significant as foods are processed, stored, and transported. Therefore attention should be given to the degree of disintegration of the initial tissue structure because of its impact on food quality, functionality, and deterioration. As the demand for functional food increases, intense research efforts for the development of new processing technologies is conducted with the ultimate goal of ensuring maximal nutritional and functional properties, as well as of improving the overall quality of a product.

Conventional food processing includes the application of technologies such as wet milling, mechanical pressing, and microfiltration that are used for the separation of solids, fats, and/or water. Other separation techniques such as alcohol precipitation, isoelectric solubilization, and ultrafiltration are able to remove macromolecules (e.g., proteins, pectin), whereas typical extraction techniques (e.g., using solvents or supercritical fluids) are used to recover small bioactive compounds like antioxidants or sugars (Galanakis, 2012, Galanakis, Goulas, Tsakona, Manganaris, & Gekas, 2013; Heng et al., 2015; Tsakona, Galanakis, & Gekas, 2012). Together with thermal technologies (e.g., pasteurization or spray drying), these techniques are often used for the fortification of foods with bioactive compounds and the preparation of functional foods. Conventional processing technologies are well documented and established, but they have shown several problems that often restrict their implementation in practice.

For instance, thermal treatments (e.g., boiling, cooking, blanching, frying, and sterilization) have been widely used to soften the plant cell wall, while inactivating the microorganism and enzyme activity that reduces the shelf-life of the food products. Additionally, many of the quality changes that occur during food storage are due to detrimental reactions catalyzed by enzymes, such as peroxidase, polyphenol oxidase, and pectin methylesterase. However, heat stress induces isomerization, oxidation, and degradation while inactivating the microorganism activity, which reduces the shelf-life of the food products. In addition, heating causes loss of functionality and compounds

with organoleptic properties due to overheating of the food matrix (Galanakis, Tornberg, & Gekas, 2010a, 2010b, 2010d, 2010e;Mujumdar & Law, 2010). Consequently, the entire stability and bioavailability of bioactive compounds is affected (Martinez-Antonio, Brahm, Gonzalez, & Stinco, 2015).

Membrane processes (e.g., nanofiltration) require increased energy consumption (Galanakis, Fountoulis, & Gekas, 2012), while others (e.g., freeze drying) have high operational costs. Additional problems like the generation of unstable products (that are difficult to preserve on the shelf) may arise during encapsulation, and extraction solvents are not always "food friendly," raising public awareness with regard to their safe utilization inside food chain.

These limitations have been a driving force for food processors to seek alternative processing technologies to preserve better bioactive compounds. In addition, consumer demands for high-quality foods with "fresh-like" characteristics, which require only a minimum amount of effort and time for preparation, has led to the introduction of convenience foods preserved by mild treatments. Novel techniques are based on volumetric forms of heating since the thermal energy is generated directly inside the food, allowing shortening of heating times and an increase in energetic and heating efficiency (Pereira & Vicente, 2010). The so-called emerging technologies can preserve foods without destroying the nutritional components and sensorial characteristics that are normally affected during heat treatment. Today the latest techniques applied in both research institutes and food industries (Deng et al., 2015; Galanakis, 2012, 2013; Galanakis, Barba, & Prasad, 2015; Galanakis & Schieber, 2014; Roselló-Soto et al., 2015a,b; Zinoviadou et al., 2015) include:

- shortening of processing and residence times,
- accelerated heat and mass transfer,
- control of Maillard reactions,
- improvement of product quality,
- enhancement of functionality,
- protection from environmental stresses, and
- extended preservation.

The 10 most popular emerging technologies examined in the broad field of food science are (Galanakis, 2013):

1. radio-frequency drying,
2. electro-osmotic dewatering,
3. cold plasma treatment,
4. high-hydrostatic pressure,
5. ultrasound-assisted extraction,
6. laser ablation,
7. high voltage electrical discharge,
8. pulsed electric field,
9. pulsed fluid bed agglomeration, and
10. nanoencapsulation.

Emerging technologies are classified as nonthermal since they avoid the use of high temperatures, in contrast to thermal processing technologies. However, they may involve heat as a result of internal energy generation (e.g., adiabatic and resistive heating during high hydrostatic pressure and

pulsed electric fields, respectively). However, this kind of treatment avoids the harmful effects of heat on the functional, nutritive, and sensory value of foods.

Radio-frequency drying is an alternative thermal technology that optimizes heat transfer, unlike classical drying with hot air. Indeed, the material is heated uniformly while the water evaporates in situ at relatively low temperatures ($<80^{\circ}C$), allowing reduction of processing time. Its main drawback is low energy efficiency (Pereira & Vicente, 2010). Electro-osmotic dewatering is another drying technique that promises the reduction of energy consumption up to two-thirds compared to traditional thermal processes. This method acts by enhancing mechanical pressure with electrochemical double layers formed at the particle−water interface of colloidal aqueous suspensions (Galanakis, 2013).

Cold (or low-temperature) plasma can be described as quasineutral particle systems of semigas and semifluid mixtures of highly energetic particles (free electrons, ions, and molecules). It is produced by a variety of electric discharges at different pressure levels since vacuum enhances conversion of liquid to gaseous phase in high-moisture food products. Cold plasma has been mainly utilized for the microbial deactivation in foods (Galanakis, 2013; Knorr et al., 2011).

Enzyme deactivation can also be conducted with high-pressure processing (e.g., up to 200 MPa), which damages membranes of microorganisms without affecting the quality of the food in terms of texture, flavor, and color (Martinez-Antonio et al., 2015). High-pressure processing has also been proposed for the modification of whey protein hydrophobicity and the solubilization of bioactive compounds, as it improves the mass transfer rate of components by increasing the plant-cell permeability, allowing diffusion in phase transition (Oroian & Escriche, 2015). Additionally, ultrasound has been shown to be effective against contaminating microorganisms of liquid foods (e.g., orange juice and guava juice) and can satisfy the requirement of a 5-log reduction of some contaminant pathogens (such as *Escherichia coli* in fruit juices) established by the US Food and Drug Administration (Zinoviadou et al., 2015). Ultrasounds have been shown to reduce the in vitro bioaccessibility of lycopene during processing of tomato pulp, due to its partial deesterification and release of pectin molecules (Anese et al., 2015).

Other technologies based on accelerated mass transfer include laser ablation and high-voltage electric discharge. The latter is a technique used for the extraction of nutraceuticals from grape seeds (Liu, Vorobiev, Savoire, & Lanoiselle, 2011). In this case, liquid substrates (e.g., extracts) are placed in a chamber between two electrodes that cause particles fragmentation by providing short pulses, i.e., 40−60 kV/cm, 2−5 μs. Pulsed electric field is another processing technology that allows preservation of fresh-like quality and nutrients in different food products (e.g., juices, milk, and liquid eggs). It enhances the mass transfer rate by reducing the cell-membrane integrity and softening plant tissues, thus influencing the texture and electroporation of the plants as well as enhancing high added-value components extraction from different fruit and vegetable tissues (Oroian & Escriche, 2015; Vorobiev & Lebovka, 2008). Pulsed electric fields have also been used for the encapsulation of nutraceuticals by intensifying fluid-bed agglomeration of instant soy protein isolate (Dacanal & Menegalli, 2010).

Modern encapsulation is typically conducted with nanotechnology and presents an effective way to deliver nanosized or nanoencapsulated nutrients and bioactive compounds, ensuring their enhanced stability and bioavailability and/or allowing their release at controlled rates to targeted sites within the body (Ofori & Peggy, 2013; Wang & Bohn, 2012).

For instance, the production of multiphase colloidal droplets of 10−100 nm (nanoemulsions) ensures the physical stability and increased bioavailability of the final product (Choi, Kim, Cho, Hwang, & Kim, 2011). Nanoemulsions preparation can be conducted using ultrasounds since they provide energy efficiency and better droplet size than classical roto-stator dispersions (Kentish et al., 2008).

Emerging technologies have disadvantages mainly concerning product safety, especially when applying the most advanced technologies. The cell-membrane permeability of cold plasma or nanotechnology and its effect on biological matrices has still not being adequately investigated. Other problems may also occur during treatment of particular food components, e.g., lipids that can be easily oxidized during processing. Some of the existing nonthermal technologies (e.g., cold plasma and ultrasound) are considered as advanced oxidation processes and cause detrimental instead of protecting effects on lipids. Thus better knowledge of their effect on food components is required. Processing of foods below commonly used temperatures allows flavors, essential nutrients, and vitamins to undergo minimal or no changes. In addition, the interactions between different food components can also be affected. For instance, the bioavailability of bioactive compounds may be modified due to interactions with other macronutrients such as fiber in low-processed foods and beverages or proteins and polysaccharides in processed food products (Dupas, Baglieri, Ordonaud, Tom, & Maillard, 2006). In any case, following market and consumer demand for tailor-made processes and products, the adaption of emerging technologies is inevitable, leading to a new state-of-the-art in functional food and nutraceutical development.

1.5 CONCLUSION

Emerging technologies represent a rapid, efficient, and reliable alternative to improve the quality of food, with the potential for developing new and enhanced functional products. However, it is important to note that most of these new technologies are still under intensive scientific research on a smaller scale, although they are fully implemented in a few cases. The current trends and practices in food processing have brought changes in the way components are incorporated into foods and consumed by humans. These changes have yet not been described integrally from the view of food components. This book discusses the impact of emerging technologies in parameters such as nutritional value, functional properties, applications, bioavailability, and the bioaccessibility characteristics of food components as well as the shelf-life, the sensory characteristics, and the profile of food products.

REFERENCES

American Dietetic Association (2004). Position of the American Dietetic Association: Functional foods. *Journal of the American Dietetic Association*, *104*(5), 814−826.

Andlauer, W., & Furst, P. (2002). Nutraceuticals: A piece of history, present status and outlook. *Food Research International*, *35*(2−3), 171−176.

Anese, M., et al. (2015). Effect of ultrasound treatment, oil addition and storage time on lycopene stability and in vitro bioaccessibility of tomato pulp. *Food Chemistry*, *172*, 685−691.

Bech-Larsen, T., & Scholderer, J. (2007). Functional foods in Europe: Consumer research, market experiences and regulatory aspects. *Trends in Food Science and Technology*, *18*, 231–234.

Benito, P., & Miller, D. (1998). Iron absorption and bioavailability: An updated review. *Nutrition Research (New York, NY)*, *18*, 581–603.

Betoret, E., Betoret, N., Vidal, D., & Fito, P. (2011). Functional foods development: Trends and technologies. *Trends in Food Science and Technology*, *22*(2011), 498–508.

Blenford, D. (1995). Bioavailability is key to nutrient effectiveness. *Food Ingredients and Process International*, *17*, 28–30.

Carbonell-Capella, J. M., Barba, F. J., Esteve, M. J., & Frigola, A. (2013). Quality parameters, bioactive compounds and their correlation with antioxidant capacity of commercial fruit-based baby foods. *Food Science and Technology International*, *20*, 479–487.

Carbonell-Capella, J. M., Buniowska, M., Barba, F. J., Esteve, M. J., & Frigola, A. (2014). Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: A review. *Comprehensive Reviews in Food Science and Food Safety*, *13*, 155–171.

Choi, A.-J., Kim, C.-J., Cho, Y.-J., Hwang, J.-K., & Kim, C.-T. (2011). Characterization of capsaicin-loaded nanoemulsions stabilized with alginate and chitosan by self-assembly. *Food and Bioprocess Technology*, *4*(6), 1119–1126.

Correia, R. T., Borges, K. C., Medeiros, M. F., & Genovese, M. I. (2012). Bioactive compounds and phenolic-linked functionality of powdered tropical fruit residues. *Food Science and Technology International*, *18*(6), 539–547.

Courraud, J., Berger, J., Cristol, J. P., & Avallone, S. (2013). Stability and bioaccessibility of different forms of carotenoids and vitamin A during in vitro digestion. *Food Chemistry*, *136*(2), 871–877.

Dacanal, C. G., & Menegalli, F. C. (2010). Selection of operational parameters for the production of instant soy protein isolate by pulsed fluid bed agglomeration. *Powder Technology*, *203*(3), 565–573.

Day, L., Seymour, R. B., Pitts, K. F., Konczak, I., & Lundin, L. (2009). Incorporation of functional ingredients into foods. *Trends in Food Science and Technology*, *20*, 388–395.

Deng, Q., Zinoviadou, K. G., Galanakis, C. M., Orlien, V., Grimi, N., Vorobiev, E., … Barba, F. J. (2015). The effects of conventional and nonconventional processing on glucosinolates and its derived forms, isothiocyanates: Extraction, degradation and applications. *Food Engineering Reviews*, *7*, 357–381.

Dupas, C., Baglieri, A. M., Ordonaud, C., Tom, D., & Maillard, M. N. (2006). Chlorogenic acid is poorly absorbed, independently of the food matrix: A Caco-2 cells and rat chronic absorption study. *Molecular Nutrition and Food Research*, *50*, 1053–1060.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterization, technological functionality and commercial applications: A review. *Food Chemistry*, *124*, 411–421.

Etcheverry, P., Grusak, M. A., & Fleige, L. E. (2012). Application of in vitro bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B 6, B 12, D, and E. *Frontiers in Physiology*, *3*, 317.

EUFIC-The European Food Information Council. (2010). Nutrient bioavailability—getting the most out of food. *Food Today. 05/2010* <http://www.eufic.org/article/en/artid/Nutrient-bioavailability-food/> Accessed 10.01.2016.

Fairweather-Tait, S. J. (1993). Bioavailability of nutrients. In R. Macrae, R. K. Robinson, & M. J. Sadler (Eds.), *Encyclopaedia of food science, food technology and nutrition* (pp. 384–388). London: Academic Press.

Fernández-García, E., Carvajal-Lérida, I., & Pérez-Gálvez, A. (2009). In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutrition Research*, *29*, 751–760.

Galanakis, C. M. (2011). Olive fruit and dietary fibers: Components, recovery and applications. *Trends in Food Science and Technology*, *22*, 175–184.

Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science and Technology*, *26*, 68–87.

Galanakis, C. M. (2013). Emerging technologies for the production of nutraceuticals from agricultural by-products: A viewpoint of opportunities and challenges. *Food and Bioproducts Processing*, *91*, 575–579.

Galanakis, C. M. (2015). Separation of functional macromolecules and micromolecules: From ultrafiltration to the border of nanofiltration. *Trends in Food Science and Technology*, *42*, 44–63.

Galanakis, C.M., Barba, F.J., & Prasad, K.N. (2015). Cost and safety issues of emerging technologies against conventional techniques. In C. M. Galanakis (Ed.), *Food waste recovery: Processing technologies and industrial techniques*.

Galanakis, C. M., Chasiotis, S., Botsaris, G., & Gekas, V. (2014). Separation and recovery of proteins and sugars from Halloumi cheese whey. *Food Research International*, *65*, 477–483.

Galanakis, C. M., Fountoulis, G., & Gekas, V. (2012). Nanofiltration of brackish groundwater by using a polypiperazine membrane. *Desalination*, *286*, 277–284.

Galanakis, C. M., Goulas, V., Tsakona, S., Manganaris, G. A., & Gekas, V. (2013). A knowledge base for the recovery of natural phenols with different solvents. *International Journal of Food Properties*, *16*, 382–396.

Galanakis, C. M., Kotanidis, A., Dianellou, M., & Gekas, V. (2015). Phenolic content and antioxidant capacity of Cypriot Wines. *Czech Journal of Food Sciences*, *33*, 126–136.

Galanakis, C. M., Markouli, E., & Gekas, V. (2013). Fractionation and recovery of different phenolic classes from winery sludge via membrane filtration. *Separation and Purification Technology*, *107*, 245–251.

Galanakis, C. M., & Schieber, A. (2014). Editorial. special issue on recovery and utilization of valuable compounds from food processing by-products. *Food Research International*, *65*, 299–230.

Galanakis, C. M., Tornberg, E., & Gekas, V. (2010a). A study of the recovery of the dietary fibres from olive mill wastewater and the gelling ability of the soluble fibre fraction. *LWT-Food Science and Technology*, *43*, 1009–1017.

Galanakis, C. M., Tornberg, E., & Gekas, V. (2010b). Dietary fiber suspensions from olive mill wastewater as potential fat replacements in meatballs. *LWT-Food Science and Technology*, *43*, 1018–1025.

Galanakis, C. M., Tornberg, E., & Gekas, V. (2010c). Clarification of high-added value products from olive mill wastewater. *Journal of Food Engineering*, *99*, 190–197.

Galanakis, C. M., Tornberg, E., & Gekas, V. (2010d). Recovery and preservation of phenols from olive waste in ethanolic extracts. *Journal of Chemical Technology and Biotechnology*, *85*, 1148–1155.

Galanakis, C. M., Tornberg, E., & Gekas, V. (2010e). The effect of heat processing on the functional properties of pectin contained in olive mill wastewater. *LWT-Food Science and Technology*, *43*, 1001–1008.

Gil-Chávez, G. J., Villa, J. A., Ayala-Zavala, F., Heredia, J. B., Sepulveda, D., Yahia, E. M., & Gonźalez-Aguilar, G. A. (2013). Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: An overview. *Comprehensive Reviews in Food Science and Food Safety*, *12*(1), 5–23.

Hasler, C. M. (2002). Functional foods: Benefits, concerns and challenges e a position paper from the American Council on Science and Health. *Journal of Nutrition*, *132*, 3772–3781.

Heaney, R. P. (2001). Factors influencing the measurement of bioavailability, taking calcium as a model. *The Journal of Nutrition*, *131*(4), 1344–1348.

Heckman, M. A., Sherry, K., & de Mejia, E. G. (2010). Energy drinks: An assessment of their market size, consumer demographics, ingredient profile, functionality, and regulations in the United States. *Comprehensive Reviews in Food Science and Food Safety*, *9*(3), 303–317.

Heng, W. W., Xiong, L. W., Ramanan, R. N., Hong, T. L., Kong, K. W., Galanakis, C. M., & Prasad, K. N. (2015). Two level factorial design for the optimization of phenolics and flavonoids recovery from palm kernel by-product. *Industrial Crops and Products*, *63*, 238–248.

Kaur, S., & Das, M. (2011). Functional foods: An overview. *Food Science and Biotechnology*, *20*, 861−875.
Kentish, S., Wooster, T. J., Ashokkumar, M., Balachandran, S., Mawson, R., & Simons, L. (2008). The use of ultrasonics for nanoemulsion preparation. *Innovative Food Science and Emerging Technologies*, *9*(2), 170−175.
Knorr, D., Forehling, A., Jaeger, H., Reineke, K., Schlueter, O., & Schoessler, K. (2011). Emerging technologies in food processing. *Annual Review of Food Science and Technology*, *2*, 203−235.
Korhonen, H. (2002). Technology options for new nutritional concepts. *International Journal of Dairy Technology*, *55*, 79−88.
Kristinsson, H. G., & Rasco, B. A. (2000). Fish protein hydrolysates: Production, biochemical, and functional properties. *Critical Reviews in Food Science and Nutrition*, *40*, 43−81.
Lesser, S., Cermak, R., & Wolffram, S. (2006). The fatty acid pattern of dietary fat influences the oral bioavailability of the flavonol quercetin in pigs. *British Journal of Nutrition*, *96*(6), 1047−1052.
Liu, D., Vorobiev, E., Savoire, R., & Lanoiselle, J. L. (2011). Intensification of polyphenols extraction from grape seeds by high voltage electrical discharges and extract concentration by dead-end ultrafiltration. *Separation and Purification Technology*, *81*(2), 134−140.
López-Varela, S., González-Gross, M., & Marcos, A. (2002). Functional foods and the immune system: A review. *European Journal of Clinical Nutrition*, *56*(3)), S29−S33.
Manach, C., Scalbert, A., Morand, C., Remesy, C., & Jimenez, L. (2004). Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition*, *79*(5), 727−747.
Manach, C., Williamson, G., Morand, C., Scalbert, A., & Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition*, *81*(1), 230s−242s.
Martinez-Antonio, J. M., Brahm, P. M., Gonzalez, A. B., & Stinco, C. M. (2015). A comprehensive review on the colorless carotenoids phytoene and phytofluene. *Archives of Biochemistry and Biophysics*, *572*(2015), 188−200.
Mcclements, D. J., & Xiao, H. (2014). Excipient foods: Designing food matrices that improve the oral bioavailability of pharmaceuticals and nutraceuticals. *Food and Function*, *5*(7), 1320−1333.
Moebus, S., & Stang, A. (2007). The metabolic syndrome e a controversial diagnostic concept. *Herz e Kardiovaskulare Erkrankungen*, *32*, 529−541.
Mollet, B., & Rowland, I. (2002). Functional foods: At the frontier between food and pharma. *Current Opinion in Biotechnology*, *13*, 483−485.
Moure, A., Cruz, J. M., Franco, D., Domínguez, J. M., Sineiro, J., Domínguez, H., ... Parajó, J. C. (2001). Natural antioxidants from residual sources. *Food Chemistry*, *72*, 145−171.
Mujumdar, A. S., & Law, C. L. (2010). Drying technology: Trends and applications in postharvest processing. *Food and Bioprocess Technology*, *3*(6), 843−852.
Ofori, J. A., & Peggy, Y.-H. (2013). *Novel technologies for the production of functional foods.* Bio-Nanotechnology. Blackwell Publishing Ltd.
Oroian, M., & Escriche, I. (2015). Antioxidants: Characterization, natural sources, extraction and analysis. *Food Research International*, *74*, 10−36.
Parada, J., & Aguilera, J. M. (2007). Food microstructure affects the bioavailability of several nutrients. *Journal of Food Science*, *72*(2), R21−R32.
Patsioura, A., Galanakis, C. M., & Gekas, V. (2011). Ultrafiltration optimization for the recovery of β-glucan from oat mill waste. *Journal of Membrane Science*, *373*, 53−63.
Pereira, R. N., & Vicente, A. A. (2010). Environmental impact of novel thermal and nonthermal technologies in food processing. *Food Research International*, *43*(7), 1936−1943.
Pogaku, R., Seng, C. E., Boonbeng, L., & Kallu, U. R. (2007). Whey protein isolate-starch system-a critical review. *International Journal of Food Engineering*, *3*, art1.

Porrini, M., & Riso, P. (2008). Factors influencing the bioavailability of antioxidants in foods: A critical appraisal. *Nutrition, Metabolism and Cardiovascular Diseases*, *18*, 647–650.

Prakash, J. (1996). Rice bran proteins: Properties and food uses. *Critical Reviews in Food Science and Nutrition*, *36*, 537–552.

Rein, M. J., Renouf, M., Cruz-Hernandez, C., Actis-Goretta, L., Thakkar, S. K., & da Silva Pinto, M. (2013). Bioavailability of bioactive food compounds: A challenging journey to bioefficacy. *British Journal of Clinical Pharmacology*, *75*(3), 588–602.

Roberfroid, M. (2002). Functional food concept and its application to prebiotics. *Digestive and Liver Disease*, *34*, S105–S110.

Roberfroid, M. B. (2007). Concepts and strategy of functional food science: The European perspective. *The American Journal of Clinical Nutrition*, *71*, 1660–1664.

Rodríguez, R., Jiménez, A., Fernández-Bolaños, J., Guillén, R., & Heredia, A. (2006). Dietary fibre from vegetable products as source of functional ingredients. *Trends in Food Science and Technology*, *17*, 3–15.

Roselló-Soto, E., Barba, F. J., Parniakov, O., Galanakis, C. M., Grimi, N., Lebovka, N., & Vorobiev, E. (2015a). High voltage electrical discharges, pulsed electric field and ultrasounds assisted extraction of protein and phenolic compounds from olive kernel. *Food and Bioprocess Technology*, *8*, 885–894.

Roselló-Soto, E., Galanakis, C. M., Brncic, M., Orlien, V., Trujillo, F. J., Mawson, R., … Barba, F. J. (2015b). Clean recovery of antioxidant compounds from plant foods, byproducts and algae assisted by ultrasounds processing. Modeling approaches to optimize processing conditions. *Trends in Food Science and Technology*, *42*, 134–149.

Schumann, K., Classen, H. G., Hages, M., Prinz-Langenhol, R., Pietrzik, K., & Biesalski, H. K. (1997). Bioavailability of oral vitamins, minerals and trace elements in perspective. *Drug Research*, *47*, 369–380.

Shahidi, F. (2009). Nutraceuticals and functional foods: Whole versus processed foods. *Trends in Food Science and Technology*, *20*, 376–387.

Tsakona, S., Galanakis, C. M., & Gekas, V. (2012). Hydro-ethanolic mixtures for the recovery of phenols from Mediterranean plant material. *Food and Bioprocess Technology*, *5*, 1384–1393.

Vaisberg, E., Lenzi, D., Hansen, R., Keon, B., & Finer, J. (2006). An infrastructure for high-throughput microscopy: Instrumentation, informatics, and integration. *Methods in Enzymology*, *414*, 484–512.

Vorobiev, E., & Lebovka, N. (2008). *Electrotechnologies for extraction from food plants and biomaterials* (pp. 39–81). Springer.

Wang, L. & Bohn, T. (2012) Health-promoting food ingredients and functional food processing, In J. Bouayed (Ed.), *Nutrition, well-being and health*.

WHO (2003). Diet, nutrition and the prevention of chronic diseases. *WHO technical report series 916*. Geneva, Switzerland. <www.fao.org/docrep/005/AC911E/AC911E00.HTM#Contents>. Accessed 21.03.16.

Wood, R. J. (2005). Bioavailability: Definition, general aspects and fortificants. In B. Caballero, A. Prentice, & L. Allen (Eds.), *Encyclopedia of Human Nutrition* (2nd ed). Oxford: Elsevier Ltd.

Zinoviadou, K. G., Barba, F. J., Galanakis, C. M., Brnčić, M., Trujillo, F., Mawson, R., & Knoerzer, K. (2015). Fruit juice sonication: Implications on food safety and physicochemical and nutritional properties. *Food Research International*, *77*(4)), 743–752.

CHAPTER

PROTEINS, PEPTIDES, AND AMINO ACIDS

2

Reza Tahergorabi[1] **and Seyed Vali Hosseini**[2]

[1]*North Carolina Agricultural & Technical State University, Greensboro, NC, United States*

[2]*University of Tehran, Karaj, Iran*

2.1 INTRODUCTION

Proteins are highly complex biochemical compounds. There are 20 different amino acids (AA), which are the building blocks of all proteins. The constituents are linked via substituted amide bonds. While it is clear that proteins have a major function in many biological processes, they also play a key role as food additives and ingredients. To signify their biological importance, these macromolecules were named proteins, derived from the Greek word *proteois*, which means of the first kind (Damodaran, 2008).

At the elemental level, proteins contain 50−55% carbon, 6−7% hydrogen, 20−23% oxygen, 12−19% nitrogen, and 0.2−3.0% sulfur. All biologically produced proteins can be used as food proteins. However, for practical purposes, food proteins may be defined as those that are easily digestible, nontoxic, nutritionally adequate, functionally useable in food products, and available in abundance. Traditionally, milk, meats (including poultry and fish), eggs, cereals, legumes, and oilseeds have been the major sources of food proteins. In addition to plants and animals, protein producers include algae (Chlorella, Scenedesmus, Spirulina spp.), yeasts, and bacteria (single-cell proteins).

Proteins differ in their nutritive value. Several factors, such as essential AA content and digestibility, contribute to these differences. The daily protein requirement therefore depends on the type and composition of proteins in a diet. Adults or children consuming only cereal or legume proteins have difficulty maintaining their health. The protein content of muscle tissue and the quality of this protein is high, since it contains kinds and ratios of AA that are similar to those required for maintenance and growth of human tissue. Of the total nitrogen content of muscle, approximately 95% is protein and 5% is smaller peptides, AA, and other compounds (Strasburg, Xiong, & Chiang, 2008).

The digestibility of protein is defined as the proportion of food nitrogen that is absorbed after ingestion. Although the content of essential AA is the primary indicator of protein quality, true quality also depends on the extent to which these AA are utilized in the body. Thus digestibility of AA can affect the quality of proteins. Food proteins of animal origin are more completely digested than those of plant origin.

The functional properties of proteins in foods are related to their structural and other physicochemical characteristics. Therefore, a fundamental understanding of the nutritional and functional

Nutraceutical and Functional Food Components. DOI: http://dx.doi.org/10.1016/B978-0-12-805257-0.00002-8

properties of proteins and the changes these properties undergo during processing is essential if the performance of proteins in foods is to be improved, and if new or less costly sources of proteins are to compete with traditional food proteins.

2.2 FUNCTIONAL AND NUTRITIONAL PROPERTIES OF PROTEINS, PEPTIDES, AND AA

2.2.1 FUNCTIONAL PROPERTIES

Functionality of food proteins is defined as "those physical and chemical properties which affect the behavior of proteins in food systems during processing, storage, preparation, and consumption" (Damodaran, 2008). The physical and chemical properties that govern protein functionality include size; shape; amino-acid composition and sequence; net charge and distribution of charges; hydrophobicity/hydrophilicity ratio; secondary, tertiary, and quaternary structures; molecular flexibility/rigidity; and ability to interact/react with other components. On an empirical level, the various functional properties of proteins can be viewed as manifestations of two molecular aspects: (1) hydrodynamic properties and (2) protein surface-related properties (Tahergorabi, Hosseini, & Jaczynski, 2011). Functional properties such as viscosity (thickening), gelation, and texturization are related to the hydrodynamic properties of proteins, which depend on size, shape, and molecular flexibility. Functional properties such as wettability, dispersibility, solubility, foaming, emulsification, and fat and flavor binding are related to the chemical and topographical properties of the protein surface.

2.2.1.1 Foam formation and foam stabilization

Foams are dispersions of gases in liquids. Proteins stabilize by forming flexible, cohesive films around the gas bubbles. In several foods, proteins function as foam-forming and foam-stabilizing components, e.g., in whipped cream, ice cream, cakes, meringue, bread, souffles, mousses, and marshmallow. This varies from one protein to another.

2.2.1.2 Gel formation

Protein gelation refers to transformation of a protein from the "sol" state to a "gel-like" state. This transformation is facilitated by heat, enzymes, or divalent cations under appropriate conditions. Most food-protein gels are prepared by heating a protein solution. In this mode of gelation, the protein in a sol state is first transformed into a "progel" state by denaturation. The progel state is usually a viscous liquid state in which some degree of protein polymerization has already occurred. There are two different types of gels, namely, the *polymeric networks* and the *aggregated dispersions*, although intermediate forms are also found. Examples of *polymeric networks* are the gels formed by gelatin and polysaccharides such as agarose and carrageenan. Examples of *aggregated dispersions* are the gels formed by globular proteins after heating and denaturation.

2.2.1.3 Emulsifying effect

Emulsions are disperse systems of one or more immiscible liquids. Several natural and processed foods, such as milk, egg yolk, coconut milk, soy milk, butter, margarine, mayonnaise, spreads,

salad dressings, frozen desserts, frankfurter, sausage, and cakes, are emulsion-type products where proteins play important roles as emulsifiers. In natural milk, the fat globules are stabilized by a membrane composed of lipoproteins. When milk is homogenized, the lipoprotein membrane is replaced by a protein film comprised of casein micelles and whey proteins. Homogenized milk is more stable against creaming than natural milk is because the casein micelle−whey protein film is stronger than the natural lipoprotein membrane.

2.2.1.4 Solubility

The solubility of a protein is the thermodynamic manifestation of the equilibrium between protein−protein and protein−solvent interactions. The functional properties of proteins are often affected by protein solubility, and those most affected are thickening, foaming, emulsifying, and gelling. Insoluble proteins have limited uses in food.

2.2.1.5 Viscosity

Consumer acceptability of several liquid and semisolid-type foods (e.g., gravies, soups, beverages) depends on the viscosity or consistency of the product. The viscosity of a solution relates to its resistance to flow under an applied force (or shear stress).

2.2.2 NUTRITIONAL PROPERTIES

Proteins are comprised of approximately 20 AA. While all of the AA are indispensable for normal growth and sustaining metabolic processes, nine of them cannot be synthesized by adult humans. Thus the nine AA are referred to as "essential" (EAA). The EAA must be obtained through the diet. Proteins differ in their nutritive value. Several factors, such as the content of EAA and digestibility, contribute to these differences. The daily protein requirement therefore depends on the type and composition of proteins in a diet.

2.2.2.1 Protein quality

The "quality" of a protein is mainly related to its EAA composition and digestibility. High-quality proteins are those that contain all the EAA at levels greater than the FAO/WHO/UNU (2001) reference levels, and a digestibility comparable to or better than those of egg-white or milk proteins. Animal proteins are better "quality" than plant proteins. Proteins of major cereals and legumes are often deficient in at least one of the EAA. While proteins of cereals, such as rice, wheat, barley, and maize, are very low in lysine and rich in methionine, the proteins of legumes and oilseeds are deficient in methionine and rich or adequate in lysine. Some oilseed proteins, such as peanut protein, are deficient in both methionine and lysine content. The EAA whose concentrations in a protein are below the levels of a reference protein are called *limiting AA*. Both animal and plant proteins generally contain adequate or more than adequate amounts of histidine (His), isoleucine (Ile), leucine (Leu), phenylalanine (Phe) + tyrosine (Tyr), and valine (Val). These AA are usually not limiting in staple foods. More often, lysine (Lys), threonine (Thr), tryptophan (Trp), or the sulfur-containing AA are the limiting AA.

The nutritional quality of a protein that is deficient in an EAA can be improved by mixing it with another protein that is rich in that EAA. For example, mixing cereal proteins with legume proteins provides a complete and balanced level of EAA. Thus diets containing appropriate amounts

of cereals and legumes (pulses) and that are otherwise nutritionally complete are often adequate to support growth and maintenance. The poor-quality protein can be nutritionally improved by supplementing it with the essential free AA that are underrepresented. To improve the quality of legumes and cereals they are usually supplemented with Met and Lys, respectively. Excessive intake of one AA often results in an increased requirement for other EAA. This is due to competition among AA for absorption sites on the intestinal mucosa. For example, when the Leu level is relatively high, this decreases absorption of Ile, Val, and Tyr even if the dietary levels of these AA are adequate. This leads to an increased dietary requirement for the latter three AA. Overconsumption of other EAA also can inhibit growth and induce pathological conditions.

2.2.2.2 Digestibility

Digestibility is defined as the proportion of food nitrogen that is absorbed after ingestion. Although the content of EAA is the primary indicator of protein quality, true quality depends on the extent to which these AA are also utilized in the body. Thus digestibility of AA can affect the quality of proteins. Animal food proteins are more completely digested than plant proteins.

2.2.2.3 Evaluation of protein nutritive value

Since the nutritional quality of proteins can vary greatly and is affected by many factors, it is important to have methods in place to evaluate quality. Quality estimates are useful for (1) determining the amount required to provide a safe level of EAA for growth and maintenance and (2) monitoring changes in the nutritive value of proteins during food processing, so that processing conditions that minimize quality loss can be created. The data from animal-feeding studies are used in several ways to evaluate protein quality. The protein efficiency ratio (PER) is the weight (in grams) gained per gram of protein consumed. This is a simple and commonly used expression. Another useful expression is the net protein ratio (NPR). Net protein ratio values provide information on the ability of proteins to support both maintenance and growth.

Although a certain protein may have a favorable AA profile and be rich in EAA, proteins are typically not 100% bioavailable. The biological value (BV) of a protein (g protein formed in the body/100 g food protein) is determined by the absolute content and relative proportions of EAA, the ratio of EAA to non-EAA, and factors such as digestibility and availability. The BV of a protein measures its efficiency in supporting the human body's needs. Egg proteins are regarded as a reference protein and have a BV of 100, meaning that 100% of the nitrogen is absorbed and retained. Milk, beef, fish, corn, and rice proteins have BVs of 93, 75, 75, 72, and 59, respectively.

2.3 BIOAVAILABILITY AND BIOACCESSABILITY OF PROTEIN, PEPTIDES, AND AA

Today, consumers are more and more aware of the benefits beyond basic nutrition provided by food and their compounds. However, the bioavailability of food compounds in human health has not been well understood. In addition, before a food component becomes bioavailable, it must be released from the food matrix and modified in the gastrointestinal (GI) tract. Therefore, before concluding on any potential health effect it is important to analyze whether the digestion process

affects bioactive compounds and their stability, as this, in turn, will affect their bioavailability and their possible beneficial effects (Carbonell-Capella, Buniowska, Barba, Esteve, & Frìgola, 2014).

The digestibility of proteins depends on, among other things, their structure. Compacting of the protein structure by cross-linking prevents unfolding of the peptide chains, which may decrease the digestibility by making some of the peptide bonds unreachable to the enzymes. Cross-linking may be caused by a decrease in the rate of protein hydrolysis due to heating, as well as by interactions of amino groups with reduced saccharides or other compounds containing carbonyl groups. In food processing, these are secondary products of lipid oxidation and aldehydes contained in smoked products. As a result of several further reactions, different unsaturated compounds are generated and interact with the amines, possibly involving different cross-linking reactions (Matthews & Laster, 1965).

In an effort to find a more accurate method to determine protein and AA digestibility of food products, several in vitro methods have been developed, simulating the physiological process of digestion. Yin, Tang, Wen, Yang, and Li (2008) studied in vitro trypsin digestibility of kidney bean protein isolate and found that the protein digestibility decreased only at pressures above 200 Mpa and for long holding times (up to 120 min). Chicón, Belloque, Recio, and López-Fandiño (2006) previously noted improved in vitro trypsin proteolysis of β-lactoglobulin as affected by pressures up to 400 MPa, regardless of the digestion carried out under pressure or over a pre-HP-treated protein. They extended their work to the pepsin digestibility of β-lactoglobulin and whey-protein isolate after high-pressure (HP) treatment and found that HP treatment at 400 MPa promoted the hydrolysis of β-lactoglobulin by pepsin, but this increased susceptibility of β-lactoglobulin to proteolysis was progressively lost during refrigerated storage (Chicón, Belloque, Alonso, & López-Fandiño, 2008).

Throughout food processing, protein sources are treated with heat, oxidizing agents such as hydrogen peroxide, organic solvents, alkalis, and acids for a variety of reasons such as to sterilize or pasteurize, to improve flavor, texture, and other functional properties, to deactivate antinutritional factors, and to prepare concentrated protein products. These processing treatments may cause the formation of Maillard compounds, oxidized forms of sulfur AA, D-AA, and cross-linked peptide chains, resulting in lower AA bioavailability and a decrease in protein quality (Bender, 1972).

Consumer preference for foods containing bioactive compounds such as peptides and free AA is also rapidly growing. The effectiveness of these compounds in providing healthful benefits depends on the ability to preserve the bioavailability of the active ingredients. However, only a small proportion of the molecules remains available following consumption for various reasons including insufficient gastric residence time, low permeability, and/or solubility within the gut, as well as instability under conditions encountered in food processing (temperature, oxygen, light) or in the GI tract (pH, enzymes, presence of other nutrients), all of which limit the activity and potential health benefits. Therefore, bioavailability is a key step in ensuring the bioefficacy of food compounds. Bioavailability is a complex process involving several different stages including liberation from a food matrix, absorption, distribution, metabolism, and elimination phases. Bioavailability includes in its definition the utilization of a nutrient and therefore can be defined as the fraction of ingested nutrient or compound that reaches the systemic circulation and is utilized (Wood, 2005). Overall, bioavailability includes GI digestion, absorption, metabolism, tissue distribution, and bioactivity. Consequently, in terms of bioavailability, when a claim is made, it must be demonstrated that the component analyzed is efficiently digested and assimilated and then, once absorbed, exerts a positive effect on human health (Carbonell-Capella et al., 2014). Food compounds need to be bioavailable and be able to be bioactive in order to exert any beneficial effects. It may be considered redundant to study the health effects of dietary food compounds if their bioavailability is not fully elucidated.

It is well known that bioefficacy may be improved through enhanced bioavailability. Therefore several technologies have been developed to improve the bioavailability of xenobiotics, including structural modifications, nanotechnology, and colloidal systems. Due to the complex nature of food compounds and also due to their different mechanisms of absorption, unraveling the bioavailability of food constituents is challenging. From a nutritional perspective, bioavailability is a fraction of a given food that the body can utilize, and is therefore a matter of nutritional efficacy (Benito & Miller, 1998; Fernàndez-Garcìa, Carvajal-Lérida, & Pérez-Gàlvez, 2009). Through a better understanding of the digestive fate of food compounds, we can impact the promotion of health and improvement of performance. Many varying factors affect bioavailability, such as bioaccessibility, food-matrix effects, transporters, molecular structures, and metabolizing enzymes. Indeed, the bioavailability of bioactive food compounds may be modified because of interactions with other macronutrients such as fiber in low-processed foods and beverages or proteins and polysaccharides in processed food products (Dupas, Baglieri, Ordonaud, Tom, & Maillard, 2006). Furthermore, when different foods come into contact with the mouth or digestive tract, various interactions may take place affecting phytochemical bioavailability (e.g., fat enhances quercetine bioavailability in meals) (Lesser, Cermak, & Wolffram, 2006).

The concept of bioaccessibility can be defined as the quantity or fraction of a compound that is released from the food matrix into the GI tract and thereby made available for intestinal absorption (Heaney, 2001; Saura-Calixto, Serrano, & Goñi, 2007). The digested food is predominantly broken down in the small intestine by bile, pancreatic, and other enzymes secreted from the intestinal mucosa (Gropper & Smith, 2009).

Assessment of bioaccessibility and bioavailability of health-associated compounds is important for understanding the relationship between food and nutrition. The rate and extent of absorption can vary widely between individuals. The interindividual variability in bioavailability depends on several key factors including diet, genetic background, gut microbiota composition, and activity. Limited bioavailability hinders the use of bioactive food compounds as functional ingredients (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; Scalbert & Williamson, 2000).

2.4 EFFECTS OF EMERGING TECHNOLOGIES ON PROTEINS, PEPTIDES, AND AA

Today there is growing interest in foods that are minimally preserved and processed. Consumers are demanding healthier, more natural foods that are less processed, with fewer preservatives and longer shelf-life without diminishing their nutritional properties. Consequently, there is currently much emphasis on the development of novel and emerging technologies for minimal preservation and processing methods in contrast to most conventional methods that are by far based on the use of thermal energy, which may alter food properties.

Due to the introduction of new and advanced technologies in the food industry, an understanding of the effects of these emerging technologies on food proteins is necessary. These new techniques allow the processing of foods below temperatures used during thermal pasteurization, so flavors, essential nutrients, and proteins undergo minimal or no changes. Foods can be nonthermally processed by ionizing irradiation, high hydrostatic pressure, ultrasounds, ohmic heating,

and electrical methods such as pulsed electric fields (PEFs) and light pulses. Therefore in this section the effect of these emerging technologies on food proteins is discussed.

2.4.1 OHMIC HEATING

Ohmic heating is an innovative heating technique employed for thermal processing. In this method, the food is placed between two electrodes that service as electrical resistors and an alternating electric current is passed through the circuit. Due to the electrical resistance, heat is generated throughout the food, and takes place volumetrically. The electrical energy is directly converted into heat, causing a temperature rise. This system is comparable to an electrical circuit, which is comprised of a resistance and a source of voltage and current. The food product acts as the resistance when placed between the two electrodes and the current passes through it. In other words, the food becomes part of an electrical circuit. This technique offers an alternate way to rapidly heat food, bypassing conventional heating systems. Ohmic heating is comparable to microwave heating without an intermediary step of converting electricity into microwaves through the magnetron before heating the product. The concept of ohmic heating technology dates back to 1897 (Jones, 1897), and the benefits of ohmic heating are numerous (Biss, Coombes, & Skudder, 1989). The most important benefit is that heating is very rapid. A large temperature gradient is not experienced within the food, i.e., heating is uniform, and the process is ideal for shear-sensitive products.

Ohmic heating is employed in pasteurizing and sterilizing of liquid and particulate foods, especially of ready-to-serve meals, fruits, vegetables, meat, poultry, or fish, and is an alternative to sterilization of foods using conventional heat exchangers or autoclaves. In addition, Naveh, Kopelman, and Mizrahi (1983) applied ohmic heating for thawing of foods. The main application of ohmic heating is to pasteurize or sterilize liquid and particulate foods, especially ready-to-serve meals, fruits, vegetables, meat, poultry, or fish. It is also an alternative to sterilization of foods using conventional heat exchangers or autoclaves. In addition, Naveh, Kopelman, and Mizrahi (1983) applied ohmic heating for thawing of foods. Furthermore, some studies of ohmic heating of seafood proteins have shown that degradation of myosin heavy chain and actin was minimized by ohmic heating, resulting in a continuous network structure of the gels (Cho, Yousef, & Sastry, 1996; Yongsawatdigul, Park, Kolbe, Abu Dagga, & Morrissey, 1995).

Park, Kim, Uemura, and Noguchi (1995) performed a study on the effect of ohmic heating on fish-protein gel. The structure of the gel was examined using scanning electron microscopy. The breaking strength and color were also measured using conventional methods, and the electrical properties were recorded. The electrode corrosion was examined by immersion in NaCl. It was found that over 100 Hz, gels had similar electrical properties, which would suggest that heating rates would be similar. However, specific heating rates increased remarkably as the frequency increased from 1 to 10 kHz. This indicates that there was a significant dielectric loss in the gel. Corrosion of aluminum electrodes increased at 50 Hz with increasing NaCl concentration. There was a negligible contamination of aluminum ions in the product as the frequency was increased to 50 kHz.

In addition, Konno and Konno (2014) evaluated the effect of ohmic heating on the gel strength of surimi obtained from squid-mantle muscle. A previous investigation had shown that squid-mantle muscle does not form elastic thermal gel. It is generally believed that myosin degradation during the heating process is the reason for this. The proteinase responsible for this an astacin-like

metalloproteinase that cleaves myosin molecule selectively into two parts of heavy meromyosin- and light meromyosin-like fragments. Moreover, addition of NaCl to dissolve myosin accelerates enzyme degradation. The activity is easily inhibited by adding in vitro chelating reagents such as ethylene diamine tetra acetic acid (EDTA). However, edible chelating reagents are difficult to find. This problem was overcome by rapid heating using an ohmic heating apparatus. Very quick and homogeneous heating of squid meat inactivated the enzyme before degradation of myosin. Initially, the heating rate was determined by using squid-meat homogenate. The heating rate of 1 degree/min was quick enough to obtain the sample with no myosin degradation. It was also found that proteinase lost the activity above 50°C. Skinned squid-mantle muscle itself was the material used for the gel production in order to preserve flavor. Chopped squid meat was further ground with 2.5% NaCl to prepare salted meat paste. The paste was stuffed into a plastic tube, and the tube was sandwiched with titanium electrodes to apply the current. The time needed to heat the paste from 0°C to 90°C was shorter than 1 min. The heating rate was sufficiently greater than the required rate. The gel produced by the method was highly elastic. It gave 650 g of breaking force and 12 mm of deformation when measured with Ø3 mm plunger. The gel was equivalent to elastic gel from Alaska pollock surimi. High elasticity of the gel was confirmed by folding the sliced gel twice, which showed no cracking.

2.4.2 HIGH-PRESSURE PROCESSING

High-pressure processing (HPP) is a simple concept. The food product is sealed in a plastic bag, inserted into a chamber, and subjected to pressures up to 7000 atmospheres (atm). Renewed interest in HP pasteurization of food has raised questions, e.g., on the pressure-temperature behavior of macromolecular food components such as proteins, lipids, and polysaccharides (Fidalgo, Saraiva, Aubourg, Vázquez, & Torres, 2014; Pazos, Méndez, Gallardo, & Aubourg, 2014; Quirós, Chichón, Recio, & López-Fandino, 2007; Torres, Saraiva, Guerra-Rodríguez, Aubourg, & Vázquez, 2014). High pressure induces unfolding of the protein structure and then aggregation with different proteins in the food, or into a different form. Both possibilities will result in textural changes in the food material. Gel formation is observed in soy, meat, fish, and egg albumin proteins. Pressure-induced gels tend to maintain their natural color and flavor, and are described as smooth, glossy, and soft, when compared with gels formed by the use of heat treatment. These results have been evaluated in relation to surimi products on an experimental scale. Interest is also focused on the possibility of unfolding the structure of lower-quality proteins, using HPP, to improve functional properties such as emulsifying and gelling capacity of high-protein food products. The effects of high pressure on protein structure have been discussed by Hendrickx, Ludikhuyze, van den Broeck, and Weemaes (1998), and the effects on protein functionality have been reviewed by Messens, Van Camp, and Huyghbaert (1997).

However, the mechanisms of protein gelation and of the sol/gel behavior of polysaccharides are not well understood. But studies on pressure-induced gelation of fish myofibrillar proteins in surimi have shown that pressure-initiated gelation may occur by using mild heat. Alternatively, HPP-aided gelation could also be enhanced by transglutaminase (Ashie & Lanier, 1999). Pressure treatment is also effective in producing highly appealing kamaboko from surimi. On the other hand, in other food products such as white wine, proteins play a significant role in colloidal stability and clarity (Sauvage, Bach, Moutounet, & Vernhet, 2010). The formation of an unattractive deposit in bottled

wine, caused by protein aggregation during storage, is a common defect of commercial wines, which makes them unacceptable to consumers. Although turbid wines do not constitute a health risk, protein haze formation is an important issue in oenology (Marangon et al., 2011; Sauvage et al., 2010). Such precipitates commonly result from denaturation of grape proteins in wine, and haze-forming proteins have been identified as pathogenesis-related (PR) grape proteins; thaumatin-like (TL) proteins and chitinases are the predominant proteins found in must and wine (Marangon et al., 2011). Both have low-molecular weight, ranging from 20 to 35 kDa. Being resistant to proteolysis and low wine pH, they are able to survive fermentation and remain in wine and potentially form haze (Marangon et al., 2011). The haze-formation phenomenon is a multifactorial process and the presence of proteins is a prerequisite. However, the wine matrix, which consists of polyphenols, polysaccharides, sulfate, pH, and ethanol, also facilitates the complex interplay that results in haze (Batista, Monteiro, Loureiro, Teixeira, & Ferreira, 2010; Pocock, Alexander, Hayasaka, Jones, & Waters, 2007).

HP technology has been used in the field of food processing because it offers several advantages over traditional methods of food conservation and hygiene (Dzwolak, Kato, & Taniguchi, 2002). Among the effects it has on food components, HP affects the noncovalent bonds (ionic, hydrophobic, and hydrogen bridges) of proteins; therefore the primary structure remains unchanged, whereas the secondary, tertiary, and quaternary structures may unfold and disassociate (Dzwolak et al., 2002). In this regard, Tabilo-Munizaga et al. (2014) suggested that HP can be used not only to attain microbiological stability but also to stabilize proteins.

The recent evolution of HP technology has allowed the development of new processes, where an enhancement of enzymatic proteolysis can be obtained by combining with HP treatment, which modifies the tertiary and quaternary structures of proteins; when the structure modification is reached in the presence of active proteases, the hydrolysis can be improved. Furthermore, by combining proteolysis and HP treatment, it is possible to produce hydrolysates with lower residual antigenicity.

There are few reports on the effects on nutritional characteristics of HPP-treated foods. Elgasim and Kennick (1980) investigated pressure treatment of meat protein at 103 MPa for 2 min. The apparent digestibility was improved, and no adverse effects on the apparent BV, net protein utilization, or PER were observed. Butz et al. (2002) studied the influence of HP on the functional properties of a choice of vegetables at around 600 MPa and in combination with elevated temperatures. Carrots, tomatoes, and broccoli were investigated and the contents of health-promoting substances were assessed. In most cases HP did not induce loss of beneficial substances in the vegetable matrices but induced changes in the structure of the products, which resulted in altered physicochemical properties such as higher glucose retardation index and water retention or reduced extractability.

2.4.3 ULTRASOUND

Ultrasound refers to sound waves and mechanical vibrations, which propagate through solids, liquids, or gases with a frequency greater than the upper limit of human hearing. Although this limit can vary from person to person, the ultrasonic frequency range is considered to be at frequencies over 20 kHz. The upper limit of the frequency range of ultrasound is mainly limited by the ability to generate ultrasonic signals (Vollmer, Everbach, Halpern, & Kwakye, 1998). As a

nonthermal technology, power ultrasound is attracting considerable interest in the food industry. Using mechanical vibrations of high enough intensity, power ultrasound can produce changes in food either by disrupting its structure or by promoting certain chemical reactions. The range of applications of power ultrasound in the food industry is vast and is growing rapidly. Some examples include emulsification (one of the earliest applications), drying, degassing, and inactivation of microorganisms. The use of this technology provides some valuable benefits such as reduced processing and maintenance costs and shorter processing times. Although ultrasound has recently attracted considerable interest in the food industry for both the analysis and processing of foods, ultrasonic techniques have been available in the industry since World War II (Povey & McClements, 1988, 1989). Typically ultrasound applications are divided into two broad categories: high- and low-intensity applications. Low-intensity applications are mainly characterized by frequencies above 100 kHz with energies below 1 W/cm^2. In the food industry, low-intensity ultrasound is used as an analytical technique either to control a process or to obtain information about different physicochemical properties such as air bubbles in aerated foods, ratio of fat in meats, vegetable, and fruit characterization, quality of eggs, cracks in cheese, texture of biscuits, milk coagulation, wine-fermentation control, or dough characterization (Benedito, Carcel, González, & Mulet, 2002; Mizrach, 2008; Resa, Bolumar, Elvira, Pérez, & Montero de Espinosa, 2007; Salazar, Turó, Chávez, & García, 2004; Simal, Benedito, Clemente, Femenia, & Rosselló, 2003).

On the other hand, high-intensity or power ultrasound applications tend to use frequencies below 100 kHz with energies above 10 W/cm^2. These applications are intended to have an effect on the material being tested generally by generation of intense cavitation (Mason & Lorimer, 1988). Cavitation is an important physical phenomenon of high-intensity ultrasound and involves the formation, growth, and implosive collapse of bubbles in liquid. Some typical examples include disruption of biological cells, emulsifying, drying, mixing materials, or microbial inactivation. In addition, many of the applications of power ultrasound are not exclusively ultrasonic processes but ultrasonically assisted processes.

It has been demonstrated that functional properties of proteins such as organoleptic, kinesthetic, hydration, interfacial, enzymatic, and rheological properties are of interest to manufacturers of pharmaceutical, food, and cosmetic products. These structural and functional properties are influenced by high-intensity ultrasound mainly due to formation of covalent and/or noncovalent bonds. The structure−function relationship in proteins may be altered during sonication either by initiating or retarding chemical or enzymatic reactions, which may include oxidation, glycosylation, hydroxylation, phosphorylation, methylation, and acylation. Structural or functional properties of proteins are influenced because of the mechanism of action of high-intensity ultrasound. The alteration of functional properties by sonication is mainly done by direct or indirect effects of sonication on enzymes such as glycosidase, invertases, and β-glucosidase. High-power ultrasound is reported to influence the functional properties of whey proteins including solubility and foaming ability by changing the temperature and conductivity of the surrounding media of the whey protein (Jambrak, Mason, Lelas, Herceg, & Herceg, 2008).

Guzey (2001) studied the effect of high-intensity ultrasound on the structure and functionality of bovine serum albumin (BSA) and found that it increases the surface activity, hydrophobicity, and charge. Potential applications of sonication may include maneuvering of protein−phospholipid and protein−protein interactions in cells and cell membranes, and alteration of functional properties such as stabilization of lipid and gas interfaces in emulsions, foams, and gels. Stadnik, Dolatowski,

and Baranowska, (2008) studied the effect of low-intensity sonication on the structural, water-holding capacity (WHC), and water compartmentalization changes in beef muscle (*m. semimembranosus*) was also investigated, showing that ultrasound treatment can accelerate the aging process.

As noted above, ultrasound has been used for many years in the study of proteins (Conway & Verrall, 1966; Owen & Simons, 1957; Pavlovskaya, McClements, & Povey, 1992; Suzuki, Tamura, & Mihashi, 1996). These studies have been used to estimate protein hydration and to infer changes in protein conformation. The latest parameters may be related to the functional properties of proteins in foods such as solubility, foaming capacity, and flexibility (Gekko & Yamagami, 1991). One of the first applications of power ultrasound was in emulsification (Wood & Loomis, 1927). This application, also based on cavitation, provides a very effective, stable, and homogenized mixing of two or more immiscible liquids without or with very few additives (Mason, Paniwnyk, & Lorimer, 1996). The great advantage of emulsification is that it can be used online for flow processes. Thus volumes up to 12,000 l/h can be processed, as is the case in the manufacture of fruit juices, tomato ketchup, and mayonnaise (Mason et al., 1996). Furthermore, high-intensity ultrasound enhances protein solubility by changing protein conformation and structure in such a way that hydrophilic parts of AA are opened toward water molecule (Moulton & Wang, 1982). In addition, Guzey (2001) reported that high-intensity ultrasonic processing improves emulsifying properties of whey-protein isolate.

The effects of power ultrasound on milk homogenization have been studied by various authors (Villamiel & de Jong, 2000; Wu, Hulbert, & Mount, 2001). It was found that sonication of fresh cow milk at 20 kHz resulted in a reduction in the size of fat globules. In particular, power ultrasound was shown to be very effective at reducing fat globule size; an average size of less than 1 μm was achieved. The application of power ultrasound also results in more uniformly distributed fat particles than the conventional homogenization method. Thus milk homogenized by the conventional homogenizer has smaller fat globules than nonhomogenized milk and milk homogenized by power ultrasound also has smaller fat globules than milk homogenized by a conventional homogenizer.

However, from the few data on negative effects, it should be noted that most of the negative effects reported in the literature could be due to experiments being carried out with an inappropriate combination of the ultrasonic parameters for the given application such as the intensity and frequency of the power ultrasound, the exposure time, the type of treatment, temperature, type of food, and volume of food to be processed. Villamiel and de Jong (2000) reported that ultrasound promotes protein denaturation. Jambrak et al. (2008), studying the effect of ultrasound treatment on solubility and foaming properties of whey-protein suspensions, concluded that although using power ultrasound in food processing can lead to advantages such as increased protein solubility, and foaming ability, disadvantages may arise when using power ultrasound, for a given frequency, without testing the right power for the treatment time, which may lead to a destructive effect of ultrasound-like protein denaturation. Milk is one of the food products more widely studied in order to analyze its functional properties after processing with power ultrasound (Bermúdez-Aguirre & Barbosa-Cánovas, 2008; Muthukumaran, Kentish, Ashokkumar, & Stevens, 2005; Villamiel & de Jong, 2000; Wu et al., 2001).

Vitamin and color changes are also of great importance. For instance, color is a key factor influencing consumer sensory acceptance. Muthukumaran et al. (2005) sonicated various whey solutions for up to 4 h and then the soluble-protein contents of the resulting solutions were analyzed using

high-performance liquid chromatography, showing identical concentration profiles in all samples. This result is in accordance with the findings of other works. However, Villamiel and de Jong (2000) did find some evidence for the denaturation of whey solutions in excess of 60°C. Muthukumaran et al. (2005) concluded that sonication does not appear to change the protein concentration profile within the whey solution when using low temperatures (22−25°C) and power levels (2 W/l). Similar observations were noted by Bermúdez-Aguirre and Barbosa-Cánovas (2008), who reported minor changes in physicochemical and nutritional properties, and no degradation of protein content or color variation was observed after treatments.

2.4.4 HIGH-INTENSITY PEFS

As noted previously, with increasing demand to obtain processed foods with better attributes than have been available to date, food researchers have pursued the discovery and development of improved preservation processes with minimal impact on the fresh taste, texture, and nutritional value of food products. Different processed food products may present a series of undesirable effects when treated by conventional, thermal methods of pasteurization. Alternatives to traditional treatment that do not involve direct heat have been investigated in order to obtain safe processed food products, but with sensory attributes resembling those of the fresh raw material. Several processing techniques have been investigated recently and include ultraviolet irradiation, gamma irradiation, ultrasound treatment, treatment with nonconventional chemical reagents, application of high-intensity magnetic fields, ultrahigh-pressure processing, use of membrane technology, and application of high-voltage/intensity PEF. Commercial applications of some alternative technologies are varied and include purification of water, pasteurization of fruit juices, and processing of milk. Application of these nonthermal technologies offers interesting opportunities for mildly processed safe products with preserved sensory and nutritional qualities. Although nonthermal technologies such as PEF have been demonstrated to have some advantages over conventional thermal technologies, they have only recently started gaining momentum in the food industry (Ortega-Rivas, 2012).

PEF processing involves the application of an externally generated electric field (typically 20−80 kV/cm) across a food product, with the intent of inactivating pathogenic microorganisms, modifying enzymes, intensifying some processes, or achieving some specific transformation in the product. The technology has long been used for cell hybridization and electrofusion in genetic engineering and biotechnology. Its application is based on the transformation or rupture of cells under a sufficiently high external electric field, resulting in increased permeability and electrical conductivity of the cellular material. When a cell is exposed to high-voltage electrical pulses, its lipid bilayer and proteins are temporarily destabilized and perforation ensues, but if the electric field is removed, the pores reseal themselves. If the intensity of the electric fields is too high, however, the microorganism will not be able to repair itself and will start leaking small compounds or even undergo lysis (Amiali, Ngadi, Raghavan, & Smith, 2006).

There are few reports on the effects of PEF on the protein components of food. Published studies mainly concern egg and milk proteins. Jeantet, Baron, Nau, Roignant, and Brule (1999) reported that PEF treatment (from 20 to 35 kV/cm strength, 100−900 Hz frequency, and 2−8 pulse number) does not induce denaturation of diaultrafiltered egg-white protein by measuring surface hydrophobicity. Fernandez-Diaz, Barsotti, Dumay, and Cheftel (2000) indicated that high-strength PEF

(31.5 kV/cm) does not cause significant or permanent conformation modification of ovalbumin protein in solution, and the same PEF were applied to dialyzed egg white without any protein precipitation or marked alterations of gelling properties. Perez and Pilosof (2004) observed that the structures of egg-white protein and ß-lactoglobulin are partially modified when subjected to long-pulse width and high-strength PEF by measuring thermal denaturation temperatures and the gelation rate. Barsotti, Dumay, Mu, Diaz, and Cheftel (2001) reported that PEF of 200 exponential decay pulses does not cause marked unfolding and aggregation of ß-lactoglobulin. However, when milk was subjected to long-duration pulses (Perez & Pilosof, 2004), or high-intensity electric fields (45−55 kV/cm) as described by Floury et al. (2005), the structure of the milk protein was apparently modified.

2.4.5 IRRADIATION (IONIZING RADIATION)

Radiation is a form of energy traveling through space as radiant energy in a wave pattern. Such an energy form can occur naturally (e.g., from the sun or rocks) or can be produced by manmade objects, such as by various electrical household appliances. The frequency or wavelength of the energy waves produced by different sources distinguishes the different types and functionality of the radiation. High-frequency radiation, e.g., gamma rays, X-rays, or UV light, poses a risk to human health.

Radiation is called ionizing radiation when it has sufficiently high frequency, such as that of gamma rays and X-rays, so that it results in the production of charged particles or ions in the material that it comes in contact with. Nonionizing radiation, such as microwaves or infrared light, does not produce ions but can create heat under moist conditions and is routinely used for purposes such as cooking and reheating of foods (Ortega-Rivas, 2012).

Ionizing radiation inactivates foodborne pathogens in food products without heat and therefore is often called "cold" pasteurization or sterilization depending on radiation dose (Jaczynski & Park, 2003a). Although irradiation is an effective means to extend the shelf-life of food products, indirect effects such as accelerated lipid oxidation, vitamin destruction, and some protein denaturation limits its application in food processing (Al-Kahtani et al., 1998; Ghadi & Venugopal, 1991; Rahman, 1999). For instance, the effects of radiation on fish-muscle proteins have been studied extensively and depend on the radiation dose (Jaczynski & Park, 2003b, 2004). Proteins treated with ionizing energy are degraded into smaller molecules that upon digestion yield the same AA as the original proteins. Gamma radiation affects the viscosity, solubility, and stability of fish-muscle proteins.

Radiation can affect all the major components of food, i.e., proteins and lipids (Stewart, 2001). However, it should be noted that even at the high doses used for sterilization, the changes are small and similar to those produced by other food-processing technologies such as pasteurization. However, other researchers have shown that irradiation can affect protein or protein-based food matrices (Bhattacharjee & Singhal, 2010; Köksel, Sapirstein, Celik, & Bushuk, 1998).

Irradiation at 1 kGy was shown to decrease gluten viscosity of commercial Mexican bread-making wheat flour (Arvizu et al., 2006). Other studies have shown the effects of gamma irradiation on the rheological behavior of mixtures of proteins (soy, caseinates, and whey) and glycerol, wherein a decrease in viscosity was observed with irradiation dose. Similar behavior was also observed for dispersions containing calcium caseinates and glycerol. At a 2:1 ratio of proteins:glycerol, 21%, 35%, and 40% reductions in viscosity were observed at 5, 15, and 25 kGy, respectively; the

corresponding values for a 1:1 ratio were 23%, 33%, and 37%. However, for these non-Newtonian dispersions, the apparent viscosities were unaffected by irradiation. Sodium caseinate shows a trend toward formation of aggregation of macromolecules at 5 kGy (Sabato & Lacroix, 2002).

The viscosity of liquid egg white decreases dramatically on irradiation regardless of irradiation doses used. The egg white becomes watery even after irradiating the shell eggs at 1.0 kGy. Ovomucin is one of the major proteins in egg white and plays an important role in its gel-like structure. Irradiation causes changes in carbohydrate and protein moieties involved in formation of ovomucin complex, resulting in a loss of gel-like structure. Irradiation may also decrease the viscosity of egg white, which is an important physical change in egg and may be useful in egg processing. Watery egg white will facilitate the separation of egg white and yolk and low viscosity can improve the flow of liquid egg white or liquid whole egg in plant facilities that break eggs (Min et al., 2005).

Several studies have suggested that irradiation below 3.5 kGy does not affect the gelation properties of liquid egg white significantly. The hardness, springiness (elasticity), cohesiveness, gumminess, and chewiness of irradiated eggs are not very different from their nonirradiated counterparts. Sensory analysis is also unable to detect any texture differences between irradiated and nonirradiated hard-cooked egg whites. Therefore, it has been suggested that irradiation of shell eggs below 2.0 kGy does not alter the thermal characteristics of egg-white proteins. However, if used for pasteurizing liquid eggs, irradiation can improve the efficiency of egg-processing steps such as adding or mixing for removal of sugars and for spray drying (Min et al., 2005).

2.5 INNOVATIVE TECHNOLOGIES FOR THE EXTRACTION OF PROTEINS FROM DIFFERENT FOOD SOURCES

As is known, proteins, together with peptides, are one of the major groups of food components, and they are found in many different organisms of both vegetal and animal origin. Peptides are also obtained during technological processes such as fermentation and storage of foods. Moreover, many experiments involve enzymatic hydrolysis of proteins from food resources such as milk, meat, fish, eggs, or plants to produce a variety of peptides (Minkiewicz et al., 2008). A wide variety of extraction tools for proteins and peptides are available based on their physicochemical and structural characteristics such as solubility, hydrophobicity, molecular weight, and isoelectric point (pI). Generally, different technologies focused on cell disruption, solubilization/precipitation, and enrichment systems are needed to obtain the protein fraction of interest. Removal of interfering compounds (mainly lipids, carbohydrates, pigments, etc.) is crucial. These procedures need to be optimized to minimize protein modification and proteolysis, as well as to be compatible with subsequent analysis (Martínez-Maqueda, Hernández-Ledesma, Amigo, Miralles, & Gómez-Ruiz, 2013). This section briefly describes the state-of-the-art of extraction and fractionation techniques for food proteins and peptides.

Mechanical homogenization can be used for any biological material, but in the case of plant tissues, where cells are covered with strong cell walls, mechanical homogenization seems to be one of the best methods for cell disruption and protein extraction (Van Het Hof et al., 2000). Anderson and Guraya (2001) evaluated the use of colloid milling and homogenization to extract rice protein and breakdown the bran. They demonstrated that the shearing actions of colloid milling and

homogenization did not result in any significant denaturation of the proteins. Sometimes a combination of mechanical homogenization with buffers is used. Examples of this are found in rice (Fukuda et al., 2003) and in olive-tree seeds (Alche, Jiménez-López, Wang, Castro-López, & Rodriguez-García, 2006). Wet milling is a physicochemical separation of the components of grain, namely, germ, bran, fiber, starch, and protein. Chemicals and enzymes can be added to the steeping water to facilitate the separation of grain components and to increase starch recovery (De Mesa-Stonestreet, Alavi, & Bean, 2010).

Ultrasound-assisted crystallization is another method that can be used for enzyme and protein extraction (Galanakis, 2012). Ultrasonic extraction is based on cavitation phenomena and has become an efficient alternative to traditional methods for solvent extraction (Karki, 2009). Cavitation breaks down biological cell walls and releases cell contents into the solvent (Mason et al., 1996). Wang (1975) studied protein extraction from defatted soybeans using a 550 W probe operating at a 20 kHz frequency, which resulted in a more efficient way to extract than any other technology. Later, the experiment was scaled up to pilot-plant level for the extraction of soybean protein (Moulton & Wang, 1982). In addition, Karki (2009) evaluated the effect of high-power ultrasound on the overall extractability of soy proteins and soy protein isolate yield and functional properties. He reported that because of the cavitation phenomenon occurring during ultrasound treatment, a change in protein native state was expected to occur, which would affect the functional properties of the soy protein isolate. Ultrasound is one of the best methods for whey-protein extraction. Whey is the liquid resulting from the coagulation of milk and is generated from cheese manufacture. Indeed, it is a potent pollutant with a biological oxygen demand of 35−45 kg/L, e.g., 4000 L of whey, the output of a small creamery, and has the polluting strength of the sewage of 1900 people. Disposal of whey by dumping into rivers frequently occurred in the United States before environmental regulations took hold. Today whey is evolving into a sought-after product because of the lactose, minerals, and proteins it contains as well as the functional properties it imparts to food. The health benefits of whey led to the development of processes to isolate solids by concentration and drying. Initial industrial attempts at concentrating and drying whey occurred in the 1920s and involved four different methods: conventional hot-roller milk driers; heating until a concentrated liquid was obtained, cooling to solidification, and then extruding in a tunnel; two-stage steam heating; and a combination of spray drying and rotary drum drying. The high cost of the process and the hygroscopic nature of the lactose in the dry product prevented much progress from being made. Roller drying, in which whey is dried on the surface of a hot drum and removed by a scraper, is still used by some processors as part of whey-powder production. Until the 1970s whey protein was available only in the heat-denatured form, a water-insoluble, gritty, yellowish-brown powder that found limited use. Membrane filtration then arrived, which allowed for the separation and fractionation of whey proteins while retaining their solubility. To accelerate whey-proteins removal, ultrasound-assisted crystallization has been used. As a result, lactose aqueous two-phase separation is accomplished. This technique is very useful for the recovery of proteins and enzymes from crude-cell extracts, and it has recently been employed for the partitioning of whey β-lactoglobulin and a-lactalbumin as well as for the isolation of citrus ascorbic acid (Galanakis, 2012).

The use of HP for the extraction of food proteins has been investigated recently. Higher pressures (40 and 80 MPa) produced approximately double-protein extraction compared to atmospheric pressure. Dong et al. (2011) suggested that HP treatment could increase the susceptibility of peanut proteins to proteolytic enzymes such as alcalase. The increase may be related to the denaturation, unfolding, or dissociation of the proteins into monomers, allowing the accessibility of enzyme to

the binding sites. High pressure revealed no alteration of protein solubility when compared with the raw protein with pH adjusted in rapeseed protein concentrates (Barbin, Natsch, & Müller, 2011).

Temperature treatments include the use of freeze−thaw and heat treatments. Freeze thawing uses the effect of ice-crystal formation in the tissue during the freezing process. Lysis of the cells or tissues is usually achieved by flash freezing the cells in liquid nitrogen and homogenizing in a mortar with a pestle. Examples of this process are found in the analysis of leaves (Wang et al., 2003), fruits (Song, Braun, Bevis, & Doncaster, 2006), and seeds (Liang, Luo, Holbrook, & Guo, 2006; Méchin, Thévenot, Le Guilloux, Prioul, & Damerval, 2007). Vincent, Wheatley, and Cramer (2006) developed a very efficient cell disruption method for grape-berry clusters, which were pulverized frozen with dry ice using a stainless-steel blender. The use of heat is common in protein processing. Heating protein solutions usually improves their solubility, emulsifying, and foaming properties, but it makes protein extraction more difficult as reported for rice bran (Khan et al., 2011; Tang, Hettiarachchy, & Shellhammer, 2002). Another approach is the application of heat during the wet-milling process. Steeping experiments have been done on temperature and holding time on sorghum grain (De Mesa-Stonestreet et al., 2010).

The *isoelectric solubilization/precipitation* (ISP) of muscle proteins with concurrent separation of lipids is a relatively new technology and can be used to efficiently recover functional proteins from fish-processing by-products and low-value muscle foods (Tahergorabi & Jaczynski, 2014). While muscle proteins are in soluble form, the insoluble components (bone, skins, scales, etc.) can be removed from solution by centrifugation, e.g., followed by protein precipitation at their isoelectric point (pH 5.5) and collection (Fig. 2.1). Isoelectric solubilization/precipitation allows efficient recovery of muscle proteins that retain gel-forming ability and concurrent separation of omega-3-rich oil. Isoelectric solubilization/precipitation processing in a continuous instead of batch mode has been applied to fish-processing by-products (Chen, Tou, & Jaczynski, 2007), krill (Chen, Tou & Jaczynski, 2009), and whole gutted carp (Taskaya, Chen, Beamer, & Jaczynski, 2009; Taskaya, Chen, & Jaczynski, 2009).

2.6 INDUCED SENSORY CHARACTERISTICS OF FOOD PROTEINS

The sensory attributes of foods such as texture, flavor, color, and appearance play a major role in food acceptance by consumers. These attributes are the net effect of complex interactions among various components of food. Proteins generally have a great influence on the sensory attributes of foods. For example, the sensory properties of bakery products are related to the viscoelastic and dough-forming properties of wheat gluten; the textural and succulence characteristics of meat products are largely dependent on muscle proteins (actin, myosin, actomyosin, and several water-soluble meat proteins); the textural and curd-forming properties of dairy products are due to the unique colloidal structure of casein micelles; and the structure of some cakes and the whipping properties of some dessert products depend on the properties of egg-white proteins.

The sensory attributes of foods are achieved by complex interactions among various functional ingredients. For instance, the sensory attributes of a cake emanate from the gelling/heat setting, foaming, and emulsifying properties of the ingredients used. Therefore for a protein to be useful as an ingredient in cakes and other such products, it must possess multiple functionalities.

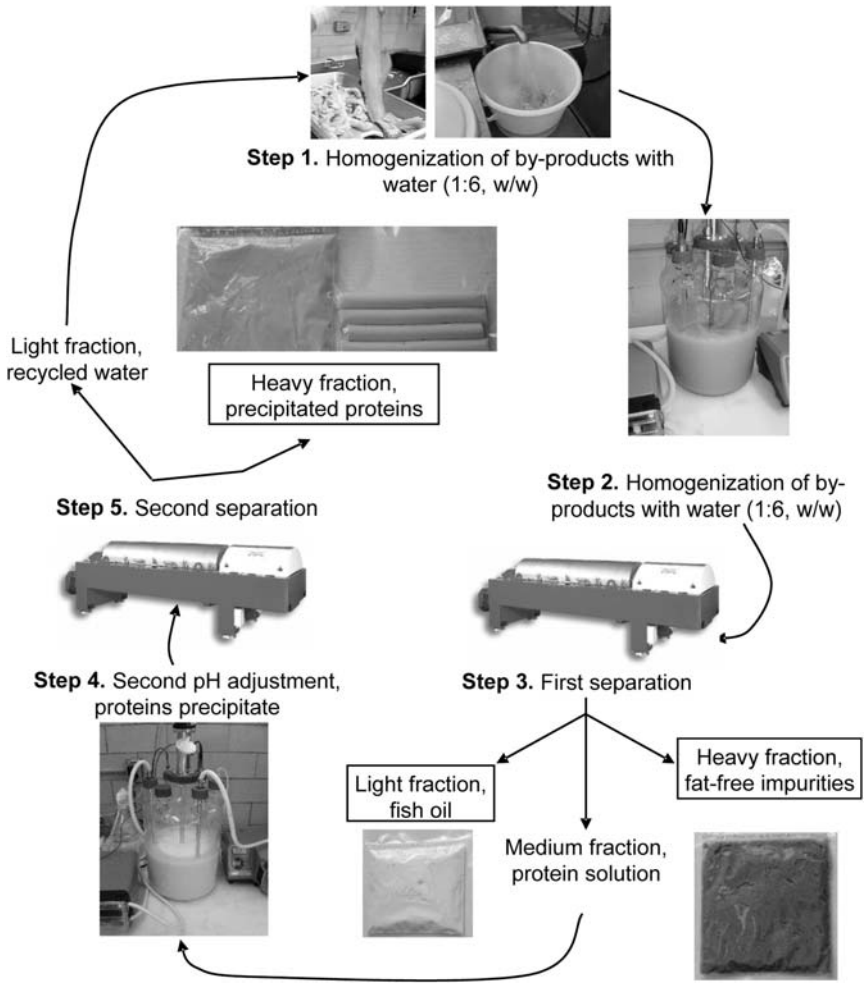


FIGURE 2.1

Diagram of the isoelectric precipitation and solubilization technology with concurrent oil separation proposed for processing fish by-products. *Note*: The materials in boxes are fractions to be further processed into food and other applications.

Adapted from Torres, J.A., Chen, Y.C., Rodrigo-Garcia, J., & Jaczynski, J. (2007). Recovery of by-products from seafood processing streams. In F. Shahidi (Ed.), Maximising the Value of Marine By-Products *(pp. 65–90). Woodhead publishing limited, Cambridge, UK.*

Proteins of animal origin, such as milk (caseins), egg, and meat proteins are widely used in fabricated foods. These proteins are mixtures of several proteins with wide-ranging physicochemical properties, and they are capable of performing multiple functions. For example, egg white has multiple functionalities such as gelation, emulsification, foaming, water binding, and heat coagulation,

which makes it a highly desirable protein in many foods. The multiple functionalities of egg white are due to complex interactions among its protein constituents, namely, ovalbumin, conalbumin, lysozyme, ovomucin, and other albumin-type proteins. Plant proteins (e.g., soy and other legume and oilseed proteins) and other proteins such as whey proteins are used to a limited extent in conventional foods. Even though these proteins are also mixtures of several proteins, they do not perform as well as animal proteins in most food products. The exact molecular properties of proteins responsible for the various desirable functionalities in food are poorly understood.

Free AA can contribute to the flavor of protein-rich foods in which hydrolytic processes occur (e.g., meat, fish, or cheese). Taste quality is influenced by the molecular configuration: sweet AA are primarily found among members of the D-series, whereas bitter AA are generally within the L-series. Consequently AA with a cyclic side chain (1-aminocycloalkane-1-carboxylic acids) are sweet and bitter. The taste intensity of a compound is reflected in its recognition threshold value. The recognition threshold value is the lowest concentration needed to recognize the compound reliably, as assessed by a taste panel. It has been shown that the taste intensity of AA is dependent on the hydrophobicity of the side chain. L-tryptophan and L-tyrosine are the bitterest AA with a threshold value of ct bitter $= 4 - 6$ mmol/L. D-tryptophan, with ct sweet $= 0.2 - 0.4$ mmol/L, is the sweetest AA. A comparison of these threshold values with those of caffeine (ct bi $= 1 - 1.2$ mmole/L) and sucrose (ct sw $= 10 - 12$ mmol/L) showed that caffeine is about 5 times as bitter as L-tryptophan and that D-tryptophan is about 37 times as sweet as sucrose. L-glutamic acid has an exceptional position. In higher concentrations it has a meat broth flavor, while in lower concentrations it enhances the characteristic flavor of a given food (flavor enhancer, cf. 8.6.1). L-methionine has a sulfur-like flavor. The bitter taste of the L-AA can interfere with the utilization of these acids, e.g., in chemically defined diets (Belitz, Grosch, & Schieberle, 2009).

2.7 CONCLUSION

Probably more than any other food component, protein is essential to human nutrition. Therefore a thorough understanding of protein functionality and its nutritional characteristics is needed. Furthermore, developing new foods involves different processing methods. Many new and emerging technologies have been introduced to the food industry but their effects on different components of food including protein are not well established. By either developing new and innovative processing methods and novel products or just reformulating existing ones, proteins enable manufacturers to meet and exceed the expectations of today's health-conscious consumer.

REFERENCES

Alche, J. D., Jiménez-López, J. C., Wang, W., Castro-López, A. J., & Rodriguez-García, M. I. (2006). Biochemical characterization and cellular localization of 11S type storage proteins in olive (*Olea europaea*) seeds. *Journal of Agricultural and Food Chemistry*, *54*, 5562–5570.

Al-Kahtani, H. A., Abu-Tarboush, H. M., Atia, M., Bajaber, A. S., Ahmed, M. A., & El-Mojaddidi, M. A. (1998). Amino acid and protein changes in tilapia and Spanish mackerel after irradiation and storage. *Radiation Physics and Chemistry*, *51*(1), 107–114.

Amiali, M., Ngadi, M. O., Raghavan, G. S. V., & Smith, J. P. (2006). Inactivation of *Escherichia coli* O157: H7 and *Salmonella enteritidis* in liquid egg white using pulsed electric field. *Journal of Food Science*, *71*, 88−94.

Anderson, A. K., & Guraya, H. S. (2001). Extractability of protein in physically processed rice bran. *Journal of the American Oil Chemists' Society*, *78*, 969−973.

Arvizu, Z. A., Fernández-Ramírez, M. V., Arce-Corrales, M. E., Cruz-Zaragoza, E., Melédrez, R., Chernov, V., & Barboza-Flores, M. (2006). Gamma irradiation effects on commercial Mexican bread making wheat flour. *Nuclear Instruments and Methods in Physics Research B*, *245*, 455−458.

Ashie, I. N. A., & Lanier, T. C. (1999). High pressure effects on gelation of surimi and turkey breast muscle enhanced by microbial transglutaminase. *Journal of Food Science*, *64*(4), 704−708.

Barbin, D. F., Natsch, A., & Müller, R. K. (2011). Improvement of functional properties of rapeseed protein concentrates produced via alcoholic processes by thermal and mechanical treatments. *Journal of Food Processing and Preservation*, *35*, 369−375.

Barsotti, L., Dumay, E., Mu, T. H., Diaz, M. D. F., & Cheftel, J. C. (2001). Effects of high voltage electric pulses on protein-based food constituents and structures. *Trends in Food Science and Technology*, *12*(3), 136−144.

Batista, L., Monteiro, S., Loureiro, V. B., Teixeira, A. R., & Ferreira, R. B. (2010). Protein haze formation in wines revisited. The stabilising effect of organic acids. *Food Chemistry*, *122*, 1067−1075.

Belitz, H. D., Grosch, W., & Schieberle, P. (2009). *Food chemistry, 4th revised and extended edn*. Berlin (DE): Springer.

Bender, A. E. (1972). Processing damage to protein food. A review. *Journal of Food Technology*, *7*, 239−250.

Benedito, J., Carcel, J. A., González, R., & Mulet, A. (2002). Application of low intensity ultrasonics to cheese manufacturing processes. *Ultrasonics*, *40*, 19−23.

Benito, P., & Miller, D. (1998). Iron absorption and bioavailability: an updated review. *Nutrition Research*, *18*, 581−603.

Bermúdez-Aguirre, D., & Barbosa-Cánovas, G. V. (2008). Study of butter fat content in milk on the inactivation of *Listeria innocua* ATCC 51742 by thermo-sonication. *Innovative Food Science and Emerging Technologies*, *9*, 176−185.

Bhattacharjee, P. ,, & Singhal, R. S. (2010). Effect of irradiation on food texture and rheology. In J. Ahmed, H. S. Ramaswamy, S. Kasapis, & J. I. Boye (Eds.), *Novel food processing (effects on rheological and functional properties)*. Boca Raton, FL: Taylor & Francis Group.

Biss, C. H., Coombes, S. A., & Skudder, P. J. (1989). The development and application of ohmic heating for the continuous heating of particulate foodstuffs. In R. W. Field, & J. A. Howell (Eds.) (Eds.), *Process engineering in the food industry* (pp. 17−260)). London: Elsevier Applied Science, Chap. 2.

Butz, P., Edenharder, R., Fernández-García, A., Fister, H., Merkel, C., & Tauscher, B. (2002). Changes in functional properties of vegetables induced by high pressure treatment. *Food Research International*, *35*, 295−300.

Carbonell-Capella, J. M., Buniowska, M., Barba, F. J., Esteve, M. J., & Frìgola, A. (2014). Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: a review. *Comparative reviews in Food Science and Food Safety*, *13*, 155−171.

Chen, Y. C., Tou, J. C., & Jaczynski, J. (2007). Amino acid, fatty acid, and mineral profiles of materials recovered from rainbow trout (*Oncorhynchus mykiss*) processing byproducts using isoelectric solubilization/precipitation. *Journal of Food Science*, *72*(9), C527−C535.

Chen, Y. C., Tou, J. C., & Jaczynski, J. (2009). Amino acid and mineral composition of protein and other components and their recovery yields from whole Antarctic krill (*Euphausia superba*) using isoelectric solubilization/precipitation. *Journal of Food Science*, *74*(2), H31−H39.

Chicón, R., Belloque, J., Alonso, E., & López-Fandiño, R. (2008). Immunoreactivity and digestibility of highpressure-treated whey proteins. *International Dairy Journal*, *18*(4), 367−376.

Chicón, R., Belloque, J., Recio, I., & López-Fandiño, R. (2006). Influence of high hydrostatic pressure on the proteolysis of β-lactoglobulin A by trypsin. *Journal of Dairy Research*, *73*, 121−128.

Cho, H. Y., Yousef, A. E., & Sastry, S. K. (1996). Growth kinetics of *Lactobacillus acidophilus* under ohmic heating. *Biotechnology and Bioengineering*, *49*(3), 334−340.

Conway, B. E., & Verrall, R. E. (1966). Partial molar volumes and adiabatic compressibilities of tetraalkylammonium and aminium salts in water. I. Compressibility behavior. *The Journal of Physical Chemistry*, *70*(12), 3952−3961.

Damodaran, S. (2008). Amino acids, peptides, and proteins. In S. Damodaran, K. L. Parkin, & O. R. Fennema (Eds.), *Fennema's food chemistry* (4th, ed., p. 268). Boca Raton, FL: CRC Press, Taylor and Francis Group.

De Mesa-Stonestreet, N. J., Alavi, S., & Bean, S. R. (2010). Sorghum proteins: The concentration, isolation, modification, and food applications of kafirins. *Journal of Food Science*, *75*, R90−R104.

Dong, X., Zhao, M., Shi, J., Yang, B., Li, J., Luo, D., & Jiang, Y. , et al (2011). Effects of combined high pressure homogenization and enzymatic treatment on extraction yield, hydrolysis and function properties of peanut proteins. *Innovative Food Science and Emerging Technologies*, *12*, 478−483.

Dupas, C., Baglieri, A. M., Ordonaud, C., Tom, D., & Maillard, M. N. (2006). Chlorogenic acid is poorly absorbed, independently of the food matrix: a Caco-2 cells and rat chronic absorption study. *Molecular Nutrition and Food Research*, *50*, 1053−1060.

Dzwolak, W., Kato, M., & Taniguchi, Y. (2002). Fourier transform infrared spectroscopy in high-pressure studies on proteins. *Biochimica et Biophysica Acta (BBA)—Molecular and Cell Biology of Lipids*, *1595*, 131−144.

Elgasim, E. A., & Kennick, W. H. (1980). Effect of pressurization of pre-rigor beef muscles on protein quality. *Journal of Food Science*, *45*, 1122−1124.

FAO/WHO/UNU (2001). *Energy and protein requirements*. Geneva: WHO, *Report of a Joint FAO/WHO/UNU expert consultation. World Health Organization technical rep. ser. 724*.

Fernandez-Diaz, M. D., Barsotti, L., Dumay, E., & Cheftel, J. C. (2000). Effects of pulsed electric fields on ovalbumin solutions and dialyzed egg white. *Journal of Agricultural and Food Chemistry*, *48*(6), 2332−2339.

Fernàndez-Garcìa, E., Carvajal-Lérida, I., & Pérez-Gàlvez, A. (2009). In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutrition Research*, *29*, 751−760.

Fidalgo, L., Saraiva, J., Aubourg, S., Vázquez, M., & Torres, A. (2014). Effect of high pressure pre-treatments on enzymatic activities of Atlantic mackerel (*Scomber scombrus*) during frozen storage. *Innovative Food Science and Emerging Technologies*, *23*, 18−24.

Floury, J., Grosset, N., Leconte, N., Pasco, M., Madec, M., & Jeantet, R. (2005). Continuous raw skim milk processing by pulsed electric field at nonlethal temperature: effect on microbial inactivation and functional properties. *Lait*, *86*, 43−57.

Fukuda, M., Islam, N., Woo, S. H., Yamagishi, A., Takaoka, M., & Hirano, H. (2003). Assessing matrix assisted laser desorption/ionization-time of flight-mass spectrometry as a means of rapid embryo protein identification in rice. *Electrophoresis*, *24*, 1319−1329.

Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: conventional, emerging technologies and commercialized applications. *Trends in Food Science and Technology*, *26*(2), 68−87.

Gekko, K., & Yamagami, K. (1991). Flexibility of food proteins as revealed by compressibility. *Journal of Agricultural and Food Chemistry*, *39*(1), 57−62.

Ghadi, S. V., & Venugopal, V. (1991). Influence of γ-irradiation and ice storage on fat oxidation in three Indian fish. *International Journal of Food Science and Technology*, *26*(4), 397−401.

Guzey, D. (2001). *High-intensity ultrasonic processing improves emulsifying properties of proteins*. Colloidal and Interfacial Food Science Laboratory, Department of Food Science and Technology, The University of Tennessee, Knoxville, TN, J. Weiss.

Heaney, R. P. (2001). Factors influencing the measurement of bioavailability, taking calcium as a model. *Journal of Nutrition*, *131*(4), 1344–1348.

Hendrickx, M. E., Ludikhuyze, L. R., van den Broeck, I., & Weemaes, C. A. (1998). Effects of high-pressure on enzymes related to food quality. *Trends of Food Science and Technology*, *9*, 197–203.

Jaczynski, J., & Park, J. W. (2003a). Microbial inactivation and electron penetration in surimi seafood during electron beam processing. *Journal of Food Science*, *68*(5), 1788–1792.

Jaczynski, J., & Park, J. W. (2003b). Physicochemical properties of surimi seafood as affected by electron beam and heat. *Journal of Food Science*, *68*(5), 1626–1630.

Jaczynski, J., & Park, J. W. (2004). Physicochemical changes in Alaska pollock surimi and surimi gel as affected by electron beam. *Journal of Food Science*, *69*(1), FCT53–FCT57.

Jambrak, A. R., Mason, T. J., Lelas, V., Herceg, Z., & Herceg, I. L. (2008). Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. *Journal of Food Engineering*, *86*, 281–287.

Jeantet, R., Baron, F., Nau, F., Roignant, M., & Brule, G. (1999). High intensity pulsed electric fields applied to egg white: Effect on *Salmonella enteritidis* inactivation and protein denaturation. *Journal of Food Protection*, *62*(12), 1381–1386.

Jones, F. (1897). Apparatus for electrically treating liquids. US Patent 1, pp. 592–735.

Karki, B. (2009). *Use of high-power ultrasound during soy protein production and study of its effect on functional properties of soy protein isolate. Graduate Thesis and Dissertations*. USA: Iowa State University, 106p.

Khan, S. H., Butt, M. S., Sharif, M. K., Sameen, A., Mumtaz, S., & Sultan, M. T. (2011). Functional properties of protein isolates extracted from stabilized rice bran by microwave, dry heat, and parboiling. *Journal of Agricultural and Food Chemistry*, *59*, 2416–2420.

Köksel, H., Sapirstein, H. D., Celik, S., & Bushuk, W. (1998). Effects of gamma-irradiation of wheat on gluten proteins. *Journal of Cereal Science*, *28*, 243–250.

Konno, K., & Konno, Y. (2014). Highly elastic thermal gel production from squid mantle muscle by using Ohmic heating. *44th WEFTA meeting*, 9–11 June, Bilbao, Spain.

Lesser, S., Cermak, R., & Wolffram, S. (2006). The fatty acid pattern of dietary fat influences the oral bioavailability of the flavonol quercetin in pigs. *British Journal of Nutrition*, *96*(6), 1047–1052.

Liang, X. Q., Luo, M., Holbrook, C. C., & Guo, B. Z. (2006). Storage protein profiles in Spanish and runner market type peanuts and potential markers. *BMC Plant Biology*, *6*, 24–32.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, *79*, 727–747.

Marangon, M., Van Sluyter, S. C., Neilson, K. A., Chan, C., Haynes, P. A., Waters, E. J., & Falconer, R. J. (2011). Roles of thaumatin-like protein and chitinases in white wine formation. *Journal of Agricultural and Food Chemistry*, *59*, 733–740.

Martínez-Maqueda, D., Hernández-Ledesma, B., Amigo, L., Miralles, B., & Gómez-Ruiz, J. A. (2013). Extraction/fractionation techniques for proteins and peptides and protein digestion. In F. Toldrá, & L. M. L. Nollet (Eds.), *Proteomics in foods: principles and applications*. New York: Springer Science + Business Media. Food Microbiology and Food Safety 2, <http://dx.doi.org/10.1007/978-1-4614-5626-1_2>.

Mason, T. J., & Lorimer, J. P. (1988). *Sonochemistry: Theory, applications and uses of ultrasound in chemistry*. New York: John Wiley.

Mason, T. J., Paniwnyk, L., & Lorimer, J. P. (1996). The uses of ultrasound in food technology. *Ultrasonics Sonochemistry*, *3*, S253–S260.

Matthews, D. M., & Laster, L. (1965). Absorption of protein digestion products: A review. *Gut*, *6*(5), 411–426.

Méchin, V., Thévenot, C., Le Guilloux, M., Prioul, J. L., & Damerval, C. (2007). Developmental analysis of maize endosperm proteome suggests a pivotal role for pyruvate orthophosphate dikinase. *Plant Physiology*, *143*, 1213–1219.

Messens, W., Van Camp, J., & Huyghbaert, A. (1997). The use of high pressure to modify the functionality of food proteins. *Trends of Food Science and Technology*, *8*, 107–112.
Min, B. R., Nam, K. C., Lee, E. J., Ko, G. Y., Trampel, D. W., & Ahn, D. U. (2005). Effect of irradiating shell eggs on quality attributes and functional properties of yolk and white. *Poultry Science*, *84*, 1791–1796.
Minkiewicz, P., Dziuba, J., Darewicz, M., Iwaniak, A., Dziuba, M., & Nalecz, D. (2008). Food peptidomics. *Food Technology and Biotechnology*, *46*, 1–10.
Mizrach, A. (2008). Ultrasonic technology for quality evaluation of fresh fruit and vegetables in pre- and post-harvest processes. *Postharvest Biology and Technology*, *48*, 315–330.
Moulton, K. J., & Wang, L. C. (1982). A pilot-plant study of continuous ultrasonic extraction of soybean protein. *Journal of Food Science*, *47*, 1127–1129.
Muthukumaran, S., Kentish, S. E., Ashokkumar, M., & Stevens, G. (2005). Mechanisms for the ultrasonic enhancement of dairy whey ultrafiltration. *Journal of Membrane Science*, *258*, 106–114.
Naveh, D., Kopelman, I. J., & Mizrahi, S. (1983). Electroconductive thawing by liquid contact. *Journal of Food Technology*, *10*, 282–288.
Ortega-Rivas, E. (2012). *High-voltage pulsed electric fields. Nonthermal food engineering operations*. New York: Springer. Food Engineering Series. <http://dx.doi.org/10.1007/978-1-4614-2038-5_13>.
Owen, B. B., & Simons, H. L. (1957). Standard partial molal compressibilities by ultrasonics. I. Sodium chloride and potassium chloride at 25. *The Journal of Physical Chemistry*, *61*(4), 479–482.
Park, S. J., Kim, D., Uemura, K., & Noguchi, A. (1995). Influence of frequency on ohmic heating of fish protein gel. *Nippon Shokuhin Kagaku Kogaku Kaishi*, *42*(8), 569–574.
Pavlovskaya, G., McClements, D. J., & Povey, M. J. W. (1992). Ultrasonic investigation of aqueous solutions of a globular protein. *Food Hydrocolloids*, *6*(3), 253–262.
Pazos, M., Méndez, L., Gallardo, J. M., & Aubourg, S. (2014). Selective-targeted effect of high pressure processing on proteins related to quality: A proteomics evidence in Atlantic mackerel (*Scomber scombrus*). *Food and Bioprocess Technology*, *7*, 2342–2353.
Perez, O. E., & Pilosof, A. M. R. (2004). Pulsed electric fields on the molecular structure and gelation of b-lactoglobulin concentrate and egg white. *Food Research International*, *37*, 102–110.
Pocock, K. F., Alexander, G. M., Hayasaka, Y., Jones, P. R., & Waters, E. J. (2007). Sulfate—a candidate for the missing essential factor that is required for the formation of protein haze in white wine. *Journal of Agricultural and Food Chemistry*, *55*, 1799–1807.
Povey, M. J. W., & McClements, D. J. (1988). Ultrasonics in food engineering. Part I: Introduction and experimental methods. *Journal of Food Engineering*, *8*, 217–245.
Povey, M. J. W., & McClements, D. J. (1989). Ultrasonics in food engineering. Part II: Applications. *Journal of Food Engineering*, *9*, 1–20.
Quirós,, A., Chichón,, R., Recio,, I., & López-Fandino,, R. (2007). The use of high hydrostatic pressure to promote the proteolysis and release of bioactive peptides from ovalbumin. *Food Chemistry*, *104*, 1734–1739.
Rahman, M. S. (1999). Irradiation preservation of foods. In M. S. Rahman (Ed.) (Ed.), *Handbook of food preservation*. New York: Marcel Dekker Inc.
Resa, P., Bolumar, T., Elvira, L., Pérez, G., & Montero de Espinosa, F. (2007). Monitoring lactic acid fermentation in culture broth using ultrasonic velocity. *Journal of Food Engineering*, *78*, 1083–1091.
Sabato, S. F., & Lacroix, M. (2002). Radiation effects on viscosimetry of protein based solutions. *Radiation Physics and Chemistry*, *63*, 357–359.
Salazar, J., Turó, A., Chávez, J. A., & García, M. J. (2004). Ultrasonic inspection of batters for on-line process monitoring. *Ultrasonics*, *42*, 155–159.
Saura-Calixto, F., Serrano, J., & Goñi, I. (2007). Intake and bioaccessibility of total polyphenols in a whole diet. *Food Chemistry*, *101*, 492–501.
Sauvage, F.-X., Bach, B., Moutounet, M., & Vernhet, A. (2010). Proteins in white wines: Thermo-sensitivity and differential adsorption by bentonite. *Food Chemistry*, *118*, 26–34.

Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition*, *130*(8S suppl.), 2073S−2085SS.

Simal, S., Benedito, J., Clemente, G., Femenia, A., & Rosselló, C. (2003). Ultrasonic determination of the composition of a meat-based product. *Journal of Food Engineering*, *58*, 253−257.

Song, J., Braun, G., Bevis, E., & Doncaster, K. (2006). A simple protocol for protein extraction of recalcitrant fruit tissues suitable for 2-DE and MS analysis. *Electrophoresis*, *27*, 3144−3151.

Stadnik, J., Dolatowski, Z. J., & Baranowska, H. M. (2008). Effect of ultrasound treatment on water holding properties and microstructure of beef (m. semimembranosus) during ageing. *LWT-Food Science and Technology*, *41*(10), 2151−2158.

Stewart, E. M. (2001). Food irradiation chemistry. In R. Molins (Ed.), *Food irradiation: Principles and applications* (pp. 37−76)). New York: John Wiley & Sons.

Strasburg, G., Xiong, Y. L., & Chiang, W. (2008). Physiology and chemistry of edible muscle tissues. In S. Damodaran, K. L. Parkin, & O. R. Fennema (Eds.), Fennema's food chemistry (4th, ed.). Boca Raton, FL: CRC Press, Taylor and Francis Group, pp. 930−935 & 949−950).

Suzuki, N., Tamura, Y., & Mihashi, K. (1996). Compressibility and specific volume of actin decrease upon G to F transformation. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, *1292*(2), 265−272.

Tabilo-Munizaga, G., Gordon, T. A., Villalobos-Carvajal, R., Moreno-Osorio, L., Salazar, F. N., Pérez-Won, M., & Acuña, S. (2014). Effects of high hydrostatic pressure (HHP) on the protein structure and thermal stability of Sauvignon blanc wine. *Food Chemistry*, *18*, 214−220.

Tahergorabi, R., Hosseini, S. V., & Jaczynski, J. (2011). Seafood proteins. In G. O. Philips, & P. A. Williams (Eds.), *Handbook of Food Proteins* (pp. 116−143). Woodhead Publishing Limited.

Tahergorabi, R., & Jaczynski, J. (2014). Isoelectric solubilization/precipitation as a means to recover proteins and lipids from seafood byproducts. In S.-K. Kim (Ed.), *Seafood processing byproducts: trends and applications* (pp. 101−123). Springer.

Tang, S., Hettiarachchy, N. S., & Shellhammer, T. H. (2002). Protein extraction from heat-stabilized defatted rice bran. 1. Physical processing and enzyme treatments. *Journal of Agricultural and Food Chemistry*, *50*, 7444−7448.

Taskaya, L., Chen, Y. C., Beamer, S., & Jaczynski, J. (2009). Texture and colour properties of proteins recovered from whole gutted silver carp (*Hypophthalmichthys molitrix*) using isoelectric solubilisation/precipitation. *Journal of the Science of Food and Agriculture*, *89*(2), 349−358.

Taskaya, L., Chen, Y. C., & Jaczynski, J. (2009). Functional properties of proteins recovered from silver carp (*Hypophthalmichthys molitrix*) by isoelectric solubilization/precipitation. *LWT-Food Science and Technology*, *42*(6), 1082−1089.

The digestive system: Mechanism for nourishing the body In S. S. Gropper, & J. L. Smith (Eds.), *Advanced nutrition and human metabolism* Belmont: Wadsworth, (5th ed., pp. 33−62).

Torres, A., Saraiva, J., Guerra-Rodríguez, E., Aubourg, S., & Vázquez, M. (2014). Effect of combining high-pressure processing and frozen storage on the functional and sensory properties of horse mackerel (*Trachurus trachurus*). *Innovative Food Science and Emerging Technologies*, *21*, 2−11.

Torres, J. A., Chen, Y. C., Rodrigo-Garcia, J., & Jaczynski, J. (2007). Recovery of by-products from seafood processing streams. In F. Shahidi (Ed.), *Maximising the Value of Marine By-Products* (pp. 65−90). Cambridge, UK: Woodhead publishing limited.

van het Hof,, K. H., de Boer,, B. C., Tijburg,, L. B., Lucius,, B. R., Zijp,, I, West,, C. E., & Weststrate,, J. A. (2000). Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride- rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption. *Journal of Nutrition*, *130*, 1189−1196.

Villamiel, M., & de Jong, P. (2000). Influence of high-intensity ultrasound and heat treatment in continuous flow on fat, proteins, and native enzymes of milk. *Journal of Agriculture and Food Chemistry*, *48*, 472−478.

Vincent, D., Wheatley, M. D., & Cramer, G. R. (2006). Optimization of protein extraction and solubilization for mature grape berry clusters. *Electrophoresis*, *27*, 1853−1865.

Vollmer, A. C., Everbach, E. C., Halpern, M., & Kwakye, S. (1998). Bacterial stress responses to 1-megahertz pulsed ultrasound in the presence of microbubbles. *Applied Environmental Microbiology*, *64*(10), 3927−3931.

Wang, L. C. (1975). Ultrasonic extraction of proteins from autoclaved soybean flakes. *Journal of Food Science*, *40*, 549−551.

Wang, W., Scali, M., Vignani, R., Spadafora, A., Sensi, E., Mazzuca, S., & Cresti, M. (2003). Protein extraction for two-dimensional electrophoresis from olive leaf, a plant tissue containing high levels of interfering compounds. *Electrophoresis*, *24*, 2369−2375.

Wood, R. J. (2005). Bioavailability: definition, general aspects and fortificants. In B. Caballero, A. Prentice,, & L. Allen (Eds.), *Encyclopedia of human nutrition* (2nd ed.).). Oxford: Elsevier Ltd.

Wood, R. W., & Loomis, A. L. (1927). The physical and biological effects of high frequency sound waves of great intensity. *Philosophical Magazine*, *4*, 417−436.

Wu, H., Hulbert, G. J., & Mount, J. R. (2001). Effects of ultrasound on milk homogenization and fermentation with yogurt starter. *Innovative Food Science and Emerging Technologies*, *1*, 211−218.

Yin, S. W., Tang, C. H., Wen, Q. B., Yang, X. Q., & Li, L. (2008). Functional properties and *in vitro* trypsin digestibility of red kidney bean (*Phaseolus vulgaris* L.) protein isolate: Effect of high-pressure treatment. *Food Chemistry*, *110*(4), 938−945.

Yongsawatdigul, J., Park, J. W., Kolbe, E., Abu Dagga, Y., & Morrissey, M. T. (1995). Ohmic heating maximizes gel functionality of Pacific whiting surimi. *Journal of Food Science*, *60*, 10−14.

CHAPTER

CARBOHYDRATES 3

Lia Noemi Gerschenson[1,2], Ana Maria Rojas[1,2] and Eliana Noemi Fissore[1,2]

[1]Buenos Aires University (UBA), Buenos Aires, Argentina [2]National Research Council of Argentina (CONICET), Buenos Aires, Argentina

3.1 DIETARY CARBOHYDRATES

Dietary carbohydrates constitute a group of chemically defined substances with a range of physical and physiological properties and health benefits. As with other macronutrients, the primary classification of dietary carbohydrates is based on chemistry, i.e., in the character of individual monomers, degree of polymerization (DP), and type of linkage (α or β) (Cummings & Stephen, 2007).

In general, carbohydrate chains with a number of carbon atoms up to nine are water-soluble and include monosaccharides (D-glucose, D-fructose, D-galactose, L-xylose, D-mannose, L-arabinose), disaccharides (sucrose, lactose), and oligosaccharides such as those derived from β-glucans, mannan oligosaccharides (MOS), galactooligosaccharides (GOS), oligofructans, xylan oligosaccharides (XOS), dextrins, and short pectins.

Carbohydrate polymers with 10 or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans, are considered as dietary fiber compounds (Philips & Cui, 2011).

In this chapter, the effect on carbohydrate properties of the use of conventional and emerging technologies for their extraction and for food preservation are discussed, with special reference to carbohydrates having a carbon chain length up to nine carbon atoms and to inulin and starch.

3.2 CHARACTERISTICS OF CARBOHYDRATES

Carbohydrates constitute the most abundant class of organic compounds found in living organisms. They are products of photosynthesis: an endothermic reductive condensation of CO_2 by the chlorophyll pigments located into the chloroplasts (the specific organelles found in vegetable cells):

$$6\,CO_2 + 6\,H_2O \xrightarrow[\text{chlorophylls}]{\text{LIGHT}} C_6H_{12}O_6 + 6\,O_2$$

Nutraceutical and Functional Food Components. DOI: http://dx.doi.org/10.1016/B978-0-12-805257-0.00003-X

In this photolysis, chemical energy is simultaneously produced as adenosine triphosphate and nicotinamide adenine dinucleotide phosphate. This energy is needed by the cells for the synthesis of proteins, lipids, and other compounds. D-glucose is the parent sugar from which most carbohydrates are derived (Nelson & Cox, 2004). Like other monosaccharides, it is a hexose (fundamentally as an aldose ring) existing in the water medium. While natural monosaccharides are comprised of a single saccharide unit of 5-, 6-, or 7-carbon atom skeleton (aldoses or ketoses), the oligosaccharides are less clearly defined and may consist of 2−10 glycosidically linked monosaccharide units (Linhardt & Bazin, 2010). Disaccharides found in foods are nonreducing sucrose and reducing lactose. Sucrose is an α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside because it does not have a free carbonyl group. It is extracted and refined from sugar cane or from sugar-beet root. Lactose is a D-glucose molecule substituted by a D-galactosyl residue at its C4 (β-D-galactopyranosyl-(1→4)-D-glucose). It is the most abundant component of most mammalian milk. On the other hand, lactulose is a semisynthetic, nonabsorbable disaccharide derived from lactose, comprised of D-galactose and D-fructose (4-O-β-D-galactopyranosyl-β-D-fructofuranose). It is easily fermented by gut microorganisms, yielding high concentrations of short-chain fatty acids (SCFA) that lead to reduced pH.

Oligosaccharides are functional food ingredients that have great potential to improve the quality of food. They have been associated with many health-promoting functions identified in many clinical studies, such as promoting *Bifidobacterium* growth in human intestine, balancing intestinal bacteria, modulating immune response, inhibiting cancers and tumors as well as stimulating mineral absorption (Benito-Román, Alonso, & Cocero, 2013a).

In the EU (European Commission directive 2008/100/EC) and other countries worldwide, some oligosaccharides (DP 3-9), including arabinoxylan-oligosaccharide (AXOS), classify as dietary fiber (De Menezes, Giuntini, Dan, Sardá, & Lajolo, 2013). The health-related importance of dietary fiber, as part of a balanced diet, has been recognized for decades. More recently, soluble fiber such as β-(1→3,1→4)-D-glucans (known as β-glucans) has been shown to have effects on the glycemic level, insulin, and cholesterol responses to foods (Brennan & Cleary, 2005; Tiwari & Cummings, 2009). Conversely, the β-glucans in the cell walls of yeast and fungi consist of (1→3) linkages with a smaller number of (1→6) linked branches. β-glucan polysaccharides of 30−50 kDa molecular weights have also been extracted through hot water from *Agaricus blazei* Murill mushrooms by Kim, Kim, Choi, and Lee (2005). The hydrolysis of these compounds with an endo-β-(1→6)-glucanase gave origin mainly to di and trisaccharides that can exert health benefits.

Together with β-glucans, MOS have been used as prebiotics to feed Nile tilapia *Oreochromis niloticus* fish (Selim & Reda, 2015) and broilers (Shendare, Gongle, Rajput, Wanjari, and Mandlekar, 2008).

Raffinose, stachyose, and verbascose are GOS found in legumes. They consist of a terminal sucrose to which one (raffinose), two (stachyose), or three (verbascose) galactose monomers are linked. Raffinose is a trisaccharide containing a galactose-linked α-(1,6), whereas the bond between galactose and the glucose unit of the terminal sucrose is α-1,3. Stachyose is a tetrasaccharide containing a galactose linked α-(1,6) to the terminal galactose unit of raffinose (Middlebos & Fahey, 2008). Humans cannot digest these oligosaccharides because they do not have α-galactosidase, which is needed for their hydrolysis.

Transgalactooligosaccharides (TGOS) are nondigestible carbohydrates that consist of chains of galactose molecules ending mainly with glucose molecule, varying in chain length (DP 2-8) and

linkage type. They are α-D-Glc*p*-(1→4)-[β-D-Gal*p*-(1→6)]$_n$ with $n = 2-5$ produced from lactose with the aid of transgalactosylase (Hammes, 2004). Like other nondigestible oligosaccharides, TGOS escape digestion in the human stomach and small intestine, consequently arriving quantitatively to the colon (Van Den Heuvel, Schoterman, & Muijs, 2000).

Xylans are a diverse group of polysaccharides belonging to the hemicellulose group of the cell-wall biopolymers of vegetables with the common feature of a β-(1→4)-linked L-xylose backbone. A common modification of xylans is the substitution with α-(1→2)-linked glucuronosyl and 4-O-methyl glucuronosyl residues. These xylans usually contain many arabinose residues attached to the backbone and are known as arabinoxylans (AXs) and glucuronoarabinoxylans. However, the distinction is not clear. Cereal endosperm AX has very little glucuronic acid, but heteroxylans in the vegetative parts of grasses are often called AXs, even though they tend to contain more glucuronic acid and 4-O-methyl glucuronosyl residues, making glucuronoarabinoxylans a more appropriate name. Branching patterns in xylan, like those in xyloglucans, correlate with taxonomy. As reported by Jayapal et al. (2013), prebiotic xylooligosaccharides (XOS) having a DP of 2−6 are naturally present in minimal concentrations in honey, fruits, vegetables, bamboo shoots, etc. Although consumption of XOS has shown to be of significant importance in maintaining the gut health and functionality of human beings, the concept of prebiotic came into light only in 1995. XOS are unusual oligosaccharides whose main constituent is xylose linked by β(1→4) linkages, which have prebiotic activity, favoring the improvement of bowel and immune functions and having antimicrobial and other health benefits (Azevedo Carvalho, De Oliva Neto, Fernandes Da Silva, & Pastore, 2013).

Specifically, Snelders, Dornez, Broekaert, Delcour, and Courtin (2013) analyzed the AXOS backbones, usually present as mixtures of different molecular entities with xylan backbones of different length, whose prebiotic properties depend on their DP and degree of arabinose substitution. AXOSs consist of a linear backbone of β-(1,4)-linked D-xylopyranosyl residues, which can be un-, mono-, or di-substituted with α-L-arabinofuranosyl units on their C-(O)-2 and/or C-(O)-3 position. Some arabinose residues are esterified with ferulic acid. Snelders et al. (2013) determined that AXOS-containing samples typically consist of a mixture of very diverse structurally related molecules, being then no possible to quantify every single molecule. Xylobiose and xylotriose backbone entities were predominant in an AXOS fraction (about 22% and 21% of the total, respectively), while they were clearly less abundant in other AXOS fractions (11% and 7%, respectively). AX oligosaccharides with a DP of the xylan backbone ranging from 4 to 9 were present at higher relative concentrations in the last fraction than in the first one. It was also found that XOS and a third AXOS fraction had a rather similar average DP of xylose monomers (2.8 and 3.4, respectively). The xylan backbones of both samples consisted of almost 50% of xylobiose. Xylotriose backbone entities were also very abundant in the XOS (34% relative to the total XOS) but less abundant in the third AXOS fraction (19%). Based on relative concentrations, xylotetraose backbone entities were present in similar levels in the XOS and in the third fraction of AXOS, while XOS and AXOS with a DP of the xylan backbone ranging from 5 to 9 were more abundant in the third AXOS fraction.

Pectin is an abundant, ubiquitous, and multifunctional component of the cell wall of all land plants (Willats, Knox, & Mikkelsen, 2006). Pectic polysaccharides consist mostly of linear polymers of galacturonic acid (GalA) called homogalacturonans (HG; α-1,4-linked GalA monomers). There are also rhamnogalacturonan-I (RG-I) regions comprised of significant amounts of rhamnose,

arabinose, and galactose, as well as by the RG-II and the xylogalacturonan domains of pectins, constituted by 13 different monosaccharides (Vincken et al., 2003). Pectin-containing agricultural by-products are potential sources of a new class of prebiotics known as pectic oligosaccharides (POS), obtained through partial hydrolysis of pectin polymers.

Cellulose is the most abundant component of plant biomass. It is found in nature almost exclusively in plant cell walls, although it is produced by some animals and a few bacteria. Despite great differences in composition and in the anatomical structure of cell walls across plant taxa, high cellulose content—typically in the range of approximately 35−50% of plant dry weight—is a unifying feature (Lynd, Weimer, Van Zyl, & Pretorius, 2002). Cellulose consists of $\beta(1\rightarrow4)$-linked glucose with a DP of between 2000 and 6000 in primary cell walls to more than 10,000 in secondary walls. Its glucan chains interact closely through hydrogen bonding, excluding water to produce areas of crystallinity (Waldron, Parker, & Smith, 2003). Only in a few cases, is cellulose present in a nearly pure state. In most cases the cellulose fibers are embedded in a matrix of other structural biopolymers, primarily hemicelluloses, and lignin (Lynd et al., 2002).

The hemicelluloses rank next to cellulose as the most abundant natural carbohydrate polymer in the biosphere. They are present in all layers of the plant cell wall but are concentrated mainly in the primary and secondary layers where they occur closely associated with cellulose and lignin (Ghose & Bisaria, 1987). They can differ greatly in different cell types, and in different species (Waldron et al., 2003). These neutral polysaccharides are polymers of xylose, galactose, mannose, arabinose, and other sugars and present a relatively long backbone chain with short branches. In general, they are classified according to the main sugar residue present: D-xylan, D-galactan, D-mannan, L-arabinan, etc., although they do not occur as homoglycans but rather as heteroglycans: L-arabino-D-xylan, L-arabino-D-glucurono-D-xylan, D-glucurono-D-xylan, etc. (Ghose & Bisaria, 1987). They have often been reported as chemically associated or cross-linked to other polysaccharides, proteins, or lignin (Wyman et al., 2005).

About 15% of flowering plant species store fructans in at least one of their organs during their lifecycle. Fructan polysaccharide is a general term used for any carbohydrate in which fructosyl-fructose links constitute the majority of the glycosidic bonds. Fructooligosaccharide (FOS) is a common name only for fructose oligomers mainly comprised of 1-kestose (GF_2), nystose (GF_3), and 1-β-fructofuranosyl nystose (GF_4), with a single D-glucose (G) moiety in which fructosyl units (F) are bound at the $\beta(2\rightarrow1)$ position of the sucrose molecule (GF) (Jaime et al., 2000). Among them, 1-kestose has better therapeutic properties than those with a high polymeric degree ($GF_{n>4}$). FOS can also be obtained by inulin hydrolysis caused by endoinulinase produced by several microorganisms, mainly fungi and some bacteria (Guío, Rodríguez, Alméciga-Diaz, & Sánchez, 2009).

Alginic acid, a natural polysaccharide harvested from brown algae, is an unbranched binary copolymer comprised of (1,4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers. These monomers can be linked to, constitute GG- and MM-blocks as well as sequences of alternating M and G units (MG block) (Jothisaraswathi, Babu, & Rengasamy, 2006). Physical and mechanical properties as well as the biocompatibility of alginate materials are highly dependent on the relative content of L-guluronic to D-mannuronic acids (De'Nobili et al., 2013; Klöck et al., 1997; Stabler, Wilks, Sambanis, & Constantinidis, 2001). Calcium ions can partially replace hydrogen bonding, zipping guluronate (but not mannuronate) chains together stoichiometrically in an "egg-box" conformation. Guluronate-chain pairing through junction zones involves three components: uronate

chains, calcium ions, and water molecules. The antiparallel arrangement is the macromolecular interaction probably favored in the gel, showing a notable contribution of hydrogen bonds to gel strength (Braccini & Pérez, 2001). Recent studies have revealed that alginate hydrolysates and their derivatives exhibit many important bioactivities, such as stimulating human keratinocytes, accelerating plant root growth, and enhancing penicillin production from cultures of *Penicillium chrysogenum* (Zhang et al., 2004). Enzymatically depolymerized alginate oligosaccharides (AlgO) can cause cytotoxic cytokine production in human mononuclear cells. Low-molecular-weight alginate derivatives such as propylene glycol alginate sodium sulfate and propylene glycol mannuronate sulfate have shown antioxidation benefits and prevention of cardiovascular and cerebrovascular diseases.

Native starch granules are found dispersed in the cytoplasm of vegetable cells, constituting the form of energy storage. Amylose and amylopectin are glucose polymers that constitute starch and serve as energy reservoirs (Delcour et al., 2010). Granules are semicrystalline and can resist hydrolysis by amylases. However, when gelatinized, they are readily hydrolyzed and converted into sugars and dextrins, thereby reducing the molecular weight of starch molecules (amylose and amylopectin). Variations in granule size ($\approx 1-100\,\mu$ in diameter), shape (round, lenticular, polygonal), size distribution (uni- or bimodal), association as individual (simple) or granule clusters (compound), and composition (α-glucan, lipid, moisture, protein, and mineral content) reflect the botanical origin. Starch granules are comprised of two types of α-glucans, amylose and amylopectin, which represent approximately 98–99% of the dry weight. The ratio of the two polysaccharides varies according to the botanical origin of the starch. They are classified as "waxy" starches, which contain less than 15% of amylose, "normal" starches, containing 20–35% of amylose, and "high" amylose starches with above 40% of amylose (Tester, Karkalas, & Qi, 2004). Amylose is defined as a linear molecule of (1→4)-linked a-D-glucopyranosyl units, but today it is well established that some molecules are slightly branched by (1→6)-α-linkages. When degraded by pure β-amylase, linear macromolecules are completely converted into maltose, whereas branched chains also give one β-limit dextrin consisting of the remaining inner core polysaccharide structure with its outer chains recessed. The β-limit dextrins of branched amyloses also show properties (iodine-binding capacity, molecular weight) close to those of the corresponding original amyloses, while remaining completely different from those of amylopectin. Amylopectin is the highly branched component of starch: it is formed through chains of α-D-glucopyranosyl residues linked together mainly by (1→4) linkages but with 5–6% of (1→6) bonds at the branch points. Amylopectin is a branched polysaccharide comprised of hundreds of short (1→4)-α-glucan chains, which are interlinked by (1→6)-α-linkages (Buleón, Colonna, Planchot, & Ball, 1998). Cereal starches contain integral lipids in the form of lysophospholipids and free fatty acids, which are positively correlated with the amylose fraction. In high amylose cereal starches, the lysophospholipids account for up to $\approx 2\%$ of starch weight (Tester et al., 2004).

Dextrins are produced by acid or enzymatic hydrolysis of starch or by a combination of both. Dextrin is one of several carbohydrates having the same general formula as starch. However, these molecules are structurally different as dextrin is a smaller and less complex molecule. The hydrolysis extent is normally expressed in terms of the "dextrose equivalent" (DE). The latest is inversely related to molecular weight, i.e., the DP, and it is an indicator of the hydrolysis degree. Dextrin is a group of low-molecular-weight carbohydrates, mixtures of polymers of D-glucose units linked by $\alpha(1\rightarrow4)$ or $\alpha(1\rightarrow6)$ glycosidic bonds. Maltodextrin is partially hydrolyzed starch that is not sweet and has a DE value lower than 20. Syrups, such as corn syrup made from cornstarch, have DE

values between 20 and 91. Dextrins are widely used for their functional properties, whereas their physicochemical properties are dependent on their molecular distribution and oligosaccharide profiles (Sun, Zhao, Zeng, Li, & Li, 2010).

Isomaltooligosaccharides (IMO) are branched oligosaccharides containing a series of α-(1,6) bonds in its structure. IMO is a mixture of glucose oligomers such as isomaltose, panose, isomaltotriose, isomaltotetrose, isomaltopentose, isomaltoshexose, and isomaltoheptose. Isomaltose (6-0-α-D-glucopyranosyl-D-glucose) is formed from two glucose monosaccharides. It is often found at the branching points of amylopectin and glycogen. IMO is the repeating of the 6-O-α-D-glucopyranosyl-D-glucose up to 4−7 units (FDA, 2005a).

Polydextrose is a polymer of glucose with sorbitol and traces of citric-acid catalyst attached to the polymer through mono- and di-ester bonds. The glucose molecules are randomly bonded, although the β-(1-6) linkage predominates. Because of the random glucose−glucose and glucose−sorbitol bonds, polydextrose is more resistant to enzymes and acidic media than other glucose polymers such as starch (FDA, 2007). Polydextrose is noncariogenic and very water-soluble and is thus used along with high-intensity sweeteners in foods low in sucrose (Auerbach et al., 2012).

3.3 OCCURRENCE AND USE OF CARBOHYDRATES

3.3.1 CARBOHYDRATES AND THE PREBIOTIC EFFECT

Low-molecular-weight carbohydrates, which include mono-, di-, and nondigestible oligosaccharides, are regularly applied in human nutrition as part of the daily diet since they are widely present in fruits and vegetables. The food industry has increased the inclusion of nondigestible oligosaccharides in their products following scientific assertions for the "bioactive" or "functional" action of carbohydrates in intestinal health (Jovanovic-Malinovska, Kuzmanova, & Winkelhausen, 2014).

To date, all known and suspected prebiotics are carbohydrate compounds, primarily oligosaccharides, known to resist digestion in the human small intestine and to reach the colon where they are fermented by the gut microflora (Slavin, 2013). Functional fiber consists of isolated, nondigestible carbohydrates that have beneficial physiological effects in animals. Although all prebiotics are fiber, not all fiber is prebiotic. The classification of a food ingredient as a prebiotic requires a scientific demonstration that the ingredient:

1. resists gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption;
2. is fermented by the intestinal microflora; and
3. stimulates selectively the growth and/or activity of intestinal bacteria associated with health and well-being (Slavin, 2013).

The colonic microbiota and their metabolic activity play a significant role in the energy homeostasis of the body and appetite regulation. The supply of substrates to the colonic microbiota has a major impact on the microbial population and the metabolites they produce, particularly short chain fatty acids (SCFA). The latter are produced when nondigestible carbohydrates, namely dietary fibers and resistant starch (RS), undergo fermentation by the colonic microbiota. Both the consumption of fermentable carbohydrates and the administration of SCFA have been correlated to a wide range of health benefits including improvements in body composition, glucose homeostasis, blood

lipid profiles, reduced body weight, and colon cancer risk (Byrne, Chambers, Morrison, & Frost, 2015). The main products of the bacterial fermentation of nondigestible carbohydrates in the gut are SCFA, heat, and gases. The process of bacterial fermentation serves as an energy-harvesting system for undigested material, rescuing energy that cannot be absorbed in the small bowel, which is used as a major energy source for some species. The main SCFA produced by bacterial fermentation are acetate, propionate, and butyrate, which are present in the approximate molar ratio of 60:20:20. It has been demonstrated that the consumption of soluble fermentable carbohydrates increases the caecal content of SCFA in animal models (Byrne et al., 2015). In particular, butyrate is also the nutrient of the colonocites. Normal colonocites are able to metabolize butyrate efficiently, which could result in a reduction of the intracellular concentration and therefore in a reduction of its growth-inhibition capacity. This butyrate-metabolizing capability could be lost in colon-cancer cell lines (Camarero, Nadal, Barrero, Haro, & Marrero, 2003).

Consumers know the importance of a healthy diet and at the same time want easy, prepared foods from the industry. The food industry has shown an increased interest in product innovation in order to satisfy consumer demand for high-quality and a variety of healthy products. Functional foods are those fortified with health-promoting additives and ingredients such as dietary fiber, natural antioxidants, and vitamin-enriched products. The latter should bring specific health benefits while satisfying the appetite and consumer sensory preferences (Redgwell, Curti, & Gehin-Delval, 2008). Prebiotics, e.g., oligosaccharides, can be used as ingredients for functional food products and nutraceuticals.

Currently available prebiotics such as inulin and its derivatives as well as GOS are relatively cheaper to manufacture and have been widely used as functional ingredients in food (Singdevsachan et al., 2016). Inulin is a natural storage carbohydrate comprised of a heterogeneous collection of fructose polymers that has been found to enhance the gastrointestinal and immune systems. In addition, it has been shown to increase the absorption of calcium and magnesium, influence the formation of blood glucose, and reduce the levels of cholesterol and serum lipids.

Oligosaccharides and polysaccharides have also gained great interest as functional food ingredients because they can manipulate the composition of colonic microbiota in the human gut by inhibition of exogenous pathogens. Inulin, FOS, GOS, lactulose, and polydextrose are recognized as well-established prebiotics, while IMO, XOS, and lactitol are categorized as emerging prebiotics (Lam & Cheung, 2013). Other nondigestible oligosaccharides and sugar alcohols such as mannitol, maltodextrin, raffinose, and sorbitol have also demonstrated prebiotic properties and health benefits. When probiotics are incorporated into food products, culture viability often decreases as a result of the pH reduction, temperature lowering, nutrients inadequacy, and oxidative stress. These challenges could be ameliorated by the addition of prebiotics into the food matrix.

Different studies have provided evidence that inulin and FOS, lactulose and resistant starch (RS) meet all aspects of the prebiotic definition, including the stimulation of *Bifidobacterium*, a beneficial bacterial genus. Other isolated carbohydrates and carbohydrate-containing foods, including GOS, TOS, polydextrose, wheat dextrin, acacia gum, psyllium, banana, whole-grain wheat, and whole-grain corn also have prebiotic effects. Finally, oligosaccharides can help to improve the organoleptic properties and nutritional value of foods (Tungland & Meyer, 2006). The functional properties of wheat flour AX-pentosans in breadmaking have been recognized (Cleemput, Roels, Vanoort, Grobet, & Delcour, 1993).

3.3.2 GOS, MOS, AND β-GLUCAN HYDROLYSIS PRODUCTS

Legume grains are a rich source of GOS such as raffinose and stachyose. Intact oligosaccharides reach the colon, where they are preferentially fermented by beneficial bifidogenic microorganisms that contain the α-galactosidase enzyme. Fermentation of nondigestible oligosaccharides results in the production of gases and SCFA, which are noteworthy due to their prebiotic activity and associated health benefits. The potential of oligosaccharides as an ingredient for functional foods promotes the search of new sources as well as of new technologies of isolation and purification.

β-glucans are water-soluble constituents of the cell walls of different cereals, such as barley (≈7 g/100 g), oat (≈5 g/100 g), rye, wheat, and sorghum (≈1−2 g/100 g) (Benito-Román et al., 2013a; Brennan & Cleary, 2005; Tiwari & Cummings, 2009). The molecular-structural characteristics of β-glucans in oats, which distinguishes them from cereal β-glucans of different botanical origins, include the ratio of tri- to tetramers, amount of cellulose oligomers, ratio of β-(1→4)/−(1→3) linkages, and molecular weight (Zhu, Du, & Xu, 2016). Soluble β-glucans and their hydrolysates have been reported to reduce plasma cholesterol and postprandial serum glucose levels in humans and animals, and they have been the subject of health claims as food constituents by the US Federal Drug Administration (FDA, 2005b) and the European Food Safe Authority (EFSA, 2010). The claimed effect attributed to β-glucans from barley and oat is that regular consumption of β-glucans contributes to maintenance of normal blood cholesterol concentrations when ingesting 3 g of β-glucan per day. Phenolics are also present in barley, mainly as benzoic and cinnamic acid derivatives. They are mainly found in the outer layers of the grain (hull) and also in the aleurone (layer rich in β-glucan) and endosperm either in free or bound forms. Ferulic acid is the main free phenolic, while *p*-hydroxybenzoic acid is the main bound phenolic in barley seed. These bound phenolics are strongly linked to other molecules, such as hemicelluloses (e.g., xylans), forming strong ester-to-ester bonds, although they can also form ether bonds with other components (Benito-Román, Alvarez, Alonso, Cocero, & Saldaña, 2015). The phenolics coextracted with polysaccharides can contribute to the reported healthy benefits of these carbohydrates (Renard, Watrelot, & Le Bourvellec, 2015). As reported by Tungland and Meyer (2006), dietary fiber includes polysaccharides, oligosaccharides, lignin as well as associated plant substances. These authors also noted that dietary fibers promote beneficial physiological effects including laxation and/or blood cholesterol attenuation and/or blood glucose attenuation.

On the other hand, Kim et al. (2005) obtained oligosaccharides through hydrolysis of the β-glucans extracted from *A. blazei* Murill mushrooms, which showed double-antidiabetic activity (in diabetic rats) compared to the oligosaccharides determined for β-glucan polysaccharides.

Together with β-glucans, MOS were used as prebiotics by Selim and Reda (2015) to feed Nile tilapia *O. niloticus* fish, significantly improving its immunological performance without affecting body weight. MOS and β-glucans were also added to a maize-based meal to feed commercial broilers, and Shendare et al. (2008) observed significantly higher body weight gain as well as improvement in feed efficiency with respect to the control diet.

3.3.3 ARABINOXYLANOLIGOSACCHARIDES AND XOS

The majority of prebiotics on the market are derived from nondigestible oligosaccharides (Zhu et al., 2016). Gibson (2004) found dietary fibers to be important prebiotics, but oligosaccharides were found to be more promising. Arabinoxylanoligosaccharides are the product of the hydrolytic

degradation of AX polysaccharides coming from the cell walls of wheat bran. They are a good example of a functional food constituent. They possess not only prebiotic properties but also potential antioxidant activity due to the present ferulic acid, which is the major phenolic acid in wheat (Snelders et al., 2013). Femia et al. (2010) determined that AXOS reduced the preneoplastic lesions in the colon of rats treated with 1,2-dimethylhydrazine. Oats can be a source of soluble dietary fiber. Oligosaccharides were obtained by Wood et al. (1991) by the action of a β-glucan hydrolase enzyme called lichenase. β-mannanase from *Penicillium oxalicum* SO efficiently hydrolyzed guar galactomannan and locust been gum to galacto-manno-oligosaccharides, with DP of 2−7 and an yield of 92% (Kurakake, Sumida, Masuda, Oonishi, & Komaki, 2006). One of the main oligosaccharides released from guar gum, with DP 7, had high galactose content (Gal/Man = 0.76) and corresponded to a blockwise galactose-substituted mannan type in galactomannan. On the other hand, lactitol-oligosaccharide (LO) was obtained from lactitol by Yanahira et al. (1995) through a transgalactosylation reaction catalyzed by *Aspergillus oryzae* β-galactosidase. The utilization of LO by human intestinal bacteria such as *Bifidobacterium*, the digestion of LO by rat jejunum mucosal homogenates, and the effects of LO on the intestinal microflora in rats were compared to those of lactitol. It was determined that only LO were utilized in vitro by *Bifidobacterium*, but LO and lactitol were not digested by rat jejunum mucosal homogenates, although a significant increase in the fecal counts of *Bifidobacterium* was observed in the LO diets. The concentration of organic acids in feces and cecal contents also significantly increased in the LO diets, while the concentration of fecal putrefactive products significantly decreased in both the LO and lactitol-loaded diets. These observations suggested that LO was effective at improving intestinal conditions. XOS are soluble dietary fibers that have prebiotic activity, favoring the improvement of bowel and immune functions and having antimicrobial and other health benefits (Azevedo Carvalho et al., 2013).

3.3.4 PECTIN-DERIVED OLIGOSACCHARIDES

Controlled hydrolysis of pectin-containing agricultural by-products like sugar beet, apple, olive, and citrus by chemical, enzymatic, and hydrothermal processes can be used to produce oligogalacturonides (GalAOS), galactooligosaccharides (GOS), rhamnogalacturonan-oligosaccharides (RGOS), etc. However, extensive research is needed to establish the role of pectin oligosaccharides (POS), both as a prebiotic and as a therapeutic agent (Babbar, Dejonghe, Gatti, Sforza, & Kathy, 2015). Strains of *P. chrysogenum* used in the early days of penicillin production liberated a yellow pigment called chrysogenin. Asilonu, Bucke, and Keshavarz (2000) determined that the addition of acid-hydrolyzed AlgO and enzyme-hydrolyzed POS to cultures of *P. chrysogenum* (ATCC 9480) led to enhanced production of the secondary metabolite yellow pigment chrysogenin by over 30−40% in shaken flasks and bioreactors. Several authors have reported that low-molecular-weight POS have a prebiotic potential better than high-molecular-weight POS (Al-Tamimi, Palframan, Cooper, Gibson, & Rastall, 2006). In vitro studies show a clear indication that POS can be successfully used to promote bifidogenic flora. Babbar et al. (2015) described olive pomace (by-product of olive-oil processing), the sugar-beet pulp remaining after sugar extraction, potato pulp, citrus waste, apple pomace, tomato, chicory roots, and cauliflower floret/leaves as potential sources of POS.

Chemical treatments such as alkaline extraction were also used by Zykwinska, Rondeau-Mouro, Garnier, and Ralet (2006) for the production of RG-I oligosaccharides from potato pulp. Hydrolytic

enzymes are used for obtaining POS. There are different enzymes active on the smooth (HG) and hairy (RG-I, RG-II) regions of pectins. The methyl esters and acetyl groups of the GalA residues are removed by pectin methyl esterase and pectin acetyl esterase, respectively. Both enzymes act before endo-polygalacturonase. Endo-polygalacturonase is able to cleave the glycosidic bond of the α-(1→4)-polygalacturonan in a random fashion. Endo-polygalacturonase generally prefers a nonesterified substrate and shows decreasing activity with increasing degree of methylation. Exopolygalacturonase attacks the substrate from the nonreducing end and is able to remove terminally (1-)-linked Gal A residues from HG chains. Other enzymes used are rhamnogalacturonan hydrolase and rhamnogalacturonanlyase (Babbar et al., 2015). For structural studies, Morris, Ralet, Bonnin, Thibault, and Harding (2010) modified an acid extracted sugar-beet (*Beta vulgaris*) pectin through enzymatic hydrolysis using fungal pectin methyl esterase and two endopolygalacturonanases, separating the RG-I fraction. On the other hand, the HG fraction was prepared by mild acid hydrolysis of the acid-extracted sugar-beet pectin. After anion exchange chromatography, oligogalacturonides were mostly found with various DP (1−10), degree of methylesterification, and of acetylation.

Partly methylated and/or acetylated oligogalacturonates were recovered after enzymatic hydrolysis (endo-polygalacturonase + pectin methyl esterase + side-chain degrading enzymes) of sugar-beet pectin by Ralet et al. (2005). Around 90% of the GalA and 75% of the acetyl groups present in the initial sugar-beet pectin were recovered as HG-derived GalAOS, with the remaining GalA and acetyl belonging to rhamnogalacturonic regions. Around 50% of the acetyl groups present in sugar-beet HGs were recovered as partly methylated and/or acetylated galacturonates of DP = 65. Lemon (*Citrus lemon*) and orange (*Citrus sinensis*) contain pectin levels from 2.5% to 5.5% (fresh weight). POS were obtained by Martínez Sabajanes et al. (2012) from orange-peel wastes submitted to enzymatic hydrolysis, using pectinases and cellulases. Galactooligosaccharides, arabinooligosaccharides (AOS), and GalAOS were found in the product mixture. In a complete investigation, POS were obtained through hydrothermal treatment (160°C optimal maximum temperature and 288 min severity factor) of orange peel wastes, purified by a two-step membrane process (discontinuous diafiltration and concentration) (Gómez et al., 2014). The prebiotic potential of the concentrate was assayed by in vitro fermentation using human fecal inocula. The POS showed the highest rate and level of production of SCFA at 48 h of fermentation compared to orange pectin, FOS used as prebiotic standard, and the control sample without any kind of oligosaccharides.

Dynamic high-pressure microfluidization is an emerging technology that generates powerful shear, turbulence, impaction, and cavitation forces simultaneously. This technology has proved to be a promising physical method to manipulate the molecular weight of polymers. Chen et al. (2013) applied a pressure of 155 MPa and six cycles to a system with 1.84% of apple pectin and observed that 32.92% of this polysaccharide was converted to POS. After this treatment, the aqueous solution was neutralized with calcium carbonate to precipitate the higher molecular weight species that were not soluble in neutral solution. The supernatant was then purified through concurrent ultrafiltration to remove the species with molecular weights higher than 5000 Da, and the permeates were collected and freeze dried. The obtained POS increased the number of *Bifidobacteria* and *Lactobacilli*, and produced a higher concentration of acetic, lactic, and propionic acids than their parent pectins when evaluated using a fecal batch culture fermentation. POS also decreased the number of bacteroides and clostridia, while their parent pectins increased them. It was determined that the effects of produced POS were comparable to those of the most studied prebiotic FOS.

3.3.5 ALGINATE-DERIVED OLIGOSACCHARIDES

As noted above, alginate is a versatile polysaccharide because of its copolymeric composition, which determines different mechanical properties and biocompatibility. Hence, AlgO with very different properties can be obtained after applying different procedures such as fungal activity or direct enzyme hydrolysis through lyases. In vitro digestion experiments demonstrated that AlgO was highly resistant to enzymes of the upper gastrointestinal tract. The alginate-derived oligosaccharides used in many studies are mixtures of unsaturated AlgO produced from enzymatic degradation of alginate by alginate lyase (Jonathan, Bosch, Schols, & Gruppen, 2013). In addition, they contain AlgO with various DP as well as isomers with the same DP but different ratios and sequences of G and M residues. Tøndervik et al. (2014) obtained through a nonreported technique a purified AlgO comprised of a high content (90−95%) of guluronate with a relatively narrow molecular-weight distribution, generated from a *Laminaria hyperborea* extracted alginate. These AlgO inhibited the fungal cell growth and increased the activity of antifungals against pathogenic strains of *Candida* and *Aspergillus* spp. Minimum inhibitory concentration screening demonstrated that AlgO (2%, 6%, 10%) increased the effects of antifungal drugs such as nystatin, amphotericin B, fluconazole, miconazole, voriconazole, or terbinafine on these strains.

Khan et al. (2012) previously found that AlgO especially reach in guluronic acid monomers were also able to perturb multidrug-resistant bacteria by modulating biofilm formation and persistence reducing the resistance to antibiotic treatment. Increasing concentrations (2%, 6%, and 10%) of AlgO were shown to have a direct effect on the quality of the biofilms produced. These results indicate that it is feasible to reduce the tolerance of wound biofilms to antibiotics with the use of specific AlgO preparations.

3.3.6 ISOMALTOSE OLIGOSACCHARIDES

Isomaltose oligosaccharide (IMO) is added to a variety of foods including baked goods and baking mixes, beverages and beverage bases, condiments, salad dressings, frozen dairy desserts and mixes, gravies, sauces, hard and soft candies, jams, meat and nut products, processed fruits and vegetables, sugar substitutes, sweet sauces, and toppings (Isomalto-Oligosaccharide VitaFiber, 2012). IMOs constitute a nondigestible, low-calorie health sweetener that supports the proliferation of the beneficial bacteria residing in the large intestine (colon). In general, IMO is used as a low-calorie sweetener mixed with a variety of other food and beverage products for the purpose of sweetening. This product is supplied to the food industries as a low-calorie, bulk sweetener and as a general food ingredient. IMO is found to be effective at increasing the number of *Bifidobacteria* and lactate and at improving the intestinal microflora in general and is therefore safe to categorize as a prebiotic (FDA, 2005a). Prebiotic active IMO was obtained by transglucosylation in the presence of maltose through a recombinant α-glucosidase from *Thermoanaerobacter ethanolicus* JW200, cloned and expressed in *Escherichia coli* by Wang, Jiang, Duan, Shao, and Li (2009). IMO is formed by enzyme-catalyzed hydrolysis of starch from different cereal crops (wheat, barley, corn), pulses (lentils, peas), rice, tapioca (cassava), potato, and other starch sources. Enzymes, including α-glucosidase, α-amylase, and pullulanase, hydrolyze the polysaccharides in starch to produce mono-, di-, tri-, and other smaller oligosaccharides with α-1,4 and α-1,6 glycoside linkages. Yeast is added to remove glucose that may be formed as a result of the enzymatic hydrolysis reactions. The final step

in the starch hydrolysis is a saccharification step that yields high maltose syrup. Maltose syrup naturally contains di- and trioligosaccharides with α-1,4 glycoside linkages. In order to convert these molecules into functional and low caloric molecules, α-1,4 linkages are enzymatically converted into α-1,6 linkages, thus forming IMO. This step is achieved by the addition of transglucosidase, which converts maltooligosaccharides into IMO (Isomalto-Oligosaccharide VitaFiber, 2012).

3.3.7 FRUCTOOLIGOSACCHARIDE

Sugar syrup and molasses from beet processing containing 620 and 570 mg/mL sucrose, respectively, were assayed by Ghazi et al. (2006) as low-cost and available substrates for the enzymatic synthesis of FOS. A commercial pectinase from *Aspergillus aculeatus*, characterized by the presence of a transfructosylating activity, was used as biocatalyst. The FOS production increased when lowering the initial pH value of syrup (7.5) and molasses (8.9) to 5.5. Sugar syrup and molasses were diluted in order to reduce substrate viscosity. Interestingly, the proportion of FOS with regard to total sugars remained almost constant, which indicated a high transferase to hydrolase ratio for this enzyme.

3.3.8 LACTOSE-DERIVED PRODUCTS

Lactose has been thoroughly studied for its physicochemical properties and crystallization behavior for food processing, as well as for its importance as a fermentation medium. Lactose is a low value-added sugar and new technologies for its transformation into more value-added products with application in food and pharmaceutical industries could lead to its valorization (Gänzel, Haase, & Jelen, 2008). Commercially produced lactose derivatives include oxidation to lactobionic acid, isomerization to lactulose, reduction to lactitol, hydrolysis to galactose and glucose, transgalactosylation to GOS [β-(1-4) or β-(1-6) linkage], and production of lactosucrose by transfructosylation.

Prebiotics are included in infant foods to substitute the bifidogenic effect of oligosaccharides that are present in human milk at a level of 1−1.3% (the milk of ruminants has much lower concentrations of oligosaccharides). While other functions cannot be mimicked by lactose derivatives, the addition of prebiotic oligosaccharides to infant formula has shown to result in levels of *Bifidobacteria* in the intestine that were comparable to those found in breast-fed infants. The transfructosylation of lactose is carried out by bacterial or fungal fructosyltransferases using sucrose or raffinose as fructosyldonor (Gänzel et al., 2008). Fructosyltransferases catalyze the hydrolysis of sucrose as well as the transfructosylation to acceptor carbohydrates. Fructosyltransferases catalyze the formation of high-molecular-weight fructan polymers and the formation of oligosaccharides in addition to sucrose hydrolysis. Depending on the type of polymer formed, fructosyltransferases are referred to as levansucrases or inulosucrases. Because the spectrum of acceptor carbohydrates is essentially comparable in all bacterial levansucrases characterized thus far, it can be assumed that the synthesis of lactosucrose from sucrose and lactose is a general property of bacterial levansucrases. Levansucrase activity is frequently found in food-fermenting lactic-acid bacteria, particularly *Lactobacillus reuteri*, *Lactobacillus pontis,* and *Lactobacillus acidophilus*. These organisms have been successfully used for the generation of high levels of oligosaccharides in food fermentations.

3.3.9 SUCROSE

Sucrose or table "sugar" as it is commonly known is one of the oldest sweetening agents and the most used caloric sweetener, both for home and commercial use. Together with starch and lactose, it is one of the three most common carbohydrates found in the diet. D-glucose and D-fructose are readily absorbed in the small intestine of humans after the sucrose is hydrolyzed by sucrase. Trehalase, sucrase-isomaltase, and maltase-glucoamylase are three integral glycoproteins of the brush-border membranes of the enterocytes, in the first part of the small intestine of mammals. Anchored to the membrane via a highly hydrophobic segment on the isomaltase subunit, the sucrase-isomaltase (EC 3.2.1.48-10) is a heterodimeric complex that accounts for about 10% of microvillous membrane proteins and about 80% of maltase activity of the small intestine (Galand, 1989).

Sucrose has the ability to disperse its particles (sometimes amorphous ones) throughout fat-phase confectionaries like chocolate, to maintain bulk, even distribution of flavor, and to contribute to food stability using water activity depression. The fact that sucrose can adapt the rheological properties of chocolate to the non-Newtonian flow characteristics that permit layering is vital for chocolate manufacture.

The dielectric constants of sucrose and other monosaccharides are much higher than those of complex carbohydrates, lipids, proteins, or other major food components. This food physical property affects its heating by microwave radiation. Because sucrose also has the property of forming a permanent dipole when its hydrogens are bonded with water, it is a suitable ingredient for the formulation of microwaveable foods. It can be used to increase the heating rate, allow surface heating only, or formulate browning and crisping conditions, as reported by Clarke (1995).

The ability of sugars to dissolve and reform crystals is used in the making of confectionaries such as toffee, brittle, fudge, and caramel. Sugar helps retain the color of the fruit through its capacity to attract and hold water. Sugar absorbs water more readily than other components, such as fruit, in preserves and jellies. Thus it prevents the fruit from absorbing water, which would cause its color to fade through dilution (Mathlouthi & Rogé, 2003). Crystalline sugar powders show very low adsorption capacity of water vapor. After processing through traditional techniques like spray or freeze drying, the sugars are present in amorphous state, which is characterized by higher water-vapor adsorption capacity than that shown by crystalline sugars. Amorphous sugars plasticized by the water adsorbed are above their respective glass transition temperature (T_g) when stored at room temperature (Foster, Bronlund, & Paterson, 2006; Roudaut, Simatos, Champion, Contreras-López, & Le Meste, 2004).

The physical state of sugars affects the stability and quality of food products. Sugar crystallization and storage stability of β-carotene in freeze-dried mango powder stored under 11.3−80.9% relative vapor pressures were studied by Harnkarnsujarit and Charoenrein (2011). A water-sorption assay revealed sugar crystallization by the loss of sorbed water. These authors showed that choosing the appropriate value of storage-water activity could prevent sugar crystallization and enhanced β-carotene stability in freeze-dried fruit powder. Increased water activity resulted in higher sugar crystallization.

3.3.10 STARCH, RESISTANT STARCH, DEXTRINS, AND MALTODEXTRINS

As a food constituent starch is the main source of energy to a human body. Commercial starch is obtained by crushing or grinding starch-containing tubers or seeds and then mixing the pulp with

water; the resulting paste is freed of its remaining impurities and then dried. Aside from their basic nutritional uses, starches are used in brewing and as thickening agents in baked goods and confectionery products (Tester et al., 2004). Starch contributes greatly to the textural properties of many foods and is widely used as a thickener, colloidal stabilizer, gelling agent, and bulking and water retention agent. The physicochemical properties and functional characteristics of starch systems and their uniqueness in various food products vary with starch biological origin (Singh, Kaur, & Mccarthy, 2007).

Starch is perceived as easily hydrolyzable to glucose under the influence of digestive enzymes. In fact, some forms of starch (called RS) do not undergo complete saccharification, but reach the large intestine and become a source of carbon to microflora colonizing it (Kapelko, Zie, & Michalski, 2012). The most common commercial preparation of RS is retrograded starch (RS3). Its properties are determined by the type of starch, amylose content, length of terminal amylopectin chains, presence of other compounds, storage conditions, and paste concentration. RS-rich whole grains are considered prebiotic in nature and it is assumed that their consumption leads to many health benefits (Patel & Goyal, 2012). They are not absorbed in the small intestine of healthy individuals but are later fermented by natural microflora of the colon to produce SCFA. Dietary fibers exhibiting high viscoelasticity impart breads with better sensory perception, lower digestible starch, and higher RS content, lowering the in vitro expected glycemic index (Angioloni & Collar, 2011). Patel and Goyal (2012) reported that roasting, baking, and boiling increased the RS content, whereas steaming and frying decreased the content. Storage also increased the RS content.

Resistant starch and partial degradation products like dextrins are promising sources of prebiotics (Śliżewska, 2013). Dextrinization has been recognized as a way to produce a new type of soluble dietary fiber called resistant maltodextrin or resistant dextrin. Resistant maltodextrins are short-chain polymers of glucose that are hardly resistant to digestion in the human digestive system. Today most of the resistant maltodextrins in food products are manufactured from starch by treatment with heat and/or acid and/or enzymes (Barczynska, Jochym, Śliżewska, Kapusniak, & Libudzisz, 2010). Dextrins are widely used due to their functional properties. Their physicochemical properties are dependent on the molecular distribution and oligosaccharide profiles. As the DE is an indicator of the hydrolysis degree, D-glucose has a DE value of 100, while intact starch has an effective DE of zero. Dextrins with the same DE can have different properties and molecular compositions, depending on the starch and its digestion. It may greatly affect the properties of dextrins such as hygroscopicity, fermentability, viscosity, sweetness, stability, gelation, solubility, and bioavailability (Sun et al., 2010). Nutriose is a soluble dextrin produced from wheat or maize starch using a highly controlled process of dextrinization followed by a chromatographic fractionation step (Fouache, Duflot, & Looten, 2003). During the dextrinization step repolymerization creates specific glycosidic bonds in addition to typical starch α-1,4- and α-1,6-linkages. Lefranc-Millot et al. (2012) confirmed the prebiotic activity of the soluble fiber Nutriose™, which can provide a beneficial effect on colonic ecology.

The ameliorating effect of therapeutic substances on the developed insulin resistance and inflammation response is a basic strategy in the management of type 2 diabetes. Through a randomized controlled clinical trial, Aliasgharzadeh, Dehghan, Pourghassem, Gargari, and Asghari-Jafarabadi (2015) determined that supplementation with prebiotic-resistant dextrin (Nutriose FB06) effectively ameliorates insulin resistance and can modulate inflammation in women with type 2 diabetes.

Preparation of RS from native potato starch, which is easily accessible in Poland and a cheap raw material, was attempted by Śliżewska (2013), who synthesized an enzyme-resistant dextrin by heating potato starch in the presence of hydrochloric (0.1% dry starch basis) and citric (0.1% dry starch basis) acids at 130°C for 3 h, obtaining a citric acid-resistant dextrin (CA-dextrin) to colon enzyme hydrolysis. Later, it was tested as a source of carbon for *Lactobacilli* and *Bifidobacteria* cultured with intestinal bacteria and showed prebiotic activity.

The products of starch hydrolysis with DE values below 20 are called maltodextrins (Sun et al., 2010). Maltodextrins with different DE can produce different yields of prebiotic glucooligosacarides by fermentation with *Gluconobacter oxydans* NCIMB 4943 (Wichienchot, Prasertsan, Hongpattarakere, & Rastall, 2009). However, Slavin (2013) reported the use of maltodextrins as placebo in studies of prebiotic effect and of weight loss. On the other hand, resistant maltodextrins made from starch are commercially available. Fibersol-2 is a well-known soluble and nondigestible starch-derived resistant maltodextrin (Barczynska et al., 2010). It is produced from cornstarch by pyrolysis and subsequent enzymatic treatment to convert a portion of the normal α-1,4 glucose linkages to random 1,2-, 1,3-, and 1,4- α or β linkages. Fibersol-2 can effectively reduce postprandial levels of blood glucose and insulin, the blood levels of triacylglycerols and serum cholesterol, and can also promote beneficial bacteria growth in the colon.

3.4 BIOACCESIBILITY, BIOAVAILABILITY, AND HEALTH EFFECTS OF CARBOHYDRATES

3.4.1 FACTORS THAT AFFECT THE BIOACCESIBILITY AND BIOAVAILABILITY

After consumption, the nutrients that are present in a food or drink are released, absorbed into the bloodstream, and transported to their target tissues. Different nutrients differ in their bioavailability, which means they are not utilized to the same extent. Release of the nutrients from the food matrix, effects of digestive enzymes in the intestine, binding and uptake by the intestinal mucosa, transfer across the gut wall to the blood or lymphatic circulation, systemic distribution and deposition, metabolic and functional use, and excretion can affect nutrient bioavailability. The bioavailability is mediated by external (e.g., characteristics of the food matrix, chemical form of the nutrient) and consumer internal (e.g., gender, age, nutrient status, and life stage) factors. The bioavailability of macronutrients (carbohydrates, proteins, and fats) is usually very high, i.e., more than 90% of the amount ingested.

Bioaccessibility is the first step in making a nutrient bioavailable. In this step, the nutrient is liberated from the food matrix and turned into a chemical form that can bind to and enter the gut cells or pass between them. Chewing, enzymatic digestion of the food in the mouth, mixing with acid and enzymes in the gastric juice, and release into the small intestine are the unit operations of the process by which the nutrients are rendered bioaccessible. The small intestine is the major site of nutrient absorption. Enzymes of the pancreatic juice continue breaking down the food matrix. Certain procedures involved in food preparation like cooking, chopping, or pureeing collaborate with mastication and enzymes in the digestibility of food matrices (EUFIC, 2010).

3.4.2 FOOD STRUCTURE AND NUTRIENTS BIOAVAILABILITY

Food structure is the organization of food constituents and is innate or built during food manufacturing. Disruption of this structure influences the release, transformation, and absorption of nutrients in the digestive tract, impacting nutritional aspects (Escamilla-García et al., 2015; Parada & Aguilera, 2007). Carbohydrates may trap bioactive molecules, such as polyphenols, decreasing the proportion that is made available or delivered to the bloodstream (Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011).

3.4.3 DIGESTION AND ABSORPTION OF CARBOHYDRATES

The main function of carbohydrates is to provide energy, but they also play an important role in the structure and function of cells, tissues, and organs, as well in the formation of carbohydrate structures on the surface of cells. Starches and sugars are the main energy-providing carbohydrate sources. Monosaccharides are absorbed by the small intestine into the bloodstream and transported to their place of use. Disaccharides are broken down into monosaccharides, while starches are broken down to their constituent sugars (using digestive enzymes), which are absorbed into the bloodstream.

The human body uses carbohydrates in the form of D-glucose. Glucose can be converted to glycogen (a polysaccharide similar to starch), which is stored in the liver and the muscles and is a readily available source of energy for the body. Glucose must be constantly maintained at an optimum level in the blood. Approximately 130 g of glucose are needed per day to cover the energy needs of the brain. Glucose may come directly from dietary carbohydrates, from glycogen stores, or from the conversion of certain amino acids resulting from protein breakdown. Several hormones, including insulin, work rapidly to regulate the flow of glucose to and from the blood to keep it at a steady level.

When a carbohydrate-containing food is eaten, there is a corresponding rise and subsequent decrease in the blood-glucose level, which is known as the glycaemic response. This reflects the rate of digestion and absorption of glucose as well as the effects of the insulin action to normalize the blood-glucose level (EUFIC, 2012).

Almost all carbohydrates are efficiently digested and absorbed into the body. However, it must be remembered that dietary fiber is not hydrolyzed by the endogenous enzymes in the small intestine of humans (Philips & Cui, 2011). Some of the remaining indigestible carbohydrates are broken down by enzymes released by bacteria in the large intestine. One of the products of bacterial digestion of these indigestible carbohydrates are SCFA, which are either used by the bacteria to make energy and grow, are eliminated in the feces, or are absorbed into cells of the colon, with a small amount being transported to the liver. Colonic cells use the SCFA to support some of their functions. The liver can also metabolize the SCFA into cellular energy (den Besten et al., 2013).

3.4.4 EFFECTS OF OLIGOSACCHARIDE ON CALCIUM AND MAGNESIUM ABSORPTION IN THE GUT

Deficiency of estrogen at menopause decreases intestinal Ca^{2+} absorption, contributing to a negative calcium balance and bone loss. Mg^{2+} deficiency has also been associated with bone loss. Nondigestible oligosaccharides may influence calcium absorption indirectly by stimulating

hypertrophy of the intestinal mucosa, thereby increasing the surface area for diffusion or directly by increasing transcellular transport through the production of SCFA (Holloway et al. 2007). These fatty acids may decrease the pH, thus allowing an additional exchange of hydrogen ions for calcium ions (van den Heuvel et al., 2000). Oligosaccharides have also shown to increase the absorption of Mg^{2+} in postmenopausal women. Although the role of Mg^{2+} in bone health is less clear, serum and bone Mg^{2+} are reduced in postmenopausal women and hence supplementation with Mg^{2+} has been shown to increase bone mass in osteoporotic women. Holloway et al. (2007) set out to determine if a spray-dried mixture of chicory oligofructose and long-chain inulin (Synergy 1) would be able to increase the absorption of both Ca^{2+} and Mg^{2+} and alter markers of the bone turnover. It was found that 6 weeks of treatment with 10 g/d of Synergy 1 in postmenopausal women significantly increased the Ca^{2+} and Mg^{2+} absorption relative to placebo treatment of the same duration. Although the markers of bone turnover did not demonstrate a clear pattern in response to the increased mineral absorption, there was a short-term decrease in the marker of bone resorption.

3.5 CONVENTIONAL EXTRACTION, RECOVERY, AND MODIFICATION OF CARBOHYDRATES

3.5.1 GLUCANS

Various technologies have been developed to obtain β-glucan concentrates. Zhu et al. (2016) reviewed the production of β-glucan technologies, such as extraction, isolation, and purification technologies from different sources, such as yeast, fungi, bacteria, and cereal, and included a list of several patents.

Although the number of publications reporting extraction procedures is large, they can be grouped into two major categories: dry and wet separation processes. Vasanthan and Temelli (2008) reviewed the different types of dry and wet technologies available for concentration of cereal β-glucan with a focus on commercial-scale processing and their impact on the physicochemical properties of β-glucan. Dry technologies include pearling, dry milling, and sieving (Kiryluk, Kawka, Gasiorowski, Chalcarz, & Aniola, 2000) and dry milling and air classification (Vasanthan & Bhatty, 1995). Doehlert and Moore (1997) studied three mechanisms of oat milling. Bran obtained with either roller milling or impact milling of groats followed by sieving to retain larger particles gave origin to products 1.7-fold enriched in β-glucan compared to bran from pearling mill. Pinball milling at different speeds, air classified, and sieved showed that the highest content of β-glucan was obtained in fractions with $>30\,\mu m$ particle size. Kiryluk et al. (2000) studied the influence of barley milling on the β-glucan content of the resulting products. It was found that the β-glucan content was two times lower in fine-grained products and about 38% higher in coarse-grained products than in dehulled barley grain.

Wet technologies are more complex, since they involve at least two or three stages and include an aqueous and aqueous/alkali extraction process, aqueous/alcohol-based enzymatic process, aqueous enzymatic process, and aqueous thermomechanical process (Vasanthan & Temelli, 2008). Cereal bran or flour is used as raw material, which is put into contact with a solvent (usually water, aqueous solvent at basic pH, or semialcoholic solution), obtaining an aqueous extract as a result. This aqueous extract contains other species apart from β-glucan (starch, proteins, fats), making a

purification step necessary. For this purpose, β-glucans are precipitated by the addition of an alcohol as antisolvent, or separated by other procedures, e.g., freeze thawing.

The nature of the extraction procedure has a profound effect on the structure and molecular weight of β-glucan. According to Tsopmo (2014), the main advantage of wet separation techniques is that the β-glucan content of the fiber concentrates obtained through these processes can reach a value of 95% (w/w). Aqueous technology results in viscosity loss due to activation of endogenous enzymes and thus generates hydrolysate products. The disadvantages include shear-induced molecular fragmentation during mixing and centrifugation as well as the high cost of production due to large volumes of water and alcohol use (Vasanthan & Temelli, 2008). The results from several studies suggest that pH and temperature have a significant effect on the extraction yield and physical, chemical, and functional properties of the β-glucans obtained (Ahmad, Anjum, Zahoor, Nawaz, & Ahmed, 2010; Burkus & Temelli, 2005; Limberger-Bayer et al., 2014; Zhu, Bals, Grimi, Ding, & Vorobiev, 2015). Benito-Roman, Alonso, and Lucas (2011) studied the influence of several operational parameters on the aqueous extraction of β-glucan from barley to find an optimal combination of factors that maximized extraction yield. Temperature, pH, extraction time, particle size, stirring rate, and solvent:flour ratio were identified as the critical factors affecting the extraction process. According to Benito-Román, Alonso, Palacio, Prádanos, and Cocero (2014), the use of water as solvent and its lack of selectivity causes the coextraction of other substances such as starch and proteins together with glucans, generating unpurified liquid extracts. Some authors have used α-amylase to hydrolyze the coextracted starch. The use of a diafiltration process, as the final step for the β-glucans purification, is appropriate, since almost complete purification is achieved.

The method reported by Wood, Weisz, Fedec, and Burrows (1989) for extracting β-glucan from oats at pilot scale is the basis of many other extraction techniques. They prepared a hexane-deffated flour that was sieved and air classified. After refluxing in ethanol to deactivate enzymes, extraction was performed with sodium carbonate at pH 10 and 35−43°C for 30 min, liquid extract was separated from residue through centrifugation, and the liquid extract was then cooled and pH adjusted to 4.5 (HCl). The resulting mixture was centrifuged to separate precipitated proteins and the supernatant was flash evaporated to reduce the volume. The liquid was cooled and mixed with isopropyl alcohol (1:1) with vigorous stirring. The precipitated gum was separated through centrifugation and air dried, obtaining a gum with 78% β-glucan content. When they compared the material obtained with that obtained at the bench, they found that the pilot-scale product exhibited significantly lower viscosity, which was attributed to viscosity losses in the centrifugation process.

Vasanthan and Temelli (2002) patented an aqueous alcohol-based enzymatic process in which barley or oat grain flour or bran is initially slurried in aqueous ethanol to remove starch and protein and screened. The retentate on the screen is reslurried in ethanol and then treated with protease and α-amylase. After screening, the β-glucan rich retentate is dried. The authors reported that with this technique, β-glucan is not solubilized and remains intact within the native cell wall, and is thus protected from shear fragmentation, showing superior physicochemical properties.

Inglett (1992) patented an aqueous alcohol-based enzymatic process in which oat bran or flour is slurried in a sufficient amount of water. The substrate is gelatinized and the pH is adjusted to about 6.0, then a thermostable α-amylase is added. The conditions of enzyme treatment are 95°C and 10−60 min. After completion of the enzymatic hydrolysis, the enzyme is inactivated by heat or acidification. The soluble fraction is separated by centrifugation and dried to yield a β-glucan concentrate containing dextrins. Later, Inglett (2000) patented an aqueous thermo-mechanical process

for β-glucan concentration. The process involves heat shearing of the oat or barley substrate (optionally in combination with other grain substrates) at a level to render the slurry sufficiently flowable that it will pass through pores of a filter, such as a sieve, or for insoluble crude fiber particles to be separated from the slurry by centrifugal forces. Oat or barley substrate is slurried with water at concentrations of about 8−18% (pH 5−7), a temperature of 90−150°C, and a time period of treatment of about 1−60 min.

Bhatty (1993, 1995) treated barley and oat bran at pilot scale using distilled water with 50% NaOH (1 hr, room temperature). After centrifugation, supernatant pH was adjusted to 6.5 (HCl) and $CaCl_2$ and α-amylase were added (1 hr, 80°C). After cooling to 50°C, the pH was adjusted to 4.5 (HCl) and the system was centrifuged to discard the pellet. Ethanol was added to the supernatant and held overnight. The resulting gum was separated through centrifugation.

Dawkins and Nnanna (1993) studied the extractability of oat gum using 12 treatment combinations in a factorial design: 2 oat products; 3 pHs (8.0−10.5); 4 temperatures (SO−70°C); and 2 replications. The extraction procedure involved alkaline treatment of flour and removal of starch residue, isoelectric precipitation of protein residue, and alcohol precipitation and collection of gum by centrifugation. Extracted oat gum ranged from 2.9% to 28% for oat bran and 1.82% to 5.24% for rolled oats, while β-glucan (in gum) ranged from 70% to 89% and 50% to 68%, respectively.

Temelli (1997) studied the effects of extraction temperature (40−55°C) and pH (7.0−10.0) on the recovery, purity, and functional properties of β-glucan from barley flour. There was a significant temperature, pH, and temperature/pH interaction effect on β-glucan recovery and the composition and functionality of the gums. β-glucan content increased with temperature but not with pH. Maximum gum yield, β-glucan content, and recovery and viscosity were achieved at pH 7.0 and 55°C. Viscosity increased with pH at constant temperature.

Ahmad, Anjum, Zahoor, Nawaz, and Din (2009) evaluated four methods of extraction of β-glucan from barley: alkaline extraction (NaOH), acidic extraction (citric acid), hot water extraction, and enzymes (α-amylase and protease) were used to facilitate extraction. Hot water treatment allowed obtaining the highest yield and recovery. This method also removed the maximum impurities from gum pellets and contained the highest amount of soluble fiber, while the alkali-extracted sample contained the highest amount of insoluble fiber.

Bae, Kim, Lee, and Lee (2012) studied the extraction of β-glucan from cauliflower mushroom. The optimal extraction conditions from cauliflower mushrooms were a pH of 6.05, an extraction time of 8 h 55 min, and a ratio of water to raw material of 19.74.

3.5.2 FRUCTOSE, OLIGOFRUCTANS, AND INULIN

The technology of inulin extraction from chicory roots is similar to saccharose extraction from sugar beets (Van Der Poel, Schiweck, & Schwartz, 1998). Classical extraction of inulin is performed on chicory by a hot water treatment (Franck, 2006). This procedure generally involves long extraction times (1.5−2 h). Franck (2006) and Moser, Agemans, and Caers (2014) reported detailed descriptions of the inulin extraction process from chicory roots. During harvesting, the roots are stored in piles on the field until transportation to the production site (Moser et al., 2014). Harvested roots are weighed, washed, and sliced. Raw inulin is extracted with hot water (70−80°C) in a countercurrent diffuser. The leached chips are dried (chicory pulp) and sold as animal feed. The obtained raw juice is filtrated before complete refining in the second phase. A first purification step

is applied to the extraction juice by liming with $Ca(OH)_2$ and carbonation at high pH. The raw juice is further refined using cationic and anionic ion-exchange resins for demineralization and active carbon for decolorization. Then, the juice is passed through a 0.2-mm filter to be sterilized, before evaporation and spray drying (Franck, 2006; Moser et al., 2014; Zhu, Bals, Grimi, & Vorobiev, 2012). During this whole process, strict control of pH and temperature is required to avoid breakdown of the inulin chain. Under acidic conditions, the inulin polymer is broken down to fructose units. The rate of this acid hydrolysis is directly proportional to temperature. Reduction in average chain length and loss of inulin also occurs at high pH (>10), where color increase is the most limiting factor. Chromatographic techniques are used to produce oligofructose in different ratios to sugars (Moser et al., 2014).

3.5.3 XYLANS

The most common methods for isolating AXs at laboratory scale are aqueous and alkaline extraction. AXs from wheat bran can be extracted in water. However, since they form hydrogen bonds with cellulose and are linked to lignin, a large portion is not easily extractable from the cell wall of wheat bran (Ebringerova & Heinze, 2000; Zhou et al., 2010). Ebringerová and Heinze (2000) described and compared various extraction procedures suitable for the isolation of xylans from different plant sources. Deutschmann and Dekker (2012) reviewed the research developments in extraction and purification methodologies and chemical modification as well as the analytical methods necessary for xylan-related research. Döring, Jekle, and Becker (2015) reviewed the commonly used methods of extraction and isolation of AX.

Sárossy, Tenkanen, Pitkänen, Bjerre, and Plackett (2013) extracted hemicelluloses from rye bran using hot water in order to avoid harsh or environmentally degrading processes. The isolated hemicelluloses resulted in a mixture of AXs and β-glucans. There is a large amount of research on the use of alkaline treatments in order to improve solubilization (Annison, Choct, & Cheetham, 1992; Bataillon, Mathaly, Nunes Cardinali, & Duchiron, 1998; Bergmans, Beldman, Gruppen, & Voragen, 1996; Beukes & Pletschke, 2011; Escarnot, Aguedo, Agneessens, Wathelet, & Paquot, 2011; García et al., 2013; Merali et al., 2013; Nilsson, Saulnier, Andersson, & Aman, 1996; Peng et al., 2010; Shiiba, Yamada, Hara, Okada, & Nagao, 1993; Sun, Fowler, Rajaratnam, & Zhang, 2010; Zhou et al., 2010). The use of xylanases (Benamrouche, Crônier, Debeire, & Chabbert, 2002; Escarnot, Aguedo, & Paquot, 2012; Moers, Celus, Brijs, Courtin, & Delcour, 2005; Zhou et al., 2010) and acid treatments is also reported (Aguedo, Vanderghem, Goffin, Richel, & Paquot, 2013; Otieno & Ahring, 2012; Wallace et al., 1995). In Table 3.1, some sources and methods used for xylan obtention are listed.

There are also some reports about the combination of enzymic and alkaline techniques. Aguedo, Fougnies, Dermience, and Richel (2014) extracted AXs from destarched wheat bran according to three different processes (an alkaline extraction, an enzyme treatment, and a hydrothermal process), aiming for production at industrial scale. The hydrothermal extraction was quick and efficient. Alkaline treatments led to one population of AXs with high Ara/Xyl ratios, with 3−4% fats, nonesterified neither by acetyl or methyl groups nor by ferulic acid, containing 0.65% uronic acids, and around 0.5% total phytates. Although the use of enzymes is much in line with a green chemistry perspective, the use of an endoxylanase leads to low extraction yields of AXs.

Table 3.1 Obtention of Xylans from Different Sources

Source	Method	Conditions	Reference
Wheat	Alkaline	0.2 M NaOH/$NaBH_4$, 80°C, 2 h	Annison et al. (1992)
Wheat	Alkaline	0.1−1 M NaOH, T: 20−80°C, *t*: 6 h	Bataillon et al. (1998)
Wheat	Alkaline	$Ba(OH)_2$ or $Ca(OH)_2$, T: 20−95°C, *t*: 16 h	Bergmans et al. (1996)
Wheat	Alkaline	80−120 g/L NaOH, T: 20−40°C, *t*: 30−60 min	García et al. (2013)
Wheat	Alkaline	0.5, 1, 4 M KOH/$NaBH_4$, T: 25°C, *t*: 2 h	Merali et al. (2013)
Wheat	Alkaline	0.2 M NaOH, T: 80°C, *t*: 1.5 h	Shiiba et al. (1993)
Wheat	Alkaline	0.15N NaOH, T: 80°C, *t*: 90 min	Zhou et al. (2010)
Wheat	Enzymatic	Xylanase, pH: 5.5, T: 60°C, *t*: 2 h	Zhou et al. (2010)
Wheat	Enzymatic	Endoxylanase, pH: 5.8, T: 60°C, *t*: 24 h	Benamrouche et al. (2002)
Wheat	Enzymatic	6 different endoxylanases, pH: 4.6, T: 30°C	Moers et al. (2005)
Wheat	Acid	HCl, T: 80°C, 100°C, pH: 1, 2, 3	Aguedo et al. (2013)
Rye	Alkaline	$Ba(OH)_2$, KOH room temperature, boiling KOH	Nilsson et al. (1996)
Sugarcane	Alkaline	3, 5, 8% NaOH, T: 50°C, *t*: 3 h	Peng et al. (2010)
Sugarcane	Alkaline	NH_4OH, NaOH, different concentrations, T and *t*	Beukes and Pletschke (2011)
Sugarcane	Acid	0.1 % H_2SO_4, T: 60°C, *t*: 12 h	Otieno and Ahring (2012)
Barley	Acid	30 mM oxalic acid, *t*: 3 h	Wallace et al. (1995)
Spelt	Alkaline	NaOH, pH: 11.5, T: 60°C, *t*: 4 h	Escarnot et al. (2011)
Spelt	Enzymatic	Different endoxylanases, *t*: 1, 4, 24 h, pH, and T according to the enzyme	Escarnot et al. (2012)

Mansberger et al. (2014) extracted AXs from rye bran in laboratory and pilot scale. For pilot-scale experiments, three protease combinations and different pH levels of the solvent (neutral and alkaline conditions) were tested. The extract was cleared by centrifugation, and protein and starch were degraded with enzyme, heat, and bentonite treatment. Finally, pentosans were separated with alcoholic precipitation or ultra diafiltration. Alkaline conditions showed better performance for extraction than pure water. Compared to precipitation with 65% ethanol, ultra diafiltration with a membrane with a cutoff-level of 8 kDa proved to be a better method for purification, because loss of AXs was much lower.

Escarnot et al. (2012) extracted water-extractable (WE) and water-unextractable (WU) AXs from micronized spelt bran after enzymic destarching and deproteinization treatments. WU−AXs were obtained by two successive extractions with 2% alkaline hydrogen peroxide at 60°C during 4 h. According to the results, 55% of the AX present in spelt bran was extracted by using the three extraction steps (WE- and WU-AXs), among AX, 13% were WE and 87% were WU.

3.5.4 STARCH AND DEXTRINS

Starch is mostly extracted from maize, wheat, and potato, but it can also be isolated from many other plants such as rice, barley, vegetables, manioc, and sweet potato. The starch industry

separates the components of the plant, starch, protein, cellulose, soluble fractions, and in the case of maize, the germ from which oil will be extracted. The methods of manufacture are specific to each plant and to a raw material.

The International Starch Institute (http://www.starch.dk/) presents detailed information on starch-manufacturing methods. Starch, Europe (http://www.starch.eu/) also presents diagrams of the main starch-production processes. Murray, Gross, and Fox (1994) describe the production process of corn, wheat, and potato starches.

3.5.4.1 Cornstarch production

The raw material is inspected and cleaned and then submitted to steeping in a continuous countercurrent process where the material is soaked in hot water ($\approx 50^{\circ}C$) containing a small concentration of sulfur dioxide, for 24–48 h, to begin breaking the starch and protein bonds. Sulfur dioxide improves fermentation by enhancing growth of favorable microorganisms, preferably lactobacillus, while suppressing detrimental bacteria, molds, fungi, and yeast. Solubles are extracted and the kernel softens. The kernel swells to more than double the size and increases its moisture content from about 15% to 45%. The steep water is drained and evaporated. The softened kernels are ground in impact or attrition mills to loosen the hull and separate the germ from the endosperm. The germ is separated by hydrocyclone separators and dried. The remainder of the kernel is submitted to additional grinding and screening. After passing through a grinding mill, the hulls are caught on screens and the slurry of starch and gluten is separated by centrifugation. The gluten is dried and the starch is washed and dewatered. With hydrocyclones and a centrifuge, the starch settles and separates from the water and fibers.

The starch slurry can go through different processes to yield starch, dextrins, or glucose. To produce starch, the starch slurry is dried in a flash drier and screened on a fine sieve to produce unmodified (native) starch. To produce modified starches, it is treated with chemicals or enzymes. By applying different reaction conditions—temperature, pH, additives—and strict process control, a variety of products with unique properties are made. To produce dextrins, the starch slurry is dried and heated or roasted with or without a catalyst (acid or alkaline). To produce glucose syrup and dextrose, it is treated with acid or enzymes and heated to break down the starch molecules. The corn syrup is refined using carbon. Water can be removed to produce regular glucose syrup or the syrup can be submitted to ion exchange to produce dextrose or high fructose syrup. Finally, to produce liquid glucose the original starch slurry is fermented and distilled.

3.5.4.2 Wheat-starch production

The most common method is to ground the wheat. The resultant flour is mixed with water to form a dough, which is then rolled or kneaded and the starch is washed off using water sprays. The gluten is separated on screens, washed, and dried. The starch slurry is submitted to a screening process and then through a series of centrifuges and/or hydrocyclones to concentrate the slurry. The starch is finally dried.

Another method is to discharge flour into a stream of warm water. Water and flour are mixed in-line and the slurry obtained is homogenized in a high-speed in-line disintegrator. The homogenized slurry is immediately separated into different fractions by a three-phase decanter. The starch fraction is the heavy phase. It is reslurred and refined by washing with fresh clean water. With hydrocyclones it is feasible to reduce fiber and solubles, including soluble protein, to low levels

with a minimum of freshwater. To save water the wash is done countercurrently. The refined starch milk is discharged to a peeler centrifuge for dewatering. The peeler filtrate is recycled to the process. The dewatered starch is peeled off and discharged by gravity to the moist starch hopper and then it is fed by a metering screw conveyor into a flash dryer and dried in hot air. The inlet air temperature is moderate. The dried starch is pneumatically transported to a starch silo ready for screening and bagging. The moisture of starch after drying is normally 12−13%. Before delivery, the starch is screened on a fine sieve to remove any scale formed in screw conveyors, etc.

3.5.4.3 Potato-starch production

Potatoes are dropped into water running flumes to remove stones and then carefully cleaned in a washer. The cleaned potatoes are grinded or crushed to disintegrate cells and release starch. A screen or a rotary sieve separates the fiber and skins, while the starch solution is purified through cycles of redispersion in water and filtration. The purified starch is then dewatered and dried.

3.5.4.4 Cassava/tapioca starch production

Cassava roots are sorted to select the wholesome ones. A rotating bar screen is used for dry cleaning of the roots prior to the washing step. Paddle or rotary washers are used for dirt removal. Roots are crushed or rasped. After rasping, the hydrogen cyanide and cyanohydrin are released and removed with the juice and processing water. Sulfur is added to prevent discoloration. Powerful flushing is needed to release the starch granules from the cells. The starch that is flushed out leaves the extraction sieves along with the fruit juice. The extraction takes place on rotating conical sieves using a countercurrent process. The starch slurry is concentrated and refined in hydrocyclones. The purified starch milk is dewatered on a continuous rotating vacuum filter or batch-operated peeler centrifuge. Finally, the moist dewatered starch is dried in a flash dryer with hot air.

3.5.4.5 Starch modification

Starch is used as a thickener, bulking agent, gelling agent, colloid stabilizer, etc. Native starches are characterized by their lack of stability under temperature, shear, and pH stresses. Modification of their physical, chemical, and enzymatic allows improving their physical and chemical properties for use in specific food applications (Liu, Hu, & Shen, 2010).

The process of starch modification involves the destructurization of the semicrystalline starch granule. In this way, the reactive sites or hydroxyl groups of the amylopectin polymer become accessible to electrophilic reactants. Glucose residues control the chemical reactivity of starch. Chemical modifications are generally achieved through etherification, esterification, cross-linking, oxidation, cationization, and grafting and changing the functional and physicochemical characteristics of starch. These modifications change gelatinization, pasting, and retrogradation behavior and the efficiency of the modification process depends on the reagent type, botanical origin of the starch, and on the size and structure of its granules (Martins Ochubiojo & Rodrigues, 2012; Simi & Abraham, 2007). The physical modification of starch is mainly done to change the granular structure and to convert native starch into cold water-soluble starch or small-crystallite starch. The methods used involve instantaneous cooking−drying of starch suspensions on heated rolls (drum-drying), puffing, continuous cooking−puffing−extruding, and spray drying (Ashogbon & Akintayo, 2014).

Under mild conditions, enzymatic methods can be more specifically controlled than chemical ones. Enzymatic modification of starches involves the use of enzymes found in plants, such as pullulanase and amylase. The first hydrolyzes α-1,6-glycoside bonds in amylopectin and dextrins when their side chains include at least two α-1,4-glycoside bonds. Isoamylase is an enzyme that totally hydrolyzes α-1,6-glycoside bonds in amylopectin, glycogen, and some branched maltodextrins and oligosaccharides, but it is characterized by lower activity than pullulanase. Starch containing a higher proportion of α-(1-6) linkages can be produced using the 1,4-α-glucan branching enzyme by itself or in combination with hydrolytic enzymes such as β-amylase, α-glucosidase, or amyloglucosidase (Kasprzak et al., 2012).

3.5.5 SUCROSE

Hugot (1986), Clarke (1988), and Chen and Chou (1993) provide detailed information about sugarcane processing. The manufacture of refined sugar from both beet and cane plants is discussed by Pennington and Baker (1990), Christodoulou (2003), and more recently, by White (2014). Juices of sugarcane and sugar beet are rich in pure sucrose, although beet sugar is generally much less sweet than cane sugar. These two sugar crops are the main sources of commercial sucrose. Other sugar crops include sweet sorghum, sugar maple, honey, and corn sugar. The types of sugar used today are white sugar (fully refined sugar), which is comprised of clear, colorless, or crystal fragments, or brown sugar, which is less fully refined and contains a greater amount of treacle residue, from which it obtains its color.

After the cane arrives at the mill yards, it is mechanically unloaded, where excessive soil and rocks are removed. The cane is cleaned by flooding with warm water or by spreading the cane on agitating conveyors that pass through strong jets of water and combing drums. Clean cane is chipped. When the beets are delivered at the refinery, they are first washed and then cut into strips.

Cleaned sugar-beet slices and sugarcane chips are shredded and milled to extract the juice and separate the fiber (bagasse). Hot water is sprayed onto the crushed material countercurrently as it leaves each mill for diluting. The disrupted material is soaked in water to which lime and sulfur dioxide are added to adjust pH, minimize subsequent color formation, and control microbial growth. The material is filtrated to improve clarity, and the clear juice undergoes vacuum evaporation to remove most of the water. Vacuum-boiling cells are arranged in series so that each succeeding cell has a higher vacuum and therefore the juice boils at lower temperature. The syrup leaves the last body continuously with about 65% solids and 35% water contents.

Next, the syrup solution is vacuum crystallized to form sugar crystals. The syrup is evaporated until saturated with sugar. As soon as the saturation point has been exceeded, small grains of sugar (seed) are added to serve as nuclei for the formation of sugar crystals. Additional syrup is added to the strike and evaporated so that the original crystals that were formed are allowed to grow in size. When sucrose concentration reaches the desired level, the dense mixture of syrup and sugar crystals is discharged into crystallizers and is slowly stirred and cooled. The remaining liquid is removed using centrifugation and drying. Damp sugar crystals are dried by being tumbled through heated air in a granulator. The dry sugar crystals are then sorted by size through vibrating screens and are packed.

3.6 EMERGING TECHNOLOGIES FOR THE EXTRACTION, RECOVERY, AND MODIFICATION OF CARBOHYDRATES IN FOOD

Conventional extraction procedures of polysaccharides are complex and inefficient, resulting in low yield and time-consuming processes that require huge amounts of energy (heating and stirring) (Benito-Román, Alonso, & Cocero, 2013b; Galanakis, 2013).

Nonthermal processing methods currently being explored for a variety of ready-to-eat products to retain fresh attributes of food while ensuring safety (Pivarnik & Worobo, 2014) include high-pressure processing (HPP), gases (ozone, chlorine dioxide, cold plasma (CP)), light (ultraviolet, pulsed light), chemicals (chlorine, surfactants), and ionizing radiation (γ-irradiation, electron beam). But other techniques have also been assayed (Deng et al., 2014; Galanakis, Barba, & Prasad, 2015; Galanakis & Schieber, 2014; Roselló-Soto et al., 2015a,b; Zinoviadou et al., 2015).

3.6.1 β-GLUCAN HYDROLYSATES

In the extraction of β-glucans, mass transfer is hindered due to the high viscosity of the extract−solution mixture formed during the process, the low concentration in the raw material, and the diffusion limitations of the solvent into the cereal flour particles. Benito-Román et al. (2011) showed that stirring rate and particle size were the variables controlling the extraction process of β-glucans. To extract bound phenolics, it is necessary to break the bond between them and the polysaccharides they are linked to. This is only possible using NaOH solutions up to 4 M. (Fry, 1986; Renard et al., 2015). To overcome the mass transfer limitations and enhance the simultaneous extraction of β-glucans and phenolics, new processes are required.

In order to maximize both the extraction yield and molecular weight of β-glucans, Benito-Román et al. (2013a) studied the effect of the amplitude, time and cycle, and their combination, which represents different amounts of energy, on ultrasound (US)-assisted extraction from barley. According to the results, the extraction yield of β-glucan hydrolysates was dependent on the amplitude and, especially, on the extraction time. Indeed, the molecular weight of β-glucan molecules was decreased by increasing the processing time. The maximum extraction yield (66.1%) was achieved when delivering the maximum amount of energy (962.5 kJ/L), leading simultaneously to the lowest molecular weight (269 kDa). Reducing the treatment intensity (energy output of 170 kJ/L) produced a decrease in the extraction yield to a minimum of 44.3%, but was accompanied by increasing β-glucan molecular weights up to 461 kDa. Cavitation created during sonication slightly improved the extraction yield of β-glucans and their molecular weight compared to that obtained in a stirred tank extraction (3 h, 55°C, 1000 rpm). However, the main effect of the US was related to the reduction in processing time (3 min against 3 h) and energy consumption (170 kJ/L against 1460 kJ/L). Both effects revealed a more efficient process, which is very interesting for scale-up of the process. In any case, energy (time, amplitude, and cycle) must be chosen as a function of the desired final product, in terms of β-glucan content and molecular weight. High-intensity treatments (>500 kJ/L) cause important depolymerization of the β-glucans, which in some cases may be the purpose. Although the effect of cycle on yield and molecular weight is limited, it has been noted as a useful mechanism to control the temperature of the system.

In order to facilitate mass transfer and diffusion, new processes like pressurized hot fluid (PHF) are promising for the extraction of β-glucan hydrolysates from cereal sources. The PHF process

involves the treatment of the milled vegetable material with a fluid in its liquid state above its boiling temperature, by the application of pressures above the normal one. Pressurized hot water and pressurized hot aqueous ethanol are the most common fluids used for phytochemical extraction (Saldaña & Valdivieso-Ramírez, 2015). The increase in temperature leads to important changes in the physicochemical properties of water related to the reduction of the relative permittivity (ε_r), viscosity, and surface tension or the increase of diffusivity. Benito-Román et al. (2015) applied a pressurized (25 bar) process to dehulled waxy barley cultivar variety "fiber" for the extraction of β-glucans and phenolic compounds through an aqueous ethanolic solution (3 g of barley:100 mL of water-ethanol). The effect of temperature (135−175°C), extraction time (15−55 min), and ethanol concentration (5−20%) was studied. It was determined that as temperature increased from 155°C to 175°C, lower yield of β-glucan was obtained (40%) due to fragmentation. However, a significant increase in the phenolic recovery was observed. High temperatures and long extraction times (>35 min) resulted in low concentrations of β-glucans and high phenolic content in the liquid extract. In addition, high temperatures tended to degrade the β-glucans, as indicated by the low extraction yields with low molecular weight as well as by the presence of sugar (hexoses) degradation compounds. Long processing times favored the extraction of β-glucans at lower temperatures (yields of 65−70%) and of phenolics at every temperature assayed. The ethanol concentration did not influence the yield of the β-glucan extraction, but helped to maintain the molecular weight of the extracted polymer. To obtain extracts rich in high-molecular-weight β-glucans (500−600 kDa) and phenolics (5 mgGAE/g barley), mild conditions of extraction at 151°C for a short period (21 min) and using 16% ethanol were needed, leading to a 51% of extractive yield for the β-glucans. Therefore concentrated solutions rich in β-glucans and antioxidants to be used as food additives and/or ingredients could be obtained. The presence of β-glucans and antioxidants in one single product would complement and reinforce the health benefits associated with both kinds of bioactive compounds.

3.6.2 XYLOGLUCANS, MANNANS, AND XYLANS

According to Minjares-Fuentes et al. (2016), the US method was used to efficiently extract hemicelluloses and their constitutive fractions (xyloglucans, mannans, and xylans) from grape pomace winery residue at mild temperatures (20°C). The extraction time, solid:liquid ratio, and potassium hydroxide (KOH) concentration were the variables studied.

The main variation was found in the KOH concentration needed for maximal yields in the isolation of hemicelluloses as a whole (7.9%), of xyloglucans (3.6%), mannans (1.1%), and xylans (1.2%), which varied between 0.4 M for hemicelluloses and 2.2−2.3 M for the rest. The optimal extraction times and solid:liquid ratios were between 2.6 and 3 h as well as 1:48 and 1:60 w/v, respectively. By US assistance, it is possible to obtain relatively high extraction yields of hemicelluloses using low KOH concentrations (0.4 M) and short extraction times (2.6 h).

3.6.3 OLIGOFRUCTANS AND INULIN

Alternative techniques for extraction of inulin and oligofructans are being investigated with the purpose of reducing the use of extraction solvents and processing times and therefore decreasing energy consumption and also increasing extraction yields.

3.6.3.1 US-assisted extraction

US-assisted extraction has recently been proposed to improve inulin extraction yield compared to traditional hot water methods, with the main independent variables being sonication amplitude, temperature, and time (Apolinário et al., 2014). Some authors have reported that the application of US could cause changes in the chemical composition of inulin. The breaking of fructan molecules, forming low-molecular-weight fragments by the direct action of US, could diminish the quality and functionality of these bioactive compounds (Apolinário et al., 2014; Narváez-Flores et al., 2015). In the direct method a probe is directly inserted into a sample vessel, while the indirect sonication is performed by immersion of the sample in an US cleaning bath and shaking it periodically in an orbital shaker (Apolinário et al., 2014).

Narváez-Flores et al. (2015) evaluated the effect of US power and temperature on the extraction of fructans from agave (*Agave tequilana* Weber var. Azul) and reported that fructan extraction was significantly increased by the use of US. Results showed a strong sonication effect in carbohydrate extraction at any extraction temperature. Microscopy analysis showed an alteration in the cellular structure and some damages in the cell wall of the agave plant at high US power at both low and high temperatures. Pourfarzad, Habibi Najafi, Haddad Khodaparast, and Hassanzadeh Khayyat (2015) studied the extraction of fructans from *Eremurus spectabilis* root powder (Serish) with conventional water extraction, direct sonication, and indirect sonication. After direct sonication, they obtained the highest amount of extraction yield and purity but a decrease in the DP when compared to indirect sonication. Therefore the authors concluded that indirect sonication extraction is a suitable method for fructan extraction. Ultrasonic technology was also applied by Milani, Koocheki, and Golimovahhed (2011) for inulin extraction from Burdock root (Arctium *lappa*) in order to increase the extraction yield. The use of high-intensity US significantly improved the extraction of inulin. It was shown that when increasing the amplitude (from 20% to 85%) and extraction time, the extraction yield increased. The optimum extraction conditions were found to be 25 min of sonication time, 83.22% of amplitude, and a temperature of 36.76°C.

3.6.3.2 Ultrasonic/microwave-assisted extraction

Lou, Wang, Wang, and Zhang (2009) studied the simultaneous ultrasonic/microwave-assisted extraction (UMAE) of inulin and the production of phenols-rich dietary fiber powder from burdock root. It was found that UMAE required much shorter extraction time than conventional stirring extraction and the recovery of inulin increased with the increase of microwave power and solvent: solid ratio. They concluded that although the recovery of inulin by UMAE was appreciably lower than that reached by conventional stirring, the extraction time was significantly shortened from 300 to 60 s through the application of this technique.

3.6.3.3 Microwave-assisted extraction

According to Chemat, Huma, and Khan (2011), microwave-assisted extraction (MAE) has attracted growing interest as it allows the efficient use of microwave energy to extract valuable compounds from solid samples. Nevertheless, the method is limited in terms of solvents and the nature of the solid material since its efficiency is typically low when the solvent lacks a significant dipole moment for microwave energy absorption. Ruiz-Aceituno, García-Sarrió, Alonso-Rodriguez, Ramos, and Sanz (2016) applied MAE and pressurized liquid extraction (PLE) methods using water

as solvent for the extraction of bioactive carbohydrates (inositols and inulin) from artichoke (*Cynara scolymus* L.) external bracts. MAE was less time consuming than PLE. Although coextraction of other interfering sugars could not be avoided, yeast treatment was shown to be effective for their subsequent removal, allowing the enrichment of extracts in these bioactive compounds. MAE applied on 0.3 g of sample at 60°C for 3 min allowed the extraction of slightly higher concentrations of inositol than PLE at 75°C for 26.7 min. In contrast, under these conditions, higher concentrations of inulin were extracted with the latter technique, considering two successive extraction cycles for both procedures.

3.6.3.4 Accelerated solvent extraction

Accelerated solvent extraction (ASE) makes use of the same solvents as traditional extraction methods while operating at elevated temperatures and pressures. Saengkanuk, Nuchadomrong, Jogloy, Patanothai, and Srijaranai (2011) used ASE for the aqueous extraction of inulin from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. It was found that the highest extraction yield of fructose and glucose was obtained by heating at 80°C for 20 min. Longer extraction times, along with higher temperatures, resulted in decreased inulin recovery. In addition, conventional extraction was also performed at its optimum condition (85°C for 1 h) and the extraction time could be reduced by at least three times in comparison to the regular method.

3.6.3.5 Pulsed electric field

The application of pulsed electric field (PEF) with very short duration (generally from several microseconds to several milliseconds) causes limited damage to cell tissue. The biological membrane is electrically pierced (electroporated) and loses its semipermeability temporarily or permanently, allowing the passage of small or bigger molecules to the surrounding liquid. Loginova, Shynkaryk, Lebovka, and Vorobiev (2010) studied the effects of thermal and PEF treatments on the efficiency of soluble solids extraction from chicory tissues. They concluded that there is clear evidence of the benefits of PEF application for enhancement of soluble-matter extraction from chicory. The activation energy of the thermal damage of the chicory tissue was noticeably reduced. The PEF pretreatment removed cell-membrane barriers and noticeably accelerated solute extraction even at low temperatures within 20−40°C. Zhu et al. (2012) studied the PEF-assisted countercurrent diffusion of inulin from chicory roots. A PEF treatment duration of 10 ms at 600 V/cm was sufficient for the effective extraction of chicory roots. According to these authors, application of this technique allowed decreasing the diffusion temperature 10−15°C, which largely covered the PEF electrical energy consumption.

3.6.3.6 Electroporation and ohmic heating

Zhu et al. (2015) studied the damage of chicory tissue by combined electroporation and ohmic heating for better solute extraction. Although combined electroporation/ohmic heating pretreatment by moderate PEF is more energy consuming than the nonthermal high PEF, the authors noted the benefits of this method for the enhancement of inulin extraction. For the same pulse parameters, combined electroporation/ohmic heating resulted in faster and more complete damage of chicory tissues as compared to the nonthermal electroporation.

3.6.3.7 Supercritical fluid extraction

Supercritical fluid extraction (SFE) is an innovative and environmentally friendly extraction process that uses a supercritical fluid as an alternative to commonly used organic solvents (Chemat et al., 2011). Mendes, Cataldo, da Silva, Nogueira, and Freitas (2005) evaluated the operational parameters of supercritical extraction technology of inulin from chicory. The maximum solute recovery was reached at 40°C and 150 bars, for 2 h of extraction. The efficiency of inulin extraction by SFE CO_2, measured by the relation between extracted mass and inulin mass present in the raw material, was inferior to 10%, probably due to the high molecular weight of inulin. The authors suggested a cosolvent could be used with the carbon dioxide in order to increase efficiency.

In Table 3.2, different emerging technologies used for the recuperation of oligofructans and inulin are listed.

3.6.4 SUCROSE

The traditional method of extracting intracellular components from plant tissues, such as sugar, pectin, pigments, is based on solid−liquid extraction. Normally, the amount of compounds released to the liquid-extracting solvent depends on the degree of disintegration of the tissue cells. Cell walls and membranes are important obstacles to the extraction of intracellular compounds (Loginova, Vorobiev, Bals, & Lebovka, 2011a; López, Puértolas, Condón, Raso, & Álvarez, 2009). For example, extraction of sucrose from sugar beet is a multistep process (slicing, extraction, purification, evaporation, crystallization). The extraction is a key step influencing the whole transformation process. Conventionally, sugar-beet slices are first rapidly (10 min) preheated at high temperature (85−90°C) to denature cells membranes. Sucrose extraction is then carried out by countercurrent diffusion in hot water (70−75°C) for about 60−90 min. The thermal aqueous extraction of sugar involves high energy consumption and promotes the coextraction of undesirable compounds such as proteins, pectins, and colloids, and increases the juice coloration. The presence of these juice impurities enormously complicates the subsequent purification, evaporation, and crystallization steps. Mhemdi, Bals, and Vorobiev (2016) used PEF (10°C, 5 kV−1 KA generator, electric field strength of 600 V/cm, and 10 ms-PEF treatment duration, 100 μs pulse duration) for electroporation of sliced beet roots before pressing at 5 bar (4 min) for juice extraction and sugar-beet sucrose production. The juice yield from untreated slices did not exceed 25%, but it attained 50% of undiluted juice (Brix $\approx$21.2%) after pressing of PEF pretreated slices for 4 min. The obtained press cake was finally introduced in a pilot diffuser (10 kg/h) to extract the residual sucrose (30°C or 70°C). Sucrose extraction from PEF pretreated and pressed slices with reduced liquid-to-solid ratios from 120% to 80−100%, at 70°C or even at 30°C, allowed substantial water and energy savings for the extraction step. It was determined that slices were better exhausted by the new extraction process, which reduced the sucrose loss in pulp from 1.2% to 0.8%. The obtained juice was more concentrated in sucrose, less colored, and purer than that conventionally extracted by diffusion. Alternative PEF pressing−diffusion technology can be very effective for sucrose extraction from sugar beets.

Sugar-beet tails and green biomass (leaves, petioles, rootlets, etc.) are recovered during sugar-beet washing before slicing. Sugar-beet tails represent about 2−6% of sugar-beet roots, which are less rich in sucrose and contain more impurities (pectins, proteins, polymers, etc.) than sugar-beet

Table 3.2 Obtention of Oligofructans and Inulin Using Different Emerging Technologies

Technology	Source	Conditions	Effect	Reference
Ultrasound	Agave	Power: 28−14 mW/mL	Alteration in cellular structure and damages in the cell-wall	Narváez-Flores et al. (2015)
Ultrasound	Jerusalem artichoke	Frequency direct sonication: 20 KHz, frequency indirect sonication: 59 KHz, *t*: 2−40 min, T: 76°C		Lingyun et al. (2007)
Ultrasound	Serish	Amplitude: 20−100%, T: 30−70°C, *t*: 5−40 min	Development of microfractures and disruption of cell walls microfractures and changes in the surface morphology of fructan granules increase in the magnitude of the zeta potential	Pourfarzad et al. (2015)
UMAE	Burdock root	Microwave power: 150−400 W, ultrasonic power: 50 W, *t*: 60 s	Microfractures and disruption of cell walls	Lou et al. (2009)
MAE	Artichoke	Power: 900 W, T: 50−120°C, *t*: 3−30 min		Ruiz-Aceituno et al. (2016)
PLE	Artichoke	Pressure: 100 bar, T: 40−120°C, *t*: 3−30 min		Ruiz-Aceituno et al. (2016)
Ultrasound	Burdock	Amplitude: 20−100%, T: 20−60°C, *t*: 5−25 min		Milani et al. (2011)
ASE	Jerusalem artichoke	Time: 20 min, T: 80°C, pressure: 1500 Psi		Saengkanuk et al. (2011)
PEF	chicory	Temperature: 20−80°C, electric field intensity: 100−600 v/cm, *t*: 10^{-3}−50 s	Removal of cell-membrane barrier Enhancement of membrane permeabilization	Loginova et al. (2010)
PEF	Chicory	T: 30−80°C, *t*: 10−50 ms, electric field intensity: 600 v/cm		Zhu et al. (2012)
PEF/ohmic heating	Chicory	Electric field strength: 400−1000 and 10,000 V/cm	Faster and more complete damage of tissues	Zhu et al. (2015)
SC CO_2	Chicory	T: 40−80°C, pressure: 62−170 bars, *t*: 2 h		Mendes et al. (2005)

slices. They are habitually separated from green biomass and mixed with sugar-beet pulp exhausted in an extractor. They can also be mixed with sugar-beet slices going to the extractor. In this case, the impurities extracted from the tails decrease the quality of the raw extracted juice and consequently complicate the subsequent purification and crystallization procedures. According to sugar makers, the valorization of sugar-beet tails with pulp is not cost effective, and their mixing with

sugar-beet slices leads to reduced extractor efficiency. Almohammed, Mhemdi, and Vorobiev (2016) used PEF as a pretreatment of sliced sugar-beet tails (12°C, 5 kV−1 kA pulse generator, electric field intensity between 0 and 600 V/cm) before submitting to solid−liquid expression at a constant pressure (1−5 bar and 10−50 bar) to produce a fermentable juice containing sucrose for bioethanol. The optimal electroporation of cell membranes involved a PEF intensity and duration of 450 V/cm and 10 ms, respectively, corresponding to an energy input $Q = 1.91$ Wh/kg. At these PEF parameters, the yield of solutes increased from 16.8% to 79.85% and the dryness of the pressed cake increased from 15% to 24% compared to the untreated tails. The PEF expressed juice was more concentrated (10% vs 5.2%) and had higher sucrose content (8.9 vs 4.5°g/100 g tails) than the juice expressed from untreated tails. The juice expressed from PEF-treated tissue was also purer (89.2% vs 86.9%) and contained less colloids such as pectins (19.6 vs 31.7 mg/g solutes) and proteins (1.5 vs 2.75 mg/g solutes) than the untreated one. Batch fermentation experiments with commercial *Saccharomyces cerevisiae* yeast showed that the higher content of fermentable sugars in PEF-pretreated expressed juice led to higher ethanol content in distillate (6.1% vs 2.95% v/v) and to a higher CO_2 weight loss (57.2 vs 28.3 g/L) than that obtained from the raw juice of unpretreated tissue. The PEF pretreatment notably improved the sucrose production and juice purity.

López et al. (2009) also used the PEF technique (1−7 kV/cm, 5−40 pulses, 1−10 Hz pulse frequency, specific energy of 0.006−0.19 kJ/kg per pulse, 2−5 ms pulse width) to enhance the solid−liquid extraction by diffusion in water (20−70°C) of sucrose from sugar-beet roots (*B. vulgaris*) (1 g sliced roots/50 mL water). At 7 kV/cm of PEF treatment, the efficiency of the solid−liquid extraction step was independent of the frequency as well as of the pulse width and pulse shape, but it was influenced by the electric-field strength applied and by the temperature of the extracting medium. The temperature of the root slices never rose above 30°C during the PEF treatment. Sucrose yield increased with field strength, time of extraction, and temperature. The effect of the field strength was higher when the extracting temperature was lower.

The application of 20 pulses at 7 kV/cm (3.9 kJ/kg) increased the maximum yield by 7 and 1.6 times, compared to untreated samples, at 20°C and 40°C, respectively. It was also observed that to achieve an 80% of sucrose extraction in 60 min, the temperature could be reduced from 70°C to 40°C when 20 pulses of 7 kV/cm were applied. This reduction in temperature would allow reducing the thermal energy consumption of the process by more than 50% along with a likely purer and more stable juice.

Loginova et al. (2011a) studied the use of PEF (100−600 V/cm electric field strength, 50 ms total time, 30−70°C temperatures) treatment previous to the countercurrent cold and mild heat extraction of sucrose from sugar beets, at a pilot-plant scale. The optimal parameters of the PEF procedure were between 600 V/cm (at 30°C) and 260 V/cm (at 60°C). The authors determined that the purity of the juice obtained by cold (30°C) and moderate (50−60°C) thermal extraction was not lower than the purity of juice obtained by conventional hot water diffusion at 70°C. In addition, the sugar-beet pulp could be well exhausted by cold or mild thermal (30−60°C) extraction of cossettes (17.8−20.8 g sucrose/100 g of cossettes) treated by PEF. The pulp obtained by cold extraction of PEF-treated cossettes had dryness >30%, which was noticeably higher than the dryness of the pulp obtained by the conventional hot water extraction procedure. In reference to the quality of the juice extracted, Loginova, Loginov, Vorobiev, and Lebovka (2011b) determined that PEF-assisted cold (30°C and 50°C) extraction produced sugar beet juices with higher purity (95.3%), in comparison to the classical hot extraction (70°C).

It was shown that application of PEF cold extraction resulted in lower concentration of colloidal impurities (mainly pectins), low coloration, and better filterability of the juice. Concentration of various colorants and their intermediates decreased significantly with the extraction temperature decrease from 70°C to 30°C. Lower temperatures are not favorable for browning development from sucrose. Treatment of the sugar-beet juices obtained through the method of lime carbon dioxide showed better purification when obtained by low-temperature aqueous extraction assisted by PEF (Loginova, Loginov, Vorobiev, & Lebovka, 2012).

The ultrafiltration of sugar-beet juices obtained by (1) conventional high temperature (70°C) diffusion and (2) the PEF-assisted (600 V/cm, monopolar pulses, 100 μs pulse duration, 200 Hz, 500 pulses) low-temperature (30°C) diffusion were compared by Loginov, Loginova, Lebovka, and Vorobiev (2011). A temperature-controlled pilot-scale diffuser was used. Noticeably low coloration and lower concentration of colloidal and polymer impurities (mainly pectins) in the diffusion juice was obtained when the extraction was performed at 30°C and assisted by the PEF pretreatment under stirring compared to the traditional diffusion process performed at 70°C. Low concentration of foulants in the juice obtained at 30°C resulted in higher filterability measured upon unstirred dead-end ultrafiltration, which was explained by the reduced formation of deposit.

Crystallization is a step involved in the process of sucrose obtention. Methods of sucrose nucleation include spontaneous nucleation, stimulated nucleation, and secondary nucleation. Nucleation of sucrose at high supersaturation is difficult to control and the nuclei easily form aggregates, whereas at low supersaturation it is difficult to achieve nucleation using traditional methods. Hu et al. (2015) analyzed the effect of the PEF treatment on crystallization and explored a feasible method to enhance sucrose nucleation at low supersaturation. The PEF treatment (10−30 kV/cm electric field strengths, 15−50 mL/min flow rates, volume of 3.0 mL) was applied on sucrose solutions at supersaturations ranging from 1.05 to 1.20. It was established that the PEF treatment had a profound influence on sucrose nucleation. In particular, the total nuclei number induced by PEF was proportional to the supersaturation degree and field strength, but varied inversely with the flow rate.

3.6.5 STARCH MODIFICATION

Starch modification through novel techniques has been reported mainly in the last 10 years.

3.6.5.1 Ultrasound

Herceg et al. (2010) studied the effects of high-power US probe and US bath (both at 24 kHz frequency) on cornstarch suspensions in water. The treatment involved different intensities and treatment times (15 and 30 min). The treatments with high-power US probes caused a significant reduction of the starting gelatinization temperatures of cornstarch. The US treatment caused disruption of starch granules by cavitational forces and made the granules more permeable to water. An increase in water solubility (20°C) was also observed, caused by the disruption of starch granules and molecules by US treatment. The texture-profile analyses of the starch gel prepared by the probe-treated suspensions presented higher hardness and higher values of adhesiveness and cohesiveness than untreated suspensions or those treated with US bath. US treatment produced the mechanical damage of the starch granules due to the collapse of cavitation bubbles, which induced high-pressure gradients and high local velocities of the liquid layers in their vicinity. They concluded that this technique can be used to modify the properties of starches. Using a similar

procedure to the one previously described. Jambrak et al. (2010) observed a decrease in enthalpy of gelatinization, a significant decrease in consistency, and an increase in the swelling power for cornstarch after US treatment. Czechowska-Biskup, Rokita, Lotfy, Ulanski, and Rosiak (2005) studied the degradation of cornstarch by 360 kHz US and reported that this technique is useful for reduction of molecular weight.

Manchun, Nunthanid, Limmatvapirat, and Sriamornsak (2012) modificated tapioca starch using a high-power ultrasonic treatment (400 W), controlling the US amplitude (50% and 100%) and the sonication time (10, 20, and 30 min). The ultrasonic treatment distorted the crystalline region of granules, especially at higher amplitude or sonication time. The starch showed a higher increase of swelling power after US treatment than after heat treatment.

Sujka and Jamroz (2013) studied potato, wheat, corn, and rice starch granule suspension in water or ethanol, sonicated at a frequency of 20 KHz and power of 170 W for 30 min. They concluded that starches were depolymerized and the degree of this phenomenon was higher in water than in ethanol. Ultrasonic treatment, especially in water, influenced the functional properties of starch, causing an increase of fat and water absorption, less gelling concentration (except potato starch), solubility, and swelling power, and a finally decrease in starch-paste viscosity.

Zheng et al. (2013) studied the effects of a single-frequency US of 25 or 80 kHz and dual-frequency US of 25 and 80 kHz on sweet-potato starch. Fourier transform infrared spectroscopy (FT-IR) studies indicated that with ultrasonic treatments, the functional groups of starch were not destroyed, but its crystal structure was damaged. The peak viscosity of starch with dual-frequency US was 14.09% lower than that of the native starch. In addition, the solubility, transmittance, and solubility increased with the treatment.

3.6.5.2 Microwaves

According to Murthy and Prasad (2005), the key features in the use of microwave heating is that the temperature and moisture gradients are in the same direction, which is opposite to that in conventional heating in which moisture should leave the matter against the temperature gradient. Microwave irradiation accelerates esterification of starch and significantly improves its yield (Lewicka, Siemion, & Kurcok, 2015). Kumoro, Retnowati, and Budiyati (2010) studied the acetylation of cassava starch with sodium hydroxide and chloroacetic acid. The maximum power of the magnetron was 650 W and the temperature ramp used was 25−150°C over 3.4 min. He concluded that the procedure used allowed obtaining the modified starch in shorter times than with conventional methods. The microwave reaction gave origin to a more random distribution of constituents over the whole starch molecule and facilitated the interaction with water and hydrophobes.

The products showed lower intrinsic viscosity and molecular weights when the proposed methods were applied. Lukasiewicz, Bednarz, Ptaszek, Bogdal, and Achremowicz (2008) studied potato-starch oxidation using microwave heating and concluded that this treatment favored starch-granule destruction and polysaccharide depolymerization. Heating and the nonthermal effects of the microwave helped to obtain higher conversion and a product with similar or better properties than the commercially available one.

3.6.5.3 Pulse electric field

Concerning the application of PEF to starch, the variables usually controlled are electric field strength and time of application. Han, Zeng, Zhang, and Yu (2009) assayed strengths up to 50 kV/cm to

evaluate the effect of PEF treatment on the properties of cornstarch. The results showed that gelatinization temperatures and enthalpy decreased with the increase of electric field strength. The PEF treatment at 50 kV/cm produced the loss of granule shape and significantly decreased the crystallinity degree. The peak, breakdown, and final viscosity of the treated starch decreased with increasing electric field strength. It was concluded that during corn treatment, starch was gelatinized. This fact provided evidence that PEF could be a useful method to modify starch properties for specific food applications.

Pulse electric field has also been used to help the acetylation of starches by increasing the substitution degree and shortening the reaction time (Hong, Zeng, Buckow, Han, & Wang, 2016). Pulse electric field-assisted acetylation of starch also markedly increased the surface roughness. In the case of potato starch (Hong, Chen, Zeng, & Han, 2016) the degree of substitution (DS) increased as a function of reaction time and strength (3−5 kV/cm), while a higher DS was observed for shorter reaction times. Acetylated starch with higher DS presented more bulges and asperities. The acetylation helped to lower retrogradation, breakdown, and setback values.

Table 3.3 summarizes some of the literature concerning the application of PEF to modify different starches. The results suggest that PEF treatments constitute a promising physical method for modifying the properties of starch to obtain additives for the food industry.

3.6.5.4 Ozone

Oxidized starch is used as an emulsifier, thickener, coating, and sealing agent in confectionaries in the food industry as well as a dough conditioner for bread. Hui, Chan, and Karim (2009) used ozone to oxidize corn, sago, and tapioca starch using different ozone generation times (1−10 min)

Table 3.3 Effect of Pulse Electric Fields on Functional Properties of Starch

Carbohydrate	Emerging Technology	Treatment Conditions	Properties Modification	Reference
Potato starch−water suspensions (8.0%, w/w)	PEF	Intensity of electric field: 30, 40, and 50 kV/cm	Gelatinization temperatures, gelatinization enthalpy, peak viscosity and breakdown viscosity decreased with increasing electric field strength	Han, Zeng, Yu, Zhang, and Chen (2009)
Maize starch−water suspensions (8.0%, w/w)	PEF	Intensity of electric field: up to 50 kV/cm. treatment time:1272 μs	Molecular weights, gelatinization temperatures and the enthalpy of gelatinization decreased with increasing electric field and treatment time	Han et al. (2012a)
Tapioca starch−water dispersions (8.0%, w/w)	PEF	Intensity of electric field: 30, 40, and 50 kV/cm Energy input of PEF treatment:(over 28.85 J/g	Dissociation and damage of PEF treated granules. Increase of amorphous structure. The temperature and enthalpy of gelatinization, the peak viscosity and the breakdown viscosity of PEF-treated samples showed a remarkable decrease with increasing electric field strengths	Han et al. (2012b)

and additional 10 min of gas application, replacing chemical techniques usually used for oxidized starch production. The content of carboxyl and carbonyl groups increased in starch with ozone generation times.

Oxidation decreased the swelling power of some starches (sago and tapioca) and the solubility of tapioca starch. The intrinsic viscosity of all oxidized starches decreased significantly, except for tapioca starch oxidized through a 5 min ozone generation time. Pasting properties were also affected. Evidently, the application of ozone to foods that have starch as an ingredient or additive may affect the characteristics of the food due to the changes that ozone might produce in this ingredient.

3.6.5.5 Irradiation

According to Bhat and Karim (2009), radiation processing is an environmentally friendly alternative to chemical modification of starches and can be employed for modification and cross-linking. Nemtanu and Brasoveanu (2010) reported that the treatment of starch with accelerated electron beam produced a decrease of the swelling power and of the gel consistency with irradiation dose increase, due to starch structural and functional changes.

Paste clarity and its stability were improved and the retrogradation phenomenon delayed with the increase in the irradiation energy absorbed. Other changes were the reduction in the temperature and enthalpy of gelatinization in a dose-dependent manner. All these radio-induced changes depended on the vegetal source of starch and were strongly correlated to the structural organization of starch, especially of amylopectin. These researchers noted the effectiveness of the applied treatment for starch modification and stated that according to the proposed application, a selection of the type of starch to be used and of the degree of the treatment is a must to obtain a product that can meet specific needs.

3.6.5.6 High pressure

Starch and its chemical derivatives are responsible for the textural and physical properties of food systems, impacting their end-use quality and/or shelf-life. Kim, Kim, and Baik (2012) studied the application of ultrahigh pressure (UHP) for the modification of starch and/or its chemical derivatives included in most processed foods as major ingredients or minor additives. It appears that UHP facilitates the hydration and swelling of starch granules in either aqueous alkaline or aqueous acidic reaction medium at 25°C and simultaneously forces infiltration of derivatizing reagents or acids into the interiors of swollen granules inducing fast modification reactions of starch. The authors reported that UHP-assisted HCl hydrolysis could be successfully achieved at 600 MPa for 30 min and hydroxypropylation, acetylation, cross-linking with $POCl_3$ and sodium trimetaphosphate could be conducted at 400 MPa for 15 min.

3.6.5.7 Cold plasma

CP is generated using electricity and a carrier gas, such as air, oxygen, nitrogen, argon, or helium. The result is the electrical discharge and subsequent ionization of the atmospheric air. Clerici, Lambert, and Chang (2012) used the CP method for the modification of cornstarch. At room temperature and CP of H_2, four processes were run: A (5 min, 30 W), B (15 min, 30 W), C (15 min, 70 W), and D (30 min, 30 W). They analyzed the moisture, color, pH, water absorption index, water-solubility index, viscosity of paste, gel firmness, and infrared spectrum of the products

obtained in each case and compared the values obtained with those of the native starch (control). All treated samples showed an increase in paste viscosity but only samples treated with processes C and D had lower retrogradation than the control. Treated starches formed stronger gels than the control starch. The infrared spectra showed changes in the position of the bands related to hydroxyls. This fact reveals the formation of intermolecular complexes in treated starches. The authors noted the importance of this technique for the starch industry as it uses no chemical reagents and the process time is very short.

3.7 USE OF EMERGING TECHNOLOGIES ON PROCESSING OF FOODS AND THEIR EFFECT ON CARBOHYDRATES

3.7.1 IONIZING RADIATION

Monosaccharides can be broken down by ionizing radiation into sugar acids and ketones (Miller, 2005). However, the effect of this treatment on fresh fruits and vegetables (live tissues) is diverse. Da Silva, Pizarro Silva, and Fillet Spoto (2007) studied the physiological and enzymatic postharvest characteristics of the pineapple cultivar smooth cayenne after γ-irradiation of fruits with absorbed doses of 100 and 150 Gy, followed by storage for 10, 20, and 30 days at 12°C and 85% relative humidity. Control specimens showed higher values of soluble pectins, total pectins, reducing sugars, sucrose, and total sugars. All the analyses indicated that the storage time was a significantly influencing factor. The 100 Gy dose and the 20-day-storage period presented the best results from the standpoint of maturation and conservation of the fruit quality. In this case, small carbohydrates decreased as a consequence of the irradiation but also as a metabolic response of the living tissues to the radiation stress (Latorre, Bonelli, Rojas, & Gerschenson, 2012).

Fan (2003) studied the irradiation-induced formation of malondialdehyde (MDA), formaldehyde, and acetaldehyde from fructose, sucrose, glucose, and malic acid solutions. MDA and formaldehyde were generated from the carbohydrate solutions upon irradiation, while little was formed from the malic-acid solution. On the other hand, a much higher amount of acetaldehyde was formed from the malic acid than from the carbohydrate solutions. As concentration of sugars in model solutions increased from 0 to 90 mg/mL, the formation of these compounds increased rapidly. A further increase in sugar concentration from 90 to 900 mg/mL resulted in a lower rate of increase in MDA and formaldehyde formation. The pH had a profound effect on the irradiation-induced formation of these compounds from carbohydrates, especially on MDA formation. The minimum amount of MDA formed from fructose and glucose solutions was observed at pH 5, while formation of MDA from sucrose solution decreased as the pH decreased from 7 to 2.

Fan (2005) studied the effect of ionizing radiation and thermal treatments on the formation of furan from sugars, ascorbic acids, and organic acids. The results showed both thermal treatments and irradiation-induced formation of furan from ascorbic acid, fructose, sucrose, or glucose. Little furan was produced from malic acid or citric acid. The pH and concentration of sugars and ascorbic-acid solutions had profound influences on furan formation due to either irradiation or thermal treatment. D-glucose was the most stable carbohydrate and a decrease in pH from 7 to 3 produced an increase in thermally induced furan formed from sucrose and ascorbic-acid solutions, but for the glucose solution less furan was formed at pH 3 than at pH 7. The levels of sugars commonly

found in fruits and fruit juices, upon irradiation, would be high enough to potentially produce low parts per billion (ppb) levels of furan. The food composition in sugars may also play a role as different sugars have varied potentials to form furan upon irradiation. For example, foods rich in fructose may produce more furan than those rich in glucose. Other factors, such as concentration of antioxidants, like ascorbic acid, may play an important role in the formation of furan. The results of this study can be used to minimize the production of furan due to thermal or irradiation processing. For example, if carbohydrates are required in formulations, glucose would be a better choice than fructose and sucrose to reduce the accumulation of furan due to irradiation. Thermal treatment (sterilization) and irradiation (5 kGy) of sugars and ascorbic acid produced similar amounts of furan.

Nonthermal processing technologies can be helpful to reduce the population of microorganisms in mesquite pod flour because heating deteriorates the characteristic flavor preferred by consumers. Fan, Felker, and Sokorai (2015) investigated the efficacy of ionizing radiation in decontaminating two types of mesquite pod flours (*Prosopis alba* and *Prosopis pallida*) that naturally contain the human pathogenic *Bacillus cereus*. The effects of irradiation on the formation of furan were also analyzed. The results showed that the populations of *B. cereus* were 3.8−5.4 log CFU/g in nonirradiated flours and populations of microflora, mesophilic spores, *B. cereus,* and *B. cereus* spores decreased with increasing radiation doses. At 6 kGy, the microorganism count was lower than 1 log CFU/g, indicating that irradiation was effective in decontaminating mesquite flour, while the content of fructose, glucose, and sucrose was not affected. Nonirradiated *P. alba* and *P. pallida* flours contained 13.0 and 3.1 ng/g of furan, respectively. The level of 3-methylbutanal was reduced or not affected by irradiation, while the hexanal level was increased. The relation of these compounds with possible changes in flavor due to irradiation constitutes an important point to be also considered when foods are irradiated.

The digestibility of starch varies among different starchy foods and plays a significant role in noninsulin-dependent diabetes treatment. Zuleta, Dyner, Sambucetti, and De Francisco (2006) studied in vitro starch digestibility of three γ-irradiated (0−3 kGy) argentinean rice flours with different amylose contents (5−25%). Their results indicated that with 3 kGy, the digestibility increased due to a loosening of the granular structure that made the molecules more accessible to enzyme activity. Campbell, Classen, and Balance (1986) performed in vivo studies by feeding animals, and also observed improved starch digestibility after cereal grain irradiation.

Irradiation of starch leads to a change in its functional attributes such as reduction of viscosity and high water solubility (Bhat & Karim, 2009). Increasing dosages of γ-irradiation creates increasing intensities of free radicals on carbohydrates. These free radicals are responsible for bringing about molecular changes and fragmentation of starch molecules. Increasing the γ-irradiation dosage causes an increase in acidity, as well as decreases the viscosity and water solubility of starches. The granule structure remains visually undamaged at low dosages of irradiation, but may suffer severe damage at higher dosages (100 kGy). According to Sokhey and Hanna (1993), irradiation of starchy foods must be controlled to bring about the required changes needed in the industry, and at the same time providing wholesome food to consumers.

3.7.2 HIGH HYDROSTATIC PRESSURE

High hydrostatic pressure (HHP) is a nonthermal process currently used for certain food products such as oysters, salsa, deli meats, guacamole and juices, targeting specific bacterial pathogens (e.g.,

Vibrio parahaemolyticus, *Vibrio vulnificus*, *Salmonella*, *Listeria*, and *E. coli* O157:H7). HPP kills microorganisms by exposing foods to very high pressures. The normal pressure used to kill pathogens in food using HHP can be as high as 6000 times the pressure we are exposed to at sea level. The high pressure causes the disruption of the microorganism's membrane. Simultaneously, the food is largely protected from this damaging force since the pressure is uniformly distributed around and throughout the food. As occurs with heat in the regular thermal sterilization process, the amount of pressure needed to kill the pathogen in the HHP processing will differ based on the microorganism and the food-containing product. This processing method can be performed on prepackaged food, minimizing the risks of recontamination. There is no commercial application of HHP on fresh products currently, and it has not been used to target viruses. In Italy, HHP is used to increase the safety and shelf-life of dry-cured and dry-fermented meat products (e.g., salami, ham), either sliced or in whole pieces, in order to preserve taste. In this sector, the pressure range used is between 500 and 600 MPa, applied at refrigeration temperature (Porto-Fett et al., 2010). Although there are many studies in relation to the effect of HHP on bacteria, the information that exists on molds and yeasts is relatively scarce. In general, yeasts and molds can be inactivated at 200−400 MPa, but when they are in the spore or ascospore state or in a food with a very high concentration of sugar, the pressure needed to inactivate them could be close to 600 MPa (Torres Bello, González Martínez, Klotz Ceberio, Rodrigo, & Martínez López, 2014).

Concerning the physicochemical effect on food, HHP technology is less detrimental than thermal treatment, since it does not break or form covalent bonds and does not create new compounds by means of molecule degradation as occurs in conventional thermal processes. However, HHP is able to break or create weak bonds (hydrogen, hydrophobic, and electrostatic interactions) only present in macromolecules such as proteins and polysaccharides (Cheftel, 1992). It allows microorganism inactivation without modifying the food nutritional and sensory quality. As a nonthermal process, the application of HHP has considerable potential as an alternative technology to heat treatments, in terms of assuring safety and quality attributes in minimally processed food products. Pasteurization is currently the main application of HPP with pressures ranging from 300 to 600 MPa using ambient or refrigerated temperature and times of 2 to 30 min. Increasing interest in HHP sterilization processes is also developing.

Since chemical reactions are also influenced by pressure according to the principle of Le Chatelier, the Maillard reaction must be taken into account during HHP processing of foods. According to Moreno, Molina, Olano, and Fandino-Lopez (2003a), the combination of HHP with elevated temperatures produces a shift of pH during treatments with a resulting impact on Maillard reaction pathways. This fact requires careful consideration when HHP is applied.

Kim, Fan, Chung, and Han (2010) studied changes in the extraction of phenolics and FOS characteristics of Jerusalem artichoke (*H. tuberosus* L.) tubers using HHP treatment alone and in combination with enzymes and/or fermentation. The HHP method (100 MPa, 50°C, 24 h) increased the soluble-solid content and decreased the turbidity compared to the control group that received hot water extraction (65°C, 24 h). This indicated improved extractability through HHP treatment either alone or in combination. Combined HHP treatment with enzymes increased the content of nonreducing sugars, FOS, and free aminoacids in the extracts with respect to those of the control, thus activating the browning reaction. Extraction of fermented Jerusalem artichoke by HHP treatment with or without enzyme showed increased polyphenol content and antioxidant activity. It was determined that HHP treatment of fermented Jerusalem artichoke with enzymes significantly improved

the extractability of bioactive molecules such as phenolics and FOS, when compared to conventional water extraction or HHP treatment alone.

Campus, Flores, Martínez, and Toldrá (2008) investigated the effects of high-pressure treatment on the chemical characteristics of dry cured loin where a reduction in the content of several flavor compounds deriving from Maillard reactions was observed in comparison to the untreated sample. This finding again underlines the fact that nonthermal processing retains fresh-like characteristics, but the reduction of the Maillard reaction may also lead to a decrease of the formation of typical color and flavor characteristics.

The conditions and benefits of HHP treatment for some plant tissues are listed in Table 3.4.

Elizondo-Montemayor et al. (2015) found a healthy effect attributed to HHP processing. HHP pasteurization reduced the glycemic index produced by the ingested fresh mango puree in healthy subjects. The mean glycemic index for consumers of HHP-pasteurized mango puree was significantly lower than that of subjects who consumed unprocessed mango puree.

Lactose consists of a glucose molecule combined with a galactose molecule. HPP causes considerable inhibition of isomerization and degradation of lactose. This plays a significant role in the final quality of thermally produced products, since the fundamental reactions leading to caramelization and furthermore to milk and dairy product quality deterioration include isomerization of aldose to ketose, fragmentation reactions, and browning (Moreno, Villamiel, & Olano, 2003b). On the other hand, HHP accelerated the Maillard reaction between lactose and lysine at alkaline pH, whereas the opposite happened in the case of pH values under 8.0 (Moreno et al., 2003a).

Liu, Li, Wang, Bi, & Liao (2014) used steam blanching, prior to HHP (600 MPa, 1 min) and high-temperature short-time (HTST) (110°C, 8.6 s) processes, for inactivation of endogenous enzymes on mango nectars. The results showed that, among other variables, redness (a^*), yellowness (b^*), and browning degree did not change after HHP or HTST treatment. After 16 weeks of storage at 4°C and 25°C, significant changes in color and browning degree of mango nectars were observed, with no differences between HHP- and HTST-treated samples except for the decrease observed in L-ascorbic acid and sodium erythorbate, which was more pronounced in HHP-treated

Table 3.4 HHP Treatment for Some Plant Tissues

Product	HHP Conditions	Conventional Processing	Changes Observed	Reference
Ginseng (*Panax ginseng*)	600 MPA, 1 min	Heating and drying	Increase of the total reducing sugar content of the red ginseng/superior coloration over heat and dried ginseng	Ghafoor, Kim, Lee, Seong, & Park, 2012
Apple purees enriched with inulin or FOS (prebiotics)	500 MPa, 1.5 min	Conventional pasteurization	In some cases, less hydrolysis of the prebiotic occurred in HPP purees than in thermal processed purees	Keenan, Brunton, Butler, Wouters, & Gormley, 2011
Mango nectars	600 MPa, 1 min	HTST, 100°C, 8.6 s	Both treatments produced a decrease in fructose, glucose and total sugar Only HPP treatment did not affect nectar viscosity	Liu et al., 2014b

samples. The increase in browning degree was attributed to the nonenzymatic reactions originating from the ascorbic-acid degradation including the Maillard reaction occurring between α-amino groups and reducing sugars.

Concerning the kinetically controlled synthesis of oligosaccharides from sucrose catalyzed by *Aspergillus niger* fructosyl-transferase, experiments performed by Vannieuwenburgh, Guibert, and Combes (2002) revealed that under high-pressure conditions, FOS yield reduction and decrease in the sucrose conversion rate were observed. This was mainly attributed to pressure inhibition of the transfer reactions while sucrose hydrolysis was little or not affected. Results demonstrated that pressure can be used to modulate the enzyme behavior and control product formation.

HHP was used by Verma, Kaushik, and Rao (2014) as a pretreatment to quicken the following osmotic dehydration of banana slices, which is an energy-consuming process. Monopropylene glycol aqueous solution (30%) was the pressure-transmitting medium. Pressure-pretreated samples (200 MPa, 5 min, 26°C) showed significantly higher water loss and solid gain during osmotic dehydration (60°Brix sucrose solution, 5 h immersion time, 40°C for the osmotic dehydration) than the non treated samples, trend that was attributed to the rupture of cell walls, making them more permeable. Superior quality osmotic dehydrated banana slices with better shelf stability coupled with reduced time and energy consumption were produced with HHP as a pretreatment. Minimized heat damage to color and flavor and less discoloration of fruit by enzymatic browning are some of the advantages of this process compared to other drying techniques. These effects can be attributed to the limited exposure of fruits to oxygen. In addition, the infusion of sugar increases the stability of pigments during further drying and subsequent storage. It was determined that the browning index increased with an increase in hydrostatic pressure because of the tissue damage produced by the HHP, by which polyphenol oxidase (PPO) and peroxidase (POD) enzymes were liberated, leading to phenolic oxidation.

HHP in the range of 800−900 MPa produced the complete inactivation of PPO and POD, according to Anese, Nicoli, and Dall (1995). The interaction of HHP and immersion temperature had a decreasing effect on the browning index of banana slices.

Oligosaccharides, including raffinose, stachyose, ciceritol, and verbascose, are commonly found in legumes and often result in flatulence in humans. The effects of soaking with US or HHP and subsequent cooking, on the oligosaccharide content of lentils, chickpeas, peas, and soybeans, were investigated by Han and Baik (2006). The total oligosaccharide content of raw legumes ranged from 70.7 mg/g in yellow peas to 144.9 mg/g in chickpeas. Soaking was effective for the reduction of oligosaccharides in the tested legumes. Compared with soaking for 3 h, soaking legumes with US for 3 h in all tested legumes or soaking legumes with HHP for 1 h, with the exception of soybeans, appeared to be more effective in the reduction of oligosaccharides. The effect of cooking on the reduction of oligosaccharide content of presoaked legumes was evident in lentils. The oligosaccharide content of chickpeas, peas, and soybeans was either unchanged or even increased by cooking after presoaking, with or without US, probably due to the leaching of other soluble components and the release of bound oligosaccharides during cooking. During soaking or cooking of legumes, raffinose leached out faster than other oligosaccharides.

Błaszczak, Bidzinska, Dyrek, Fornal, and Wenda (2008) determined that the presence of water and the high-pressure (650 MPa) pretreatment of suspensions of starches (native, waxy maize starch, Hylon VII rich in amylose) both resulted in the reduction of the amount of thermally generated radicals, upon thermal treatment at 180−230°C.

UHP processing is an attractive nonthermal technique for shelf-life extension associated with nonthermal sterilization and a reduction or increase in enzymatic activity. When starch is present in foods, the possibility of granular structure destruction must be considered because the reversible hydration of the amorphous phase followed by irreversible distortion of the crystalline region of starch granules and the breaking of secondary and tertiary structures can occur due to high pressures. It was observed that most high-pressure-treated starch granules suffer little leaching of amylase and conserve the shape of intact starch granules but the original smooth surface turns rough after treatment (Kim et al., 2012).

Santos, Saraiva, and Gomes (2015) studied the pasting of maize starch after HPP. Starch suspensions (20% w/v) in water were treated at 400 MPa, 5 min, 23°C. After treatment, the samples were freeze dried. The decrease of gelatinization enthalpy with treatment was observed.

Liu et al. (2010) reviewed the applications of HHP as nonthermal technology. They reported that HHP treatments produced lower gelatinization onset temperature, indicating great potential in energy saving for the baking industry if such treated starches are used in bakery products. They also noted that the impact on the sensory attributes of the final products needs to be studied.

3.7.3 FOAM-MAT DRYING

The foam-mat drying process is a relatively simple and inexpensive technique. However, one difficulty that has been previously experienced with this process is the lack of foam stability during the heating cycle. If the foam does not remain stable, cellular breakdown might occur, causing serious impairment of the drying operation. Variables affecting foam formation, density, and stability include chemical nature of the fruit, soluble solids, pulp fraction, type, and concentration of foaming agent and type, and concentration of foam stabilizer (Karim & Wai, 1999).

Foam-mat drying allows processing of hard-to-dry materials such as tomato paste and a variety of fruit pulps and juices, i.e., high-viscosity liquid or semiliquid food materials. Preferential product quality stems from accelerated drying at generally lower drying temperatures. This method has received attention because of its ability to obtain products of desired properties with favorable rehydration and controlled density and with high preservation of volatiles. However, the reduced density of foamed materials leads to decreased dryer load, which has to be compensated for by shorter drying time to maintain the dryer throughput. The shortcoming of this drying method is the poor heat transfer of air wrapped in the foamed materials.

In order to obtain powders, Karim and Wai (1999) used the foam-mat drying process to dehydrate starfruit (*Averrhoa carambola* L.) pureé (7°Brix; pH = 3.80−3.95), previously pasteurized for 3 min at 87°C. Methyl cellulose (Methocel 65HG, Sigma Aldrich) was used as foaming agent in 1−5% w/w aqueous solutions, which were mixed (20 mL) with 180 g of pureé (0.1−0.5% w/w methyl cellulose). Foam was obtained by high-speed stirring and spread (5.0 mm thick) onto a plastic wire mesh followed by drying (70°C or 90°C) under air flow. Overrun and stability of foams (lower syneresis) increased with the methyl cellulose concentration up to a maximum at 0.4% w/w. It was determined that the drying period decreased from 90 to 60 min as the temperature increased to 90°C, but unfavorable color (slight browning by sugar caramelization and Maillard reactions) and flavor changes accompanied this shortened drying. The foam-mat dried products were free flowing, finely divided powders, which were readily rehydrated in cold water. A sensory panel

found some flavor loss in the rehydrated foam-mat powders in comparison to the rehydrated freeze-dried powder developed, especially for powders obtained by drying at 90°C.

In order to produce high-quality crisp banana chips, Thuwapanichayanan, Prachayawarakorn, and Soponronnarit (2008) used the foam-mat method to dry banana puree (800 g) beaten with different proportions of fresh egg albumen as foam agent (2%, 5%, 10% on a wet puree basis), at maximum speed and for enough time (20 min) to achieve foam densities of 0.3, 0.5, and 0.7 g/cm^3. Banana foam mats with 5 mm thickness were then dried to the final moisture content of 0.03 kg/kg (dry basis), at 60°C, 70°C, and 80°C and superficial air velocity of 0.5 m/s. As expected, the highest egg albumen concentrations (5% and 10%) produced the lowest banana foam density (0.3 g/cm^3). The average effective moisture diffusivity (D_{eff}) increased with the drying (dry bulb) temperature ($1.02-3.60\times10^{-9}$ m^2/s) and, at a given temperature, notably decreased with the increase in the initial foam density.

Lower foam density (0.3 g/cm^3) corresponding to larger void area and larger pore sizes of banana foams provided higher values of effective diffusivity. Activation energies of D_{eff} (temperature dependence of the drying process) slightly increased (21.08−25.19 kJ/mol) with the foam density. In spite of an efficient moisture transport, the very porous banana foams led to lower values of hardness and crispness. Shrinkage was also higher, except for the samples with initial foam densities beyond 0.5 g/cm^3. The drying temperature had no significant effect on the textural properties, morphology, and shrinkage. Therefore the initial foam density of 0.5 g/cm^3 and a drying temperature of 80°C were recommended for obtaining crispy banana chips.

3.7.4 PULSED ELECTRIC FIELD

The chemical structure of carbohydrates determines their reactivity. Sugars containing aldehyde or ketone groups (such as aldoses and ketoses) that can be oxidized to carboxylic acids are classified as reducing sugars. Nonreducing sugars have acetal or ketal groups that cannot be oxidized into carboxylic acid. Maltose is a reducing disaccharide, while sucrose is a nonreducing one (Hernoux-Villière, 2013). Reducing sugars are able to condense with amines in a Maillard reaction. Gänzel et al. (2008) noted that lactose can participate in Maillard reactions when heated at sterilization conditions. Thermal processing is performed to prevent microbial spoilage and enzymatic browning with the aim of extending the shelf-life of foods. However, the product undergoes physical and chemical changes during processing that impair the organoleptic properties and may also reduce the content or bioavailability of some bioactive compounds. In fruit products, accumulation of brown color (α,β-unsaturated carbonyl, β-hydroxy carboxyl) compounds during processing occurs due to enzymatic and nonenzymatic browning reactions. Nonenzymatic browning involves caramelization, ascorbic-acid degradation, and Maillard reactions. While caramelization occurs on heat treatment of sugars at high temperatures, ascorbic-acid degradation occurs by oxidative and nonoxidative (hydrolytic) paths in citrus juices. The Maillard reaction, taking place between the α-amino groups of peptides and proteins and the reducing sugars, is the most important cause of browning in juices and other food products. This reaction also produces volatile and nonvolatile flavor compounds such as furans, furanones, furaneol, and sotolon, which are responsible for the organoleptic quality of processed foods (Kumar et al., 2015). Not only the temperature and composition of foods determine the prevalent nonenzymatic browning reactions, but also the pH, relative

humidity, oxygen partial pressure, and packaging affect the degree of browning. Furfural is a typical product of the nonenzymatic browning reaction derived from the ascorbic-acid destruction.

In juices with high sugar content, 5-hydroxy methyl furfural (HMF) is mainly found as a consequence of the nonenzymatic browning. There is an interaction between the glucose and ascorbic-acid browning reaction chains. The velocity of the browning reactions for monosaccharides is slow at pH 3.5 and increases with pH, from 4.1 to 5.0 (Rojas & Gerschenson, 1997a), because the proportion of the acyclic structure of D-glucose increases (Van Dam, Kieboom, & Bekkum, 1986). Davies and Wedzicha (1994) proposed that ascorbate anion can suffer addition of a α,β-unsaturated carbonyl compound through Michael's mechanism (Fodor, Arnold, Mohacsi, Karle, & Flippen-Anderson, 1983). Rojas and Gerschenson (1997b) proposed that 3,4-dideoxy-3-ene-hexulose produced from D-glucose can act as that carbonyl compound producing melanoidins. Ascorbic acid can also enter into the browning scheme of Maillard as a highly reactive α-dicarbonyl compound, leading to the formation of a wide variety of end products (Rojas & Gerschenson, 1997a).

Mango (*Mangifera indica* L.) is a rich source of carotenoids, with high ascorbic-acid and phenolic-compound content. Because of the short shelf-life of fresh mango, which limits its marketability, any excess supply must be processed into various shelf-stable mango products in order to minimize postharvest losses (Liu, Wang, Li, Bi, & Liao, 2014b). Mango nectar is a commercially familiar and preferred product. The traditional processing of mango nectar has been performed by thermal processing, which results in the alteration of flavor due to the effect of the high temperature. The thermal processing of the nectar also results in the production of nonenzymatic browning by-products such as HMF, all affecting both the nutritive and sensory attributes of the fruit product, making it less preferable (Kumar et al., 2015). Therefore, emerging technologies like nonthermal processes (e.g., PEF, HHP) constitute viable alternatives to be studied in order to minimize the loss of volatiles and the formation of HMF.

As reported by Ngadi, Bajwa, and Alakali (2012), decreased nonenzymatic browning was observed in carrot juice treated by PEF. Strawberry, tomato, and water melon juices also showed a decrease in the level of nonenzymatic browning. Kumar et al. (2015) combined PEF (38 kV/cm, 120 Hz, 24 μs) with thermal processing (96°C for 90 s) to obtain a significant reduction in the volatile compounds of the processed mango nectar. The level of HMF formed during processing increased during storage and after the thermal treatment. When the PEF pretreatment was applied, lower negative effects on the retention of volatile compounds were observed. The HMF concentration was also lower than in the unique thermal processing and was no different than the level in the unprocessed mango nectar, enhancing the fresh-like character of the product. It can then be inferred that PEF processing does not significantly promote browning reactions. Hence, this nonthermal process helps to inactivate microorganisms without promoting carbohydrate decompositions and reactions between carbohydrates and proteins.

Mixtures of lactose and caseinate were studied by Pan and Melton (2007) at 60°C for up to 96 h at humidities ranging from 29% to 95%. They observed that the Maillard reaction was the major pathway at all humidities. According to Michalac, Alvarez, Ji, and Zhang (2003), optimized PEF processing conditions allow to inactivate spoilage bacteria in milk without altering the quality of milk. The combined effect of the use of a temperature of 60°C and 400 MPa high-pressure treatments (3 h) was studied by Moreno et al. (2003b) in lactose systems. They observed the formation of isomeric disaccharides (lactulose and epilactose) and galactose at alkaline pHs and atmospheric pressures, which decreased at high pressures. This gave origin to a reduction of caramelization of lactose.

Under the effect of PEF, the biological membrane is electrically pierced and temporarily or permanently loses its selective semipermeability. The electrical permeabilization of biological membranes (called electroporation) allows introducing small or large molecules into cells or extracted from cells. PEF pretreatment of fruit slices can facilitate the access of antibrowning agents into the cells via electroporation (Shayanfar, Chauhan, Toepfl, & Heinz, 2014). Aguiló-Aguayo et al. (2014a) analyzed the impact of PEF processing conditions on the distribution of water in carrot tissue and on the extractability of soluble sugars. After PEF treatment, an increase in water permeability of tissues and/or a loss of integrity in the tonoplast was found to be proportional to the electric-field intensity. Moreover, an increase in sucrose, β- and α-glucose, and fructose concentrations in carrot-slice extracts after PEF treatment suggests increases in both cell-wall and vacuole permeability as a result of tissue exposure.

Aguiló-Aguayo et al. (2014b) examined the feasibility of PEF as a pretreatment method for enhancing the sugar content in carrot puree and studied its effect on carotenoid retention and color. Increases in redness (a^* values) in purees treated at 0.25 kV/cm for 10 ms (7.5 J/kg) correlated with the increases in their total carotenoid contents. This effect can actually be ascribed to the best delivery of carotenoids from the cells in the rest of the tissue that formed the puree, due to electroporation and tissue softening. The observed enhancement in sugar extraction seems to be due to the PEF processing conditions that induce electroporation. Higher free sugar levels of sucrose, β-glucose, α-glucose, and fructose in PEF-treated carrot purees than in untreated samples were determined for treatment energies of 29 and 86 J/kg, which indicated that PEF processing enhanced the bioavailability of sugars in carrot purees, improving the overall health benefits of the vegetable product with relatively low input energies.

In order to improve the expression and quality of apple juice, Turk, Vorobiev, and Baron (2012) combined a continuous PEF treatment module (650 V/cm, 23.2 ms-total time, 32 kJ/kg of energy) and a continuous pressing system of the apple smash (4400 kg/h flow rate) on an industrial scale. It led to an increased juice yield (711−763 g/kg) due to the PEF treatment, as well as the polyphenol level. Juice color was significantly altered by the PEF treatment. The yellow saturation increased due to the increase in oxidation products derived from polyphenols, in relation to the PPO activity. The juice from PEF-treated mash was less turbid and showed higher odor intensity and typical odor of apple than the untreated mash. The overall and typical tastes of apple juice were also significantly more intense. The fructose, glucose, and malic-acid content of juice did not vary despite a significant increase of the dry matter for treated samples. However, a significant decrease in fructose and glucose as well as in polyphenol levels was noted for the pomace from treated mash with respect to the control. The increase in juice yield and the decrease in water amounts determined in pomace were positive factors for the economic balance of this process because the pomace drying required less energy. However, PEF treatment had a positive impact on the sensory attributes of the raw juices that showed a higher intensity of the typical apple odor.

Stress responses of potato tissue subjected to PEF with strengths ranging from 200 to 400 V/cm with a single rectangular pulse of 1 ms were assayed by Gómez Galindo et al. (2009). Metabolic profiling of data obtained through gas chromatography with time-of-flight mass spectrometric detection (GC/TOF-MS) and ultra-performance liquid chromatography combined with time-of-flight mass spectrometry detection (UPLC/TOF-MS) and clustering analysis showed that 24 h after the application of PEF, potato metabolism showed PEF-specific responses characterized by changes in the hexose pool, a trend that may involve starch and ascorbic-acid degradation.

Zeng, Gao, Han, Zeng, and Yu (2016) submitted waxy rice starch to PEF treatments at intensities of 30−50 kV/cm. Gelatinization onset temperature, peak temperature, conclusion temperature, and enthalpy value of PEF-treated starches were lower than those of native starch. PEF-treated starches showed an increase in rapidly digestible starch level and a decrease in slowly digestible starch level, showing that structural changes in waxy rice starch significantly affected its digestibility. It can be concluded that PEF application to foods with starch could affect its nutritional properties.

Ben Ammar, Lanoisellé, Lebovka, Van Hecke, and Vorobiev (2010) studied the effect of PEF and osmotic pretreatments on potato-tissue structure and on the freezing and freeze-drying behaviors of this tissue using strengths of 400 V/cm for PEF. The osmotic treatment was performed in an aqueous solution of NaCl. The samples were either frozen in an air-blast freezer at an air temperature of −80°C and a velocity of 2 m/s or freeze-dried at 0°C and 0.04 mbar pressure. Sequential PEF and osmotic pretreatment of potato tissue resulted in starch granules with rougher surface. The faster freezing and freeze drying and visually better quality of the dried samples were observed for samples submitted to PEF or PEF followed by osmotic pretreatments.

3.7.5 ULTRASOUND

US is the energy generated by sound waves of 20,000 or more vibrations per second. Presently, most developments of ultrasonics (sonication) for food applications are nonmicrobial in nature (USDA, 2015). For classification of US applications in the food industry, the amount of energy of the generated sound field, characterized by the sound power (W) and the sound intensity (W/m^2), is the most important criterion (Knorr, Zenker, Heinz, & Lee, 2004). The uses of US are broadly classified into two groups (Dolatowski, Stadnik, & Stasiak, 2007). Low-energy (low-power, low-intensity) US applications involve the use of frequencies higher than 100 kHz at intensities below 1 W/cm^2, which are generally nondestructive. They are successfully used for noninvasive monitoring of food processes and as an analytical technique for providing information about the physicochemical properties of foods, such as composition, structure, and physical state. The other group is high-energy (high-power, high-intensity) US, which uses intensities higher than 1 W/cm^2 (typically in the range 10−1000 W/cm^2), at frequencies between 18 and 100 kHz. High-intensity US has been used for many years to generate emulsions, disrupt cells, and disperse aggregated materials. More recently, various areas have been identified with greater potential for future development, such as modification and control of crystallization processes, degassing of liquid foods, enzyme inactivation, enhanced drying and filtration, and induction of oxidation reactions (Dolatowski et al., 2007).

US exerts stresses that can be classified as thermal and nonthermal (mechanical). The thermal stress originates mainly from part of the input energy absorbed by the solution and is reflected as an increase in temperature. Mechanical stresses can be either cavitational or noncavitational. Cavitational stress can be attributed to the liquid jets produced by collapsing cavities in case of inertial cavitation.

Buldakov et al. (2009) examined the US mechanism and its impact on chemical and biological effects in vitro as a function of changing pulse-repetition frequency (PRF) from 0.5 to 100 Hz using a 1 MHz generator at low intensities and 50% duty factor. The extent of sucrose hydrolysis in irradiated aqueous solutions showed no correlation to the PRF change. Instead, it was only dependent on time of sonication. Raising the intensity from 0.3 to 0.8 W/cm^2 did not seem to induce more reaction. On the other hand, the continuous wave mode exerted opposing effects on sucrose

hydrolysis depending on the intensity used. At 0.3 W/cm^2, the absorbance was about half the value of 0.5 Hz, but at 0.8 W/cm^2, the situation was reversed. The increase in temperature in the experiments was not expected to significantly account for any of the outcomes, especially under pulsed--mode treatments. Biological effects were assessed by measuring the extent of cell killing and apoptosis induction in U937 cells using Trypan blue dye exclusion test and flow cytometry, respectively. The results showed significant PRF dependence with respect to $OH^{\bullet}$ formation, cell killing, and apoptosis induction. The lowest free-radical formation and cell killing and the highest cell viability were found at 5 Hz (100 ms pulse duration). On the other hand, no correlation was found between sucrose hydrolysis and PRF. Kohno, Mokudai, Ozawa, and Niwano (2011) only detected $OH^{\bullet}$ in the O_2-dissolved water samples after sonication.

Lactose can be in the glassy amorphous, in the rubbery amorphous, or in the crystalline phase. The transition from the rubbery to the crystalline phase is strongly affected by the moisture content and water activity of the system as they determine both mobility and reactivity, influencing different reactions that give origin to browning, the crystallization of amorphous lactose during storage of dairy ingredients, the glass transition temperatures, and stickiness and caking (Huppertz & Gazi, 2015). Bund and Pandit (2007) studied the process of crystallization in lactose solutions with the help of US in the presence of ethanol at ambient temperature. They observed that US enabled rapid crystallization and a relative uniformity of crystal size distribution as well as prevention of agglomeration in comparison to nonsonicated samples.

3.7.6 COLD PLASMA

As noted earlier, CP is the result of an electrical discharge in a carrier gas that produces ionization of the atmospheric air. As a consequence, the microbial inactivation effect of plasma treatment can be attributed to the presence and formation of a number of antimicrobial products in the air such as UV radiation, ozone, charged particles, and "supercharged" oxygen (plasma ions). All of these products work together to kill pathogens like bacteria and viruses (Thirumdas, Sarangapani, & Annapure, 2015). CP at atmospheric pressure can be generated by transforming argon gas into plasma at a radio frequency of 27 MHz or by electric discharge between two electrodes separated by dielectric barriers (Mahajan, Caleb, Singh, Watkins, & Geyer, 2014).

In the past, CP was used for sterilization of sensitive materials and now it is extended to food industries as a novel, emerging, nonthermal technology that could potentially decontaminate the surfaces of fresh produce (fruits, meat products, cheese, etc.). It offers the advantage of being chemical and water-free because the antimicrobial products are formed in the air. While not currently available for commercial use, this nonthermal processing technique is environmentally friendly and sustainable, as it does not require storage of chemicals or use of large volumes of processing water. Since CP is a waterless process and can be implemented in open air, it can be integrated into a forced air-cooling process (Pivarnik & Worobo, 2014). The modifications caused by CP on substrates involve cross-linking, etching, and deposition (grafting). Although it is a surface active technology, CP is used to inactivate endogenous enzymes that are responsible for browning reactions, particularly PPO and PODs. Several research investigations have shown reduced growth of microorganisms via different modes of actions by etching phenomenon, cell disruption by electrophoration, etc. The reactive species in plasma have been widely associated with the direct oxidative effects on the outer surface of microbial cells. The effect of plasma is highly dependent on the

presence of water in the substrate (food material). The highest effect was observed in moist organisms compared to dry organisms (Thirumdas et al., 2015). The use of cold atmospheric pressure gas-phase plasma for the treatment of sour cherry juice produced a higher percentage of phenolic acid and anthocyanin content than observed for the pasteurized product. This trend was linked to the liberation of these compounds due to the dissociation of aggregates or particles by the action of the plasma treatment (Garofulić et al., 2015; Renard et al., 2015). In this work, the cold atmospheric plasma jet was generated in argon by applying a 25 kHz electric field through a single-electrode atmospheric jet. Optical emission spectroscopy of Ar plasma jet in the region from 200 to 1000 nm was performed using a miniature fiber spectrometer of 0.8 nm spectral resolution. The light was collected from the region near the capillary tube exit using a quartz lens and a solar-resistant optical fiber. The existence of excited $NO^{\bullet}$, $OH^{\bullet}$, $O^{\bullet}$ radicals within the plasma jet was shown as well as excited N_2 and Ar.

Péroval et al. (2003) grafted omega-3 fatty acids on AX polymeric films by using CP associated with electron beam irradiation, reaching a surface hydrophobicity in the modified films higher than that observed for a waxy coating or for a low-density polyethylene film. Casted films were preactivated by contact for 3 min with the oxygen plasma produced into a vacuum chamber (0.08 mBar) at 900 W of power (electromagnetic field). The hot electrons generated and accelerated by the applied field collided with gas molecules and thereby transferred energy through ionization, bond breakage, and other forms of excitation. Excited molecules and atoms in the plasma could emit photons in the ultraviolet region. The equipment included a microwave oscillator and a generator (4.33 GHz, 900 W), a quartz glass tube, a reaction chamber (capacity 50 L), an oil rotary pump, a diffusion pump, and a mass-flow controller. The polymeric film was deposited on a sample holder at 57 cm from the center of the plasma source. Once the pressure of 0.08 mBar was reached and remained constant, oxygen gas was introduced at the desired flow rate into the plasma chamber, and the glow discharge was initiated. The exposure time was about 3 min. During this phase, peroxides were formed on the surface of film. Oxygen plasma-treated films were immediately immersed in oil (fish and linseed oils) and were kept for 2 h at room temperature. Electron-beam exposure induced the grafted mechanism by producing the energy required for breaking reactive bonds. Hence, a molecule in contact with the "activated" material could copolymerize. The first molecule was grafted on the activated polymeric chain by opening of its reactive double bond. The activated molecule could therefore react forward with another neighboring molecule, initiating the copolymerization.

The grafted mechanism is close to a lipid-oxidation process with an initiation phase followed by propagation and termination phases. Plasma technology is considered as a modern nonconventional technique used for the preparation of modified starches, altering its physical and chemical properties. CP is also used to alter the germination rate of seeds (Thirumdas et al., 2015).

3.7.7 OZONE

Ozone is a powerful sanitizer, similar to chlorine or common bleach. It has a major advantage over other sanitizers in that it leaves no residue and has no risk of causing undesirable organochloride cancer-causing by-products. Ozone is the sweet smell in the air after a lightning strike, and it is commonly formed when a photocopy machine is operating. While the air we breathe contains two atoms

of oxygen, O_2, ozone contains three atoms of oxygen, O_3, making it very reactive. Ozone is able to kill pathogens, extend food shelf-life, and is only toxic to humans at very high doses. A big advantage is that the toxicity does not stay with the food. Once the food is treated, the ozone decomposes quickly into oxygen, leaving fresh, safe food. However, ozone should not be used to treat high-fat foods since it will cause oxidation of fat, resulting in development of rancid off-flavors. Ozone is a gas, but it can be bubbled into water and used as a washing method. Ozone itself and the by-products of ozone decomposition work to make solid or liquid foods safe. Ozone is a very potent sanitizer that decomposes to ordinary oxygen that is safe to breathe. Nonetheless, both ozone formation and high concentrations are dangerous to humans so worker safety training is important. There are commercial applications for ozone use for specific foods. Ozone can oxidize carbohydrates, giving origin to structures that are able to participate in other oxidative or browning reactions. Ozone may not be suitable for products rich in unsaturated fats and soluble proteins (Perry & Yousef, 2011).

Perez, Sanz, Rios, Olias, and Olias (1999) studied the effect of ozone treatment on the postharvest quality of strawberry fruits (*Fragaria ananassa* Duch. cv. Camarosa), which were stored at 2°C in an atmosphere-containing ozone (0.35 ppm). Fruits were moved to 20°C after three days. The sucrose content decreased with storage time in both treated (T) and nontreated (NT) fruits, but the authors noted that the pattern of sugar changed during storage was different between treatments. The lower content of sucrose in NT strawberries on day 3 was correlated with the increase in glucose and fructose but for T fruits, low sucrose, glucose, and fructose contents were detected, probably due to activation of another sucrose degradation pathway in response to oxidative stress caused by ozone. It can be concluded that the processing can impair nutritional value and organoleptical acceptance because the balance of sugars and organic acids has a great influence on strawberry flavor.

3.8 CONCLUSION

Carbohydrates constitute a group of chemically defined substances with a range of physical and physiological properties and health benefits for consumers. Their main function is to provide energy, but they also play an important role in the structure and function of cells, tissues, and organs. Some carbohydrates have specific nutritional actions. For example, inulin acts as a prebiotic, which means it resists gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption, it is fermented by the intestinal microflora, and stimulates selectively the growth and/or activity of intestinal bacteria associated with health and well-being. In relation to food products, carbohydrates are components, ingredients, and additives that contribute not only to their nutritional value but also to their organoleptical properties. In this chapter, the diversity of carbohydrate occurrence and uses and the conventional and emerging techniques used for their isolation and modification were revised along with the effect of new processing technologies on potential changes that can affect bioavailability, bioaccessibility, and quality of food. It can be concluded that emerging technologies can help: (1) to increase yield and minimize time and energy used, optimizing carbohydrate extraction and modification; (2) to industrialize food, creating innovative products and food ingredients with new physicochemical and functional properties, including physiological functionality, due to the changes these technologies induce. The scarce background of systematic information on the effect of novel techniques on carbohydrate changes and on their impact on organoleptic characteristics and health issues shows the need for studies on the subject.

ACKNOWLEDGMENTS

The authors acknowledge the financial support of University of Buenos Aires (UBACyT 20020130100550BA/2014-2017), National Agency of Scientific and Technical Research (PICT 2012-1941 and 2013-2088), and CONICET (PIP 11220120100507/2013-2015).

REFERENCES

Aguedo, M., Fougnies, C., Dermience, M., & Richel, A. (2014). Extraction by three processes of arabinoxylans from wheat bran and characterization of the fractions obtained. *Carbohydrate Polymers*, *105*, 317−324.

Aguedo, M., Vanderghem, C., Goffin, D., Richel, A., & Paquot, M. (2013). Fast and high yield recovery of arabinose from destarched wheat bran. *Industrial Crops and Products*, *43*, 318−325.

Aguiló-Aguayo, I., Downey, G., Keenan, D. F., Lyng, J. G., Brunton, N., & Rai, D. K. (2014a). Observations on the water distribution and extractable sugar content in carrot slices after pulsed electric field treatment. *Food Research International*, *64*, 18−24.

Aguiló-Aguayo, I., Hossain, M. B., Brunton, N., Lyng, J., Valverde, J., & Rai, D. K. (2014b). Pulsed electric fields pre-treatment of carrot purees to enhance their polyacetylene and sugar contents. *Innovative Food Science and Emerging Technologies*, *23*, 79−86.

Ahmad, A., Anjum, F., Zahoor, T., Nawaz, H., & Ahmed, Z. (2010). Extraction and characterization of β-D-glucan from oat for industrial utilization. *International Journal of Biological Macromolecules*, *46*, 304−309.

Ahmad, A., Anjum, F. M., Zahoor, T., Nawaz, H., & Din, A. (2009). Physicochemical and functional properties of barley β-glucan as affected by different extraction procedures. *International Journal of Food Science and Technology*, *44*, 181−187.

Aliasgharzadeh, A., Dehghan, P., Pourghassem, Gargari, B., & Asghari-Jafarabadi, M. (2015). Resistant dextrin, as a prebiotic, improves insulin resistance and inflammation in women with type 2 diabetes: A randomized controlled clinical trial. *British Journal of Nutrition*, *113*, 321−330.

Almohammed, F., Mhemdi, H., & Vorobiev, E. (2016). Pulsed electric field treatment of sugar beet tails as a sustainable feedstock for bioethanol production. *Applied Energy*, *162*, 49−57.

Al-Tamimi, M. A. H. M., Palframan, R. J., Cooper, J. M., Gibson, G. R., & Rastall, R. A. (2006). In vitro fermentation of sugar beet arabinan and arabino-oligosaccharides by the human gut microflora. *Journal of Applied Microbiology*, *100*, 407−414.

Anese, M., Nicoli, M. C., & Dall, A. G. (1995). Effect of high pressure treatments on peroxidase and polyphenoloxidase activities. *Journal of Food Biochemistry*, *18*, 285−293.

Angioloni, A., & Collar, C. (2011). Physicochemical and nutritional properties of reduced-caloric density high fiber breads. *LWT-Lebensmittel-Wissenschaft und-Technologie*, *44*, 747−758.

Annison, G., Choct, M., & Cheetham, N. (1992). Analysis of wheat arabinoxylans from a large-scale isolation. *Carbohydrate Polymers*, *19*, 151−159.

Apolinário, A. C., De Lima Damasceno, B. P. G., De Macêdo Beltrão, N. E., Pessoa, A., Converti, A., & Da Silva, J. A. (2014). Inulin-type fructans: A review on different aspects of biochemical and pharmaceutical technology. *Carbohydrate Polymers*, *101*, 368−378.

Ashogbon, A., & Akintayo, E. (2014). Recent trend in the physical and chemical modification of starches from different botanical sources: A review. *Starch−Stärke*, *66*(1−2), 41−57.

Asilonu, E., Bucke, C., & Keshavarz, T. (2000). Enhancement of chrysogenin production in cultures of *Penicillium chrysogenum* by uronic acid oligosaccharides. *Biotechnology Letters*, *22*(11), 931−936.

Auerbach, M. H., Mitchell, H., & Moppett, F. K. (2012). Polydextrose. In Lyn O' Brien Nabors (Ed.), *Alternative Sweeteners* (4th ed, p. 490). Boca Raton, Florida, USA: CRC Press, Taylor & Francis Group LLC, Chapter 27.

Azevedo Carvalho, A. F., De Oliva Neto, P., Fernandes Da Silva, D., & Pastore, G. M. (2013). Review. Xylo-oligosaccharides from lignocellulosic materials: Chemical structure, health benefits and production by chemical and enzymatic hydrolysis. *Food Research International*, *51*, 75–85.

Babbar, N., Dejonghe, W., Gatti, M., Sforza, S., & Kathy, E. (2015). Pectic oligosaccharides from agricultural byproducts: Production, characterization and health benefits. *Critical Reviews in Biotechnology*, *26*, 1–13.

Bae, I., Kim, K., Lee, S., & Lee, H. (2012). Response surface optimization of β-glucan extraction from cauliflower mushrooms (*Sparassis crispa*). *Food Science and Biotechnology*, *21*(4), 1031–1035.

Barczynska, R., Jochym, K., Śliżewska, K., Kapusniak, J., & Libudzisz, Z. (2010). The effect of citric acid-modified enzyme-resistant dextrin on growth and metabolism of selected strains of probiotic and other intestinal bacteria. *Journal of Functional Foods*, *2*, 126–133.

Bataillon, M., Mathaly, P., Nunes Cardinali, A. P., & Duchiron, F. (1998). Extraction and purification of arabinoxylan from destarched wheat bran in a pilot scale. *Industrial Crops and Products*, *8*, 37–43.

Ben Ammar, J., Lanoisellé, J., Lebovka, N., Van Hecke, E., & Vorobiev, E. (2010). Effect of a pulsed electric field and osmotic treatment on freezing of potato tissue. *Food Biophysics*, *5*, 247–254.

Benamrouche, S., Crônier, D., Debeire, P., & Chabbert, B. (2002). A chemical and histological study on the effect of (1→4)--endo-xylanase treatment on wheat bran. *Journal of Cereal Science*, *36*, 253–260.

Benito-Román, Ó., Alonso, E., & Cocero, M.J. (2013a). Glucans from pressurized hot water extraction of barley. *III iberoamerican conference on supercritical fluids cartagena de Indias (Colombia)*. Available from <http://www.nupeg.ufrn.br/prosciba/prosciba2013/Papers/T2–40.pdf> Accessed 30.01.2016.

Benito-Román, O., Alonso, E., & Cocero, M. J. (2013b). Ultrasound-assisted extraction of β-glucans from barley. *LWT-Lebensmittel-Wissenschaft und-Technologie*, *50*, 57–63.

Benito-Roman, O., Alonso, E., & Lucas, S. (2011). Optimization of the β-glucan extraction conditions from different waxy barley cultivars. *Journal of Cereal Science*, *53*, 271–276.

Benito-Román, O., Alonso, E., Palacio, L., Prádanos, P., & Cocero, M. J. (2014). Purification and isolation of β-glucans from barley: Downstream process intensification. *Chemical Engineering and Processing*, *84*, 90–97.

Benito-Román, O., Alvarez, V. H., Alonso, E., Cocero, M. J., & Saldaña, M. D. A. (2015). Pressurized aqueous ethanol extraction of β-glucans and phenolic compounds from waxy barley. *Food Research International*, *75*, 252–259.

Bergmans, M. E. F., Beldman, G., Gruppen, H., & Voragen, A. G. J. (1996). Optimisation of the selective extraction of (glucurono) arabinoxylans from wheat bran: Use of barium and calcium hydroxide solution at elevated temperatures. *Journal of Cereal Science*, *23*, 235–245.

Beukes, N., & Pletschke, B. (2011). Effect of alkaline pre-treatment on enzyme synergy for efficient hemicellulose hydrolysis in sugarcane bagasse. *Bioresource Technology*, *102*, 5207–5213.

Bhat, R., & Karim, A. (2009). Impact of radiation processing on starch. *Comprehensive Reviews in Food Science and Food Safety*, *8*, 44–58.

Bhatty, R. S. (1993). Physicochemical properties of roller-milled barley bran and flour. *Cereal Chemistry*, *70*, 397–402.

Bhatty, R. S. (1995). Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat brans. *Journal of Cereal Science*, *2*, 163–170.

Błaszczak, W., Bidzinska, E., Dyrek, K., Fornal, J., & Wenda, E. (2008). Effect of high hydrostatic pressure on the formation of radicals in maize starches with different amylose content. *Carbohydrate Polymers*, *74*, 914−921.

Braccini, I., & Pérez, S. (2001). Molecular basis of Ca2+-induced gelation in alginates and pectins: The egg-box model revisited. *Biomacromolecules*, *2*, 1089−1096.

Brennan, C. S., & Cleary, L. J. (2005). Review. The potential use of cereal (1→3,1→4)-β-D-glucans as functional food ingredients. *Journal of Cereal Science*, *42*, 1−13.

Buldakov, M. A., Hassan, M. A., Zhao, Q. L., Feril, L. B., Jr., Kudo, N., Kondo, T., & Riesz, P. ,etal (2009). Influence of changing pulse repetition frequency on chemical and biological effects induced by low-intensity ultrasound in vitro. *Ultrasonics Sonochemistry*, *16*, 392−397.

Buleón, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: Structure and biosynthesis. *International Journal of Biological Macromolecules*, *23*, 85−112.

Bund, R., & Pandit, A. (2007). Sonocrystallization: Effect on lactose recovery and crystal habit. *Ultrasonics Sonochemistry*, *14*, 143−152.

Burkus, Z., & Temelli, F. (2005). Rheological properties of barley b-glucan. *Carbohydrate Polymers*, *59*, 459−465.

Byrne, C. S., Chambers, E. S., Morrison, D. J., & Frost, G. (2015). The role of short chain fatty acids in appetite regulation and energy homeostasis. *International Journal of Obesity*, *39*, 1331−1338.

Camarero, N., Nadal, A., Barrero, M. J., Haro, D., & Marrero, P. F. (2003). Histone deacetylase inhibitors stimulate mitochondrial HMG-CoA synthase gene expression via a promoter proximal Sp1 site. *Nucleic Acids Research*, *31*(6), 1693−1703.

Campbell, G. L., Classen, H. L., & Balance, G. M. (1986). Gamma irradiation treatments of cereal grains for chick diets. *Journal of Nutrition*, *116*, 560−590.

Campus, M., Flores, M., Martínez, A., & Toldrá, F. (2008). Effect of high pressure treatment on colour, microbial and chemical characteristics of dry cured loin. *Meat Science*, *80*(4), 1174−1181.

Cheftel, J. C. (1992). Effects of high hydrostatic pressure on food constituents: An overviewIn C. Balny, R. Hayashi, K. K. Heremans, & P. Masson (Eds.), *High pressure and biotecnology* (224, pp. 195−209)). *colloque INSERM*.

Chemat, F., Huma, Z., & Khan, M. (2011). Application of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry*, *18*, 813−835.

Chen, J., & Chou, C. (1993). *Cane sugar handbook: A manual for cane sugar manufacturers and their chemists* (12th ed.)Hoboken: John Wiley and Sons.

Chen, J., Liang, R., Liu, W., Li, T., Liu, C., Wu, S., & Wang, Z. (2013). Pectic-oligosaccharides prepared by dynamic high-pressure microfluidization and their in vitro fermentation properties. *Carbohydrate Polymers*, *91*, 175−182.

Christodoulou, P. (2003). Sugars and carbohydrates. In G. Liadakis, & C. Tzia (Eds.), *Extraction optimization in food engineering*. Boca Raton: CRC Press.

Clarke, M. A. (1988). Sugarcane processing: Raw and refined sugar manufacture. In M. A. Clarke, & M. A. Godshall (Eds.), *Chemistry and processing of sugarbeet and sugarcane*. Amsterdam: Elsevier Science Publishing Co., Inc.

Clarke, M. A. (1995). Technological value of sucrose in food products. In M. Mathlouthi, & P. Reiser (Eds.), *Sucrose: properties and applications*. Dordrecht: Springer Science + Business Media.

Cleemput, G., Roels, S. P., Vanoort, M., Grobet, P. J., & Delcour, J. A. (1993). Heterogeneity in the structure of water-soluble arabinoxylans in European wheat flours of variable bread-making quality. *Cereal Chemistry*, *70*, 324−329.

Clerici, M., Lambert, C., & Chang, Y. (2012). Modification of starch using cold plasma technology. Book of abstracts of the AACC international meeting 2012. *Cereal Food World*, *57*(4), 42.

Cummings, J. H., & Stephen, A. M. (2007). Carbohydrate terminology and classification. *European Journal of Clinical Nutrition*, *61*(Suppl. 1), S5–S18.

Czechowska-Biskup, R., Rokita, B., Lotfy, S., Ulanski, P., & Rosiak, J. (2005). Degradation of chitosan and starch by 360-kHz ultrasound. *Carbohydrate Polymers*, *60*, 175–184.

Da Silva, J. M., Pizarro Silva, J., & Fillet Spoto, M. H. (2007). Physiological and enzymatic analyses of pineapple subjected to ionizing radiation. *Ciência e Tecnologia de Alimentos, Campinas*, *27*(3), 602–607.

Davies, C. G. A., & Wedzicha, B. L. (1994). Ascorbic acid browning. The incorporation of C(1) from ascorbic acid into melanoidins. *Food Chemistry*, *49*(2), 165–167.

Dawkins, N. L., & Nnanna, I. A. (1993). Oat gum and β-glucan extraction from oat bran and rolled oats: Temperature and pH effects. *Journal of Food Science*, *58*, 562–566.

Delcour, J., Bruneel, C., Derde, L., Gomand, S., Pareyt, B., Putseys, J., & Lamberts, L. ,etal (2010). Fate of starch in food processing: From raw materials to final food products. *Annual Review of Food Science and Technology*, *1*, 87–111.

De Menezes, E. W., Giuntini, E. B., Dan, M. C. T., Sardá, F. A. H., & Lajolo, F. M. (2013). Codex dietary fiber definition. Justification for inclusion of carbohydrates from 3 to 9 degrees of polymerisation. *Food Chemistry*, *140*, 581–585.

Den Besten, G., Van Eunen, K., Groen, A., Venema, K., Reijngoud, D., & Bakker, B. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, *54*(9), 2325–2340.

Deng, Q., Zinoviadou, K. G., Galanakis, C. M., Orlien, V., Grimi, N., Vorobiev, E., & Barba, F. J. ,etal (2014). The effects of conventional and nonconventional processing on glucosinolates and its derived forms, isothiocyanates: Extraction, degradation and applications. *Food Engineering Reviews*, *7*, 357–381.

De'nobili, M. D., Curto, L. M., Delfino, J. M., Soria, M., Fissore, E. N., & Rojas, A. M. (2013). Performance of alginate films for retention of L-(+)-ascorbic acid. *International Journal of Pharmaceutics*, *450*, 95–103.

Deutschmann, R., & Dekker, R. (2012). From plant biomass to bio-based chemicals: Latest developments in xylan research. *Biotechnology Advances*, *30*, 1627–1640.

Doehlert, D. C., & Moore, W. (1997). Composition of oat bran and flour prepared by three different mechanisms of dry milling. *Cereal Chemistry*, *74*, 403–406.

Dolatowski, Z. J., Stadnik, J., & Stasiak, D. (2007). Applications of ultrasound in food technology. *Acta Scientiarum Polonorum, Technologia Alimentaria*, *6*(3), 89–99.

Döring, C., Jekle, M., & Becker, T. (2015). Technological and analytical methods for arabinoxylan quantification from cereals. *Critical Reviews in Food Science and Nutrition*, . Available from http://dx.doi.org/10.1080/10408398.2012.749207.

Ebringerova, A., & Heinze, T. (2000). Xylan and xylan derivatives–biopolymers with valuable properties, 1. Naturally occurring xylans structures, isolation procedures and properties. *Macromolecular Rapid Communications*, *21*, 542–556.

EFSA Panel On Dietetic Products, Nutrition And Allergies. (2010). Scientific opinion on the substantiation of a health claim related to oat beta-glucan and lowering blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006. *EFSA Journal*, *8*(12), 1885.

Elizondo-Montemayor, L., Hernández-Brenes, C., Ramos-Parra, P. A., Moreno-Sánchez, D., Nieblas, B., Rosas-Pérez, A. M., & Lamadrid-Zertuche, A. C. (2015). High hydrostatic pressure processing reduces the glycemic index of fresh mango puree in healthy subjects. *Food and Function*, *6*, 1352–1360.

Escamilla-García, M., Alvarado-González, J., Calderón-Domínguez, G., Chanona-Pérez, J., Méndez-Méndez, J., Perea-Flores, M., & Farrera-Rebollo, R. (2015). Tools for the study of nanostructures. In H. Hernández-Sánchez, & G. F. Gutiérrez-López (Eds.), *Food Nanoscience and Nanotechnology*. New York: Springer.

Escarnot, E., Aguedo, M., Agneessens, R., Wathelet, B., & Paquot, M. (2011). Extraction and characterization of water-extractable and water-unextractable arabinoxylans from spelt bran. Study of the hydrolysis conditions for monosaccharides analysis. *Journal of Cereal Science*, *53*, 45–52.

Escarnot, E., Aguedo, M., & Paquot, M. (2012). Enzymatic hydrolysis of arabinoxylans from spelt bran and hull. *Journal of Cereal Science*, *55*, 243–253.

EUFIC-The European Food Information Council. (2010). Nutrient bioavailability—getting the most out of food. *Food Today*, *05/2010*, Available from <http://www.eufic.org/article/en/artid/Nutrient-bioavailability-food/> Accessed 10.01.2016.

EUFIC-The European Food Information Council. (2012). The basics. Carbohydrates. Available from <http://www.eufic.org/article/en/expid/basics-carbohydrates/> Accessed 10.01.2016.

Fan, X. (2003). Ionizing radiation induces formation of malondialdehyde, formaldehyde, and acetaldehyde from carbohydrates and organic acid. *Journal of Agricultural and Food Chemistry*, *51*, 5946–5949.

Fan, X. (2005). Impact of ionizing radiation and thermal treatments on furan levels in fruit juice. *Journal of Food Science*, *7*, E409–E414.

Fan, X., Felker, P., & Sokorai, K. J. (2015). Decontamination of mesquite pod flour naturally contaminated with *Bacillus cereus* and formation of furan by ionizing irradiation. *Journal of Food Protection*, *78*(5), 954–962.

FDA. (2005a). *Generally recognized as safe (GRAS) notice. GRAS exemption claim for isomalto-oligosaccharide or IMO*. Available from <http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/documents/document/ucm268863.pdf> Accessed on 02.02.2016.

FDA. (2005b). Food labeling, health claims; soluble dietary fiber from certain foods and coronary heart disease, 21 CFR Part 101. *Federal Register*, *70*(246), 76150–76162.

FDA. (2007). Generally recognized as safe (GRAS). Determination for the addition of polydextrose to infant formula as a prebiotic ingredient in combination with galactooligosaccharides. *GRAS noted 000233*. Available from <http://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM269127> Accessed on 02.02.2016.

Femia, A. P., Salvadori, M., Broekaert, W. F., François, I. E. J. A., Delcour, J. A., Courtin, C. M., & Caderni, G. (2010). Arabinoxylan-oligosaccharides (AXOS) reduce preneoplastic lesions in the colon of rats treated with 1,2-dimethylhydrazine (DMH). *European Journal of Nutrition*, *49*(2), 127–132.

Fodor, G., Arnold, R., Mohacsi, T., Karle, I., & Flippen-Anderson, J. (1983). A new role for l-ascorbic acid: Michael donor to α,β-unsaturated carbonyl compounds. *Tetrahedron*, *39*(13), 2137–2145.

Foster, K. D., Bronlund, J. E., & Paterson, A. H. J. (2006). Glass transition related cohesion of amorphous sugar powders. *Journal of Food Engineering*, *77*(4), 997–1006.

Fouache, C., Duflot, P., & Looten, P. (2003). US. Patent 6630,586.

Franck, A. (2006). Inulin. In A. M. Stephen, G. O. Phillips, & P. A. Williams (Eds.), *Food polysaccharides and their applications*. Boca Raton: CRC Press.

Fry, S. C. (1986). Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Annual Review in Plant Physiology*, *37*, 165–186.

Galanakis, C. M. (2013). Emerging technologies for the production of nutraceuticals from agricultural by-products: A viewpoint of opportunities and challenges. *Food and Bioproducts Processing*, *91*, 575–579.

Galanakis, C.M., Barba, F.J., & Prasad, K.N. (2015). Cost and safety issues of emerging technologies against conventional techniques, In C. M. Galanakis (Ed.), *Food waste recovery: processing technologies and industrial techniques*.

Galanakis, C. M., & Schieber, A. (2014). Editorial. Special issue on recovery and utilization of valuable compounds from food processing by-products. *Food Research International*, *65*, 299–230.

Galand, G. (1989). Brush border membrane sucrase-isomaltase, maltase-glucoamylase and trehalase in mammals. *Comparative Biochemistry and Physiology B*, *94*(1), 1–11.

Gänzel, M. G., Haase, G., & Jelen, P. (2008). Lactose: Crystallization, hydrolysis and value-added derivatives. *International Dairy Journal*, *18*, 685–694.

García, J. C., Díaz, M. J., Garcia, M. T., Feria, M. J., Gómez, D. M., & López, F. (2013). Search for optimum conditions of wheat straw hemicelluloses cold alkaline extraction process. *Biochemical Engineering Journal*, *71*, 127–133.

Garofulić, I. E., Jambrak, A. R., Milošević, S., Dragović-Uzelac, V., Zorić, Z., & Herceg, Z. (2015). The effect of gas phase plasma treatment on the anthocyanin and phenolic acid content of sour cherry Marasca (*Prunus cerasus* var. Marasca) juice. *LWT-Lebensmittel-Wissenschaft und-Technologie*, *62*, 894–900.

Ghafoor, K., Kim, S. O., Lee, D. U., Seong, K., & Park, J. (2012). Effects of high hydrostatic pressure on structure and colour of red ginseng (*Panax ginseng*). *Journal of the Science of Food and Agriculture*, *92*(15), 2975–2982.

Ghazi, I., Fernandez-Arrojo, L., Gomez De Segura, A., Alcalde, M., Plou, F. J., & Ballesteros, A. (2006). Beet sugar syrup and molasses as low-cost feedstock for the enzymatic production of fructo-oligosaccharides. *Journal of Agricultural and Food Chemistry*, *54*(8), 2964–2968.

Ghose, T. K., & Bisaria, V. S. (1987). Measurement of hemicellulase activities. Part 1: Xylanases. *Pure and Applied Chemistry*, *59*(12), 1739–1752.

Gibson, G. R. (2004). From probiotics to prebiotics and healthy digestive system. *Journal of Food Science*, *69* (5), 141–143.

Gómez, B., Gullón, B., Remoroza, C., Schols, H. A., Parajó, J. C., & Alonso, J. L. (2014). Purification, characterization, and prebiotic properties of pectic oligosaccharides from orange peel wastes. *Journal of Agricultural and Food Chemistry*, *62*, 9769–9782.

Gómez Galindo, F., Dejmek, P., Lundgren, K., Rasmusson, A., Vicente, A., & Moritz, T. (2009). Metabolomic evaluation of pulsed electric field-induced stress on potato tissue. *Planta*, *230*, 469–479.

Guío, F., Rodríguez, M. A., Alméciga-Diaz, C. J., & Sánchez, O. F. (2009). Recent trends in fructooligosaccharides production. *Recent Patents on Food, Nutrition and Agriculture*, *1*, 221–230.

Hammes, W. P. (2004). How selective are the prebiotics? In Senate Commission on Food Safety (Ed.), *Functional food: safety aspects*. Weinheim, Germany: Wiley VCH Verlag GmbH & Co. KGaA.

Han, I. H., & Baik, B. K. (2006). Oligosaccharide content and composition of legumes and their reduction by soaking, cooking, ultrasound, and high hydrostatic pressure. *Cereal Chemistry*, *83*(4), 428–433.

Han, Z., Yu, Q., Zeng, X., Luo, D., Yu, S., Zhang, B., & Chen, X. (2012a). Studies on the microstructure and thermal properties of pulsed electric fields (PEF)-treated maize starch. *International Journal of Food Engineering*, *8*(1). Article15, <http://dx.doi.org/10.1515/1556–3758.2375>.

Han, Z., Zeng, X., Fua, N., Yu, S., Chen, X., & Kennedy, J. F. (2012b). Effects of pulsed electric field treatments on some properties of tapioca starch. *Carbohydrate Polymers*, *89*, 1012–1017.

Han, Z., Zeng, X., Yu, S., Zhang, B., & Chen, X. (2009). Effects of pulsed electric fields (PEF) treatment on physicochemical properties of potato starch. *Innovative Food Science and Emerging Technologies*, *10*, 481–485.

Han, Z., Zeng, X., Zhang, B., & Yu, S. (2009). Effects of pulsed electric fields (PEF) treatment on the properties of cornstarch. *Journal of Food Engineering*, *93*, 318–323.

Harnkarnsujarit, N., & Charoenrein, S. (2011). Effect of water activity on sugar crystallization and β-carotene stability of freeze-dried mango powder. *Journal of Food Engineering*, *105*(4), 592–598.

Herceg, I., Jambrak, A., Šubarić, D., Brnčić, M., Brnčić, S., Badanjak, M., & Herceg, Z. ,etal (2010). Texture and pasting properties of ultrasonically treated corn starch. *Czech Journal of Food Science*, *28*(2), 83–93.

Hernoux-Villière, A. (2013). *Catalytic depolymerisation of starch-based industrial waste use of nonconventional activation methods and novel reaction media*. OULU 2013 A 611. Acta Universitatis Ouluensis. Available from: <http://herkules.oulu.fi/isbn9789526201634/isbn9789526201634.pdf> Accessed 01.02. 2016.

Holloway, L., Moynihan, S., Abrams, S. A., Kent, K., Hsu, A. R., & Friedlander, A. L. (2007). Effects of oligofructose-enriched inulin on intestinal absorption of calcium and magnesium and bone turnover markers in postmenopausal women. *British Journal of Nutrition*, *97*, 365–372.

Hong, J., Chen, R., Zeng, X., & Han, Z. (2016). Effect of pulsed electric fields assisted acetylation on morphological, structural and functional characteristics of potato starch. *Food Chemistry*, *192*, 15–24.

Hong, J., Zeng, X., Buckow, R., Han, Z., & Wang, M. (2016). Nanostructure, morphology and functionality of cassava starch after pulsed electric fields assisted acetylation. *Food Hydrocolloids*, *54*, 139–150.

Hu, B., Huang, K., Zhang, P., Zeng, X., Han, Z., & Yu, S. (2015). Pulsed electric field effects on sucrose nucleation at low supersaturation. *Sugar Technology*, *17*(1), 77–84.

Hugot, E. (1986). *Handbook of cane sugar engineering* (3rd edAmsterdam: Elsevier Science Publishing Co., Inc.

Hui, T., Chan, R., & Karim, A. (2009). Physicochemical and functional properties of ozone-oxidized starch. *Journal of Agricultural and Food Chemistry*, *57*, 5965–5970.

Huppertz, T., & Gazi, I. (2015). Lactose in dairy ingredients: Effect on processing and storage stability. *Journal of Dairy Science*, *98*, 1–10.

Inglett, G.F. (1992). *Method of making soluble dietary fiber compositions from cereals*. United States Patent # 5,082,67.

Inglett, G.F. (2000). *Soluble hydrocolloid food additives and method of making*. United States Patent # 6,060,519.

International Starch Institute, Science Park Aarhus, Denmark (2014). Available from: <http://www.starch.dk/> Accessed 05.01.2016.

Isomalto-Oligosaccharide Vitafiber™ (2012). *Health Canada*. Available from <http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/isomalto-oligosaccharide-eng.php> Accessed 28.01.2016.

Jaime, L., Martinez, F., Martin-Cabrejas, M. A., Molla, E., Lopez-Andreu, F. J., Waldron, K. W., & Esteban, R. M. (2000). Study of total fructan and fructooligosaccharide content in different onion tissues. *Journal of the Science of Food and Agriculture*, *81*, 177–182.

Jambrak, A., Herceg, Z., Šubarić, D., Babic, J., Brnčić, M., Brnčić, S., Gelo, J., et al. (2010). Ultrasound effect on physical properties of cornstarch. *Carbohydrate Polymers*, *79*, 91–100.

Jayapal, N., Samanta, A. K., Kolte, A. P., Senani, S., Sridhar, M., Suresh, K. P., & Sampath, K. T. (2013). Value addition to sugarcane bagasse: Xylan extraction and its process optimization for xylooligosaccharides production. *Industrial Crops and Products*, *42*, 14–24.

Jonathan, M. C., Bosch, G., Schols, H. A., & Gruppen, H. (2013). Separation and identification of individual alginate oligosaccharides in the feces of alginate-fed pigs. *Journal of Agricultural and Food Chemistry*, *61*, 553–560.

Jothisaraswathi, S., Babu, B., & Rengasamy, R. (2006). Seasonal studies on alginate and its composition II: Turbinaria conoides (J.Ag.) Kütz. (Fucales, Phaeophyceae). *Journal of Applied Phycology*, *18*, 161–166.

Jovanovic-Malinovska, R., Kuzmanova, S., & Winkelhausen, E. (2014). Oligosaccharide profile in fruits and vegetables as sources of prebiotics and functional foods. *International Journal of Food Properties*, *17*(5), 949–965.

Kapelko, M., Zie, T., & Michalski, A. (2012). Effect of the production method on the properties of RS3/RS4 type resistant starch. Part 2. Effect of a degree of substitution on the selected properties of acetylated retrograded starch. *Food Chemistry*, *135*, 2035–2042.

Karim, A. A., & Wai, C. C. (1999). Foam-mat drying of starfruit (*Averrhoa carambola* L.) pureé. Stability and air drying characteristics. *Food Chemistry*, *64*, 337–343.

Kasprzak, M., Lærke, E., Hofmann Larsen, F., Knudsen, K., Pedersen, S., & Jørgensen, A. (2012). Effect of enzymatic treatment of different starch sources on the *in vitro* rate and extent of starch digestion. *International Journal of Molecular Science*, *13*, 929–942.

Keenan, D. F., Brunton, N., Butler, F., Wouters, R., & Gormley, R. (2011). Evaluation of thermal and high hydrostatic pressure processed apple purees enriched with prebiotic inclusions. *Innovative Food Science and Emerging Technologies*, *12*, 261–268.

Khan, S., Tøndervik, A., Sletta, H., Klinkenberg, G., Emanuel, C., Onsøyen, E., & Thomas, D. W. , et al (2012). Overcoming drug resistance with alginate oligosaccharides able to potentiate the action of selected antibiotics. *Antimicrobial Agents and Chemotherapy*, *56*(10), 5134–5141.

Kim, D., Fan, J. P., Chung, H. C., & Han, G. D. (2010). Changes in extractability and antioxidant activity of Jerusalem artichoke (*Helianthus tuberosus* L.) tubers by various high hydrostatic pressure treatments. *Food Science and Biotechnology*, *19*(5), 1365–1371.

Kim, H., Kim, B., & Baik, M. (2012). Application of ultra high pressure (UHP) in starch chemistry. *Critical Reviews in Food Science and Nutrition*, *52*(2), 123–141.

Kim, Y. W., Kim, K. H., Choi, H. J., & Lee, D. S. (2005). Anti-diabetic activity of beta-glucans and their enzymatically hydrolyzed oligosaccharides from *Agaricus blazei*. *Biotechnology Letters*, *27*(7), 483–487.

Kiryluk, J., Kawka, A., Gasiorowski, H., Chalcarz, A., & Aniola, J. (2000). Milling of barley to obtain b-glucan enriched products. *Nahrung*, *44*(4), 238–241.

Klöck, G., Pfefferrnann, A., Ryser, C., Gröhn, P., Kuttler, B., Hahn, H. J., & Zimmermann, U. (1997). Biocompatibility of mannuronic acid-rich alginates. *Biomaterials*, *18*, 707–713.

Knorr, D., Zenker, M., Heinz, V., & Lee, D. U. (2004). Applications and potential of ultrasonics in food processing. *Trends in Food Science and Technology*, *15*, 261–266.

Kohno, M., Mokudai, T., Ozawa, T., & Niwano, Y. (2011). Free radical formation from sonolysis of water in the presence of different gases. *Journal of Clinical Biochemistry and Nutrition*, *49*(2), 96–101.

Kumar, R., Bawa, A. S., Rajeswara Reddy, K., Kathiravan, T., Subramanian, V., & Nadanasabapathi, S. (2015). Pulsed electric field and combination processing of mango nectar: Effect on volatile compounds and HMF formation. *Croatian Journal of Food Science and Technology*, *7*(2), 58–67.

Kumoro, A., Retnowati, D., & Budiyati, C. (2010). Microwave assisted synthesis and characterization of acetate derived cassava starch. *American Journal of Food Technology*, *5*(2), 100–110.

Kurakake, M., Sumida, T., Masuda, D., Oonishi, S., & Komaki, T. (2006). Production of galacto-manno-oligosaccharides from guar gum by β-mannanase from Penicillium oxalicum SO. *Journal of Agricultural and Food Chemistry*, *54*(20), 7885–7889.

Lam, K.-L., & Cheung, P. C.-K. (2013). Nondigestible long chain beta-glucans as novel prebiotics. *Bioactive Carbohydrates and Dietary Fiber*, *2*, 45–64.

Latorre, M. E., Bonelli, P. R., Rojas, A. M., & Gerschenson, L. N. (2012). Microwave inactivation of red beet (*Beta vulgaris* L. var.conditiva) peroxidase and polyphenoloxidase and the effect of radiation on vegetable tissue quality. *Journal of Food Engineering*, *109*, 676–684.

Lefranc-Millot, C., Gruerin-Deremaux, L., Wils, D., Neut, C., Miller, L. E., & Saniez-Degrave, M. H. (2012). Impact of a resistant dextrin on intestinal ecology: How altering the digestive ecosystem with Nutriose, a soluble fiber with prebiotic properties, may be beneficial for health. *The Journal of International Medical Research*, *40*, 211–224.

Lewicka, K., Siemion, P., & Kurcok, P. (2015). Chemical modifications of starch: Microwave effect. *International Journal of Polymer Science*, 2015. Article ID 867697. Available from <http://dx.doi.org/10.1155/2015/867697>.

Limberger-Bayer, V., De Francisco, A., Chan, A., Oro, T., Ogliari, P., & Barreto, P. (2014). Barley β-glucans extraction and partial characterization. *Food Chemistry*, *154*, 84–89.

Lingyun, W., Jianhua, W., Xiaodong, Z., Da, T., Yalin, Y., Chenggang, C., & Fan, Z. ,etal (2007). *Studies on the extracting technical conditions of inulin from Jerusalem artichoke tubers*. *Journal of Food Engineering* (79, pp. 1087–1093).

Linhardt, R. J., & Bazin, H. G. (2010). Properties of carbohydrates. In B. Fraser-Reid, K. Tatsuta, & J. Thiem (Eds.), *Glycoscience: chemistry and chemical biology*. Heidelberg: Springer-Verlag.

Liu, F., Li, R., Wang, Y., Bi, X., & Liao, X. (2014). Effects of high hydrostatic pressure and high-temperature short-time on mango nectars: Changes in microorganisms, acid invertase, 5-hydroxymethylfurfural, sugars, viscosity, and cloud. *Innovative Food Science and Emerging Technologies*, *22*, 22–30.

Liu, F., Wang, Y., Li, R., Bi, X., & Liao, X. (2014). Innovative effects of high hydrostatic pressure and high temperature short time on antioxidant activity, antioxidant compounds and color of mango nectars. *Food Science and Emerging Technologies*, *21*, 35–43.

Liu, P. L., Hu, X. S., & Shen, Q. (2010). Effect of high hydrostatic pressure on starches: A review. *Starch/Starke*, *62*, 615–628.

Loginov, M., Loginova, K., Lebovka, N., & Vorobiev, E. (2011). Comparison of dead-end ultrafiltration behaviour and filtrate quality of sugar beet juices obtained by conventional and "cold" PEF-assisted diffusion. *Journal of Membrane Science*, *377*, 273–283.

Loginova, K., Loginov, M., Vorobiev, E., & Lebovka, N. I. (2011). Quality and filtration characteristics of sugar beet juice obtained by "cold" extraction assisted by pulsed electric field. *Journal of Food Engineering*, *106*, 144–151.

Loginova, K., Loginov, M., Vorobiev, E., & Lebovka, N. I. (2012). Better lime purification of sugar beet juice obtained by low temperature aqueous extraction assisted by pulsed electric field. *LWT-Lebensmittel-Wissenschaft und-Technologie*, *46*, 371–374.

Loginova, K. V., Shynkaryk, M. V., Lebovka, N. I., & Vorobiev, E. (2010). Acceleration of soluble matter extraction from chicory with pulsed electric fields. *Journal of Food Engineering*, *96*, 374–379.

Loginova, K. V., Vorobiev, E., Bals, O., & Lebovka, N. I. (2011a). Pilot study of countercurrent cold and mild heat extraction of sugar from sugar beets, assisted by pulsed electric fields. *Journal of Food Engineering*, *102*, 340–347.

López, N., Puértolas, E., Condón, S., Raso, J., & Álvarez, I. (2009). Enhancement of the solid-liquid extraction of sucrose from sugar beet (*Beta vulgaris*) by pulsed electric fields. *LWT-Lebensmittel-Wissenschaft und-Technologie*, *42*, 1674–1680.

Lou, Z., Wang, H., Wang, D., & Zhang, Y. (2009). Preparation of inulin and phenols-rich dietary fiber powder from burdock root. *Carbohydrate Polymers*, *78*, 666–671.

Lukasiewicz, M., Bednarz, S., Ptaszek, A., Bogdal, D., & Achremowicz, B. (2008). Microwave assisted starch oxidation–a 'green' way for polysaccharide modification, *12th international electronic conference on synthetic organic chemistry (ECSOC-12)*. Available from <http://www.mdpi.org/ecsoc-12> Accessed 30.01.2016.

Lynd, L. R., Weimer, P. J., Van Zyl, W. H., & Pretorius, I. S. (2002). Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, *66*(3), 506–577.

Mahajan, P. V., Caleb, O. J., Singh, Z., Watkins, C. B., & Geyer, M. (2014). Postharvest treatments of fresh produce. *Philosophical Transactions of the Royal Society A*, *372*, 1–19.

Manchun, S., Nunthanid, J., Limmatvapirat, S., & Sriamornsak, P. (2012). Effect of ultrasonic treatment on physical properties of tapioca starch. *Advanced Materials Research*, *506,*, 294–297.

Mansberger, A., D'amico, S., Novalin, S., Schmidt, J., Tömösközi, S., Berghofer, E., & Schoenlechner, R. (2014). Pentosan extraction from rye bran on pilot scale for application in gluten-free products. *Food Hydrocolloids*, *35*, 606–612.

Martinez Sabajanes, M., Yañez, R., Alonso, J. L., & Parajo, J. C. (2012). Pectic oligosaccharides production from orange peel waste by enzymatic hydrolysis. *International Journal of Food Science and Technology*, *4*, 747–754.

Martins Ochubiojo, E., & Rodrigues, A. (2012). Starch: From food to medicine. In B. Valdez (Ed.), *Scientific, health and social aspects of the food industry*. Rijeka, Croatia: InTech Europe. (Chapter 18. pp. 355–380). Available from <http://www.intechopen.com/books/scientific-health-and-social-aspects-of-the-food-industry/starch-from-food-to-medicine>.

Mathlouthi, M., & Rogé, B. (2003). Water vapour sorption isotherms and the caking of food powders. *Food Chemistry*, *82*, 61–71.

Mendes, M.F., Cataldo, L.F., da Silva, C.A., Nogueira, R.I., & Freitas, S.P. (2005). Extraction of the inuline from chicory roots (*Chicorium intybus* L.) using supercritical carbon dioxide, *2nd mercosur congress on chemical engineering. ENPROMER*. Costa Verde, Brasil.

Merali, Z., Ho, J. D., Collins, S. R. A., Le Gall, G., Elliston, A., Käsper, A., & Waldron, K. (2013). Characterization of cell wall components of wheat straw following hydrothermal pretreatment and fractionation. *Bioresource Technology*, *131*, 226–234.

Mhemdi, H., Bals, O., & Vorobiev, E. (2016). Combined pressing-diffusion technology for sugar beets pretreated by pulsed electric field. *Journal of Food Engineering*, *168*, 166–172.

Michalac, S., Alvarez, V., Ji, T., & Zhang, Q. (2003). Inactivation of selected microorganisms and properties of pulsed electric field processed milk. *Journal of Food Processing Preservation*, *27*, 137–151.

Middlebos, I. S., & Fahey, G. C., Jr. (2008). Soybean carbohydrates. In L. A. Johnson, P. J. White, & R. Galloway (Eds.), Soybeans: Chemistry, production, processing, and utilization. *Urbana, IL*. AOCS Press, Monograph Series on Oilseeds.

Milani, E., Koocheki, A., & Golimovahhed, Q. (2011). Extraction of inulin from Burdock root (*Arctium lappa*) using high intensity ultrasound. *International Journal of Food Science and Technology*, *46*, 1699–1704.

Miller, R. B. (2005). *Electronic irradiation of foods: An introduction to the technology. Food engineering serie*. New York: Springer.

Minjares-Fuentes, R., Femenia, A., Garau, M. C., Candelas-Cadillo, M. G., Simal, S., & Rosselló, C. (2016). Ultrasound-assisted extraction of hemicelluloses from grape pomaceusing response surface methodology. *Carbohydrate Polymers*, *138*, 180–191.

Moers, K., Celus, I., Brijs, K., Courtin, C. M., & Delcour, J. A. (2005). Endoxylanase substrate selectivity determines degradation of wheat water-extractable and water-unextractable arabinoxylan. *Carbohydrate Research*, *340*, 1319–1327.

Moreno, F. J., Molina, E., Olano, A., & Fandino-Lopez, R. (2003a). High-pressure effects on Maillard reaction between glucose and lysine. *Journal of Agricultural and Food Chemistry*, *51*, 394–400.

Moreno, F. J., Villamiel, M., & Olano, A. (2003b). Effect of high pressure on isomerisation and degradation of lactose in alkaline media. *Journal of Agricultural and Food Chemistry*, *51*, 1894–1896.

Morris, G. A., Ralet, M. C., Bonnin, E., Thibault, J. F., & Harding, S. E. (2010). Physical characterisation of the rhamnogalacturonan and homogalacturonan fractions of sugar beet (*Beta vulgaris*) pectin. *Carbohydrate Polymers*, *82*, 1161–1167.

Moser, M., Agemans, A., & Caers, W. (2014). Production and bioactivity of oligosaccharides from chicory roots. In F. J. Moreno, & M. L. Sanz (Eds.), *Food Oligosaccharides: Production, Analysis and Bioactivity*. Hoboken: Wiley-Blackwell.

Murray, B., Gross, D., & Fox, T. (1994). *Starch manufacturing: A profile*. Research Triangle Institute, United States Environmental Protection Agency. Available from <http://www3.epa.gov/ttnecas1/regdata/IPs/Starch%20Manufacturing_IP.pdf> Accessed 05.01.2016.

Murthy, G.S., & Prasad, S. (2005). A completely coupled model for microwave heating of foods in microwave oven. *Abstracts of the ASAE Meeting 2005*. Presentation Paper Number, 05, pp.60–62.

Narváez-Flores, M., Sánchez-Madrigal, M., Quintero-Ramos, A., Paredes-Lizárraga, M., González-Laredo, R., Ruiz-Gutiérrez, M., & Meléndez-Pizarro, A. ,etal (2015). Ultrasound assisted extraction modeling of fructans from agave (*Agave tequilana* Weber var. Azul) at different temperatures and ultrasound powers. *Food and Bioproducts Processing*, *96*, 232–239.

Nelson, D. L., & Cox, M. M. (2004). *Lehninger principles of biochemistry* (4th ed.)USA:: Freeman, W. H. & Company.

Nemtanu, M., & Brasoveanu, M. (2010). Functional properties of some nonconventional treated starches. In M. Elnashar (Ed.), *Biopolymers*. Rijeka, Croatia: InTech Europe. (Chapter 17, pp. 320–344), Available from <http://www.intechopen.com/books/biopolymers/functional-properties-of-some-nonconventional-treatedstarches>.

Ngadi, M. O., Bajwa, S. S. S., & Alakali, J. (2012). Minimally processed foods. In B. K. Simpson (Ed.), *Food biochemistry and food processing* ((2nd ed.)). Oxford: Wiley Blackwell. John Wiley & Sons, Inc.

Nilsson, M., Saulnier, L., Andersson, R., & Aman, P. (1996). Water unextractable polysaccharides from three milling fractions of rye grain. *Carbohydrate Polymers*, *30*, 229–231.

Otieno, D., & Ahring, B. (2012). A thermochemical pretreatment process to produce xylooligosaccharides (XOS), arabinooligosaccharides (AOS) andmannooligosaccharides (MOS) from lignocellulosic biomasses. *Bioresource Technology*, *112*, 285–292.

Palafox-Carlos, H., Ayala-Zavala, J., & González-Aguilar, G. (2011). The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. *Journal of Food Science*, *76*(1), R6–R15.

Pan, G. G., & Melton, L. D. (2007). Nonenzymatic browning of lactose and caseinate during dry heating at different relative humidities. *Journal of Agricultural and Food Chemistry*, *55*(24), 10036–10042.

Parada, J., & Aguilera, J. M. (2007). Food microstructure affects the bioavailability of several nutrients. *Journal of Food Science*, *72*(2), R21–R32.

Patel, S., & Goyal, A. (2012). The current trends and future perspectives of prebiotics research: A review. *Biotechnology*, *2*, 115–125.

Peng, F., Ren, J., Xu, F., Bian, J., Peng, P., & Sun, R. (2010). Fractional study of alkali-soluble hemicelluloses obtained by graded ethanol precipitation from sugar cane bagasse. *Journal of Agricultural and Food Chemistry*, *58*, 1768–1776.

Pennington, N. L., & Baker, C. (1990). *Sugar: a users' guide to sucrose*. Berlin: Springer.

Perez, A., Sanz, C., Rios, J., Olias, R., & Olias, J. (1999). Effects of ozone treatment on postharvest strawberry quality. *Journal of Agricultural and Food Chemistry*, *47*, 1652–1656.

Péroval, C., Debeaufort, F., Seuvre, A. M., Chevet, B., Despré, D., & Voilley, A. (2003). Modified arabinoxylan-based films. Part B. Grafting of omega-3 fatty acids by oxygen plasma and electron beam irradiation. *Journal of Agricultural and Food Chemistry*, *51*, 3120–3126.

Perry, J., & Yousef, A. (2011). Decontamination of raw foods using ozone-based sanitization techniques. *Annual Reviews in Food Science and Technology*, *2*, 281–298.

Philips, G. O., & Cui, S. W. (2011). An introduction: Evolution and finalisation of the regulatory definition of dietary fiber. *Food Hydrocolloids*, *25*, 139–143.

Pivarnik, L.F., & Worobo, R. (2014). *Nonthermal or alternative food processing methods to enhance microbial safety and quality*. Frequently asked questions (USDA-NIFA #2011–68003–30005). Available from <http://ucfoodsafety.ucdavis.edu/files/227891.pdf> Accessed 03.02.2016.

Porto-Fett, A. C. S., Call, J. E., Shoyer, B. E., Hill, D. E., Pshebniski, C., Cocoma, G. J., & Luchansky, J. B. (2010). Evaluation of fermentation, drying, and/or high pressure processing on viability of *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp., and *Trichinella spiralis* in raw pork and Genoa salami. *International Journal of Food Microbiology*, *140*, 61–75.

Pourfarzad, A., Habibi Najafi, M., Haddad Khodaparast, M., & Hassanzadeh Khayyat, M. (2015). Characterization of fructan extracted from *Eremurus spectabilis* tubers: A comparative study on different technical conditions. *Journal of Food Science and Technology*, *52*(5), 2657–2667.

Ralet, M. C., Cabrera, J. C., Bonnin, E., Quéméner, B. C., Hellin, P., & Thibault, J. F. (2005). Mapping sugar beet pectin acetylation pattern. *Phytochemistry*, *66*, 1832–1843.

Redgwell, R. J., Curti, D., & Gehin-Delval, C. (2008). Physicochemical properties of cell wall materials from apple, kiwifruit and tomato. *European Food Research and Technology*, *227*, 607–618.

Renard, C.M.G.C., Watrelot, A.A., & Le Bourvellec, C. (2015). Interactions between polyphenols and polysaccharides: Mechanisms and consequences in food processing and digestion, *29th EFFoST international conference proceedings*. 10–12 November, Athens, Greece. Available from <http://prodinra.inra.fr/ft?id = 028F925F-CA7A-4B0A-A553–47038E96F030> Accessed 21.01.2016.

Rojas, A. M., & Gerschenson, L. N. (1997a). Ascorbic acid destruction in sweet aqueous model systems. *LWT-Lebensmittel-Wissenschaft und-Technologie*, *30*, 567–572.

Rojas, A. M., & Gerschenson, L. N. (1997b). Influence of system composition on ascorbic acid destruction at processing temperatures. *Journal of the Science of Food and Agriculture*, *74*, 369–378.

Roselló-Soto, E., Barba, F. J., Parniakov, O., Galanakis, C. M., Grimi, N., Lebovka, N., & Vorobiev, E. (2015a). High voltage electrical discharges, pulsed electric field and ultrasounds assisted extraction of protein and phenolic compounds from olive kernel. *Food and Bioprocess Technology*, *8*, 885–894.

Roselló-Soto, E., Galanakis, C. M., Brncic, M., Orlien, V., Trujillo, F. J., Mawson, R., & Barba, F. J. ,etal (2015b). Clean recovery of antioxidant compounds from plant foods, byproducts and algae assisted by ultrasounds processing. Modeling approaches to optimize processing conditions. *Trends in Food Science and Technology*, *42*, 134–149.

Roudaut, G., Simatos, D., Champion, D., Contreras-López, E., & Le Meste, M. (2004). Molecular mobility around the glass transition temperature: A mini review. *Innovative Food Science and Emerging Technology*, *5*, 127–134.

Ruiz-Aceituno, L., García-Sarrió, J., Alonso-Rodriguez, B., Ramos, L., & Sanz, M. L. (2016). Extraction of bioactive carbohydrates from artichoke (*Cynara scolymus* L.) external bracts using microwave assisted extraction and pressurized liquid extraction. *Food Chemistry*, *196*, 1156–1162.

Saengkanuk, A., Nuchadomrong, S., Jogloy, S., Patanothai, A., & Srijaranai, S. (2011). A simplified spectrophotometric method for the determination of inulin in Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. *European Food Research and Technology*, *233*, 609–616.

Saldaña, M. D. A., & Valdivieso-Ramírez, C. S. (2015). Pressurized fluid systems: Phytochemical production from biomass. *Journal of Supercritical Fluids*, *96*, 228–244.

Santos, M., Saraiva, J., & Gomes, M. (2015). Pasting of maize and rice starch after high pressure processing: Studies based on an acoustic wave sensor. *Sensors and Actuators B*, *209*, 323–327.

Sárossy, Z., Tenkanen, M., Pitkänen, L., Bjerre, A. B., & Plackett, D. (2013). Extraction and chemical characterization of rye arabinoxylan and the effect of β-glucan on the mechanical and barrier properties of cast arabinoxylan films. *Food Hydrocolloids*, *30*, 206–216.

Selim, K. M., & Reda, R. M. (2015). Beta-glucans and mannan oligosaccharides enhance growth and immunity in nile. *North American Journal of Aquaculture*, *77*(1), 22–30.

Shayanfar, S., Chauhan, O. P., Toepfl, S., & Heinz, V. (2014). Effect of nonthermal hurdles in extending shelf life of cut apples. *Journal of Food Science and Technology*, *51*(12), 4033–4039.

Shendare, R. C., Gongle, M. A., Rajput, A. B., Wanjari, B. V., & Mandlekar, S. M. (2008). Effect of supplementation of manno-oligosaccharide and β-glucans on maize based meal on commercial broilers. *Veterinary World*, *1*(1), 13–15.

Shiiba, K., Yamada, H., Hara, H., Okada, K., & Nagao, S. (1993). Purification and characterization of two arabinoxylans from wheat bran. *Cereal Chemistry*, *70*, 209–214.

Simi, C., & Abraham, T. (2007). Hydrophobic grafted and cross-linked starch nanoparticles for drug delivery. *Bioprocess and Biosystems Engineering*, *30*, 173–180.

Singdevsachan, S. K., Auroshree, P., Mishra, J., Baliyarsingh, B., Tayung, K., & Thatoi, H. (2016). Mushroom polysaccharides as potential prebiotics with their antitumor and immunomodulating properties: A review. *Bioactive Carbohydrates and Dietary Fibre*, *7*, 1–14.

Singh, J., Kaur, L., & Mccarthy, O. J. (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications−A review. *Food Hydrocolloids*, *21*, 1−22.

Slavin, J. (2013). Fiber and prebiotics: Mechanisms and health benefits. *Nutrients*, *5*, 1417−1435.

Śliżewska, K. (2013). The citric acid-modified, enzyme-resistant dextrin from potato starch as a potential prebiotic. *Acta Biochimica Polonica (ABP)*, *60*(4), 671−675.

Snelders, J., Dornez, E., Broekaert, W. F., Delcour, J. A., & Courtin, C. M. (2013). Determination of the xylan backbone distribution of arabinoxylan-oligosaccharides. *Bioactive Carbohydrates and Dietary Fibre*, *2*, 84−91.

Sokhey, A. S., & Hanna, M. A. (1993). Properties of Irradiated Starches. *Food Structure*, *12*(4). Article 2. Available from <http://digitalcommons.usu.edu/foodmicrostructure/vol12/iss4/2> Accessed 10.01.2016.

Stabler, C., Wilks, K., Sambanis, A., & Constantinidis, I. (2001). The effects of alginate composition on encapsulated TC3 cells. *Biomaterials*, *22*, 1301−1310.

Starch, Europe. Available from <http://www.starch.eu/extraction-and-processing>. Accessed 03.01.2016.

Sujka, M., & Jamroz, J. (2013). Ultrasound-treated starch: SEM and TEM imaging, and functional behaviour. *Food Hydrocolloids*, *31*, 413−419.

Sun, J., Zhao, R., Zeng, J., Li, G., & Li, X. (2010). Characterization of dextrins with different dextrose equivalents. *Molecules*, *15*, 5162−5173.

Sun, X., Fowler, P., Rajaratnam, M., & Zhang, G. (2010). Extraction and characterisation of hemicelluloses from maize stem. *Phytochemical Analysis*, *21*, 406−415.

Temelli, F. (1997). Extraction and functional properties of barley β-glucan as affected by temperature and pH. *Journal of Food Science*, *62*, 1194−1201.

Tester, R. F., Karkalas, J., & Qi, X. (2004). Starch-composition, fine structure and architecture. *Journal of Cereal Science*, *39*, 151−165.

Thirumdas, R., Sarangapani, C., & Annapure, U. S. (2015). Cold plasma: A novel nonthermal technology for food processing. *Food Biophysics*, *10*(1), 1−11.

Thuwapanichayanan, R., Prachayawarakorn, S., & Soponronnarit, S. (2008). Drying characteristics and quality of banana foam mat. *Journal of Food Engineering*, *86*, 573−583.

Tiwari, U., & Cummings, E. (2009). Review. Factors influencing β-glucan levels and molecular weight in cereal-based products. *Cereal Chemistry*, *86*(3), 290−301.

Tøndervik, A., Sletta, H., Klinkenberg, G., Emanuel, C., Powell, L. C., Pritchard, M. F., & Hill, K. E. (2014). Alginate oligosaccharides inhibit fungal cell growth and potentiate the activity of antifungals against *Candida* and *Aspergillus* spp. *Plos One*, *9*(11), 1−10 , *e112518*.

Torres Bello, E. F., González Martínez, G., Klotz Ceberio, B. F., Rodrigo, D., & Martínez López, A. (2014). High pressure treatment in foods. *Foods*, *3*, 476−490.

Tsopmo, A. (2014). Processing oats and bioactive components. In V. R. Preedy (Ed.), *Processing and impact on active components in food*. London: Academic Press.

Tungland, B. C., & Meyer, D. (2006). Nondigestible oligo- and polysaccharides (dietary fiber): Their physiology and role in human health and food. *Comprehensive Reviews in Food Science and Food Safety*, *1*, 90−109.

Turk, M. F., Vorobiev, E., & Baron, A. (2012). Improving apple juice expression and quality by pulsed electric field on an industrial scale. *LWT-Lebensmittel-Wissenschaft und-Technologie*, *49*, 245−250.

USDA. (2015). *Food and drug administration. protecting and promoting your health*. Available from http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm103342.htm.

Van Dam, H. E., Kieboom, A. P. G., & Bekkum, H. (1986). The convertion of fructose and glucose in acidic media: Formation of hydroxymethylfurfural. *Starck/Stärke*, *38*(3), 95−101.

Van Den Heuvel, E. G. H. M., Schoterman, M. H. C., & Muijs, T. (2000). Transgalactooligosaccharides stimulate calcium absorption in postmenopausal women. *The Journal of Nutrition*, *130*(12), 2938–2942.

Van Der Poel, P. W., Schiweck, H., & Schwartz, T. (1998). *Sugar Technology . Beet and Cane Sugar Manufacture* (1st edBerlin: Verlag Dr Albert Bartens KG.

Vannieuwenburgh, C., Guibert, A., & Combes, D. (2002). Kinetic studies and mathematical model for sucrose conversion by *Aspergillus niger* fructosyl-transferase under high hydrostatic pressure. *Bioprocess and Biosystems Engineering*, *25*, 13–20.

Vasanthan, T., & Bhatty, R. S. (1995). Starch purification after pin milling and air classification of waxy, normal, and high amylose. *Cereal Chemistry*, *72*(4), 379–384.

Vasanthan, T., & Temelli, F. (2002). *Grain fractionation methods and products.* PCT International Publication Number WO 02/027011 A3.

Vasanthan, T., & Temelli, F. (2008). Grain fractionation technologies for cereal b-glucan concentration. *Food Research International*, *41*, 876–881.

Verma, D., Kaushik, N., & Rao, P. S. (2014). Application of high hydrostatic pressure as a pretreatment for osmotic dehydration of banana slices (Musa, avendishii) finish-dried by dehumidified air drying. *Food and Bioprocess Technology*, *7*, 1281–1297.

Vincken, J. P., Schols, H. A., Oomen, R. J. F. J., Mccann, M. C., Ulvskov, P., Voragen, A. G. J., et al. (2003). If homogalacturonan were a side chain of rhamnogalacturonan I. Implications for cell wall architecture. *Plant Physiology*, *132*, 1781–1789.

Waldron,, K. W., Parker,, M. L., & Smith,, A. C. (2003). Plant cell walls and food quality. *Reviews in Food Science and Food Safety,*, *2*, 101–119.

Wallace, G., Russell, W. R., Lomax, J. A., Jarvis, M. C., Lapierre, C., & Chesson, A. (1995). Extraction of phenolic-carbohydrate complexes from graminaceous cell walls. *Carbohydrate Research*, *272*, 41–53.

Wang,, Y.-H., Jiang, Y., Duan, Z.-Y., Shao, W.-L., & Li, H.-Z. (2009). Expression and characterization of a glucosidase from *Thermoanaerobacter ethanolicus* JW200 with potential for industrial application. *Biologia*, *64*, 1053–1057.

White, J. (2014). Sucrose, HFCS, and fructose: history, manufacture, composition, applications, and production. In J. M. Rippe (Ed.), *Fructose, High Fructose Corn Syrup, Sucrose and Health*. Berlin: Springer.

Wichienchot, S., Prasertsan, P., Hongpattarakere, T., & Rastall, R. A. (2009). Manufacture of gluco-oligosaccharide prebiotic by *Gluconobacter oxydans* NCIMB 4943. *Songklanakarin Journal of Science and Technology (SJST)*, *31*(6), 597–603.

Willats, W., Knox, J., & Mikkelsen, J. (2006). Pectin: New insights into an old polymer are starting to gel. *Trends in Food Science and Technology*, *17*, 97–104.

Wood, P. J., Weisz, J., Fedec, P., & Burrows, V. D. (1989). Large-scale preparation and properties of oat fractions enriched in (1–3)(1–4)-beta-D-glucan. *Cereal Chemistry*, *66*(2), 97–103.

Wood, P. J., Weisz, J., & Blackwell, B. A. (1991). Molecular characterization of cereal b-D-glucans. Structural analysis of oat b-D-glucan andd rapid structural evaluation of b-D-glucans from different sources by high-perfomance liquid chromatography of oligosaccharides released by lechinase. *Cereal Chemistry*, *68*, 31–39.

Wyman, C. E., Decker, S. R., Himmel, M. E., Brady, J. W., Skopec, C. E., & Viikari, L. (2005). Hydrolysis of Cellulose and Hemicellulose. In S. Dumitriu, (Ed.), *Polysaccharides: Structural diversity and functional versatility* (2nd ed). NY: Marcel Dekker.

Yanahira, S., Morita, M., Aoe, S., Suguri, T., Nakajima, I., & Deya, E. (1995). Effects of lactitol-oligosaccharides on the intestinal microflora in rats. *Journal of Nutritional Science and Vitaminology (Tokyo)*, *41*(1), 83–94.

Zeng, F., Gao, Q., Han, Z., Zeng, X., & Yu, S. (2016). Structural properties and digestibility of pulsed electric field treated waxy rice starch. *Food Chemistry,*, *94*, 1313–1319.

Zhang, Z., Yu, G., Guan, H., Zhao, X., Du, Y., & Jiang, X. (2004). Preparation and structure elucidation of alginate oligosaccharides degraded by alginate lyase from Vibro sp. 510. *Carbohydrate Research*, *339*, 1475−1481.

Zheng, J., Li, Q., Hu, A., Yang, L., Lu, J., Zhang, X., & Lin, Q. (2013). Dual-frequency ultrasound effect on structure and properties of sweet potato starch. *Starch/Starke*, *65*, 621−627.

Zhou, S., Liu, X., Guo, Y., Wang, Q., Peng, D., & Cao, L. (2010). Comparison of the immunological activities of arabinoxylans from wheat bran with alkali and xylanase-aided extraction. *Carbohydrate Polymers*, *81*, 784−789.

Zhu, F., Du, B., & Xu, B. (2016). Review. A critical review on production and industrial applications of beta-glucans. *Food Hydrocolloids*, *52*, 275−288.

Zhu, Z., Bals, O., Grimi, N., Ding, L., & Vorobiev, E. (2015). Better damage of chicory tissue by combined-electroporation and ohmic heating for solute extraction. *Food and Bioproducts Processing*, *94*, 248−254.

Zhu, Z., Bals, O., Grimi, N., & Vorobiev, E. (2012). Pilot scale inulin extraction from chicory roots assisted by pulsed electric fields. *International Journal of Food Science and Technology*, *47*(7), 1361−1368.

Zinoviadou, K. G., Barba, F. J., Galanakis, C. M., Brnčić, M., Trujillo, F., Mawson, R., & Knoerzer, K. (2015). Fruit juice sonication: Implications on food safety and physicochemical and nutritional properties. *Food Research International*, *77*(4), 743−752.

Zuleta, A., Dyner, L., Sambucetti, M. E., & De Francisco, A. (2006). Effect of gamma irradiation on the functional and nutritive properties of rice flours from different cultivars. *Cereal Chemistry*, *83*, 76−79.

Zykwinska, A., Rondeau-Mouro, C., Garnier, C., & Ralet, M. C. (2006). Alkaline extractability of pectic arabinan and galactan and their mobility in sugar beet and potato cell walls. *Carbohydrate Polymers*, *65*, 510−520.

This page intentionally left blank

CHAPTER

LIPIDS

4

Anet Režek Jambrak and Dubravka Škevin
University of Zagreb, Zagreb, Croatia

4.1 INTRODUCTION

Lipids are usually referred to as fats and oils. Fats are materials that are solid at ambient temperature and oils are those liquid at ambient temperature. Lipids [characterized as oils, greases, fats, and fatty acids (FAs)] are one of the most important components of natural foods and many synthetic compounds and emulsions. The contribution of bioactive lipids to health is determined by their compositional factors. FA composition (especially levels of omega-3, omega-6, and omega-9 FAs) and other high-value minor lipid compounds (e.g., glycolipids, phospholipids, tocols, phytosterols, aroma compounds, and phenolics) have been shown to exhibit health-promoting properties and positively affect the physiological functions of our body. Oils and fats derived from different sources (plant, animals, and microorganisms) generally differ from one another both in terms of their major and minor constituents.

Lipid oxidation in foods follows a chain reaction that has three different phases: initiation, propagation, and termination. In the presence of initiators, unsaturated lipids (LH) form carbon-centered alkyl radicals (L•). The formed radical from the initiation step abstracts hydrogen from other lipid molecules and reacts with hydrogen to form hydroperoxides (LOOH) and another lipid alkyl radicals (L•). Hydroperoxides can break down to form free radical products (alkoxyde or hydroxyl) or even a peroxy free radical, hydroxyl free radical, and water. These steps lead to a proliferation that can catalyze oxidation reaction by propagation steps and thus the reaction becomes autocatalytic (Pingret, Fabiano-Tixier, & Chemat, 2013).

Lipid oxidation is a thermodynamically governed phenomenon: in the absence of catalytic agents, heat has a marked effect in diminishing the activation energy in endothermic reactions. This phenomenon is strongly encouraged by the presence of inorganic and biological catalysts, such as metal ions and enzymes, normally present as their constituent. The goal of emerging technologies is to limit the extent of lipid degradation by avoiding high temperature for long periods, or controlling such catalysts by inactivation (enzymes) or removal (metal ions). Lipid systems are the most pressure-sensitive biological components, since lipid assemblies are governed by hydrophobic interactions that are very susceptible to high pressure (HP) ($\Delta V\# \leq -10$ mL/mol). The melting temperature (T_m) of triglycerides increases by more than 10°C per 100 MPa, so lipids present in a liquid state at room temperature will crystallize under pressure (Gabriela, Meza, Barnaba, et al., 2016). Next to lipid oxidation kinetics, other reactions such as polymerization, thermal degradation,

Nutraceutical and Functional Food Components. DOI: http://dx.doi.org/10.1016/B978-0-12-805257-0.00004-1

cyclization, Maillard reactions, Strecker degradation, denaturation, or oxygen depletion could occur at such high temperatures (Vandamme et al., 2015). Alternative food-processing techniques like ultrasound (US), cold plasma (CP), and high-pressure processing (HPP) are used in food preservation today. However, these technologies may lead to deterioration and modification of food compounds (e.g., carbohydrates, proteins, and lipids). Therefore particular caution should be taken during the selection of processing parameters.

For instance, HPP at 800 MPa and 60°C significantly reduces the concentration of linoleic acid (18:2) in muscle, and thiobarbituric acid reactive substances (TBARS) was shown to increase linearly with treatment pressure after chill storage under vacuum (Russell, 2002). HPP of "muscle" foods results in greater lipid oxidation following treatment at higher pressures (>400 MPa) and this appears to be more likely in 'red meats' where a higher content of myoglobin has been suggested. Indeed, depending on pressure and treatment duration, the treatment can be as damaging as heat (Simonin, Duranton, & de Lamballerie, 2012). Using CP instead, the interactions between antioxidant and plasma-immanent species leads to the formation of oxidative compounds, which in turn leads to the prooxidative effect and cascade reactions. The reactions between hydroxyl radicals and organic species are extremely fast and nonspecific, and are almost always controlled by the mass transfer of hydroxyl radicals to the organic species (Korachi et al., 2015). Similarly, UV or ionizing radiation (IR) initiate free radical reactions and generate singlet oxygen that swiftly reacts with unsaturated FAs. On the other hand, application of high-intensity pulsed electric field (PEF) may cause temporary or permanent permeabilization of cell membranes, while FA and lipid oxidation reactions can be attributed to the mechanism of electropermeabilization. These processes are very important in food processing, as degradation of fats decreases the nutritional quality and safety of food products.

4.2 STABILITY OF LIPIDS IN FOOD PRODUCTS

The physical properties of fats are extremely important. They are the consequence of the triacylglycerol (TAG) composition, which influences the nature, stability, and structure of ordered phases. This is related to the number of carbon atoms, unsaturation, and conformation of the FAs, or to the TAG structure itself. Food lipids are foods components that are very susceptible to oxidation processes and therefore oxidation reactions are one of the major sources of deterioration that occurs during manufacturing, storage, distribution, and final preparation of foods. Water activity is partially responsible for minimizing nonenzymatic browning reactions and spontaneous autocatalytic lipid oxidization reactions, prolonging the activity of enzymes and vitamins, as well as optimizing the physical properties of products such as moisture migration, texture, flavor, odor, and shelf-life. With a water activity of 0.3, the product is most stable with respect to lipid oxidation, nonenzymatic browning, enzyme activity, and the various microbial parameters. As water activity increases, the probability of the food product deteriorating increases. All foods that contain lipids, even at a very low level ($<1\%$), are susceptible to oxidation, leading to rancidity. Deleterious changes in foods caused by lipid oxidation include loss of flavor, color, and nutrient value, development of off-flavors, as well as accumulation of unhealthy compounds (Shirey & Sidisky, 1998; Vandamme et al., 2015).

One of the most effective ways of retarding lipid oxidation in foods is to incorporate antioxidants. Endogenous plant antioxidants are capable of inhibiting lipid peroxidation and protecting

against oxidative damage in biological systems. Lipid-oxidation products are very reactive with food components and can cause unwanted nutritional and sensory effects. Lipid peroxides are also able to decompose lipid-soluble vitamins, such as vitamins E, A, C, and carotenes. Lipid free radicals can interact with several amino acids of protein molecules to induce protein free radicals. The resultant end-products may be complexes formed by protein−protein and lipid−protein cross-linking. The overall effect of these reactions is damage to amino acid residues. Active oxygen species (AOS), which are generated during decomposition of lipid peroxides, are also capable of reacting with amino acids.

Hydrogenation of fats is produced by the addition of hydrogen to double bonds of FA chains in TAGs. This process has a vital role in the fats and oils industry because it achieves two main goals. First, it permits the transformation of liquid oils into semisolid fats for specific applications (e.g., margarine and shortenings), and second, it results in materials with improved stability. Regarding to emulsion stability against fat droplet aggregation or coalescence, homogenization conditions related to droplet-size characteristics are critical. For instance, lipid nanoparticles may be produced by HP homogenization, which is able to contribute to the emulsification process by rupturing bulk fat into small droplets. Emulsifiers get associated on newly formed fat droplet surface, making a protective membrane that prevents fat droplets from coalescence. Due to their small droplet size (from 100 to 500 nm) and narrow particle size distribution, nanoemulsions present long-term stability against creaming or sedimentation.

4.3 NUTRITIONAL AND FUNCTIONAL PROPERTIES OF LIPIDS

The nutritional properties of lipids allow food scientists to consider them during functional foods design, e.g., to control digestion and adsorption of triglycerides as well as to affect the bioaccessibility of hydrophobic bioactives. For instance, TAG containing 6−12 carbons atoms are very attractive because of their low caloric content and the fact that their fat content is not stored in body tissues. During digestion, the medium-chain FAs released by TAG (following the action of the sn-1,3-specific pancreatic lipase) are preferentially transported via the portal vein to the liver. This occurs because these FAs are more soluble than long-chain FAs (LCFA) and are metabolized as rapidly as glucose. TAGs are thought to provide less metabolizable energy per gram than traditional fats and oils and thus are an efficient food source for patients with pancreatic insufficiency and other forms of malabsorption. On the other hand, TAGs that contain high amounts of very long LCFAs are poorly absorbed, partly because they have a melting point that is higher than body temperature. In addition, they exhibit poor emulsion formation and micellar solubilization. The decreased absorption capacity of very LCFA and saturated FA makes them potential substrates for low-calorie structured lipids with industrial applications. An example of very long LCFAs is behenic acid (docosanoic acid), a saturated FA with 22 carbons (22:0).

The potential gastrointestinal fate of oil-in-water emulsions (like in food) containing lipid phases from different sources was examined by Zhang and McClements (2015) using medium-chain triglycerides, vegetable (corn, olive, sunflower, and canola oil), marine (fish and krill oil), and flavor oils (orange and lemon oil). The lowest rates and extents of lipid digestion were observed for emulsified flavor oil, followed by emulsified krill oil. According to the results, differences in the digestibility of emulsions prepared using different oils were attributed to differences in

their compositions (FA chain length and unsaturation). This study focused on the potential gastrointestinal fate of protein-coated oil droplets prepared using different lipid phases. As expected, the flavor oils were not digested under simulated gastrointestinal tract conditions because they did not contain TAGs that could be hydrolyzed by lipase. All of the emulsions containing TAGs were rapidly and fully digested under simulated gastrointestinal tract conditions, regardless of their initial free fatty acid (FFA) compositions. This may be important for functional food applications where a food manufacturer can select a variety of different oil sources and get the same gastrointestinal effect. The rate and extent of lipid digestion in emulsions prepared from krill oil was appreciably lower than that of the other oils, which may have been because they contained appreciable amounts of phospholipids and predigested TAGs (Zhang & McClements, 2015).

The beneficial effects of $n-3$ polyunsaturated fatty acids (PUFA) on human health have also been recognized. An enormous number of epidemiological and clinical studies have focused on the health effects of $n-3$ PUFA, particularly eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6), which are the two most important $n-3$ PUFA. These studies have shown that these acids play an important role in the prevention and treatment of cardiovascular diseases, hypertension, diabetes, cancer, etc., and are essential for normal growth and development. Dietary oils, which are recommended in healthful nutrition, often contain high amounts of PUFA, which are particularly sensitive to oxidation. Therefore the oxidation potential of fats and oils rich in PUFA (e.g., vegetable oils) should be considered in the processing of these products (Speranza & Macedo, 2012).

4.3.1 PHYTOSTEROL

Phytosterols or cholesterol must be incorporated into micelles for absorption. Once inside, the enterocyte, cholesterol, and a small percentage of phytosterols are esterified by acyl cholesterol acyl transferase, packaged into chylomicrons, and secreted into the lymphatic system (Nicolson & Ash, 2014).

4.3.2 OMEGA-3 PUFA

The two most important metabolically active PUFA are the linoleic acid and α-linolenic acid of the $n-6$ and the $n-3$ families. Linoleic acid and α-linolenic acid are elongated and desaturated in animal cells, forming the metabolically active longer chain $n-6$ (arachidonic acid) and $n-3$ (eicosapentaenoic, docosapentaenoic, and docosahexaenoic acid) PUFA. α-Linolenic acid is a key constituent of dark green leafy vegetables (e.g., broccoli, cabbage, and spinach), many seed oils, and cereal products. The long-chain omega-3 FAs (eicosapentaenoic, docosapentaenoic, and docosahexaenoic acid) are predominantly found in oil-rich seafood including tuna and salmon. Omega-3 FAs are pleiotropic molecules with a broad variety of biological actions including antiaggregatory, antiinflammatory, and antiarrhythmic responses. These FAs play a role in the management of hypertension and hyperlipidemia and in the prevention of several diseases such as coronary heart disease, type 2 diabetes, and insulin resistance. The latter effects are mediated by alterations in circulating plasma lipids, eicosanoids, cytokines, and physicochemical properties in the phospholipid membrane (Nicolson & Ash, 2014).

4.3.3 DIGESTION

Oral phospholipids are dispersed, partially or completely degraded, and absorbed in the gastrointestinal tract. The first part of hydrolysis takes place in the stomach (10−30%), whereas most fat hydrolysis and absorption occurs in the upper small intestine. Phospholipids can be oxidized and degraded during their ingestion, digestion, and eventual adsorption by the intestinal lumen. Once phospholipid micelles and liposomes are protected by binding specific oligosaccharides, they can be taken intact by the gastrointestinal brush border cells (Fig. 4.1). Most micellar phospholipids are absorbed intact by mucosal cells as unoxidized, undegraded phospholipids. When uncomplexed dietary membrane phospholipids are directly absorbed by mucosal cells, most of these molecules are partially degraded by pancreatic phospholipases, usually by removal of one or even both of the acyl FA chains and further degradation (Nicolson & Ash, 2014).

The key building blocks of most lipid nanoparticles are lipophilic substances (lipids) and surface-active substances (surfactants). A variety of different lipophilic substances may be utilized, including TAGs, diacylglycerols, monoacylglycerols, flavor oils, mineral oils, fat substitutes, waxes, oil-soluble vitamins (vitamins A, D, and E), and nutraceuticals (carotenoids, curcuminoids, and flavonoids). A variety of different surface-active substances can also be utilized, including small-molecule surfactants (Tweens), phospholipids, proteins (casein), and polysaccharides. The lipophilic and surface-active substances used to fabricate lipid nanoparticles have different susceptibilities to digestion within the different stages of the human gastrointestinal tract, which plays an important role in determining their biological fate (Zeeb, Lopez-Pena, & McClements, 2015).

In a recent study, the stability of emulsified oil droplets (rather than the initial layer properties) was shown to play a major role in the rate and extent of lipid digestion (Zeeb et al., 2015). These results suggest that directed destabilization of emulsified lipids using interfacial engineering approaches has the potential to be used for modifying lipid digestibility and/or to release bioactive agents at specific sites within the gastrointestinal tract (Fig. 4.2). In this study, biopolymer coatings were displaced from the lipid droplet surfaces within the gastrointestinal tract or were permeable to digestive enzymes and lipid-digestion products. Consequently, the digestive enzymes accessed the lipids and converted them into FFA and monoacylglycerols. Multilayer coatings may be used to improve the stability of lipid droplets to aggregation within a food product (Zeeb et al., 2015).

Liposomes consist of a molecular lipid bilayer that separates the inner aqueous phase from the external continuous water phase. Liposomes are formed via hydrophilic−hydrophobic interactions between an amphiphilic agent (e.g., phospholipids) and water molecules. They play an important role in delivering hydrophobic drugs (Singh & Thompson, 2012). For microencapsulation, the bioactive compound is entrapped either in the inner aqueous phase (low loading capacity) or within the membrane (higher loading capacity), with the size of carriers varying from 30 nm to a few μm (Cocero, Martín, Mattea, & Varona, 2009). Due to the limited chemical and physical stability of liposomes (they easily undergo aggregation, coalescence, phospholipid hydrolysis, and oxidation), the formation of large unilamellar vesicles is preferred. Upscaling liposome production in their dry state (proliposomes) is particularly relevant for the food industry: combining liposome formation with drying methods (e.g., spray drying, freeze drying, supercritical fluid precipitation) can be a cost-effective and sustainable alternative for encapsulation (Soukoulis and Bohn, 2015).

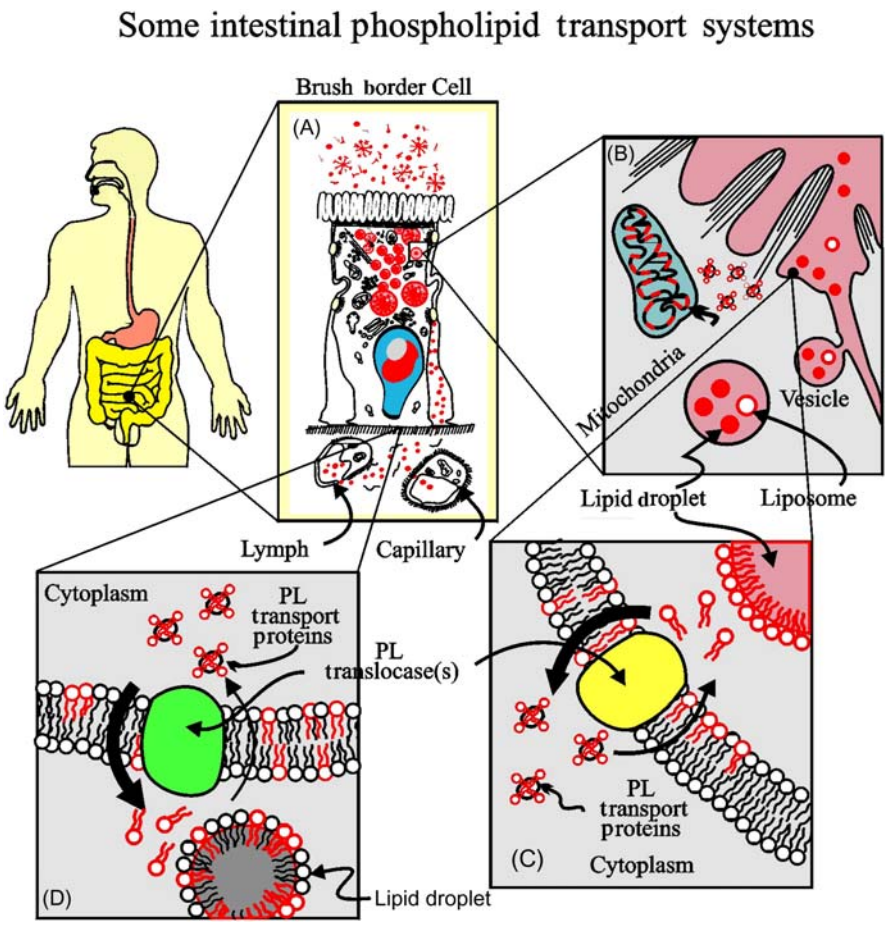


FIGURE 4.1

(A) Lipid Replacement Therapy (LRT) phospholipids are usually protected from complete disruption and enzymatic degradation by bound oligosaccharides, permitting transportation into cells as small lipid droplets and vesicles by endocytotic processes. At the distal or basolateral regions of the brush border cells excess lipid vesicles and droplets can be extruded by a reverse exocytotic process and eventually transported to lymph and blood vessels. (B) The endocytotic transport of lipid droplets and vesicles into brush border epithelial cells is shown in more detail. In addition, some of the phospholipids are absorbed by the epithelial cell plasma membrane and transported as simple phospholipids or their degradation products to organelle membranes, including mitochondria, by phospholipid transport systems. (C) After absorption by the brush border epithelial plasma membrane, phospholipids are flipped to the inner plasma membrane surface by phospholipid translocases. At the inner plasma membrane surface the phospholipids can be absorbed by phospholipid transport and carrier proteins and moved to other membranes. (D) At the brush border cell basolateral surface excess phospholipids are delivered by transport proteins to the inner surface of the plasma membrane (Nicolson & Ash, 2014).

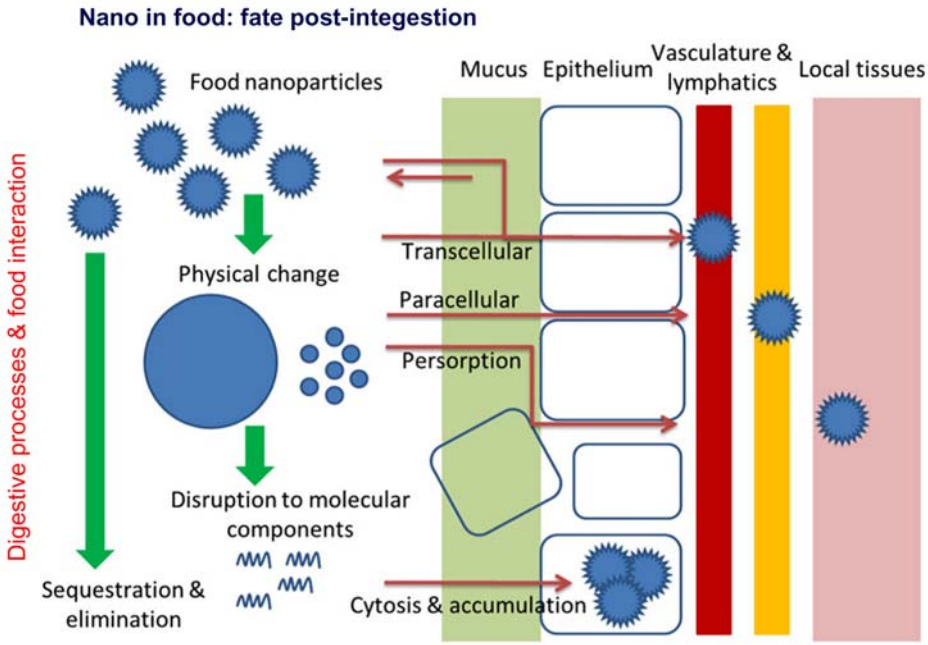


FIGURE 4.2

Potential fate of ingested food ingredients illustrating nanomaterials that enter the GI tract can either change from within the lumen or be absorbed via a number of potential routes (Yada et al., 2014).

4.3.4 OXIDATIVE STRESS

Oxidative stress occurs when the production of reactive oxygen species (ROS), such as superoxide anion radicals, hydroxyl radicals and hydrogen peroxide, and reactive nitrogen species (RNS), such as peroxynitrite anion, are in excess of the cell's ability to destroy these molecules using natural antioxidants. Cellular targets of ROS/RNS include nucleic acids, proteins and lipids, and mitochondrial structures, which are especially sensitive to oxidative damage.

The reaction of ROS/RNS with cellular membranes is particular damaging, causing oxidation of double bonds in phospholipid unsaturated FA to aldehyde products, such as malondialdehyde, 4-hydroxynonenal, 4-oxo-2-nonenal, and acrolein. These reactive products bind covalently to protein thiol groups and other cellular materials, altering their function. They are also markers of neurodegeneration, inflammation, diabetes, atherogenesis, and other pathogenic processes linked to oxidative stress and lipid peroxidation (Nicolson & Ash, 2014).

4.4 THE ROLE OF PROCESSING IN THE BIOACCESSIBILITY OF LIPIDS

Lipid oxidation is one of the main limiting factors in the quality and acceptability of food products. This process leads to changes in color, generation of off-odors and off-flavors, as well as the

production of potentially toxic compounds. Natural antioxidants, such as tocopherol, vitamin C, and phenolic compounds from plants, have been shown to decrease lipid oxidation as effectively as synthetic antioxidants. Despite the many advantages of increasing the monounsaturated FAs and PUFA content in food matrices, their high susceptibility to lipid oxidation is a major issue. This oxidative phenomenon leads to loss of shelf-life, consumer acceptability, functionality, nutritional value, organoleptic properties, and safety.

During processing of lipids, at high temperatures, there is a formation of radicals. Antioxidants present in the system (e.g., α-tocopherol) react with peroxyl radicals (ROO•) and alkoxyl radical intermediates (RO•) to form hydroxy compounds. In a study by Zou et al. (2015), the influence of lipid droplet size of excipient emulsions on the solubility and bioaccessibility of powdered curcumin was investigated. Corn oil-in-water emulsions with different initial mean droplet diameters were prepared. Emulsions were mixed with powdered curcumin and the resulting mixtures were incubated at either 30°C (to simulate a salad dressing) or 100°C. The amount of curcumin transferred to the excipient emulsions was higher at 100°C than at 30°C after incubation, and increased with decreasing droplet size at the higher temperature. The curcumin concentration in the mixed micelle phase and total digest depended on the droplet size of the original excipient emulsions: large $>$ medium $\approx$ small. However, the bioaccessibility of curcumin did not depend strongly on size. These effects were attributed to the competing behavior of oil droplets. Curcumin was solubilized and chemically degraded under simulated gastrointestinal conditions. The influence of droplet size on curcumin transfer was found to be more complex. This behavior was attributed to the competing effects of droplet surface area on chemical degradation and mass transfer rates. These effects have important implications for specifying optimum processing conditions and emulsion microstructures for enhancing curcumin bioavailability. Both effects increase as a function of increasing temperature and decreasing droplet size (Zou et al., 2015).

4.5 EFFECT OF EMERGING TECHNOLOGIES ON LIPID OXIDATION

The effect of emerging (nonthermal) processing technologies (e.g., HPP, PEFs, CP, US, irradiation, HP homogenization) on lipids is particularly noteworthy. Lipids can be very easily oxidized during processing, and some of the existing nonthermal technologies (e.g., CP and US) are considered as advanced oxidation processes. Indeed, they are conducted near ambient temperature, based on highly reactive radicals, and thus the stability of lipids requires special attention in some modern food-processing methods.

4.5.1 MECHANISM OF OXIDATION USING NONTHERMAL FOOD-PROCESSING TECHNIQUES

US has been extensively used as an advanced oxidation process for wastewater treatment. This is due to the production of $OH\cdot$ radicals in aqueous solutions and subsequent oxidation of pollutants in the presence of US. The formation of $OH\cdot$ radicals takes place by pyrolysis. The latest process occurs inside the cavity in the presence of US, due to the very high temperatures reached (Mahamuni & Adewuyi, 2010). Advanced oxidation processes rely on free radicals, mainly

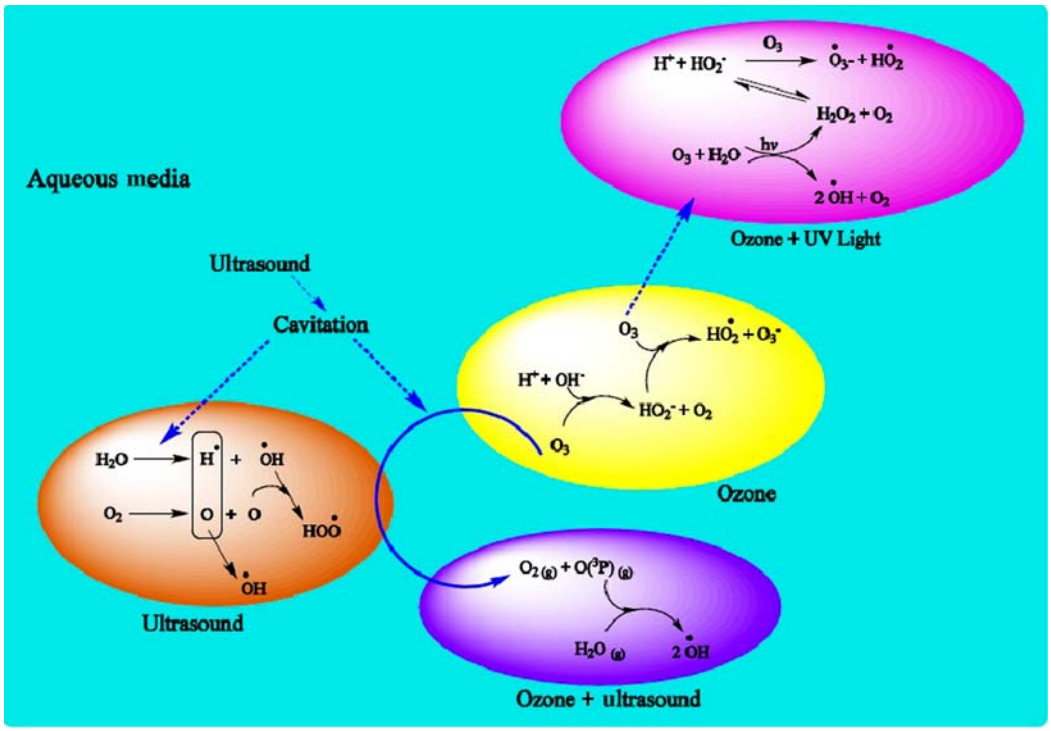


FIGURE 4.3

Principal reactions governing nonthermal technologies based on advanced oxidation processes in aqueous liquid medium (Misra, 2015).

hydroxyl radicals (Fig. 4.3; Misra, 2015). Hydroxyl radicals are highly unstable and therefore highly reactive because one of their electrons is unpaired. They are relatively nonselective species that can readily attack a wide group of organic chemicals (including lipids), simplifying their chemical structures. Since the reactions between hydroxyl radicals and organic species are extremely fast and nonspecific, these reactions are always controlled by the mass transfer of hydroxyl radical to the organic species. For Fenton processes, oxidation occurs via hydroxyl radicals that are rapidly generated through the decomposition of H_2O_2 catalyzed by ferrous (Fe (II)) or ferric ions (Fe (III)). Fenton system efficiency depends on both H_2O_2 and Fe (II) dosages, concentration, and properties of effluent organic matter as well as on the solution pH, which should be kept in the acid range. In combination with UV−vis light, oxidation rates are strongly increased likely due to the photolysis of iron aqua complex $(Fe(OH)^{2+})$, providing a new important source of hydroxyl radicals.

4.5.2 IONIZING RADIATION

Food irradiation has shown to be one of the most efficient methods for improving hygienic quality, increasing shelf-life, and enhancing the functional properties of different foods. Considering its

various applications, this technique can play a noteworthy role in the food industry. Low doses of irradiation (<1 kGy) may be used to retard sprouting or rooting of bulbs, tubers, and nuts during storage. High doses of irradiation (>10 kGy) have potential applications in developing sterilized food for special purposes. The irradiation alone (with a dose range of 1−10 kGy) or in combination with other effective technologies can reduce microbial load, kill insect eggs and larva, affect enzyme activity, and alter sensory properties. Ultraviolet or IR initiate free radical reactions and generate singlet oxygen, which swiftly reacts with unsaturated FAs.

The radiolytic products of lipids and lipid-containing foods depend mainly on the FA composition of foods. Radiation-induced hydrocarbons and 2-alkylcyclobutanones are the detection markers in characterizing the irradiation history of fat-containing foods, where the concentrations of these major radiolytic compounds show a linear relationship with the irradiation dose and temperature during processing. The stability of these radiation-induced chemical changes is usually time-dependent and significantly affected by postirradiation storage and processing conditions. Radiation-induced off-flavors in different meat products are ascribed mainly to enhanced lipid oxidation. Isooctane-soluble carbonyl compounds in the lipid part and low-molecular-weight, acid-soluble carbonyls in the protein part of meat could be formed upon irradiation. The 1-heptene and 1-nonene are the most important volatile components showing pronounced effects of irradiation (Akram, Ahn, & Kwon, 2012).

DNA has been considered as the main cellular target of deleterious effects of IR. Molecular signals initiated at cellular membranes are now identified as critical events in a large spectrum of radiation-induced cellular processes. IR provokes DNA damage directly by energy deposit on the DNA double-helix and indirectly by reactive species. Generation of ROS/RNS-inducing proteins and lipids modifications seems to be the prevalent mechanism. Plasma-membrane fluidity and permeability are directly affected by radiation-induced lipid damage. Lipid peroxidation and fragmentation represent the principal lipid modifications observed after exposure to IR. Lipid fragmentation appears in both hydrophilic and hydrophobic sections of lipids. Increased lipid peroxidation in the first hours after radiation exposure is a process of lipid degradation affecting mostly PUFA, leading to fragmentation of their polar component with consequent loss of membrane-barrier function, which is critical for cell integrity (Niaudet, Corre, Niaudet, & Paris, 2016).

Electron-beam irradiation is a novel food decontamination technology that uses low-dose IR for the treatment of food. The main purpose is to eliminate microbial contamination. Electron-beam irradiation inhibits the germination of crops and controls the ripening rate of vegetables and fruits. Better food preservation can be achieved by using electron-beam irradiation as a hurdle technology, in combination with other traditional or nontraditional food-processing technologies. The primary principle for radiation detection in fat-containing food is the detection of specific lipid molecules produced after the irradiation of lipids. For example, 2-alkylcyclobutanones are produced from irradiated FAs and glycerides. The reduction in meat quality during storage depends mainly on the degree of lipid peroxidation and associated changes. Irradiation may promote lipid peroxidation (because of the production of superoxide and hydroxyl radicals), causing the development of unfavorable odors and adverse color changes and reduction in shelf-life (Yang et al., 2015).

4.5.3 HIGH HYDROSTATIC PRESSURE

HPP is a nonthermal technology that has been widely incorporated in the food industry. It is used for fruit, vegetable, meat, and beverages processing. It has an impact on lipid oxidation, depending

on pressure applied, temperature, and time of processing. Bolumar (2015) described the modification of the lipid fraction of dry-cured fermented sausage through fat reduction (35%) and fat replacement of animal fat with olive oil (up to 10%). HPP-treated meat has been used to reduce the fat content by 35% in dry-cured fermented sausages, enabling the stable incorporation of olive oil, without affecting consumer acceptability. The addition of HPP-treated meat as a fat replacer resulted in good physical and sensory properties. On the other hand, the incorporation of olive oil either by direct addition (4.3% oil) or within a HPP-created protein network (10% oil) resulted in unacceptable products since the oil was not properly retained inside the sausage matrix.

HP and carbon dioxide could be used for more than microbial inactivation. For instance, it can extract cholesterol and lipids from meats (King, Johnson, & Friedrich, 1989). However, HPP treatments of raw or heat-processed meat above 300 MPa may induce lipid oxidation most often occurring during subsequent storage (Simonin et al., 2012). Two mechanisms have been proposed to explain the pressure-induced lipid oxidation: increased accessibility of iron from heme proteins and disruption of membranes. In the presence of ethylendiaminetetraacetic acid (a metal-ion chelator) lipid oxidation is reduced, due to the transition metal-ion catalysis. The critical pressure that triggers lipid oxidation is between 300 and 500 MPa, depending on processing factors such as temperature and time and composition of the lipid and nonlipid fractions of the treated material (Gabriela, Meza, Barnaba, et al., 2016).

Physicochemical properties like color, fat, lipid oxidation, moisture and protein content, pH, and texture of HP treated, freshly prepared rennet-coagulated soft cheese were studied by Okpala, Piggott, and Schaschke (2010). Treatment was up to 291 MPa and 29 min. HP treatment significantly influenced ($p < 0.05$) fat and lipid oxidation of the fresh cheese. Fat content increased apparently as moisture decreased significantly after HP treatment above 100 MPa. Increased pressures reduced lipid oxidation in the HP-treated fresh cheese. The lipid oxidation (TBA) on HP fresh cheese were measured in days 1 and 8. Mean TBA values obtained for HP fresh cheese samples for Days 1 and 8 ranged from 1.3 to 6.4 and from 1.6 to 7.5 μg malonaldehyde g^{-1} sample. Processing time was not shown to be significant for lipid oxidation. The TBA value (lipid oxidation) decreased with increasing pressure. This fact implies that HPP has the potential to reduce the rate of rancidity in fresh cheese at increased pressure levels irrespective of processing time. HP treatment decreased the rate of rancidity because lipid oxidation decreased at increased pressures.

The effects of HPP on the physicochemical characteristics of an oil-based spinach sauce model system was investigated by Medina-Meza, Barnaba, Villani, and Barbosa-Cánovas (2015). Color, chlorophylls, ascorbic acid, polyphenoloxidase activity, and lipid oxidation (measured as percentage of oleic acid, peroxide value, and p-anisidine content) were evaluated. Both pressure and time had a significant effect, but HPP was not very effective at triggering lipid oxidation. During storage for 21 days at 4°C, lipid oxidation was drastically inhibited in HPP-treated samples. These results demonstrate the reliability of HPP to preserve the physicochemical characteristics of oil-based vegetable sauces. Oil-based sauces are susceptible to auto-oxidation and thermo-oxidation, and the extent of these reactions could be mitigated or triggered by the presence of antioxidants or prooxidants.

Kruk et al. (2014) studied the combined effect of HP with the addition of soy sauce and/or olive oil on the quality and safety of chicken breast. Using an increased pressure of 600 MPa, the oleic acid and total unsaturated FA content of olive oils increased. Lipid oxidation was retarded by the addition of olive oil combined with HP, but increased with increasing storage time (up to 7 days) for all the treatments, including the pressurized control of 300 MPa. Conclusively, the use of olive

oil was found to be beneficial for suppressing the lipid oxidation induced by HP. In contrast, the addition of soy sauce promotes lipid oxidation perhaps because of salts and other reactive compounds in the sauce.

Tume, Sikes, and Smith (2010) studied postrigor, paired muscle samples subjected to pressures of 0.1 (atmospheric), 200, or 800 MPa for 20 min at 60°C, after α-tocopherol addition. Intramuscular lipid was similar for each group (4.02% vs 4.26%), but lipids from the muscle-containing medium concentrations of α-tocopherol were more saturated and less monounsaturated than these obtained at higher concentrations. HPP at 800 MPa and 60°C did not reduce the amount of α-tocopherol but significantly reduced the concentration of linoleic acid (18:2) in muscle. TBARS increased linearly with treatment pressure only in 6-day chilled storage under vacuum. Muscle from the high α-tocopherol cattle had greater accumulation of lipid peroxides by day 6. HPP of "muscle" foods results in greater lipid oxidation following treatment at higher pressures (>400 MPa) and this appears to be more likely in 'red meats' where a higher content of myoglobin has been suggested. The release of free metal ions as a result of pressure treatment is likely to lead to greater possibility of lipid oxidation of the polyunsaturated membrane lipids. The authors showed that lipid oxidation (TBARS) was only slightly affected by pressures up to 800 MPa at 60°C on day 0 but became significant during 6 days of chilled storage under vacuum. Lipid oxidation was relatively low after 6 days of storage when pressure−heat treated at 200 MPa. Finally, low concentrations of α-tocopherol demonstrated a protective effect of α-tocopherol against lipid oxidation during HPP (Tume et al., 2010).

Several other studies concerning lipid oxidation in fish and meat products have been performed using TBA (or TBARS) or peroxide value tests (Table 4.1). Similar to cold isostatic pressing of metals and ceramics, HPP demands much higher pressures (from 100 to above 800 MPa), faster cycling, high capacity, and sanitation. Pressure treatment had little effect on lipid oxidation below 300 MPa, but increased linearly at pressures above this value. It appears that 300−400 MPa is a critical pressure for inducing marked changes in meat. The addition of citric acid (0.02%) inhibited the increased rate of lipid oxidation of pork meat, thus eliminating the catalytic effect of pressure

Table 4.1 Impact of HPP on Lipids

Induced Effect	Reference
Very HPs (above 800 MPa) can lead to the formation of free radicals, promoting lipid peroxidation.	Andres, Muller, Adamsen, and Skibsted (2004)
The extent of posttreatment oxidation can be reduced by applying protective strategies such as the addition antioxidants and chelators.	Andres et al. (2004)
Processed meat (above 300 MPa) may induce lipid oxidation, most often occurring during subsequent storage.	Andres et al. (2004)
Lipid oxidation: increased accessibility of iron from heme proteins and disruption of membranes.	Simonin et al. (2012)
Depending on processing factors such as temperature and time, and composition of the lipid and nonlipid fractions.	Gabriela, Meza, Barnaba, et al. (2016)
For treatment at higher pressures (>400 MPa) oxidation is more likely to occur in 'red meats' where is a higher content of myoglobin.	Tume et al. (2010)

treatment. It is possible that metal ions ($Fe^{+2/+3}$, $Cu^{+2/+3}$) were released during HPP, becoming available to generate free radicals (via Fenton's reaction), whereas the addition of citric acid effectively chelated the released ions. In chicken breast muscle, no effect of HPP on lipid degradation was observed until reaching 500 MPa, but treatment at 800 MPa gave an oxidation extent statistically equal to heat treatment at 80°C. In fish products, lipid autoxidation occurs at lower pressure. For instance, a slight increase of the TBARS value was observed using 200 MPa in turbot fillets. However, no triglyceride hydrolysis was revealed (Chevalier, Le Bail, & Ghoul, 2001), suggesting that the pressure treatment applied did not affect the hydrolysis mechanisms.

Application of very HPs (above 800 MPa) can lead to the formation of free radicals, promoting lipid peroxidation (Andres et al., 2004), which could induce cholesterol oxidation. Hydroperoxides of PUFA may initiate cholesterol oxidation (Andres et al., 2004). At this level of pressure, initiation of oxidation becomes more important than degradation of secondary lipid oxidation products. This fact defines a critical threshold pressure that triggers lipid oxidation. It is set between 300 and 500 MPa, a value closely related not only to process variables such as temperature and/or time, but also to the composition of lipid and nonlipid fractions of the treated food. In the case of foods of marine origin, the high presence of PUFA promotes the initiation of radical mechanisms, which accelerate oxidation in subsequent storage periods. The extent of posttreatment oxidation can be reduced by applying protective strategies such as the addition antioxidants and chelators.

A HP homogenization process was used to prepare oil-in-water nanoemulsions at 300, 800, or 1200 bar (Shukat & Relkin, 2011). This study showed that hot homogenization carried out using HP can be applied for the preparation of lipid droplets to functionalize them as carrier matrices of α-tocopherol.

4.5.4 PULSED ELECTRIC FIELD

PEF is a nonthermal method of food preservation that uses short pulses of electricity for microbial inactivation and causes minimal detrimental effect on food-quality attributes. PEF treatment is conducted at ambient, subambient, or slightly above ambient temperature for less than 1 s in order to minimize energy loss due to foods heating. PEF technology is considered superior to traditional heat treatment of foods because it avoids or greatly reduces the detrimental changes of the sensory and physical properties of foods (DeVito, 2006). Electric fields in the range of 5−50 kV/cm generated by the application of short high-voltage pulses (μs) between two electrodes cause microbial inactivation at temperatures below those used in thermal processing. Application of PEF of high intensity and duration from microseconds to milliseconds may cause temporary or permanent permeabilization of cell membranes. By applying the dielectric rupture theory, it is postulated that membrane rupture is caused by an induced transmembrane potential approximately 1 V larger than the natural potential of the cell membrane (Mahnič-Kalamiza, Vorobiev, & Miklavčič, 2014). Electroporation of a cell membrane can be reversible or irreversible, depending on factors such as intensity of the electric field, number of pulses, and duration of the pulses. The plasma membranes of cells become permeable to small molecules after being exposed to an electric field. Permeation then causes swelling and eventually rupture of the cell membrane (Table 4.2).

Oxidation of lipids is often related to ROS. ROS are very strong oxidants including O_3 (ozone), $^1O_2$ (single oxygen), $O_2^{\bullet -}$ (superoxide radical), $HO_2\bullet$ (hydroperoxide radical), •OH (hydroxyl radical), H_2O_2, etc. PEF treatment has been studied using NaCl solutions, showing significant effects on H_2O_2

Table 4.2 Influence of PEFs on Lipids

Induced Effect	Reference
The oxidation of lipids is often related to ROS.	Zhao et al. (2012)
PEFs of high intensity and duration from microseconds to milliseconds may cause temporary or permanent permeabilization of cell membranes.	Mahnič-Kalamiza et al. (2014)
FA and lipid-oxidation reactions can also be attributed to the mechanism of electropermeabilization.	Sotelo et al. (2015)
When external electric field is applied on biological cells (animal, plant, or microbial), disruption of the cell membrane increases enzyme reaction and volatile-compound release.	Sotelo et al. (2015)

concentration (Zhao, Yang, & Zhang, 2012). The increment of H_2O_2 concentration was a function of the applied electric field strength and pH of NaCl solution. At each pH of NaCl solution, H_2O_2 was detected until the applied electric field strength exceeded a critical value. AOS is a generic name given to a variety of molecules and free radicals derived from molecular oxygen. O_3 is an allotrope of O_2; it showed a higher oxidative ability, and was attained from the discharge of O_2. In the water environment, H_2O_2 was produced through O_2 photolysis and further reacted with O_3 to form hydroxyl radical (•OH) with high reactivity. H_2O_2 was accumulated in the processing chamber with electric field strength increasing during PEF treatment. It is believed that many more reactive oxygens such as singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\bullet -}$), and hydroxyl radical (•OH) would be produced in the PEF treatment chamber, which is vital for food lipid oxidation (Zhao et al., 2012).

Sotelo et al. (2015) noted that the C6 aldehydes and alcohols are generated by the consecutive action of the enzymes lipoxygenase and alcohol dehydrogenase on PUFA. In addition, it was found that production of C6 aldehydes and alcohols was enhanced by the electroporation effects of PEF application. Other studies have reported that C6 aldehydes are produced from enzymatic reactions, which are attributed to the quantities of precursor molecules already present in the fruit. Hexanal and (*E*)-2-hexenal are products of FA (linoleic and linolenic acid) oxidation in the presence of lipoxygenase, while (*Z*)-2-hexen-1-ol is a secondary compound from these oxidation reactions. FA and lipid-oxidation reactions can also be attributed to the mechanism of electropermeabilization by PEF. When an external electric field is applied on biological cells (animal, plant, or microbial), disruption of the cell membrane increases enzyme reaction and volatile-compound release (Sotelo et al., 2015).

4.5.5 ULTRASOUND

The US technique presents several advantages over conventional methods in terms of energy consumption, time, and higher throughput. US methods are used for numerous processes on high lipid-containing food products such as milk, yogurt, and cheese, presenting great results in cooking, cutting, emulsification/homogenization, and microbial inactivation (Pingret et al. 2013). Cavitation is the formation, growth, and sometimes, the implosion of microbubbles created in a liquid when US waves propagate through it. The collapse of the bubbles leads to energy accumulation in hotspots where temperatures of above 5000°C and pressures of approximately 500 MPa have been measured (Jambrak, Mason, Lelas,

Herceg, & Herceg, 2008; Jambrak, Mason, Paniwnyk, & Lelas, 2007a). This phenomenon can cause lipid oxidation through three mechanisms that act alone or in combination:

1. the first one is purely thermal, due to the high temperatures achieved during cavitation;
2. the second one is due to free radicals generated by sonolysis; and
3. the third one is due to the mechanical forces (shear forces) created by microstreaming and shockwaves (Chemat, Grondin, Sing, & Smadja, 2004).

Fats and their emulsions are important food materials used in many products. The solid-fat content of food products contains significant amounts of fats (e.g., chocolate, butter), and lipid degradation could occur by hydrolysis or oxidation. TAGs may be hydrolyzed into FFA (hydrolytic rancidity) and could be oxidized to volatile and nonvolatile compounds, influencing odor and taste development, respectively. Lipid oxidation occurs not only during processing steps but also in raw materials and during product storage. The oil itself contains metals such as iron, copper, and nickel, which are catalysts for oxidation (Mason et al., 2011). During storage, light and oxygen are the major oxidative agents (Table 4.3).

Emulsification is the process of mixing two immiscible phases (e.g., oil and water) with the aid of a surface-active agent (emulsifier) into homogeneous dispersion or emulsion. Unless the mixing is spontaneous (e.g., formation of microemulsions), the process requires an energy input using mechanical agitation or ultrasonication to facilitate the formation of small droplets (Awad, Moharram, Shaltout, Asker, & Youssef, 2012). With ultrasonication, the collapse of cavitation releases different forms of high energy microjets near interfaces and facilitate emulsification.

Table 4.3 Influence of US on Lipids

Induced Effect	Reference
Cavitation phenomenon causes lipid oxidation through three mechanisms, which act alone or in combination: the first one is purely thermal, due to the high temperatures achieved during cavitation; the second one is due to free radicals generated by sonolysis; and the third one is due to the mechanical forces (shear forces) created by microstreaming and shockwaves.	Chemat et al. (2004)
Lipid degradation could occur by hydrolysis or oxidation.	Mason et al. (2011)
Oxidation in oil emulsions is present after sonication, even when in indirect contact with the US source.	Chemat et al. (2004)
Lipid oxidation follows a radical chain reaction mechanism through initiation, propagation, and termination stages.	Chemat et al. (2004)
The highest concentration of hydroxyl radical formation in the sonicated whey was found to be between 400 and 1000 kHz.	Torkamani et al. (2014)
Volatile-compound generation results most likely through US-induced pyrolysis (24 kHz, 2.5−20 min).	Reiner et al. (2009a, 2009b)
Pyrolysis reactions lead to homolytic fission of O−H bonds and ^{-}OH, H^{+} formation.	Reiner et al. (2009a, 2009b)
Ultrasonication did not promote lipid-oxidative reactions beyond detectable odor thresholds for volatile compounds, even at the highest specific energy input value (390 kJ/kg) or when the highest level of free radical formation occurred near 1000 kHz.	Torkamani et al. (2014)

Compared to mechanical agitation, the use of US required less surfactant, but produced smaller and more stable droplets. Another study showed that increasing irradiation time and/or ultrasonic irradiation power increases the dispersed phase volume and decreases droplet size, and these effects were strongly dependent on viscosity of the oil and interfacial tension (Bapat & Pandit, 2008).

US emulsification systems are cost saving and easy to use. In addition, they integrate to existing industrial lines to improve the quality of emulsified products, e.g., milk homogenization before cheese-making to improve the yield of cheese (Soria & Villamiel, 2010), and thermosonication to simultaneously pasteurize milk and disintegrate large milk fat globules (Bermúdez-Aguirre & Barbosa-Cánovas, 2008). The different ways in which cavitation can be beneficially used in food processing applications, are the reduction of reaction time, the increase in the reaction yield, and using appropriate conditions (lower temperature and pressure) compared to the conventional routes. The reduction period of the desired reactions is reduced in addition to enhanced selectivity of the reaction pathways. With cavitation, the water molecules can be broken down into form free radicals, which intensifies the chemical reactions and induces cross-linking of protein molecules in an aqueous medium. Hydroxide radicals (OH^-) and hydrogen atoms are generated from the dissociation of water molecules in aqueous solution as a result of the high temperature and pressure of the collapsing gas bubbles associated with cavitation (sonolysis) (Awad et al., 2012).

Within the collapsing cavitation bubble, the extreme temperature and pressure conditions can induce the dissociation of water into hydroxyl radicals and hydrogen atoms, which can trigger chain reactions at the interface of the bubble or in the surrounding liquid. Besides changes in lipids, US causes flavor impairments (Chemat et al., 2004). The effects of high-power US treatment (20 kHz) on some components of refined sunflower oil were studied by Chemat, Huma, and Khan (2011) with the aim of verifying if and to what extent modifications in the lipidic fraction occur. When sunflower oil was treated by US, a modification of sensory and organoleptic qualities of the oil (development of fishy, rancid, and metallic odor) was observed without visual modification. However, when the sonicated sunflower oil was stored in darkness for 24 h at least, a cloudy medium appeared inside the oil. Oxidation of edible oils is attributed to cavitation (shockwaves), which affects structural and functional components up to the point of lipid oxidation and deterioration. Metals (e.g., copper) occurring naturally in edible oils may also form oxy radical species in combination with US cavitation. Oxy radical species generate the formation of volatile compounds and nonvolatile products including oxymonomers and oxypolymers (Chemat, Lagha, Amar, & Chemat, 2004).

More recently, Torkamani, Juliano, Ajlouni, and Singh (2014) used an US reactor at different frequencies (20–2000 kHz) and specific energies (8.0–390 kJ/kg) to examine oxidation of lipids in Cheddar cheese whey. Polar lipid (PL), FFA and bound FAs, and lipid oxidation-derived compounds were identified and quantified before and after US processing. The highest concentration of hydroxyl radical formation in the sonicated whey was found to be between 400 and 1000 kHz. Lipid-oxidation volatile compounds were detected in both nonsonicated and sonicated whey. In another study, Riener, Noci, Cronin, Morgan, and Lyng (2009a, 2009b) identified volatile-compound generation most likely through US-induced pyrolysis (24 kHz, 2.5–20 min, nominal power 400 W) and lipid oxidation as the main factors causing rubbery flavor in milk. That was explained by the occurrence of cavitation, leading to local high temperatures and pressures upon violent collapse of the cavitation bubbles (Jambrak et al., 2010; Jambrak, Lelas, Mason, Krešić, & Badanjak, 2009; Jambrak et al. 2008). Pyrolysis reactions led to homolytic fission of O–H bonds and ^-OH, H^+ formation, resulting from the intense local temperature and pressure conditions caused during bubble collapse. The effect of ultrasonication on oxidation of lipids in a range

of US frequencies (20−2000 kHz) and specific energies (8−390 kJ/kg) applied to whey was also explored. Ultrasonication as a standalone treatment did not promote lipid-oxidative reactions beyond detectable odor thresholds for volatile compounds, even at the highest specific energy input value (390 kJ/kg) or when the highest level of free radical formation occurred near 1000 kHz. Variation of frequency, power input, and processing time did not result in a significant change in the concentration of oxidative volatile compounds, PL species, or FFA (Torkamani et al., 2014).

The effect of ultrasonication on lipid oxidation has also been investigated in various types of milk (Juliano & Watkins, 2014). Four batches of raw milk (up to 2 L) were sonicated at various frequencies (20, 400, 1000, 1600, and 2000 kHz) using different temperatures (4, 20, 45, and 63°C), sonication times, and US energy inputs (up to 409 kJ/kg). Pasteurized skim milk was sonicated at low and high frequency for comparison. The cavitational yield, characterized in all systems in water, was highest between 400 and 1000 kHz. Volatile compounds from milk-lipid oxidation were detected and exceeded their odor threshold values at 400 and 1000 kHz at specific energies greater than 271 kJ/kg in raw milk. No oxidative volatile compounds were detected below 230 kJ/kg in batch systems at the tested frequencies under refrigerated conditions. Skim milk showed a lower energy threshold for oxidative volatile formation.

The same oxidative volatiles were detected after various passes of milk through a 0.3 L flow cell enclosing a 20 kHz horn and operating above 90 kJ/kg. This study showed that lipid oxidation in milk can be controlled by decreasing the sonication time and temperature in the system. The collapse of cavitation bubbles resulting from localized pressure gradients (caused by a series of compression and rarefaction cycles during US processing) is responsible for the localized generation of hotspots, which lead to temperature increase in the liquid (Jambrak et al., 2007a, 2007b). Extreme temperature and shear conditions created by cavitation result in the generation of hydroxyl and hydrogen radicals, promoting lipid oxidation. Formation of lipid-oxidation volatiles in milk depends on many US processing factors including frequency, power levels, processing time, temperature of the milk sample, and fat content (Herceg et al., 2012a, 2012b). One of these volatiles is nonanal, an aldehyde derived from oxidative reactions of FA in milk. The highest concentrations were seen after 1000 kHz treatment above 271 kJ/kg for raw milk and 102 kJ/kg for pasteurized skim milk. Other volatiles detected under these values were mostly related to biochemical/hydrolytic and esterification reactions naturally seen in milk.

A temperature effect was seen at extended sonication time up to 20 min at 400 and 1000 kHz (271 and 490 kJ/kg, respectively) where higher formation of nonanal was seen at 45°C than at refrigerated conditions. Low-temperature sonication of milk better controlled the formation of oxidative volatile compounds below threshold concentrations. Milk oxidation, created in a high-intensity flowthrough cell at 20 kHz, was related to the localized high-intensity near the sonotrode in a reduced sample volume. Lipid oxidation could be controlled by decreasing the sonication time and temperature in the system (Juliano & Watkins, 2014).

Essential oils (EO) are considered as suitable antioxidants to preserve sunflower oils against oxidation (Hashemi et al., 2015). Indeed, they contain components such as thymol and carvacrol, which show antioxidant activity against autoxidation UV and US oxidation. EO are more active during autoxidation, while the antioxidant activity of EO reduces after sample treatment with UV and US. During common conditions, degradation of commercial edible oils generates unpleasant flavor and polymer formation after a few storage months. When the oil itself contains oxidation catalysts (e.g., copper and iron), polymer and oxidation compounds appear more rapidly (Hashemi et al., 2015). In these cases, oxidation is attributed to cavitation, which affects structural and

functional components up to the point of lipid deterioration. This phenomenon can cause lipid oxidation by purely thermal, sonolysis (generated free radicals), and shear forces (Chemat et al., 2004).

4.5.6 COLD PLASMA

Plasma, the fourth state of matter, is an ionized gas that can be generated using a range of gases or gas mixtures (e.g., argon, helium, nitrogen, air, or oxygen). Plasma generated in air consists of a reactive form of atoms, excited molecules, charged particles, ROS, RNS, and UV photons (Pankaj et al., 2014). Nonthermal gas-phase plasma treatment (NPT) offers distinct advantages for the decontamination of foods. The electron temperature is much higher than the ion and neutral temperatures and are typically close to room temperature ("cold" or nonthermal). The gas is at atmospheric pressure. NPT or cold-plasma treatment is known as nonthermal because it has electrons at hotter temperature than the heavy particles that are at room temperature. It has been shown that plasmas generated outside thermal equilibrium at atmospheric pressure produce an antimicrobial effect (Laroussi, 2005; Thirumdas, Sarangapani, & Annapure, 2014; Ziuzina, Patil, Cullen, Keener, & Bourke, 2014). This effect results from the interaction of the organic media with a wide variety of active oxidizing species, excited atoms, and molecules, as well as UV radiation produced during plasma interaction with air (Sun, 2014). Although much work has already been done in an effort to understand the effect of plasma on microorganisms (Jambrak, Stulić, Mrvčić, Milošević, & Šimunek, 2015; Laroussi, 2005; Niemira, 2012; Ziuzina et al., 2014) little is known about its interaction with food components, especially with lipids. Addition of antioxidants to protect cells against the damaging effects of ROS and RNS can protect lipids.

Dielectric barrier discharge plasma jet (Ar/0.6% O_2) was used on fish-oil samples as a faster and more realistic accelerated lipid-oxidation method. Both thermal and nonthermal plasma (NTP)-based accelerated oxidation protocols have been evaluated using naturally aged fish oil as reference (Vandamme et al., 2015) (Fig. 4.4). Experiments were conducted using both virgin-oil and α-tocopherol-enriched fish-oil samples. Secondary lipid-oxidation volatiles were measured using head space−solid phase microextraction−gas chromatography−mass spectroscopy. These accelerated oxidation techniques induced the formation of typical lipid-oxidation markers (e.g., 2-propenal, (*E*)-2-pentenal, heptanal), but in both cases significant differences were observed compared to the naturally aged fish oil. On the other hand, NTP correctly predicted an antioxidative effect when 1000 μg/g α-tocopherol was added to the fish oil, while thermal-based tests resulted in the induction of prooxidative chemistry.

There are some limitations of plasma processing such as increase in lipid oxidation and degradation in color. Cold-plasma technology has been successfully applied to decontaminate the surface of walnuts and peanuts (Thirumdas et al., 2014). The main problem encountered was an increase in peroxide value by 20% in walnuts at higher power and treatment time. Similar results were observed in the case of plasma-treated peanuts samples. This may be due to the fact that radicals produced by plasma are capable of oxidizing lipid molecules, resulting in peroxide value increase. Until now no investigation has been carried out on the formation of any toxic compounds after the application of CP in food products. Any technology used for food processing should not affect allergenicity of food constituents (Table 4.4).

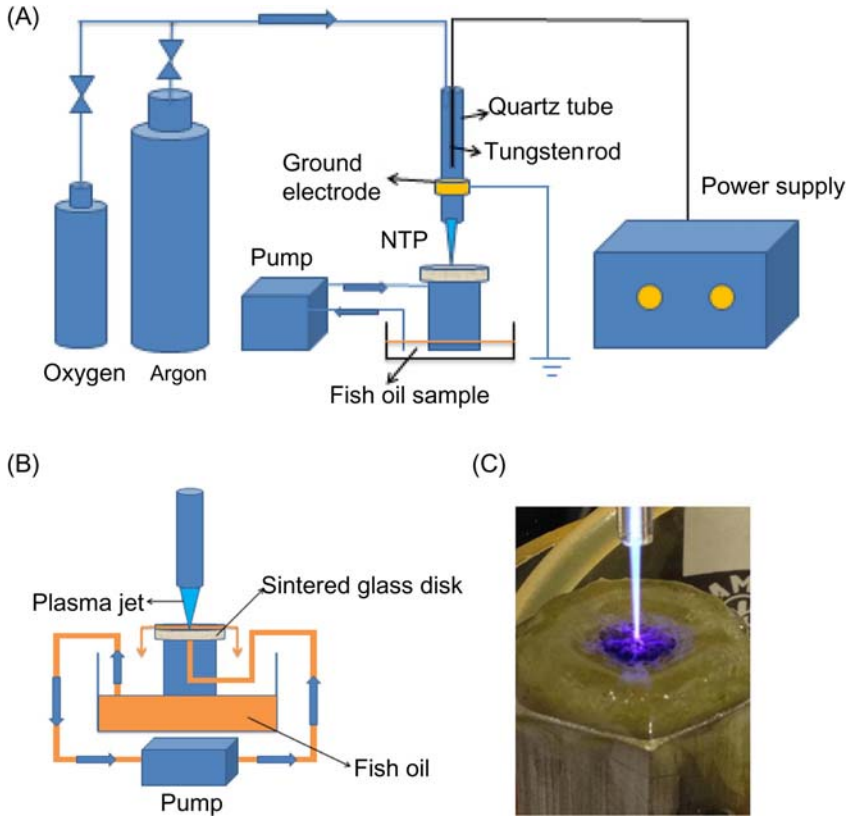


FIGURE 4.4

Cold-plasma treatment of fish oil: (A) overall NTP configuration; (B) NTP—treatment of oil sample (detail); (C) NTP—contact with fish-oil sample (Vandamme et al., 2015).

The intensity of lipid-oxidative deterioration of PUFAs-enriched foodstuffs depends on different factors, e.g., the degree of unsaturation of FA as well as the presence of external factors (oxygen and light), metallic ions, or high temperatures. Food products enriched with healthier unsaturated FAs are more sensitive to lipid oxidation, leading to overall quality deterioration and development of unwanted aroma properties. In the case of CP treatment, lipid peroxidation could be due to interactions between antioxidant and plasma-immanent species leading to the formation of oxidative compounds, which in turn lead to the prooxidative effect and cascade reactions.

For example, the saturated FAs concentration decreased from 64.4% to 63.6% within the first 3−5 min of CP treatment (Korachi et al., 2015). This change in FA may be attributed to the dehydrogenation of stearic acid caused by the oxygen radicals, resulting in an increase in oleic acid. The decrease in oleic acid after 20 min could be indicative of an opposing or reversible reaction produced by the H and OH plasma species. Comparison of levels of C18:0, C12:0, and C10:0 showed C18:0 to decrease, while short-chain FA (C10:0 and C12:0) increased following plasma application, indicating

Table 4.4 Influence of CP on Lipids

Induced Effect	Reference
The effect of CP results from the interaction of the organic media with a wide variety of active oxidizing species, excited atoms, and molecules, as well as UV radiation that are produced during plasma interaction with air.	Sun (2014)
Secondary lipid oxidation volatiles detected are formation of typical lipid-oxidation markers (e.g., 2-propenal, (*E*)-2-pentenal, heptanal).	Vandamme et al. (2015)
Increased peroxide value by 20% in walnuts at higher power and treatment time.	Thirumdas et al. (2014)
Approximately 64% of FFA were saturated FAs. Their concentration decreased from 64.4% to 63.6% within the first 3−5 min of plasma application. Following 5 min CP treatment, the total saturated FAs gradually increased to 65.8% (20 min).	Korachi et al., (2015)
Application of CP showed a larger effect on PUFA in milk, which decreased from 3.0% to 2.8% after only 3 min treatment and further decreased to 2.5% following 20 min of treatment.	Korachi et al., (2015)

a hydrolytic effect on long-chain saturated FA (Korachi et al., 2015). Plasma treatment produces free radicals such as hydroperoxyl radicals, superoxide radicals, and singlet oxygen that are described as attacking PUFA, which generate shorter FA. The formed active species can initiate lipid peroxidation and produce hydroperoxide (Korachi et al., 2015). This suggests that the CP system does not significantly affect the FA composition of milk for treatment times up to 20 min.

In the work by Durme Van, Sint-lieven, Nikiforov, and Vandamme (2014), NTP was investigated as a potential technology for the controlled and standardized accelerated induction of lipid oxidation in complex food matrices. Three different radio frequencies (RF-25 W with a gas flow of 2 slm) and plasma types (Ar; 0.3% O_2/Ar; 0.3% H_2O/Ar) were studied. Chemical profiling revealed that a short plasma treatment at ambient temperature conditions resulted in the formation of several volatiles that were also present in naturally deteriorated oil samples (Durme Van et al., 2014). The applicability of NTP was investigated as a new innovative accelerated lipid-oxidation test using fish oil (Horn et al., 2009). A natural aging test of 11 weeks resulted in the formation of many lipid-oxidation volatiles, of which aldehydes proved to be the most important group. Compounds such as (*E*,*E*)-2,4-heptadienal, (*E*,*Z*)-2,6-nonadienal, 1-octen-3-ol, (*E*)-2-decenal, and others proved to be important oxidation compounds. The NTP treatment resulted in the formation of several lipid-oxidation products, which were also all found in the naturally aged fish oil, such as 2-propenal, (*E*)-2-pentenal, heptanal, and 1-penten-3-one. Other lipid-oxidation markers found in the naturally aged fish oil, e.g., (*E*,*E*)-2,4-heptadienal and (*E*,*E*)-2,4-decadienal, did not form during the NTP treatment. This was attributed to the fact of presence of highly turbulent atmosphere near the reaction zone, which is able to accelerate volatiles formation to be stripped from the oil sample. NTP proved to be able to accelerate the oxidation process in the fish oil, with a more accurate prediction of the antioxidative properties of α-tocopherol. One important advantage of this plasma technique is the high steerability. Many parameters, such as voltage, treatment time, oxygen concentration, configuration, water concentration, and carrier gas can be altered, resulting in other plasma characteristics. For example, when water is doped in the argon jet, a high concentration of hydroxyl radicals can be expected in the plasma. Since these highly reactive species (ROS) are also responsible for natural oxidation processes, the oxidation chemistry could be closer to natural

oxidation. The problem with the NTP configuration technique is the stripping of volatiles during the treatment, but this could be overcome by treating the oil in a reaction chamber.

4.5.7 OTHER PROCESSING TECHNOLOGIES

Electrohydrodynamic processing uses the application of an electrical potential to a flowing fluid. This technique can be used in food processing. Control of the potential and flow rate allows the formation of a jet, which subsequently breaks up to generate droplets with diameter in the nanometer to micrometer size range. One of the emerging technologies in food formulations is the application of electrohydrodynamic technology for encapsulating active ingredients such as emulsifiers and flavors (Yoshii et al., 2001). Electrohydrodynamic spray technology has advantages, e.g., scale and morphology can be easily varied according to the requirement of the food materials (Luo, Loh, Stride, & Edirisinghe, 2011). The size distribution of the particles can be near monodisperse, leading to better flavor perception (Gañán-Calvo, 1999). In the study by Eltayeb, Bakhshi, Stride, and Edirisinghe (2013) electrohydrodynamic technology was used to encapsulate water-soluble maltol flavor within solid-lipid matrices of stearic acid (SA) and ethylcellulose (EC). The reason for using a SA−EC matrix to encapsulate maltol was to limit flavor degradation or loss during processing and storage. This solid matrix was able to provide more protection against chemical reactions such as oxidation.

Extrusion processing and antinutritional compounds were studied by Imran, Anjum, Ahmad, Khan, and Mushtaq (2015). In this study, the extrusion cooking significantly decreased the antinutritional compounds in raw flaxseed meal, and was shown to impart a strong effect on reduction of antinutritional compounds in flaxseed meal. The reduction rate of antinutritional compounds in flaxseed was slow at low-temperature range during the initial point (100°C) of extrusion. The samples of extruded flaxseed meal were considered to be of good quality as indicated by initially low levels of FFA. No significant ($p \leq 0.05$) changes in FFA were found at the end of 30 days for extruded samples, but the FFA content increased when operating temperature, moisture level of initial feed material, and duration of the storage increased. It was generally observed that the FFA value of the samples increased with storage time and increasing temperature. The FFA values above 2% were considered as onset of rancidity. The oxidation and high values of FFA indicate that the toxic compounds have been produced in raw or processed material and the oxidized products may be associated with the rancid taste and development of cancer and atherosclerosis in biological systems.

4.6 CONCLUSION

In order to understand lipid oxidation, we must know the causes of peroxidation and possible ways of protecting and stabilizing the desired product. When using emerging (alternative) techniques, careful attention of processing parameters is required, including:

1. temperature, pressure, treatment time for HPP;
2. power, amplitude, temperature, and treatment time for US processing;
3. flow, frequency, and treatment time for CP; and
4. intensity of electric field, strength, treatment time, and temperature for PEF.

In addition, lipid oxidation can be autocatalytic and thus could be triggered by the presence of metals, light, increased temperature, and the presence of radicals or other compounds. Protective actions include the addition of antioxidants, or other compounds that can "silence" the presence of free radicals and cascade reactions. Optimization of processing parameters is also of great importance to suppress, avoid, or minimize production of free radicals and triggering of lipids.

REFERENCES

Akram, K., Ahn, J.-J., & Kwon, J.-H. (2012). Identification and characterization of gamma-irradiated dried Lentinus edodes using ESR, SEM, and FTIR analyses. *Journal of Food Science*, *77*(6), C690−6. Available from http://dx.doi.org/10.1111/j.1750-3841.2012.02740.x.

Andres, A., Muller, J. S., Adamsen, C., & Skibsted, L. (2004). High pressure treatment of dry-cured Iberian ham. Effect on radical formation, lipid oxidation and colour. *European Food Research and Technology*, *219*(3). Available from http://dx.doi.org/10.1007/s00217-004-0939-9.

Awad, T. S., Moharram, H. A., Shaltout, O. E., Asker, D., & Youssef, M. M. (2012). Applications of ultrasound in analysis, processing and quality control of food: A review. *Food Research International*, *48*(2), 410−427. Available from http://dx.doi.org/10.1016/j.foodres.2012.05.004.

Bapat, P. S., & Pandit, A. B. (2008). Thermodynamic and kinetic considerations of nucleation and stabilization of acoustic cavitation bubbles in water. *Ultrasonics Sonochemistry*, *15*(1), 65−77. Available from http://dx.doi.org/10.1016/j.ultsonch.2007.01.005.

Bermúdez-Aguirre, D., & Barbosa-Cánovas, G. V. (2008). Study of butter fat content in milk on the inactivation of Listeria innocua ATCC 51742 by thermo-sonication. *Innovative Food Science and Emerging Technologies*, *9*(2), 176−185. Available from http://dx.doi.org/10.1016/j.ifset.2007.07.008.

Bolumar, T. (2015). *Fat reduction and replacement in dry-cured fermented sausage by using high pressure processing meat as fat replacer and olive oil* (October). http://dx.doi.org/10.1515/pjfns-2015-0026.

Chemat F., Grondin I., Costes P., Moutoussamy L., Sing A.S., Smadja J. et. al. (2004). *High power ultrasound effects on lipid oxidation of refined sunflower oil sunflower oil* (January 2016). http://dx.doi.org/10.1016/j.ultsonch.2003.07.004.

Chemat, F., Grondin, I., Sing, A. S. C., & Smadja, J. (2004). Deterioration of edible oils during food processing by ultrasound. *Ultrasonics Sonochemistry*, *11*(1), 13−15. Available from http://dx.doi.org/10.1016/S1350-4177(03)00127-5.

Chemat, F., Zill-E-Huma, & Khan, M. K. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry*, *18*(4), 813−835. Available from http://dx.doi.org/10.1016/j.ultsonch.2010.11.023.

Chemat, S., Lagha, A., Amar, H. A., & Chemat, F. (2004). Ultrasound assisted microwave digestion. *Ultrasonics Sonochemistry*, *11*(1), 5−8. Available from http://dx.doi.org/10.1016/S1350-4177(03)00128-7.

Chevalier, D., Le Bail, A., & Ghoul, M. (2001). Effects of high pressure treatment (100−200 MPa) at low temperature on turbot (*Scophthalmus maximus*) muscle. *Food Research International*, *34*(5), 425−429. Available from http://dx.doi.org/10.1016/S0963-9969(00)00187-3.

Cocero, M. J., Martín, Á., Mattea, F., & Varona, S. (2009). Encapsulation and co-precipitation processes with supercritical fluids: Fundamentals and applications. *Journal of Supercritical Fluids*, *47*(3), 546−555. Available from http://dx.doi.org/10.1016/j.supflu.2008.08.015.

DeVito, F. (2006). Application of pulsed electric field (PEF) techniques in food processing. *Chemical Engineering*, 1–116.

Durme Van, J., Sint-lieven, K.K., Nikiforov, A., & Vandamme, J. (2014). *Accelerated lipid oxidation using nonthermal plasma technology: Evaluation of volatile compounds* (October 2015). http://dx.doi.org/10.1016/j.foodres.2014.04.043.

Eltayeb, M., Bakhshi, P. K., Stride, E., & Edirisinghe, M. (2013). Preparation of solid lipid nanoparticles containing active compound by electrohydrodynamic spraying. *Food Research International*, *53*(1), 88–95. Available from http://dx.doi.org/10.1016/j.foodres.2013.03.047.

Gabriela, I., Meza, M., Barnaba, C., Medina-meza, I.G., Barnaba, C., & Barbosa-cánovas, G.V. (2016). *Effects of high pressure processing on lipid oxidation: A review Effects of high pressure processing on lipid oxidation: A review* (January). http://dx.doi.org/10.1016/j.ifset.2013.10.012.

Gañán-Calvo, A. M. (1999). The electrohydrodynamic atomization of liquids today. *Journal of Aerosol Science*, *30*(30), S547–S548. Available from http://dx.doi.org/10.1016/S0021-8502(99)80285-7.

Hashemi, S. M. B., Khaneghah, A. M., Tavakolpour, Y., Asnaashari, M., & mehr, H. M. (2015). Effects of Ultrasound Treatment, UV Irradiation and Avishan-e-Denaei Essential Oil on Oxidative Stability of Sunflower Oil. *Journal of Essential Oil Bearing Plants*, *18*(5), 1083–1092. http://doi.org/10.1080/0972060X.2015.1039218.

Herceg, Z., Jambrak, A. R., Lelas, V., & Thagard, S. M. (2012a). The effect of high intensity ultrasound treatment on the amount of Staphylococcus aureus and Escherichia coli in milk. *Food Technology and Biotechnology*, *50*(1), 46–52.

Herceg, Z., Juraga, E., Sobota-Šalamon, B., & Režek-Jambrak, A. (2012b). Inactivation of mesophilic bacteria in milk by means of high intensity ultrasound using response surface methodology. *Czech Journal of Food Sciences*, *30*(2), 108–117.

Horn, A. F., Nielsen, N. S., & Jacobsen, C. (2009). Additions of caffeic acid, ascorbyl palmitate or γ-tocopherol to fish oil-enriched energy bars affect lipid oxidation differently. *Food Chemistry*, *112*(2), 412–420. http://doi.org/10.1016/j.foodchem.2008.05.094.

Imran, M., Anjum, F.M., Ahmad, N., Khan, M.K., & Mushtaq, Z. (2015). *Impact of extrusion processing conditions on lipid peroxidation and storage stability of full-fat flaxseed meal Impact of extrusion processing conditions on lipid peroxidation and storage stability of full-fat flaxseed meal* (November). http://dx.doi.org/10.1186/s12944-015-0076-4.

Jambrak, A. R., Herceg, Z., Šubarić, D., Babić, J., Brnčić, M., Brnčić, S. R., … Gelo, J. (2010). Ultrasound effect on physical properties of corn starch. *Carbohydrate Polymers*, *79*(1), 91–100. Available from http://dx.doi.org/10.1016/j.carbpol.2009.07.051.

Jambrak, A. R., Lelas, V., Mason, T. J., Krešić, G., & Badanjak, M. (2009). Physical properties of ultrasound treated soy proteins. *Journal of Food Engineering*, *93*(4), 386–393. Available from http://dx.doi.org/10.1016/j.jfoodeng.2009.02.001.

Jambrak, A. R., Mason, T. J., Lelas, V., Herceg, Z., & Herceg, I. L. (2008). Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. *Journal of Food Engineering*, *86*(2), 281–287. Available from http://dx.doi.org/10.1016/j.jfoodeng.2007.10.004.

Jambrak, A. R., Mason, T. J., Paniwnyk, L., & Lelas, V. (2007a). Accelerated drying of button mushrooms, Brussels sprouts and cauliflower by applying power ultrasound and its rehydration properties. *Journal of Food Engineering*, *81*(1), 88–97. http://doi.org/10.1016/j.jfoodeng.2006.10.009.

Jambrak, A. R., Mason, T. J., Paniwnyk, L., & Lelas, V. (2007b). Ultrasonic effect on pH, electric conductivity, and tissue surface of button mushrooms, Brussels sprouts and cauliflower. *Czech Journal of Food Sciences*, *25*(2), 90–100.

Jambrak, A. R., Stulić, V., Mrvčić, J., Milošević, S., & Šimunek, M. (2015). The effect of high power ultrasound and cold gas-phase plasma treatments on selected yeast in pure culture. *Food and Bioprocess Technology*, *8*(4), 791–800. Available from http://dx.doi.org/10.1007/s11947-014-1442-3.

Juliano, P., & Watkins, P. (2014). *Lipid oxidation volatiles absent in milk after selected ultrasound processing* (January 2016). http://dx.doi.org/10.1016/j.ultsonch.2014.03.001.

Juliano, P., & Watkins, P. (2014b). Lipid oxidation volatiles absent in milk after selected ultrasound processing. *Ultrasonics Sonochemistry* (June 2015). http://dx.doi.org/10.1016/j.ultsonch.2014.03.001.

King, J. W., Johnson, J. H., & Friedrich, J. P. (1989). Extraction of fat tissue from meat products with supercritical carbon dioxide. *Journal of Agricultural and Food Chemistry*, *37*(4), 951–954. Available from http://dx.doi.org/10.1021/jf00088a027.

Korachi, M., Ozen, F., Aslan, N., Vannini, L., Guerzoni, M. E., Gottardi, D., & Ekinci, F. Y. (2015). Biochemical changes to milk following treatment by a novel, cold atmospheric plasma system. *International Dairy Journal*, *42*, 64–69. http://doi.org/10.1016/j.idairyj.2014.10.006.

Kruk, Z. A., Kim, H. J., Kim, Y. J., Rutley, D. L., Jung, S., Lee, S. K., & Jo, C. (2014). Combined effects of high pressure processing and addition of soy sauce and olive oil on safety and quality characteristics of chicken breast meat. *Asian-Australasian Journal of Animal Sciences*, *27*(2), 256–265. Available from http://dx.doi.org/10.5713/ajas.2013.13417.

Laroussi, M. (2005). Low temperature plasma-based sterilization: Overview and state-of-the-art. *Plasma Processes and Polymers*, *2*(5), 391–400. Available from http://dx.doi.org/10.1002/ppap.200400078.

Luo, C. J., Loh, S., Stride, E., & Edirisinghe, M. (2011). Electrospraying and electrospinning of chocolate suspensions. *Food and Bioprocess Technology*, *5*(6), 2285–2300. Available from http://dx.doi.org/10.1007/s11947-011-0534-6.

Mahamuni, N. N., & Adewuyi, Y. G. (2010). Advanced oxidation processes (AOPs) involving ultrasound for waste water treatment: A review with emphasis on cost estimation. *Ultrasonics Sonochemistry*, *17*(6), 990–1003. Available from http://dx.doi.org/10.1016/j.ultsonch.2009.09.005.

Mahnič-Kalamiza, S., Vorobiev, E., & Miklavčič, D. (2014). Electroporation in food processing and biorefinery. *The Journal of Membrane Biology*, *247*(12), 1279–1304. Available from http://dx.doi.org/10.1007/s00232-014-9737-x.

Mason, T. J., Chemat, F., & Vinatoru, M. (2011). The extraction of natural products using ultrasound or microwaves. *Ultrasound*, *44*(0), 237–247.

Medina-Meza, I. G., Barnaba, C., Villani, F., & Barbosa-Cánovas, G. V. (2015). Effects of thermal and high pressure treatments in color and chemical attributes of an oil-based spinach sauce. *LWT - Food Science and Technology*, *60*(1), 86–94. Available from http://dx.doi.org/10.1016/j.lwt.2014.09.033.

Misra, N. N. (2015). The contribution of non-thermal and advanced oxidation technologies towards dissipation of pesticide residues. *Trends in Food Science & Technology*, *45*(2), 229–244. http://doi.org/10.1016/j.tifs.2015.06.005.

Niaudet, C., Corre, I., Niaudet, C., & Paris, F. (2016). *Plasma membrane signaling induced by ionizing radiation plasma membrane signaling induced by ionizing radiation* (April 2010). http://dx.doi.org/10.1016/j.mrrev.2010.01.014.

Nicolson, G. L., & Ash, M. E. (2014). Biochimica et Biophysica Acta Lipid Replacement Therapy: A natural medicine approach to replacing damaged lipids in cellular membranes and organelles and restoring function. *BBA - Biomembranes*, *1838*(6), 1657–1679. Available from http://dx.doi.org/10.1016/j.bbamem.2013.11.010.

Niemira, B. A. (2012). Cold plasma reduction of *Salmonella* and *Escherichia coli* O157: H7 on almonds using ambient pressure gases. *Journal of Food Science*, *77*(3), 171–175. Available from http://dx.doi.org/10.1111/j.1750-3841.2011.02594.x.

Okpala, C.O.R., Piggott, J.R., & Schaschke, C.J. (2010). *Influence of high-pressure processing (HPP) on physico-chemical properties of fresh cheese* (January 2016). http://dx.doi.org/10.1016/j.ifset.2009.10.003.

Pankaj, S. K., Bueno-Ferrer, C., Misra, N. N., Milosavljević, V., O'Donnell, C. P., Bourke, P., … Cullen, P. J. (2014). Applications of cold plasma technology in food packaging. *Trends in Food Science & Technology*, *35*(1), 5–17. Available from http://dx.doi.org/10.1016/j.tifs.2013.10.009.

Pingret, D., Durand, G., Fabiano-Tixier, A. S., Rockenbauer, A., Ginies, C., & Chemat, F. (2012). Degradation of edible oil during food processing by ultrasound: Electron paramagnetic resonance, physicochemical, and sensory appreciation. *Journal of Agricultural and Food Chemistry*, *60*(31), 7761−7768. Available from http://dx.doi.org/10.1021/jf301286f.

Pingret, D., Fabiano-Tixier, A. S., & Chemat, F. (2013). Degradation during application of ultrasound in food processing: A review. *Food Control*, *31*(2), 593−606. Available from http://dx.doi.org/10.1016/j.foodcont.2012.11.039.

Riener, J., Noci, F., Cronin, D. A., Morgan, D. J., & Lyng, J. G. (2009a). Characterisation of volatile compounds generated in milk by high intensity ultrasound. *International Dairy Journal*, *19*(4), 269−272. Available from http://dx.doi.org/10.1016/j.idairyj.2008.10.017.

Riener, J., Noci, F., Cronin, D. A., Morgan, D. J., & Lyng, J. G. (2009b). The effect of thermosonication of milk on selected physicochemical and microstructural properties of yoghurt gels during fermentation. *Food Chemistry*, *114*(3), 905−911. Available from http://dx.doi.org/10.1016/j.foodchem.2008.10.037.

Russell, N. J. (2002). Bacterial membranes: The effects of chill storage and food processing. An overview. *International Journal of Food Microbiology*, *79*(1-2), 27−34. Available from http://dx.doi:10.1016/S0168-1605(02)00176-9.

Shirey, R. E., & Sidisky, L. M. (1998). *Analysis of flavors and off-flavors in foods and beverages using SPME* (Vol. 32), Bellefonte, PA: Supelco/Sigma-Aldrich.

Shukat, R., & Relkin, P. (2011). *Lipid nanoparticles as vitamin matrix carriers in liquid food systems: On the role of high- pressure homogenisation, droplet size and adsorbed materials* (November 2015). http://dx.doi.org/10.1016/j.colsurfb.2011.03.028.

Simonin, H., Duranton, F., & de Lamballerie, M. (2012). New insights into the high-pressure processing of meat and meat products. *Comprehensive Reviews in Food Science and Food Safety*, *11*(3), 285−306. Available from http://dx.doi.org/10.1111/j.1541-4337.2012.00184.x.

Singh, H., & Thompson, A. (2012). *Liposomes as food ingredients and nutraceutical delivery systems. Encapsulation technologies and delivery systems for food ingredients and nutraceuticals* (pp. 287−318). Cambridge: Woodhead Publishing. Available from http://dx.doi.org/10.1016/B978-0-85709-124-6.50011-1.

Soria, A. C., & Villamiel, M. (2010). Effect of ultrasound on the technological properties and bioactivity of food: A review. *Trends in Food Science and Technology*, *21*(7), 323−331. Available from http://dx.doi.org/10.1016/j.tifs.2010.04.003.

Sotelo, K. A. G., Hamid, N., Oey, I., Gutierrez-Maddox, N., Ma, Q., & Leong, S. Y. (2015). Effect of pulsed electric fields on the flavour profile of red-fleshed sweet cherries (*Prunus avium* var. Stella). *Molecules (Basel, Switzerland)*, *20*(3), 5223−5238. Available from http://dx.doi.org/10.3390/molecules20035223.

Soukoulis, C., & Bohn, T. (2015). *A Comprehensive Overview on the Micro- and Nano-technological Encapsulation Advances for Enhancing the Chemical Stability and Bioavailability of Carotenoids*. http://doi.org/10.1080/10408398.2014.971353.

Speranza, P., & Macedo, G. A. (2012). Lipase-mediated production of specific lipids with improved biological and physicochemical properties. *Process Biochemistry*, *47*(12), 1699−1706. Available from http://dx.doi.org/10.1016/j.procbio.2012.07.006.

Sun, D.-W. (2014). *Emerging technologies for food processing*. Elsevier Science. Retrieved from https://books.google.com/books?id = mgJ0AwAAQBAJ&pgis = 1.

Thirumdas, R., Sarangapani, C., & Annapure, U. S. (2014). Cold plasma: A novel nonthermal technology for food processing. *Food Biophysics*, *10*(1), 1−11. Available from http://dx.doi.org/10.1007/s11483-014-9382-z.

Torkamani, A. E., Juliano, P., Ajlouni, S., & Singh, T. K. (2014). Impact of ultrasound treatment on lipid oxidation of Cheddar cheese whey. *Ultrasonics Sonochemistry*, *21*(3), 951−957. Available from http://dx.doi.org/10.1016/j.ultsonch.2013.11.021.

Tume, R.K., Sikes, A.L., & Smith, S.B. (2010). *Enriching M. sternomandibularis with—tocopherol by dietary means does not protect against the lipid oxidation caused by high-pressure processing* (January 2016). http://dx.doi.org/10.1016/j.meatsci.2009.08.013.

Vandamme, J., Nikiforov, A., Dujardin, K., Leys, C., De Cooman, L., & Van Durme, J. (2015). Critical evaluation of nonthermal plasma as an innovative accelerated lipid oxidation technique in fish oil. *Food Research International*, *72*, 115−125. Available from http://dx.doi.org/10.1016/j.foodres.2015.03.037.

Yada, R. Y., Buck, N., Canady, R., DeMerlis, C., Duncan, T., Janer, G., ... Thurmond, S. (2014). Engineered Nanoscale Food Ingredients: Evaluation of Current Knowledge on Material Characteristics Relevant to Uptake from the Gastrointestinal Tract. *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 730−744. http://doi.org/10.1111/1541-4337.12076.

Yang, B.B., Wang, C., Lung, H., Cheng, Y., Huang, H., Yang, B.B., & Wang, C. (2015). *Microbial decontamination of food by electron beam irradiation Microbial decontamination of food by electron beam irradiation* (January 2016). http://dx.doi.org/10.1016/j.tifs.2015.03.005.

Yoshii, H., Soottitantawat, A., Liu, X.-D., Atarashi, T., Furuta, T., Aishima, S., ... Linko, P. (2001). Flavor release from spray-dried maltodextrin/gum arabic or soy matrices as a function of storage relative humidity. *Innovative Food Science & Emerging Technologies*, *2*(1), 55−61. Available from http://dx.doi.org/10.1016/S1466-8564(01)00019-4.

Zeeb, B., Lopez-pena, C.L., & Mcclements, D.J. (2015). *Controlling lipid digestion using enzyme-induced crosslinking of biopolymer interfacial layers in multilayer emulsions* (January 2016). http://dx.doi.org/10.1016/j.foodhyd.2014.12.018.

Zhang, Z., & Mcclements, D.J. (2015). *Influence of lipid type on gastrointestinal fate of oil-in-water emulsions: In vitro digestion study* (May). http://dx.doi.org/10.1016/j.foodres.2015.05.014.

Zhao, W., Yang, R., & Zhang, H. Q. (2012). Recent advances in the action of pulsed electric fields on enzymes and food component proteins. *Trends in Food Science and Technology*, *27*(2), 83−96. Available from http://dx.doi.org/10.1016/j.tifs.2012.05.007.

Ziuzina, D., Patil, S., Cullen, P. J., Keener, K. M., & Bourke, P. (2014). Atmospheric cold plasma inactivation of *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* inoculated on fresh produce. *Food Microbiology*, *42*, 109−116. Available from http://dx.doi.org/10.1016/j.fm.2014.02.007.

Zou, L., Zheng, B., Liu, W., Liu, C., Xiao, H., & McClements, D. J. (2015). Enhancing nutraceutical bioavailability using excipient emulsions: Influence of lipid droplet size on solubility and bioaccessibility of powdered curcumin. *Journal of Functional Foods*, *15*, 72−83. Available from http://dx.doi.org/10.1016/j.jff.2015.02.044.

CHAPTER

MINERALS

5

Silvina Rosa Drago

UNL, Santa Fe, Ciudad Autónoma de Buenos Aires, Argentina; Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

5.1 INTRODUCTION

In the 1990s, growing interest in preventing specific micronutrient deficiencies in vulnerable groups was added to previous concerns about caloric-protein malnutrition in developing countries. The International Conference on Nutrition organized by the FAO (1992) emphasized the importance of "hidden hunger" due to deficiencies in micronutrients. Globally, about 2 billion people suffer from a chronic deficiency of micronutrients (WHO, 2008). These forms of malnutrition can affect growth, physical and cognitive development of infants, toddlers, and school children, work performance and productivity of adults as well as reproductive functions in women (Muthayya et al., 2013). Micronutrient deficiencies are not only determined by low intake, but also by the amount available for absorption and the utilization of certain foods or diets. Mineral bioaccessibility depends on promoters and inhibitors from the diet, which must be taken into account when fortifying or developing a nutritionally improved food. Since low bioavailability is responsible for iron, zinc, and calcium deficiencies, this chapter addresses only these three minerals.

The iron content of food is variable, both in terms of quantity and chemical forms. For example, in meat iron content ranges between 2 and 4 mg/100 g, whereas 50% or more is in heme-iron form. In the liver, iron exists in higher amounts (14−20 mg/100 g), and ferritin and hemosiderin predominate. Fish, poultry, and seafood contain less than 2 mg/100 g, eggs contain 0.3 mg/100 g, and milk contains less than 0.1 mg/100 g. In vegetables, the highest concentration of iron can be found in legumes (7−10 mg/100 g). Cereals have a content of 2−4/100 g mg, whereas fruits and vegetables show small and variables amounts of iron. Regarding zinc, foods of animal origin constitute the main source, e.g., 20−60 mg/kg in meat, 3−5 mg/L in milk, and >15 mg/kg in fish and seafood. In cereals, the content decreases with milling and refined flours contain less than 5 mg/kg, slightly less than 30% of the whole grain. The zinc content of vegetables varies according to the content of the soil. Calcium is not an abundant mineral in most Western diets. The main contributors are dairy products (120 mg/100 mL). Butter and cream also contain minimal amounts of calcium (13 mg/100 g), whereas the calcium content in cheese depends on the process (98−1290 mg Ca/100 g). Some leafy vegetables (chard, spinach, 110 mg/100 g), fish consumed with bones (sardines, canned), and some seeds (almonds, sesame) can also be important contributors of calcium.

Nutraceutical and Functional Food Components. DOI: http://dx.doi.org/10.1016/B978-0-12-805257-0.00005-3

Calcium intake may considerably increase by calcium incorporation by culinary practices (jams, nixtamal) or through water in rural areas (Portela, 2015).

Today fortified foods contribute to essential mineral intake, particularly those involved in micronutrient deficiencies. Consequently, essential minerals play an important role in nutraceutical food development. In this chapter, the effects of food processing, consumer trends, and factors related to the host are discussed. Methodologies for measuring bioavailability in humans, methods using laboratory animals, and in vitro methodologies for estimating mineral bioaccessibility are also discussed.

5.2 NUTRIENT BIOAVAILABILITY

Bioavailability can be defined as the proportion of a nutrient in a food, diet, or dietary supplement that is absorbed and used for normal body functions. This involves the absorption and transport of nutrients to the relevant body tissues and its conversion to the physiologically active compound, in such a way the nutrient can be used to maintain normal metabolic functions (Davidsson, 1994; O'Dell, 1989).

In the case of minerals, the amount available for absorption (bioaccessible) depends on the composition and physical characteristics of the diet, the content and the chemical species of the mineral, the presence of ligand promoters or inhibitors of the absorption, luminal gastrointestinal secretions, and interactions that occur as a result of the interplay of these factors. The proportion of available minerals that is taken up by cells of the mucosa and degree of utilization in the body depends on physiological factors related to the host, such as the body's reserves, physiological demands (growth, pregnancy, lactation), and the presence of infections, diseases, or when certain medications are taken (Fairweather-Tait, 1992; Milner, 1990). The stages of digestion and absorption are fundamentally important in nutrient bioavailability (Ekmekcioglu, 2000). Knowledge of the bioavailability of essential minerals is important to establish the recommended dietary intake, to assess the adequacy of intake recommendations, to select the most effective fortification compounds, and to establish recommendations for the restoration, fortification, or enrichment of food or formulated foods (Hurrell, Furniss, et al., 1989).

5.3 IRON

Iron is an essential micronutrient that is part of hemoglobin, which is required for the transport of oxygen and CO_2 in the blood. It is also a component of tissue enzymes such as cytochromes (critical for energy production) and enzymes involved in the immune system (Martínez, Ros, Periago, & López, 1999). About 70−95% of iron is part of the functional compartment (mostly from the heme group) and the rest belongs to the reserve (Portela, 2015). Iron deficiency is the most common nutritional disorder in the world (Cook & Reusser, 1983; Fairweather-Tait, 1995). More than 500 million people have iron-deficiency anemia and many more are deficient without anemia (ACC/CN, 1992). Deficiency results because the amount of absorbed iron is insufficient to meet the requirements. This situation is more common in cases where increased amounts are needed,

(e.g., as a result of pregnancy, growth, menstrual loss, parasitic infections) or when food constituents alter absorption or the amount provided by the diet is insufficient.

The marked decrease in hemoglobin concentration reduces job performance in adults (Finch & Cook, 1984). It is also associated with behavioral abnormalities and cognitive and motor development in infants (Andraca, Castillo, & Walter, 1997). For instance, Halterman, Kaczorowski, Aligne, Auinger, and Szilagyi (2001) demonstrated lower standardized math scores among iron-deficient school-aged children and adolescents, including those with iron deficiency without anemia.

5.3.1 DIETARY SOURCES AND ABSORPTION OF IRON

Iron is present in foods, both in inorganic and organic forms. It is absorbed mainly in the duodenum. Losses are essentially exfoliation of epithelial cells (12−14 mg/day in the adult male). The excretion of iron in the body is limited and there are no mechanisms that regulate it to control its homeostasis. Therefore control of total body iron balance is performed on the site of absorption (Benito & Miller, 1998; Bothwell, 1995; Finch & Cook, 1984). During digestion, dietary iron is distributed in two pools (Fig. 5.1). The quantitatively most important pool is nonheme iron, which is present in the iron in vegetables (cereals, vegetables, fruits, etc.), soluble inorganic iron, iron of dairy products and eggs, and iron of animal tissue that is not heme-iron. The other pool, with different absorptive behavior is heme iron, which includes hemoglobin and myoglobin from red meat, fish, and poultry (Cook, 1983). Exceptions to this model of pools are dietary iron in the form of ferritin, hemosiderin, ferric hydroxides, and oxides, as well as iron from contamination (from grinding or from containers used in food preparation). These iron forms are less soluble than the common pool of nonheme iron and therefore only the soluble part participates (Benito & Miller, 1998; Hallberg, 1981a, 1981b).

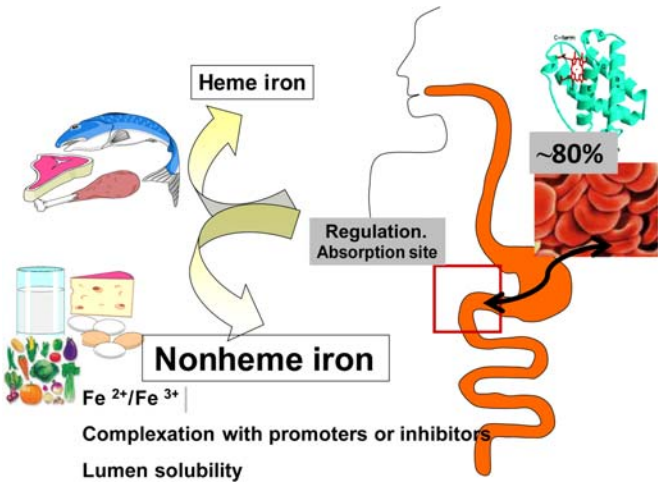


FIGURE 5.1

Iron bioavailability.

The absorption of nonheme iron is determined by individual factors (the state of iron reserves of the individual and its particular requirements) and dietary factors involving qualitative and quantitative characteristics of food iron and the presence of other constituents of the diet (Finch & Cook, 1984; Hallberg, 1981b). The combination of all the factors present in the intestinal lumen exerts a powerful influence on the body's ability to extract iron from the luminal nonheme iron pool (Lynch, 1997).

Heme iron is highly bioavailable (15−35%), because the iron porphyrin complex is absorbed intact and the iron is released intracellularly from porphyrin ring, avoiding the interaction with diet's inhibitory ligands (Hallberg, 1981b). In contrast, nonheme iron comes into a pool of exchange at the gastrointestinal level, since it is subject to the action of exogenous ligands, promoters, and inhibitors. Subsequently, its absorption depends on the composition of the diet and other factors operating in the lumen of the stomach and proximal small intestine (Finch & Cook, 1984). Therefore the absorption rate can vary a lot, e.g., from $<1\%$ up to 20−30%, depending on the ligands present, the iron's nutritional status, and individual requirements.

5.3.2 IRON CHEMISTRY

The different absorption of nonheme iron from various foods depends partially on its chemical properties. The bioavailability of dietary iron is influenced by chemical factors, valence, solubility, and its ability to form complexes (Hurrell, 1984; Lee & Clydesdale, 1979; Smith, 1983).

Food iron exists in two oxidation states (ferric and ferrous) and has a distinct tendency to hydrolyze. At acidic pH both forms exist as hydrates. When the pH increases, iron hydrolyzes and forms insoluble polymers of high molecular weight (Smith, 1983). Most of the inorganic iron from food is in the ferric form, which precipitates at pH >3 if it is not chelated (Benito & Miller, 1998). In the absence of suitable ligands, the Fe^{2+} is more bioavailable than Fe^{3+} because it is more soluble at physiological pHs and has lower affinity for ligands that can inhibit its absorption (South & Miller, 1998).

When inorganic iron is ingested alone, its absorption is a function of its solubility and charge density, which are affected by the conditions of the gastrointestinal tract. When the same source of iron is taken with food, absorption also depends on the reactivity of the iron source with the matrix provided by the food, the alteration of its chemical state by environmental factors in the body, and the presence of promoters and inhibitors of absorption. During the digestive process, nonheme iron can change its valence and form complexes with dietary ligands. The latter are affected by the food's preparation and processing as well as the conditions in the gastrointestinal tract. These changes can affect the chemical status of both intrinsic and extrinsic iron. Solubility is a key requirement for iron absorption. Soluble-iron species remaining stable throughout the variable conditions imposed by the passage through the gastrointestinal tract will be the most bioaccessible. Complexing agents preventing iron precipitation in the neutral-basic environment of the intestine favor its absorption (Lee & Clydesdale, 1980). The stability of iron complexes increases as a function of chelating ligand concentration. The strength of the bond, the solubility of the complex, its stability, and environmental factors (e.g., pH and the presence of competing ligands) determine whether or not iron will be available for absorption by the cells of the mucosa (Van Dokkum, 1992).

Table 5.1 Absorption Promoters and Inhibitors of Dietary Nonheme Iron

Promoters	Inhibitors
Ascorbic acid	Tannins and polyphenols
Animal tissues (meat factor)	Phytates
Amino acids	Vegetable, dairy, and egg proteins
Organic acids: citric, lactic, etc.	Calcium

5.3.3 BIOAVAILABILITY OF NONHEME IRON

In the acidic digestive environment of the stomach, most of the nonheme iron is released from the complexes with food components and enters the common pool of nonheme iron. Upon entering the duodenum, increasing pH toward neutrality occurs, which favors the formation of new complexes. At this point, a competition occurs between the different ligands to form complexes of iron with absorption inhibitors and promoters (Bothwell, 1995).

Promoters or enhancers are those ligands that form soluble chelates with iron, preventing its precipitation and allowing its release for absorption by the mucosa. An effective promoter must have a sufficiently high affinity for iron to compete with other diet ligands able to decrease absorption (South & Miller, 1998). Inhibitors are those ligands that bind iron in insoluble or very high affinity complexes and therefore do not release it for absorption (Table 5.1).

5.3.3.1 Inhibitors of iron absorption

Phytates occurring in legumes, cereals, and vegetables are responsible for the low bioavailability of iron (Gillooly et al., 1983; Hurrell, 2004). When taken with meals, tannins and polyphenols from tea, coffee, and yerba may also have an inhibitory effect on absorption (Lim, Riddell, Nowson, Booth, & Szymlek-Gay, 2013; Thankachan, Walczyk, Muthayya, Kurpad, & Hurrell, 2008; Wolfgor, Rodríguez, Pellegrino, & Valencia, 1995) by forming insoluble complexes with iron (Smith, 1983). Polyphenols are also known to reduce iron bioavailability from sorghum foods (Cercamondi, Egli, Zeder, & Hurrell, 2014).

In addition, calcium salts may interact with iron and other dietary components forming polymineral−ligand complexes, which are insoluble and not available for absorption (Clydesdale, 1988; Hatton, Muntzel, Absalon, Lashley, & Mccarron, 1991). The mechanism of inhibition involves an effect at the level of intestinal mucosal cells (Hallberg, Brune, Erlandsson, Sandberg, & Rossanser-Hultén, 1991; Hallberg, Rossanser-Hultén, Brune, & Gleerup, 1992). In general, the inhibitory effect on iron uptake depends on the type of food, natural calcium content, or calcium source added. Some proteins of both animal (milk protein, bovine albumin, egg proteins) and vegetable origin (soy protein, wheat, etc.) also exert an inhibitory effect. Casein is a more inhibitory factor than whey protein (Drago & Valencia, 2004). The food industry uses soy-protein isolates in the manufacture of numerous products (e.g., infant formula, bakery, dairy, and meat products) as it is a good-quality protein, abundant, and inexpensive, showing good functionality (Galán & Drago, 2014). However, soy-protein isolates markedly reduce iron bioavailability not

only due to the effect of the protein itself (Lynch, Dasenko, Cook, Juillerat, & Hurrell, 1994), but also due to their high content of phytic acid (Cook, Morck, & Lynch, 1981; Hallberg & Rossander, 1982; Hurrell et al., 1992).

5.3.3.2 Promoters of iron absorption

Ascorbic acid is a remarkable enhancer acting by improving absorption through ferric ion reduction and forming stable chelates with iron in the stomach. Thus solubility is maintained when food enters the neutral environment of the duodenum (Lee & Clydesdale, 1981). This latter effect can be explained by the fact that ascorbic acid forms soluble complexes with iron at pHs lower than the inhibitory ligands, which means it acts at stomach level where the pH conditions are unfavorable for complexation with other ligands (Hurrell, 1984). All this counteracts the inhibitory effects of compounds such as tannins (Cercamondi et al., 2014) and phytates (Hurrell, 2004; Teucher, Olivares, & Cori, 2004). When ascorbic acid is added to high-iron bioavailability foods, its effect is much less pronounced. Its promoter action, which has been demonstrated in numerous studies (Clydesdale, 1983; Davidsson, Walczyk, Morris, & Hurrell, 1998; Gillooly et al., 1984), is related to its concentration and is more evident in the presence of inhibitors. However, Clydesdale and Nadeau (1984) observed several solubilization patterns of different iron sources and showed that the optimum ratio of ascorbic acid per iron is dependent on the iron source used. In general, the impact of ascorbic acid on iron absorption has been evaluated in isolated meals. Although there is less information regarding the long-term effect of ascorbic acid on iron absorption at population level, after conducting a fortification program for 15 months, Stekel et al. (1986) observed a reduction in the prevalence of anemia in Chilean infants fed with infant formulas fortified with ferrous sulfate (15 mg Fe/L) and ascorbic acid (100 mg/L). Other organic acids (e.g., citric, malic, tartaric, and lactic) also favor iron bioavailability (Ballot et al., 1987; Derman et al., 1980; Gillooly et al., 1983). The use of Na_2EDTA (in an EDTA:Fe molar ratio less than or equal to 1) has been proposed to enhance absorption of intrinsic or added iron in diets containing absorption inhibitors (Mcphail, Patel, Bothwell, & Lamparelli, 1994). The promoter effect of muscle protein (meat, fish, pork, and poultry) on iron (mainly the nonheme form) absorption is well known (Cook & Monsen, 1976; Hallberg, Bjorn-Rasmussen, Howard, & Rossander, 1979; Hurrell, 1984). The mechanism of action of muscle proteins is unclear but the promoting effect is called the "meat factor." The gastrointestinal release of cysteine and cysteine-containing peptides with stable thiol groups, which would act as complexing and reducing agents may be involved in the promoting effect (Layrisse, Martínez-Torres, Leets, Taylor, & Ramírez, 1984; Martinez-Torrez, Romano, & Layrisse, 1981; Taylor, Martinez-Tórrez, Romano, & Layrisse, 1986), may be involved Additionally, peptides derived from the digestion of the major myofibrillar protein (Kane & Miller, 1984), or the interaction of nonheme iron with carboxyl groups of amino acids (Shears, Ledward, & Neale, 1987), could participate in muscle protein-promoting effect. The experimental designs used in human studies are often selected to maximize the inhibitory or promoter effect of a food factor in a single meal. Indeed, several researchers suggest that such studies may overstate the effects of specific food components, and in complete diets, the influence of inhibitors and promoters may be diluted (Benito & Miller, 1998; Cook, Dassenko, & Lynch, 1991). However, in the case of groups who have less varied diets, such as infants and children, the results obtained this way would probably be more reliable (Hurrell, Davidsson, Reddy, Kastenmayer, & Cook, 1998).

5.3.4 EFFECTS OF FOOD PROCESSING

Food processing can decrease or increase the bioavailability of iron. For example, heating and storing of cooked food can destroy or decrease ascorbic acid content and consequently reduce iron absorption. Martínez-Torres et al. (1986) demonstrated that prolonged cooking reduces iron absorption from meat, probably because the integrity of the "meat factor" is affected. The extent of extraction during the milling process also increases the bioavailability of iron, since it eliminates some of the phytates contained in the tegument of cereals. However, it decreases the content of iron and other minerals contained in the bran and germ.

Some traditional food-preparation processes, such as leavened bread, soaking, germination, and fermentation of cereals can be used to reduce the amount of inhibitors. These processes improve iron absorption, mainly due to activation of endogenous phytase, which degrade the hexa and penta-phosphate inositols to inorganic phosphorus and inositols containing fewer phosphates, which do not interfere with mineral absorption. Phytase activity is favored by pH decrease occurring during fermentation, since its optimum pH is equal to 5.15 (Svanberg, Lorri, & Sandberg, 1993). In the case of fermented soy products, iron bioavailability improves due to the degradation of phytic acid (produced by the action of phytase), protein hydrolysis, and other changes in the food components produced by the process (Macfarlane, Van Der Riet, Bothwell, Baynes, & Siengenberg, 1990). Phytate hydrolysis can also be achieved by the addition of exogenous phytase (Hurrell et al., 1992; Phillippy & Wyatt, 2001). Thus dephytinization can potentially enhance mineral absorption in high-phytate foods (Gibson, Bailey, Gibbs, & Ferguson, 2010) and low-tannin sorghum porridge (Hurrell, Reddy, Juillerat, & Cook, 2003).

Lactic fermentation of vegetables has been reported to increase iron bioavailability. The mechanism for the increased bioavailability is likely an effect of the increase in ferric-iron species caused by the lactic fermentation (Scheers, Rossander-Hulthen, Torsdottir, & Sandberg, 2016). Bernardi, Drago, Sánchez, and Freyre (2006) showed that *Prosopis alba* (Algarrobo) pulp cookies formulated with simultaneous additions of ascorbic acid and citric acid resulted in better iron bioaccessibility than those obtained with ascorbic acid or citric acid alone.

5.3.5 CONSUMER TRENDS

In developing countries, micronutrient nutritional deficiencies are due to the lower dietary quality of ingested iron. Indeed, poor populations consume more cereals and tubers, restricting the intake of animal products, fruits, and vegetables that are more expensive. Inhibitory factors, commonly present in diets rich in vegetables and low in heme iron, are responsible for the high incidence of iron-deficiency anemia (Whittaker & Vanderveen, 1990). Therefore although the total iron intake of these populations can be high, their bioavailability is low and increased consumption of affordable foods would not be enough to meet the needs of some vulnerable groups.

5.3.6 FACTORS RELATED TO THE HOST

Iron nutritional status, increased requirements for pregnancy or growth in infants and children, micronutrient deficiencies, and certain medical conditions affect the absorption or utilization of dietary or fortified iron (Bothwell, 1995). For instance, vitamin A deficiency alters the use of iron for

hemoglobin synthesis, not allowing the use of liver and spleen stores (Portela, 2015). Iron-deficient individuals absorb iron more efficiently than those whose iron stores are adequate (Bothwell, 1995; Hallberg, 1981b). Individuals with hypochlorhydria or achlorhydria also have lower absorption of iron than those who have a normal gastric acid secretion (Benito & Miller, 1998). Some parasitic infections can both, increase iron absorption due to intestinal blood loss, or lead to deficiency due to generalized malabsorption (Allen & Ahluwalia, 1997; Hallberg, 1981a).

5.4 ZINC

Zinc is an essential nutrient that plays a part in growth, reproduction, tissue repair, and cellular immunity. It is also involved in metabolism, as a component of metallo-protease enzymes fulfilling catalytic, structural, and regulatory functions, for the stabilization of membranes, and for various ionic cell functions (Milner, 1990). Zinc deficiency increases the risk and severity of a variety of infections, restricts physical growth, and affects specific outcomes of pregnancy (Hess, Lönnerdal, Hotz, Rivera, & Brown, 2009). Marginal deficiency of zinc has been recognized in numerous groups, both in industrialized and developing countries. Since there is no single indicator for detecting marginal deficiencies (Milner, 1990), assessment of nutritional status with respect to zinc is not easy to perform (Gibson & Ferguson, 1998). It is detected when clinical symptoms or stunting appear, which are reversed by zinc supplementation (Wise, 1995). Although zinc deficiency can be caused in many cases by inadequate dietary intake, the presence of absorption inhibitors is probably the most common cause of deficiency (Lönnerdal, 2000). Other causes include malabsorption or high losses from diarrhea. In a population, the risk of zinc deficiency is estimated using three types of indicators: the prevalence of low serum zinc, inadequate zinc intakes, and the prevalence of low height-for-age (De Benoist, Darnton-Hill, Davidsson, Fontaine, & Hotz, 2007)

5.4.1 DIETARY SOURCES AND ABSORPTION OF ZINC

The zinc content of foods is highly variable. Sources with higher content are oysters, herring (Solomons & Ruz, 1997), and lean red meat (Coyle, Philcox, & Rofe, 1998). Other protein sources such as pork, poultry, fish, milk, and dairy products have lower zinc content. Plant-based foods have still lower amounts, except the embryonic portion of cereals such as wheat germ and legumes (Portela, 2015). Zinc is mainly absorbed in the small intestine, especially in the duodenum and jejunum. Current theories maintain that the modulation of absorption of dietary zinc and the intestinal conservation of endogenous zinc secreted postprandially in the intestinal lumen are the two main mechanisms that regulate total body zinc homeostasis. Both processes can be affected by dietary factors (Hambidge, Krebs, & Miller, 1998; Villa, Arizmendiarrieta, & Morán, 1990). The solubility in the intestinal lumen is a major factor in determining the uptake by enterocytes (Cousins, 1997). Finally, fractional zinc absorption decreases when the amount of zinc in a food increases, probably due to a saturation of transport mechanisms (Sandström & Cederblad, 1980).

5.4.2 ZINC BIOAVAILABILITY: DIETARY FACTORS, PROMOTERS, AND INHIBITORS

Most of the zinc found in foods is attached to proteins and nucleic acids, usually in the form of stable complexes, so the digestive process must be efficient to make it bioavailable. Zinc bound to

proteins from animal tissues has better bioavailability than plant zinc. Thus increasing the intake of proteins increases zinc intake. However, bioavailability depends on the protein type. Meat proteins counteract the inhibitory effect of phytate on zinc absorption from a single meal. This effect is attributed to the release of amino acids that bound zinc and keep it in solution (Sandström & Cederblad, 1980) and cysteine-containing peptides released during the digestion of animal protein, forming soluble ligands with zinc (Nair, Augustine, & Konapur, 2016).

Ligands and low-molecular-weight compounds that chelate zinc and prevent its precipitation at physiological pHs are called absorption enhancers (Pabón & Lönnerdal, 1993). Examples of such ligands are amino acids such as histidine and cysteine (Fairweather-Tait, 1992), organic acids derived from the metabolism of carbohydrates, and citrate (Pabón & Lönnerdal, 1993).

The exogenous factor identified as the most important absorption inhibitor is phytic acid (Fairweather-Tait, 1992), which is mainly found in cereals and legumes. Phosphate groups of hexaphosphate and pentaphosphate inositol can form strong complexes with zinc, lowering its solubility, while tetra and triphosphate forms do not significantly influence it. In the absence of calcium or magnesium, the reaction between phytate and zinc appears to depend on phytate/zinc molar ratio (Wise, 1995).

Calcium content can also affect zinc absorption if the diet contains phytates. This is because calcium binds with phytate and zinc forming insoluble poly-mineral-phytate complexes, which consequently cannot be absorbed (O'Dell, 1989). Regardless of if dietary phytate is low or high, calcium does not impair zinc absorption in women with conventional diets (Hunt & Beiseigel, 2009). Indeed, if the calcium/phytate molar ratio is high, zinc absorption is increased as a result of calcium−zinc competition by phytate (Lönnerdal, Cederblad, Davidsson, & Sandström, 1984). On the other hand, sorghum polyphenols inhibit zinc absorption in the presence (but not absence) of phytic acid (Brnić, Wegmüller, Zeder, Senti, & Hurrell, 2014).

Iron can also alter zinc absorption, since they have many similar absorption and transport mechanisms and may therefore compete for absorption. The mineral present in molar excess will exclude the other present in smaller proportion from the membrane transporter (Solomon & Ruz, 1997). This is a competing concept as the primary interaction is antagonistic. The Fe−Zn interaction has been evaluated in rats and humans using metabolic-balance studies and zinc-absorption tests. Epidemiological observations in children and pregnant women have also been performed (Hambidge et al., 1983; Yip, Reeves, Lönnerdal, Keen, & Dallman, 1985). Solomon and Ruz (1997) concluded that such interaction and competitive inhibition of zinc's uptake by iron overload occurs when the Fe/Zn ratio is 2:1 or greater and if the total amount of ionic species is greater than 25 mg. The physiological basis would be the competition of chemically similar ions by a common absorptive via (Lönnerdal, 1991). However, Faiweather-Tait (1995) and Whittaker (1998) reported that the amounts of iron required to inhibit zinc absorption are only achieved with the use of iron supplements. Aggett and Comerford (1995) reported that the amounts of iron required to inhibit zinc absorption are only achieved with the use of iron dietary supplements, which may exert this effect if consumed along with food, and food iron fortification probably does not affect zinc absorption. Other components present in foods exerting an inhibitory effect are oxalates and polyphenols (Aggett and Comerford 1995). The latter exist in commonly used infusions like tea, coffee, and yerba mate, and decrease the bioavailability of dietary zinc, although there are no systematic studies of their long-term effect.

5.4.3 PROCESSING INFLUENCE

Processing of cereals influences the bioaccessibility of dietary zinc. Grain milling allows separation of bran and germen, reducing its phytate content. The fermentation of leavened bread substantially reduces its phytate content through the activation of endogenous phytases in cereals. Soaking grains and germination also reduce phytate content of legumes and cereals by the same mechanism (Gibson, Yeudall, Drost, Mtitimini, & Cullinan, 1998). As noted above, another alternative for reducing phytate content is treating these foods with exogenous food-grade phytase. Moreover, high temperatures, such as those achieved in thermoplastic extrusion, also hydrolyze inositol hexaphosphate in less phosphorylated forms that have lower metal-binding capacity (Watzke, 1998). Milk fermentation (yogurt) increased iron, zinc, and calcium bioaccessibility from iron-fortified dairy products (Drago & Valencia, 2002). The addition of citric acid also proved to be a good enhancer of iron and calcium bioaccessibility in algarrobo pulp cookies formulated with 1:50 and 1:100 Fe: citric acid/molar ratios (Bernardi et al., 2006).

5.4.4 CONSUMER TRENDS

There are two widespread dietary patterns that are likely to be the main factors involved in the etiology of dietary zinc deficiency: diets with low-zinc bioaccessibility and diets with low total zinc. The first are cereal-based or unfermented bean-based diets and therefore potentially high in phytic acid content. The latter are diets based on starchy roots and tubers. When these two types of diets are combined with low intake of red meat, poultry, or fish (as is the case in many developing countries) the intake of bioaccessible zinc is likely inadequate. These dietary patterns can be used to identify populations at risk of inadequate zinc nutrition.

5.4.5 FACTORS RELATED TO THE HOST

Previous intake of zinc can affect absorption. Diets low in zinc increase its absorption in all age groups, because the homeostatic mechanisms regulate increasing the absorption and retention (Davidsson, 1994; Lönnerdal, 2000). Moreover, humans absorb zinc more efficiently from low-zinc diets. In response to low-zinc intake (<11 mg/d) for several weeks (4−8), zinc absorption was upregulated if the diets were low in phytate, but such adaptation did not occur with higher phytate diets (Hunt, Beiseigel, & Johnson, 2008). The absorption processes are affected by diseases such as malabsorption and diarrhea (Hambidge et al., 1998). Inhibition of acid secretion in humans may also reduce zinc absorption (Cousins, 1997).

5.5 CALCIUM

Calcium is the most abundant mineral inside the body. More than 99% is found in bones where it plays an important role in their structure and strength. Another very small portion is involved in regulating critical functions, including muscle contraction, nerve-impulse transmission, the activity of enzymes, and maintenance of cell membranes. Its role in metabolic regulation is so critical

that it is necessary that the blood concentration remains within a very narrow range. If the calcium is insufficient in the diet for this purpose, bones act as a reserve to maintain constant blood level. Therefore long-term calcium deficiency can lead to gradual skeletal demineralization and the onset of adult osteoporosis (Portela, 2015). Calcium reserves reach peak levels between puberty and 30 years old (Hallberg et al., 1992). Such reserves are increased in this first stage of life and used in the second part. If there has been good nutrition and adequate supplies of calcium, deposits will be sufficient to maintain adequate bone density in the second stage of life. Unfortunately, if stocks are low, there is no way to increase them. It is therefore critical to follow a diet rich in calcium in the first stage of life and maintain a moderate level of exercise to ensure good bone density later in life.

5.5.1 DIETARY SOURCES

Dairy products are an excellent source of this mineral, both in spite of quantity and bioavailability. Other foods such as soy products, legumes, leafy vegetables, sardines, mackerel, dried fruits, and seaweed provide lower amounts of calcium that are less bioaccessible. Nixtamal is an important source of calcium in some countries (Portela, 2015).

5.5.2 BIOAVAILABILITY OF CALCIUM FROM FOOD: INFLUENCE OF DIETARY AND ASSOCIATED HOST FACTORS

The efficiency of calcium absorption depends on its intake, e.g., it is higher at lower intake. In turn, the proportion of dietary calcium that is absorbed depends on its bioaccessibility in the food and is influenced by substances in the intestinal lumen that may increase or inhibit its absorption. In addition, certain quantities of calcium are endogenously secreted in the saliva, bile, and pancreas, which are dumped into the intestine and get lost in part, not being fully resorbed. The homeostatic regulation of blood-calcium concentration is achieved by complex interactions between the processes of absorption, urinary excretion, and bone resorption, coordinated by several hormones and vitamins. As an example, adequate vitamin D is essential for the efficient utilization of calcium, and low levels of estrogen (postmenopausal) increase its losses. Calcium absorption and utilization is greater during adolescence and pregnancy and tends to decline with age. The effect of dietary factors on calcium absorption is poorly understood (Kernefick & Cashman, 2000). In general, calcium is absorbed better when it is in soluble ionic form. All factors that increase the solubilization tend to increase absorption.

While it is considered that stomach acidity has a solubilizing function and reduced gastric secretion decreases the amount of calcium absorbed, this is not a general rule. The insoluble calcium carbonate is absorbed efficiently, even with low acidity (Bo-Linn et al., 1984). Heaney, Smith, Recker, and Hinders (1989) observed that the absorption of calcium from different sources in humans and rats was higher when calcium was taken in the presence of food. The effect of food was more evident in the presence of an insoluble calcium salt. In particular, food causes increased secretion and slower emptying of the stomach, allowing better dispersion and dissolution of the insoluble salts. In addition, other dietary components could act as absorption promoters through

complex formation. For example, Knox et al. (1991) postulated that stomach acidity is needed to solubilize the insoluble calcium carbonate in the fasting state but is not required for calcium absorption, if its source is food or a supplement consumed with meals. Thus the complexation of calcium with various food compounds would be the most important determinant of dietary calcium absorption.

Various compounds such as phytates (Knox et al., 1991) and oxalic acid are capable of acting as inhibitors by forming insoluble complexes in the gut and reducing calcium absorption (Heaney, Weaver, & Recker, 1988). The main sources of oxalic acid are vegetables such as chard, spinach, artichokes, and almonds. Foods rich in fiber also reduce absorption when containing high contents of phytic acid. However, the fermentation of fiber in the colon increases the acidity and calcium solubility, which can counteract the adverse effects of the absorption occurring in the small intestine. Studies in rats and children with short bowel syndrome have shown that ingestion of very high fat levels (especially if it induces steatorrhea) increased fecal losses of calcium, because insoluble soaps form. However, the importance of these data has not been established in adults consuming a mixed diet (Greger, 1988). Other food components affect the bioavailability of calcium, primarily modifying urinary excretion and not calcium absorption. Protein, sodium, and chloride have a hypercalciuric effect, while phosphorus has a hypocalciuric effect (Greger, 1988). Certain medications such as anticoagulants and steroids also increase calcium losses acting as antagonists.

Casein–phosphopeptides from casein digestion and milk lactose are considered promoters of dietary calcium absorption (Naito, Gunshin, & Noguchi, 1989). However, the mechanism of action of lactose is not completely elucidated. Its stimulatory effect would be through an interaction with absorptive cells (increasing their permeability) or nonspecific and mediated by an increase in water flow. The effect of different carbohydrates in calcium balance has not been adequately tested, since there are conflicting results and the different methodologies make it difficult to obtain concrete conclusions (Kaup, 1998). Although the primary carbohydrate of human milk is lactose, infant formula may contain other components such as sucrose or maltodextrins. However, it has been observed that after the second month of life, infant formulas based on soy protein, which are lactose-free, generally produce less calcium retention than adapted bovine milk formulations (Greer, 1989). It has also been suggested that any carbohydrate that escapes proximal absorption increases calcium absorption in the distal portions of the intestine (Greger, 1988). For these reasons, although various dietary factors can affect bioaccessibility and absorption, calcium balance is a very complex process, not only dependent on the solubility of calcium in the intestinal lumen, but also of various homeostatic mechanisms and dietary modulators that do not act at the absorption site.

5.6 METHODOLOGIES FOR MEASURING BIOAVAILABILITY AND MINERAL BIOACCESSIBILITY

The methodology for determining the bioavailability of iron, zinc, and calcium involves in vivo methods, both in animals and humans (Van Campen, 1983). On the other hand, bioaccessibility is estimated using in vitro methods.

5.6.1 DETERMINATIONS IN HUMANS

Measuring iron bioavailability in humans has been extensively reviewed by several researchers (Hallberg, 1981b; Van Campen, 1983). The main techniques used to assess the bioavailability of iron, zinc, and calcium in humans are shown in Table 5.2. Traditionally, balancing techniques have been used to study the absorption of nutrients. They are based on the subtraction of the amount of a measured nutrient in urinary excreta and/or fecal and dietary intake over a specific period. However, problems in collecting stool (incomplete collection) tend to overestimate the absorption (Ehrenkranz, Ackerman, Nelly, & Janghorbani, 1984) and lack of measurement of endogenous secretion tends to underestimate it (Davidsson, 1994; Ehrenkranz et al., 1984). This technique reflects the absorbed mineral and not the bioavailability. Moreover, it does not consider the adaptation of the organism to a particular diet. Its low precision is probably responsible for the conflicting data from the human studies found in literature (Heaney, Recker, & Hinders, 1988).

Techniques for the assessment of absorption-measuring plasma levels after food ingestion are not used for iron. Regarding zinc, they only serve to evaluate differences in absorption of various salts at drug levels, but not for foods (Sandström, Arvidsson, Cederblad, & Björn-Rasmussen, 1980). For calcium, they are not useful since hormones have constant serum calcium levels (Greger, 1988).

Research on iron bioavailability was supplied by the demonstration of three key milestones. The first was the development of an intrinsic tag approach to measure iron absorption (Moore & Dubach, 1951) in which radioisotopes of iron (^{55}Fe and ^{59}Fe) were incorporated biosynthetically in live animals and plants during their growth and development. The second was a series of experiments using this proposal to demonstrate that food constituents had a great influence on iron absorption in humans (Allen & Ahluwalia, 1997). The third was the verification that extrinsic tags could be used to measure the absorption of iron from foods, by adding isotopes (Bjorn-Rasmussen, Hallberg, & Walker, 1973). This development made the measurement of iron absorption from individual foods, diets, and fortified sources possible. However, extrinsic tags involve the underlying assumption that the added iron radioisotopes are completely exchanged with the intrinsic nonheme iron of foods, reaching the same distribution. This can be demonstrated if the fractional absorption of intrinsic and extrinsic tags is highly correlated and does not differ significantly, which depends on the food matrix. Furthermore, the extrinsic tag is representative of the absorption of intrinsic mineral if the amount of extrinsic tag is small compared to the amount of intrinsic mineral, and the physiological conditions are not disturbed by a significant change in mineral intake. This is more difficult to achieve with the stable isotope tag because of the amounts used (Serfass, Ziegler,

Table 5.2 Assessment of the Bioavailability of Iron, Zinc, and Dietary Calcium in Humans

Non isotopic methods	Metabolic balance Changes in hemoglobin and serum ferritin (for Fe) Plasma/serum levels (Zn)	
Isotopic methods Radioisotopic (a) Stable isotopes (b)	– Metabolic balance – Simple isotopic techniques – Double-isotope technique	Radioactive count in whole body (Zn, Ca) (a) Incorporation of isotopes in blood or hemoglobin (Fe) (a,b)

Edwards, & Houk, 1989). Regarding zinc, Serfass et al. (1989) demonstrated that it is possible to use the extrinsic tag for infant formula because they proved there is a total isotope exchange between zinc and dietary minerals. The applicability of the extrinsic tag to radioactive calcium has also been validated in absorption studies of this mineral in human milk (Liu et al., 1989). Different mineral isotopes can be used in simple tag techniques or double-isotopic methods (Davidsson, 1994).

In the case of iron, since both radioisotopes (^{55}Fe and ^{59}Fe) can be used as tags and distinguished in analysis, it is possible to evaluate the same subject using two different meals labeled with different isotopes in a period of 14−16 days (time of incorporation of iron in red blood cells), allowing simultaneous comparisons in the same individual (Forbes et al., 1989). Another possibility is that one of the isotopes is incorporated into test food and the other in the reference dose, with both administered orally. After 14 days of administration, the fraction of each isotope incorporated into erythrocytes is compared (Abrams, Wen, & Stuff, 1996). Because the absorption in iron-deficient individuals is greater than in replete ones, a reference dose is used to assess iron bioavailability. In individuals without iron stores, but without anemia (borderline), iron bioavailability from a reference dose containing 3 mg of ferrous sulfate and 30 mg of ascorbic acid is about 40%. Then, iron absorption in test meal is usually expressed as the absorption value corresponding to the 40% absorption of a reference dose (Hallberg, 1981a, 1981b). This relative absorption is known as standardized absorption and reduces interindividual differences due to different iron nutrition status. It is important to note that, when radioisotope incorporation in hemoglobin is measured, iron absorption and utilization are measured, making it a true measure of bioavailability.

Unlike iron, in the case of zinc there is no isotope incorporation into specific tissues or body compartments where it can be measured (Davidsson, 1994). Therefore the fractional absorption is determined through the body retention of this mineral. In simple radioisotope techniques, extrinsically labeled food with ^{65}Zn is supplied and 14 days later total body retention of radioisotope is measured. This period ensures that the unabsorbed tag fraction has been excreted in feces (Lönnerdal et al., 1984; Sandström, Keen, & Lönnerdal, 1983). The application of stable isotope tracer techniques are also used to assess zinc physiology, metabolism, and homeostasis. Their linking with saturation response and compartmental modeling could assist the continued search for simple markers of zinc status (Tran, Gopalsamy, Mortimer, & Young, 2015). The double-isotope technique, which applies both radioactive and stable isotopes, is currently used to measure fractional absorption of Zn (Brnić et al., 2016). Fractional absorption of Zn is determined by the relative ratio of the isotope orally versus intravenously administered (Abrams et al., 1996). One of the isotopes is used to label the studied food and the other one is injected intravenously. The isotope bloodshot represents a standard equivalent to 100% absorption and the isotope ratio ingested to this standard is a measure of mineral absorption (Sparacino, Shames, Vicini, King, & Cobelli, 2002).

Regarding calcium, absorption can be estimated by the fractional retention of ^{47}Ca. The evaluation of retained calcium can be made using a test meal label with ^{47}Ca, prior to measuring the total body counts (Knox et al., 1991). Currently, the dual-isotope technique is considered the best method for studying the absorption of calcium. This technique can be applied to individual foods, showing high accuracy (Abrams et al., 1996; Griessen et al., 1989). Isotopic techniques in humans constitute the benchmark for comparison with any other methodology (Hurrell, 1997). However, it is only possible to determine the bioavailability in the case of iron, as in the case of zinc and calcium, the absorbed or retain mineral is evaluated. In addition, radioisotope techniques in humans

are costly, time consuming, and often quite complex to perform (Davidsson, 1994; Luten et al., 1996), due to the large interindividual variations (Ekmekcioglu, 2000). Moreover, there is an increasing reluctance to use them internationally due to ethical reasons. This type of evaluation is performed in adult volunteers and does not apply to the most susceptible populations suffering iron deficiency (e.g., infants, school children, and pregnant or lactating) (Bosscher, Zhengli, et al., 2001). In the last decade, stable isotopes have been used to study bioavailability of iron in these vulnerable groups in the same way as radioisotopes (Kastenmayer et al., 1994; Sandström, Fairweather-Tait, Hurrell, & Van Dokkum, 1993; Wienk, Marx, & Beynen, 1999). In these studies, individuals are not exposed to ionizing radiation. However, during the early years of their employment, large quantities of isotopes were required to enrich meals tests, resulting in larger amounts than which naturally occurs. This was inconvenient due to high costs, and isotopes were not truly supplied in trace amounts. Thus the validity of their use as extrinsic markers for bioavailability studies was unclear (Fairweather-Tait, 1992). Subsequently, the sensitivity of stable isotopes measurement was improved, to the point that small doses are sufficient for absorption studies in humans. However, the determination of stable isotope is more difficult than the radioisotope and requires a more laborious sample preparation (Davidsson, 1994). In addition, it is necessary to have special equipment (thermal ionization mass spectrometer, inductively coupled plasma mass spectrometer, or fast atom bombardment) and trained personnel, which limits its application to studies with small numbers of subjects and to only a few laboratories around the world.

5.6.2 METHODS USING LABORATORY ANIMALS

Rat models have also been used in numerous studies (Galdi, Bassi, Barrio Rendo, & Valencia, 1988; Langini et al., 1988; Lönnerdal, Yuen, & Huang, 1994) to evaluate human bioavailability. However, this method is restricted by interspecies differences in growth rate, intestinal and microbial enzyme activities, as well as intestinal anatomy and physiology (Sandström, 1997).

Repletion assay hemoglobin has been used to compare the relative efficiency of iron sources to restore the content of hemoglobin in anemic rats (Forbes et al., 1989). This animal model was selected for its accessibility in the laboratory. It could be useful to know the bioavailability of a particular iron source. For this assay it is necessary to study the bioavailability of the studied iron source and another taken as reference (ferrous sulfate) in various levels for comparison (AOAC, 2000). Therefore numerous lots of animals are necessary. However, rodent studies do not allow determining the quantitative importance of absorption inhibitors or promoters to the bioavailability of iron (Ekmekcioglu, 2000). Reddy and Cook (1991) conducted a comparative study of iron's bioavailability in humans and rats using identical methodology and the same foods in both cases. Iron absorption in the rat was influenced little by promoter or inhibitor factors present in the diet and was not affected by food matrices such as meat or tea having effects on human iron bioavailability. This result brought into question the effectiveness of rat models to evaluate iron bioavailability in humans. A similar conclusion was extracted for the promoting effect of ascorbic acid on human iron bioavailability (Whittaker & Vanderveen, 1990).

On the other hand, studies performed using laboratory animals have shown that zinc bioavailability depends on the animal's age and nutritional status. It is also affected by the source and the amount of zinc consumed (Hunt, Johnson, & Swan, 1989). Furthermore, unlike the human, rat is able to synthesize ascorbic acid and possesses intestinal phytase activity, which limits its validity

as a model (Allen, 1998; Wienk et al., 1999). With regard to the absorption and utilization of calcium, one of the techniques used to monitor the effect of various dietary factors is the measurement of calcium bone levels using animal models. However, this is a slow process, in which the animals must adapt to dietary regimes (Greger, 1988), and there might be other nutritional factors affecting bioavailability.

Lönnerdal et al. (1994) have proposed the use of rat pups as a model to study the bioavailability of iron and other minerals. However, the differences obtained studying human milk and infant formulas were minimal, indicating that this method is not suitable because of its low sensitivity. Rat pups highly absorb minerals and trace elements and thus have a greater capacity to capture nutrients than human infants. Thus the respective absorptive mechanisms differ, e.g., passive diffusion dominates the process mediated by carriers in these animals (Sandström, 1997). This fact indicates that extrapolation from rat studies to humans is invalid, and thus this animal model should not be used for iron and zinc bioavailability studies.

5.6.3 IN VITRO METHODOLOGIES FOR ESTIMATING MINERAL BIOACCESSIBILITY

In vitro techniques are quick, relatively simple, and less expensive than in vivo techniques, allowing better control of experimental variables (Wienk et al., 1999). Various methods have been developed to estimate the fraction of mineral or trace element that is bioaccessible. These techniques evaluate solubility, dialyzability, and mineral uptake by cell cultures of Caco-2 line and have also been used to identify possible physicochemical properties of foods, which may explain differences in the absorption of minerals (Shen, Robberecht, Van Dael, & Deelstra, 1995).

5.6.3.1 Mineral solubility

Solubility has been used to predict bioavailability, since the former is a prerequisite for absorption (Wienk et al., 1999). Inorganic iron salts, frequently used as fortifiers, have solubility characteristics defined under standardized conditions (pH, redox potential, etc.). However, these measurements under standard conditions cannot be extrapolated to varying conditions present in various foods (Clydesdale, 1983). In the food matrix, the solubility can be altered due to chemical changes or the presence of certain constituents such as phosphates, proteins, phytate, carboxylic acids and dietary fiber (Clydesdale & Nadeau, 1984).

Intrinsic iron solubility has been studied on gastrointestinally digested samples (Hunt et al., 1989; Pushpanjali & Santosh Khokhar, 1996), or samples subjected to different pH values (Clydesdale & Nadeau, 1984; Forbes et al., 1989). It has also been used to evaluate the effect of processing and storage time on "iron profile" (ferrous, ferric, or complex) from different sources (Galdi, Valencia, & Sambucetti, 1987; Lee & Clydesdale, 1980). However, the use of solubility to predict iron bioavailability was unsatisfactory, since iron complexes can be soluble but nonabsorbable (e.g., iron complexed with milk protein) (Jackson, 1992). In addition, these techniques showed poor correlation with in vivo studies (Forbes et al., 1989). The solubility technique has also been applied to other minerals. For instance, solubility at specific pHs after enzymatic digestion simulating the stomach and intestinal processes has been applied to measure zinc availability. Hunt et al. (1989) conducted comparative studies of zinc uptake in rats and zinc solubility after an in vitro gastrointestinal digestion and concluded that solubility did not correspond to the measured bioavailability.

Some researchers have used solubility to predict calcium bioavailability (Keane, Potter, & Sherbon, 1988). Roig, Alegría, Barberá, Farré, and Lagarda (1999) noted that this measure is more relevant to the results found in rats than dialyzability. However, while the solubility of different calcium sources can be very different, the percentage of calcium absorption of these products may not be different in humans (Sheikh, Santa Ana, Nicar, Schiller, & Fordtran, 1987). While some authors indicate that there is a good relationship between in vitro solubility and in vivo bioavailability (Pantako & Amiot, 1994; Roig et al., 1999), others support the opposite position (Ekmekcioglu, 2000; Schwartz, Belko, & Wien, 1982; Wien & Schwartz, 1983; Zemel, 1984). Zemel (1984) suggested the use of ion-soluble calcium from in vitro gastrointestinal digestion as a better bioavailability indicator than total soluble calcium. According to the results, the discrepancy between solubility and in vivo methods was attributed to the formation of very stable calcium-soluble complexes that are not absorbed.

5.6.3.2 Mineral dialyzability

Mineral dialyzability is one of the most frequently used in vitro methods. It involves pepsin digestion at acid pH (gastric digestion) followed by digestion with pancreatin and bile salts (intestinal digestion) at higher pH. The proportion of the element that diffuses through a semipermeable membrane during intestinal digestion stage represents the dialyzability of the element and is used as an estimator of the element proportion available for absorption (Miller, Schricker, Rasmussen, & Van Campen, 1981). Dialyzability methods differ from the solubility ones, since the diffusion step differentiates soluble high- and low-molecular-weight compounds. Miller and Berner (1989) concluded that dialyzable iron involving low-molecular-weight complexes is a better indicator of bioavailability than solubility. Since the bioavailability of nonheme iron depends on the food characteristics, the presence of other food constituents, and the conditions in the gastrointestinal tract, this in vitro technique can reproduce intraluminal conditions affecting mineral absorption. Other methods using continuous flow dialysis have also been developed (Larsson, Minekus, & Havenaar, 1997; Wolters et al., 1993). The latter is more representative of physiological conditions than the equilibrium dialysis method, since the dialyzable components are continuously removed from the intestinal digestion mixture. However, more investigations are needed.

Equilibrium dialysis can be used to estimate many iron-absorption promoter or inhibitor factors (Schricker, Miller, Rasmussen, & Van Campen, 1981). In addition, it has been used to examine the influence of processes on the bioavailability of food iron (Ummadi, Chenoweth, & Uebersax, 1995). Hurrell, Lynch, Trinidad, Dassenko, and Cook (1989) observed that the dialysis method correctly predicted the direction of the response but not its magnitude. Although no in vitro method can reproduce the prevailing physiological conditions of in vivo studies, dialyzability showed similar results to those obtained in human studies for iron (Schricker et al., 1981). While it has only been validated for iron, it is being used to measure availability of other minerals, such as Zn, Ca, Mg, and Cu.

Regarding zinc and calcium, several authors have noted that this technique has shown good relationship with in vivo studies (Bosscher, Van Caillie-Bertrand, et al., 2001; Bosscher, Zhengli, et al., 2001; García, Alegría, Barberá, Farré, & Lagarda, 1998; Shen, Luten, Robberecht, Bindels, & Deelstra, 1994, 1995). In this line, Kernefick and Cashman (2000) found that the dialyzability can be a useful screening method to evaluate the effect of certain dietary factors (e.g., phytates, oxalate, fiber, lactose, and caseino−phosphopeptides) that affect calcium absorption. However,

correlation with in vivo studies regarding the effect of casein and polyalcohols was not good. In a study of low-fat dairy products, Reykdal and Lee (1991) concluded that the dialyzability method is more suitable than the solubility one for the estimation of calcium bioavailability.

In any case, dialyzability requires precise standardization because it has shown intra- and interlaboratory variability (Forbes et al., 1989; Luten et al., 1996). The final pH at which the dialysis is performed and pH adjustment during gastrointestinal digestion can be a source of result variability (Bosscher, Zhengli, et al., 2001). Depending on iron's oxidation state and the presence of complexing agents, pH can cause iron precipitation and prevent its in vivo absorption and in vitro dialyzability. Optimization of this technique to achieve accurate pH adjustment and decreasing variability was performed by Wolfgor, Drago, Rodríguez, Pellegrino, and Valencia (2002), who defined the way to calculate the molarity of PIPES [piperazine-NN′-bis(2-ethane-sulfonic acid)] buffer to assure a final pH of 6.5 ± 0.2 independent of the food matrix. Thereafter, Drago, Binaghi, and Valencia (2005) proposed a pH-adjusting method of a 0.15 mol/L PIPES buffer with the same purpose.

5.6.3.3 Using cell cultures

Cultures of Caco-2 cells can be used to measure mineral uptake. This line (from human colon adenocarcinoma) undergoes spontaneous differentiation in cell culture. Once confluence is reached, a polarized monolayer of epithelial cells with many characteristics of mature enterocytes, such as brush border microvillous membrane, enzymes associated to brush border, specialized tight junctions between cells, and desmosomes is formed (Alvarez-Hernánadez, Nichols, & Glass, 1991; Pinto et al., 1983). Caco-2 cultures have been used to study iron uptake and transport in model systems (Alvarez-Hernández et al., 1991; Glahn et al., 1995; Halleux & Schneider, 1994) and in food matrices (García, Flowers, & Cook, 1996; García, Leets & Layrisse, 2000; Glahn et al., 1998; Glahn, Lee, & Miller, 1999; Glahn, Wien, Van Campen, & Miller, 1996).

Assays for studying mineral uptake from food matrices include three basic steps: digestion/sample preparation, the proper uptake assay, and measurement of captured mineral. At the stage of sample preparation, food is subjected to enzymatic digestion with stomach pepsin and intestinal pancreatin-bile digestion in order to reproduce physiological conditions. For performing capture or transport tests, the monolayer must not be damaged, so the digestive enzymes should be eliminated or their enzymatic activity reduced. Several methodological alternatives have been tested for this purpose, but this stage should still be improved.

During the uptake assay, pH should remain within a certain range in order not to alter the integrity of the monolayer. Furthermore, depending on the pH, uptake (Garcia et al., 1996) and the effect of promoters or inhibitors (Glahn et al., 1995) can be modified. The use of substances that remove the insoluble iron nonspecifically bound to the surface may be necessary (Glahn et al., 1995, 1996). Another critical factor is the level of iron used in the culture media that affects avidity of cells. The amount of iron in the test sample must be within certain levels. The concentration must not exceed the value at which the uptake is maximum, to not saturate the transport mechanisms of cells. This aspect is generally not discussed or considered in the literature. In addition, the level at which the uptake is maximized for different sources of iron should be defined (Glahn et al., 1995). The measurement of captured mineral may be performed by atomic absorption spectroscopy, through the use of radioisotopes (^{59}Fe), or by measuring ferritin after 24 h of dialysate contact with the monolayer cells. This is possible since the formation of ferritin (intracellular iron-storage protein) occurs

in response to its capture and provides a measure of its cellular uptake. According to Wienk et al. (1999), Caco-2 also cells present other disadvantages, such as:

1. They are cells of transformed nature (as they are derived from a colon carcinoma) and it remains questionable as to what extent normal metabolic processes are maintained.
2. There is an absence of the mucin layer, which is naturally present in vivo.
3. They have much higher transepithelial resistance than small intestine cells.
4. They have low expression of carriers, resulting in low transport speed.

This technique is promising, but still needs to be optimized, standardized, and validated for use in assessing mineral availability or bioaccessibility.

5.7 EFFECTS OF EMERGING TECHNOLOGIES ON MINERALS

5.7.1 HIGH-PRESSURE PROCESS

Consumers increasingly demand convenience foods of the highest quality in terms of natural flavor and taste, and which are free from additives and preservatives. This demand has triggered the need for the development of a number of nonthermal approaches to food processing, of which high-pressure (HP) technology has proven to be very valuable (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). However, the impact of HP on minerals has been little studied. Andrés, Villanueva, and Tenorio (2016) studied untreated and HP (450−650 MPa for 3 min at 20°C) milk and soy smoothies and observed that low-molecular-weight compounds like soluble sugars (glucose and fructose), organic acids (citric, malic, tartaric, oxalic, and quinic), and minerals (sodium, potassium, calcium, magnesium, iron, copper, zinc, and manganese) showed no significant changes after the treatment and storage. However, Huppertz, Kelly, and Fox (2002) reported pressure-induced shifts in mineral balance in milk. Later, Huppertz, Fox, and Andkelly (2004) demonstrated that HP treatment of milk (100−600 MPa) resulted in considerable solubilization of α1- and β-casein, probably due to solubilization of colloidal calcium phosphate and disruption of hydrophobic interactions.

On the other hand, calcium was used to improve texture in processed carrots. Sila, Smout, Vu, and Hendrickx (2004) observed that HP pretreatment (200−500 MPa for 15 min, 20−60°C) of carrots combined with $CaCl_2$ infusion improved texture during thermal processing by retarding the rate of thermal softening. Speroni, Jung, and De Lamballerie (2010) also studied the effect of calcium (2−25 mM) and HP treatment on heat gelation of soybean proteins. HP treatment had different effects on heat-induced gelation depending on the presence of calcium and on the nature of the proteins. In the absence of calcium, gels with low stiffness were formed after HP treatment, compared with untreated samples. But in the presence of calcium, gel stiffness was increased after HP treatment of dispersions containing β-conglycinin, while the opposite effect was observed for glycinin fraction.

5.7.2 HP HOMOGENIZATION

Minerals, particularly calcium, have an effect on milk proteins when dairy proteins are subjected to the effect of HP homogenization. Zamora, Trujillo, Armaforte, Waldron, and Kelly (2012) reported that conventional and ultrahigh-pressure homogenization triggered the incorporation of unbound

whey proteins in the curd and of caseins through ionic bonds involving calcium salts. These treatments provoked changes in protein interactions within rennet curds. In whey protein-depleted milk concentrate solutions, dynamic ultrahigh-pressure homogenization treatment (300 MPa) increased viscosity more pronouncedly at low mineral level (5% vs 10%, w/w). This treatment only affected protein aggregation and not casein micelle structure (Sørensen et al., 2015).

5.8 CONCLUSION

The bioavailability of minerals in foods is affected by processing, dietary factors, promoters, and inhibitors. In addition, the methodologies measuring mineral bioavailability and bioaccessibility are of critical importance. On the other hand, there are very few studies on the effect of emerging technologies on food minerals. Like other processes, emerging technologies do not affect minerals, but have an effect on the macromolecules associated with them (e.g., structure, physical properties). The impact of such physical changes on mineral bioavailability has not yet been fully elucidated, but more information is provided in Chapter 10, Interaction of Compounds.

ACKNOWLEDGMENT

To Dr. Mirta E. Valencia.

REFERENCES

Abrams, S. A., Wen, H., & Stuff, J. E. (1996). Absorption of calcium, zinc and iron from breast milk by five- to seven-month-old infants. *Pediatric Research*, *39*(3), 384–390.

ACC/SCN (1992). *Second report on the world nutrition situation*. Geneva: ACC/SCN.

Aggett, P. J., & Comerford, J. G. (1995). Zinc and human health. *Nutrition Review*, *2*, S16–S22.

Allen, L. H. (1998). Zinc and micronutrient supplements for children. *American Journal of Clinical Nutrition*, *68*(S), 495S–498S.

Allen, L.H. & Ahluwalia, N. (1997) *Improving iron status through diet. The application of knowledge concerning dietary iron bioavailability in human populations*. pdf.usaid.gov/pdf_docs/pnacb908.pdf.

Alvarez-Hernánadez, X., Nichols, G. M., & Glass, J. (1991). Caco-2 cell line: A system for studying intestinal iron transport across epithelial cell monolayers. *Biochimica et Biophysica Acta*, *1070*, 205–208.

Andraca, I., Castillo, M., & Walter, T. (1997). Desarrollo psicomotor y conducta en lactantes anémicos por deficiencia de hierro. In A. M. O'Donnell, F. E. Viteri, & E. Carmuega (Eds.), *Deficiencia de Hierro. Desnutrición oculta en América Latina*. Buenos Aires: CESNI.

Andrés, V., Villanueva, M. J., & Tenorio, M. D. (2016). Influence of high pressure processing on microbial shelf life, sensory profile, soluble sugars, organic acids, and mineral content of milk- and soy-smoothies. *LWT - Food Science and Technology*, *65*, 98–105.

AOAC (Association of Official Analytical Chemists). (2000). In W. Horwitz (Ed.), *Official methods of analysis* (17th ed). Washington, DC: AOAC.

Ballot, D., Baynes, R. D., Bothwell, T. H., Gillooly, M., Macfarlane, B. J., Macphail, A. P., . . . Bothwell, J. E. (1987). The effect of fruit juices and fruits on the absorption of iron from a rice meal. *British Journal of Nutrition*, *57*, 331–343.

Benito, P., & Miller, D. D. (1998). Iron absorption and bioavailability: And updated review. *Nutrition Research*, *18*(3), 581–603.

Bernardi, C., Drago, S. R., Sánchez, H., & Freyre, M. (2006). Formulation and sensory evaluation of *Prosopis alba* (Algarrobo) pulp cookies with increased iron and calcium dialyzabilities. *Plants Foods for Human Nutrition*, *61*(1), 37–42.

Bjorn-Rasmussen, E., Hallberg, L., & Walker, R. B. (1973). Food iron absorption in man. II Isotopic exchange of iron between labelled foods and between a food and an iron salt. *American Journal of Clinical Nutrition*, *26*, 1311–1319.

Bo-Linn, G. W., Davis, G. R., Buddrus, D. J., Morawski, S. G., Santa Ana, C., & Fordtran, J. S. (1984). An evaluation of the importance of gastric acid secretion in the absorption of dietary calcium. *Journal of Clinical Investigation*, *73*, 640–647.

Bosscher, D., Van Caillie-Bertrand, M., Robberecht, H., Van Dyck, K., Van Cauwenbergh, R., & Deelstra, H. (2001). *In vitro* availability of calcium, iron, and zinc availability from first age infant formula and human milk. *Journal of Pediatric Gastroenterology and Nutrition*, *32*, 54–58.

Bosscher, D., Zhengli, L., Van Cauwenbergh, R., Van Caillie-Bertrand, M., Robberecht, H., & Deelstra, H. (2001). A method for *in vitro* determination of calcium, iron, and zinc availability from first age infant formula and human milk. *International Journal of Food Science and Nutrition*, *52*, 173–182.

Bothwell, T. H. (1995). Overview and mechanisms of iron regulation. *Nutrition Reviews*, *53*(9), 237–245.

Brnić, M., Wegmüller, R., Melse-Boonstra, A., Stomph, T., Zeder, C., Tay, F. M., & Hurrell, R. F. (2016). Zinc absorption by adults is similar from intrinsically labelled zinc-biofortified rice and from rice fortified with labelled zinc sulfate. *Journal of Nutrition*, *146*(1), 76–80.

Brnić, M., Wegmüller, R., Zeder, C., Senti, G., & Hurrell, R. F. (2014). Influence of phytase, EDTA, and polyphenols on zinc absorption in adults from porridges fortified with zinc sulfate or zinc oxide. *Journal of Nutrition*, *144*, 1467–1473.

Cercamondi, C. I., Egli, I. M., Zeder, C., & Hurrell, R. F. (2014). Sodium iron EDTA and ascorbic acid, but not polyphenol oxidase treatment, counteract the strong inhibitory effect of polyphenols from brown sorghum on the absorption of fortification iron in young women. *British Journal of Nutrition*, *111*(3), 481–489.

Clydesdale, F. M. (1983). Physicochemical determinants or iron bioavailability. *Food Technology*, *37*(10), 133–138.

Clydesdale, F. M. (1988). Mineral interactions in foods. In C. E. Bodwell, & J. W. Erdman (Eds.), *Nutrient interactions* (pp. 73–113). New York and Basel: Marcel Dekker, Inc..

Clydesdale, F. M., & Nadeau, D. B. (1984). Solubilization of iron in cereals by milk and milk fractions. *Cereal Chemistry*, *61*(4), 330–335.

Cook, J. D. (1983). Determinants of nonheme iron absorption in man. *Food Technology*, *37*(10), 124–126.

Cook, J. D., Dassenko, S. A., & Lynch, S. R. (1991). Assessment of the role of non heme-iron availability in iron balance. *American Journal of Clinical Nutrition*, *54*, 717–722.

Cook, J. D., & Monsen, E. R. (1976). Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. *American Journal of Clinical Nutrition*, *29*, 859–867.

Cook, J. D., Morck, T. A., & Lynch, S. R. (1981). The inhibitory effect of soy products on nonheme iron absorption in man. *American Journal of Clinical Nutrition*, *34*(12), 2622–2629.

Cook, J. D., & Reusser, M. E. (1983). Iron fortification: an update. *American Journal of Clinical Nutrition*, *38*, 648–659.

Cousins, R. J. (1997). Zinc. In E. E. Ziegler, & L. J. Filer (Eds.), *Conocimientos actuales sobre nutrición* (7ma Edición). Cap 29 Washington, DC: Organización Panamericana de la Salud-Instituto Internacional de Ciencias de la Vida.

Coyle, P., Philcox, J. C., & Rofe, A. M. (1998). Zinc in man. *Clinical Biochemist Reviews*, *19*, 107–112.

Davidsson, L. (1994). Minerals and trace elements in infant nutrition. *Acta Paediatric. Suppl*, *395*(Suppl), 38–42.

Davidsson, L., Walczyk, T., Morris, A., & Hurrell, R. F. (1998). Influence of ascorbic acid on iron absorption from an iron-fortified, chocolate-flavored milk drink in Jamaican children. *American Journal of Clinical Nutrition*, *67*(5), 873–877.

De Benoist, B., Darnton-Hill, I., Davidsson, L., Fontaine, O., & Hotz, C. (2007). Conclusions of the joint WHO/UNICEF/IAEA/IZiNCG interagency meeting on zinc status indicators. *Food Nutrition Bulletin*, *28*(3 Suppl), S480–4.

Derman, D. P., Bothwell, T. H., Torrance, J. D., Bezwoda, W. R., Macphail, A. P., Kew, M. C., ... Charlton, R. W. (1980). Iron absorption from maize (*Zea mays*) and sorghum (*Sorghum vulgare*) beer. *British Journal of Nutrition*, *43*, 271–279.

Drago, S. R., Binaghi, M. J., & Valencia, M. E. (2005). Effect of gastric digestion pH on iron, zinc and calcium availability from preterm and term starting infant formulas. *Journal of Food Science*, *70*, 2, S107–112.

Drago, S. R., & Valencia, M. E. (2002). Effect of fermentation on iron, zinc and calcium availability from iron fortified dairy products. *Journal of Food Science*, *67*(8), 3130–3134.

Drago, S. R., & Valencia, M. E. (2004). Influence of components of infant formulas on in vitro iron, zinc and calcium availability. *Journal of Agriculture and Food Chemistry*, *52*, 3202–3207.

Ehrenkranz, R. A., Ackerman, B. A., Nelly, C. M., & Janghorbani, M. (1984). Determination with stable isotopes of the dietary bioavailability of zinc in premature infants. *American Journal of Clinical Nutrition*, *40*, 72–81.

Ekmekcioglu, C. (2000). Intestinal bioavailability of minerals and trace elements from milk and beverages in humans. *Nahrung*, *44*(6), S390–S397.

Fairweather-Tait, S. J. (1992). Bioavailability of trace elements. *Food Chemistry*, *43*, 213–217.

Fairweather-Tait, S. J. (1995). Iron–zinc and calcium–Fe interactions in relation to Zn and Fe absorption. Symposium on "Micronutrient interactions". *Proceedings of the Nutrition Society*, *54*, 465–473.

FAO & OMS (1992) *Conferencia Internacional sobre Nutrición. Elementos principales de estrategias nutricionales*. Roma, 5–11 de diciembre. http://www.fao.org/docrep/017/z9730s/z9730s.pdf.

Finch, C. A., & Cook, J. D. (1984). Iron deficiency. *American Journal of Clinical Nutrition*, *39*, 471–477.

Forbes, A. L., Adams, A. E., Arnaud, M. J., Chichester, C. O., Cook, J. D., Harrison, B. N., ... Wittaler, P. (1989). Comparison of in vitro, animal, and clinical determinations of iron availability: International Nutritional Anemia Consultative Group Task Force report on iron bioavailability. *American Journal of Clinical Nutrition*, *49*, 225–238.

Galán, M. G., & Drago, S. R. (2014). Effects of soy protein and calcium levels on mineral bioaccessibility and protein digestibility from enteral formulas. *Plant Foods for Human Nutrition*, *69*(3), 283–289.

Galdi, M., Bassi, M., Barrio Rendo, M. E., & Valencia, M. E. (1988). Ferric glycinate iron bioavailability determined by haemoglobin regeneration method. *Nutrition Reports International*, *38*, 591–598.

Galdi, M., Valencia, M. E., & Sambucetti, M. E. (1987). Changes in the physicochemical state of iron and nutritional deterioration in powdered infant formulas during storage. *Nutrition Reports International*, *35*, 1279–1284.

García, M. N., Flowers, C., & Cook, J. D. (1996). The Caco-2 cell culture system can be used as a model to study food iron availability. *Journal of Nutrition*, *126*, 251–258.

García, M. N., Leets, I., & Layrisse, M. (2000). β-Carotene and inhibitors of iron absorption modify iron uptake by Caco-2 cells. *Journal of Nutrition*, *130*(1), 5–9.

García, R., Alegría, A., Barberá, R., Farré, R., & Lagarda, M. J. (1998). Dialyzability of iron, zinc and cooper of different types of infant formula marketed in Spain. *Biological Trace Element Research*, *65*, 7–17.

Gibson, R. S., Bailey, K. B., Gibbs, M., & Ferguson, E. L. (2010). A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food Nutrition Bulletin*, *31*(2 Suppl), S134−46.

Gibson, R. S., & Ferguson, E. L. (1998). Assessment of dietary zinc in a population. *American Journal of Clinical Nutrition*, *68*(S), 430S−434S.

Gibson, R. S., Yeudall, F., Drost, N., Mtitimini, B., & Cullinan, T. (1998). Dietary interventions to prevent zinc deficiency. *American Journal of Clinical Nutrition*, *68*(2 Suppl), 484S−487S.

Gillooly, M., Bothwell, T. H., Torrance, J. D., Macphail, A. P., Derman, D. P., Bezwoda, W. R., . . . Mayet, F. (1983). The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. *British Journal of Nutrition*, *49*, 331−342.

Gilloly, M., Torrance, J. D., Bothwell, T. H., Macphail, A. P., Derman, D., Mills, W., & Mayet, F. (1984). The relative effect of ascorbic acid on iron absorption from soy-based and milk-based infant formulas. *American Journal of Clinical Nutrition*, *40*(3), 522−527.

Glahn, R. P., Gangloff, M. B., Van Campen, D. R., Miller, D. D., Wien, E. M., & Norvell, W. A. (1995). Bathophenanthrolenen disulfonic acid and sodium dithionite effectively remove surface-bound iron from Caco-2 cells monolayers. *Journal of Nutrition*, *125*, 1833−1840.

Glahn, R. P., Lai, C., Hsu, J., Thompson, J. F., Guo, M., & Van Campen, D. R. (1998). *Decreased citrate improves iron availability from infant formula: Application of an* in vitro *digestion/Caco-2 cell culture model*, . *Journal of Nutrition* (128, pp. 257−264).

Glahn, R. P., Lee, O. A., & Miller, D. D. (1999). In vitro digestion/Caco-2 cell culture model to determine optima ascorbic acid to iron ratio in rice cereal. *Journal of Food Science*, *64*(5), 925−929.

Glahn, R. P., Wien, E. M., Van Campen, D. R., & Miller, D. D. (1996). Caco-2 cell iron uptake from meat and casein digests parallel *in vivo* studies: use of a novel *in vitro* method for rapid estimation of iron bioavailability. *Journal of Nutrition*, *126*, 332−339.

Greer, F. R. (1989). Calcium, phosphorous, and magnesium: How much is too much for infant formulas? *Journal of Nutrition*, *119*, 1846−1851.

Greger, J. L. (1988). Calcium availability. *Cereal Food World*, *33*(9), 796−800.

Griessen, M., Cochet, B., Infante, F., Jung, A., Bartholdi, P., Donath, A., . . . Courvoisier, B. (1989). Calcium absorption from milk in lactase-deficient subjects. *American Journal of Clinical Nutrition*, *49*, 377−384.

Hallberg, L. (1981a). Bioavailability of dietary iron in man. *Annual Review of Nutrition*, *1*, 123−147.

Hallberg, L. (1981b). Bioavailable nutrient density: A new concept applied in the interpretation of food iron absorption data. *American Journal of Clinical Nutrition*, *34*, 2242−2247.

Hallberg, L., Bjorn-Rasmussen, E., Howard, L., & Rossander, L. (1979). Dietary heme iron absorption. A discussion of possible mechanisms for the absorption promoting effect of meat and for the regulation of iron absorption. *Scandinavian Journal of Gastroenterology*, *14*, 769−779.

Hallberg, L., Brune, M., Erlandsson, M., Sandberg, A. S., & Rossanser-Hultén, L. (1991). Calcium: Effect of different amounts on nonheme- and heme-iron absorption in humans. *American Journal of Clinical Nutrition*, *53*, 112−119.

Hallberg, L., & Rossander, L. (1982). Effect of soy protein on nonheme iron absorption in man. *American Journal of Clinical Nutrition*, *36*, 514−520.

Hallberg, L., Rossanser-Hultén, L., Brune, M., & Gleerup, A. (1992). Calcium and iron absorption: Mechanism of action and nutritional importance. *European Journal of Clinical Nutrition*, *46*, 317−327.

Halleux, C., & Schneider, I. J. (1994). Iron absorption by Caco 2 cells cultivated in serum-free medium as in vitro model of the human intestinal epithelial barrier. *Journal of Cellular Physiology*, *158*, 17−28.

Halterman, J. S., Kaczorowski, J. M., Aligne, C. A., Auinger, P., & Szilagyi, P. G. (2001). Iron deficiency and cognitive achievement among school-aged children and adolescents in the United States. *Pediatrics*, *107* (6), 1381−1386.

Hambidge, K. M., Krebs, N. F., Jacobs, M. A., Favier, A., Guyette, L., & Ickle, D. N. (1983). Zinc nutritional status during pregnancy: A longitudinal study. *American Journal of Clinical Nutrition*, *37*, 429–442.

Hambidge, K. M., Krebs, N. F., & Miller, L. (1998). Evaluation of zinc metabolism with use of stable-isotope techniques: Implications for the assessments of zinc status. *American Journal of Clinical Nutrition*, *68*(2 Suppl), 410S–413S.

Hatton, D., Muntzel, M., Absalon, J., Lashley, D., & Mccarron, D. A. (1991). Dietary calcium and iron: Effects on blood pressure and hematocrit in young spontaneously hypertensive rats. *American Journal of Clinical Nutrition*, *53*, 542–546.

Heaney, R. P., Recker, R. R., & Hinders, S. M. (1988). Variability of calcium absorption. *American Journal of Clinical Nutrition*, *47*, 262–264.

Heaney, R. P., Smith, K. T., Recker, R. R., & Hinders, S. M. (1989). Meal effects on calcium absorption. *American Journal of Clinical Nutrition*, *49*, 372–376.

Heaney, R. P., Weaver, C. M., & Recker, R. R. (1988). Calcium absorbability from spinach. *American Journal of Clinical Nutrition*, *47*, 707–709.

Hess, S. Y., Lönnerdal, B., Hotz, C., Rivera, J. A., & Brown, K. H. (2009). Recent advances in knowledge of zinc nutrition and human health. *Food Nutrition Bulletin*, *30*(1 Suppl), S5–11.

Hunt, J. R., & Beiseigel, J. M. (2009). Dietary calcium does not exacerbate phytate inhibition of zinc absorption by women from conventional diets. *American Journal of Clinical Nutrition*, *89*(3), 839–843.

Hunt, J. R., Beiseigel, J. M., & Johnson, L. K. (2008). Adaptation in human zinc absorption as influenced by dietary zinc and bioavailability. *American Journal of Clinical Nutrition*, *87*(5), 1336–1345.

Hunt, J. R., Johnson, P. E., & Swan, P. B. (1989). The dynamic nature of zinc availability from foods in vivo. *Biological Trace Element Research*, *19*, 119–127.

Huppertz, T., Fox, P. F., & Andkelly, A. L. (2004). High-pressure treatment of bovine milk: Effects on casein micelles and whey proteins. *Journal of Dairy Research*, *71*(1), 97–106.

Huppertz, T., Kelly, A. L., & Fox, P. F. (2002). Effects of high pressure on constituents and properties of milk. *International Dairy Journal*, *12*(7), 561–572.

Hurrell, R. F. (1984). Bioavailability of different iron compounds used to fortify formulas and cereals: Technological problems. In A. Stekel (Ed.), *Iron nutrition in infancy and childhood*. New York: Raven Press.

Hurrell, R. F. (1997). Bioavailability of iron. En: Assessment of the bioavailability of micronutrients. Proceedings of an ILSI Europe Workshop. *European Journal of Clinical Nutrition*, *51*(1), S4–S8.

Hurrell, R. F. (2004). Phytic acid degradation as a means of improving iron absorption. *International Journal for Vitamin and Nutrition Research*, *74*(6), 445–452.

Hurrell, R. F., Davidsson, L., Reddy, M., Kastenmayer, P., & Cook, J. D. (1998). A comparison of iron absorption in adults and infants consuming identical infants formulas. *British Journal of Nutrition*, *79*, 31–36.

Hurrell, R. F., Furniss, D. E., Burri, J., Whittaker, P., Lynch, S. R., & Cook, J. D. (1989). Iron fortification of infant cereals: A proposal for the use of ferrous fumarate or ferrous succinate. *American Journal of Clinical Nutrition*, *49*, 1274–1282.

Hurrell, R. F., Juillerat, M. A., Reddy, M. B., Lynch, S. P., Dassenko, A. A., & Cook, J. D. (1992). Soy protein, phytate, and iron absorption in humans. *American Journal of Clinical Nutrition*, *56*, 573–578.

Hurrell, R. F., Lynch, S. R., Trinidad, P. T., Dassenko, S. A., & Cook, J. D. (1989). Iron absorption in humans as influenced by bovine milk proteins. *American Journal of Clinical Nutrition*, *49*, 546–552.

Hurrell, R. F., Reddy, M. B., Juillerat, M. A., & Cook, J. D. (2003). Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *American Journal of Clinical Nutrition*, *77*(5), 1213–1219.

Jackson, L. S. (1992). The effect of dairy products on iron availability. *Critical Reviews in Food Science and Nutrition*, *31*(4), 259–270.

Kane, A. P., & Miller, D. D. (1984). *In vitro* estimation of the effects of selected proteins on iron bioavailability. *American Journal of Clinical Nutrition*, *39*, 393–401.

Kastenmayer, P., Davidsson, L., Galan, P., Cherouvrier, F., Hercberg, S., & Hurrell, R. F. (1994). A double stable isotope technique for measuring iron absorption in infants. *British Journal of Nutrition*, *71*, 411–424.

Kaup, S. M. (1998). Aspects of mineral bioavailability in infant nutrition. *International Dairy Journal*, *8*, 435–441.

Keane, L. A., Potter, N. N., & Sherbon, J. W. (1988). Estimation of calcium status in selected food systems. *Journal of Food Science*, *53*, 1111–1112.

Kernefick, S., & Cashman, K. D. (2000). Investigation of an in vitro model for predicting the effect of food components on calcium availability from meals. *International Journal of Food Science and Nutrition*, *51*, 45–54.

Knox, T. A., Kassrjian, Z., Dawson-Hughes, B., Golner, B. B., Dallal, G. E., Arora, S., & Russell, R. M. (1991). Calcium absorption in elderly subjects on high- and low-fiber diets: effect of gastric acidity. *American Journal of Clinical Nutrition*, *53*, 1480–1486.

Langini, S., Carbone, N., Galdi, M., Barrio Rendo, M. E., Portela, M. L., & Valencia, M. E. (1988). Ferric glycinate iron bioavailability for rats as determined by extrinsic radioisotopic labelling of infant formulas. *Nutrition Reports International*, *38*, 729–735.

Larsson, M., Minekus, M., & Havenaar, R. (1997). Estimation of the bioavailability of iron and phosphorous in cereal using a dynamic in vitro gastrointestinal model. *Journal of the Science of Food and Agriculture*, *74*, 99–106.

Layrisse, M., Martínez-Torres, C., Leets, I., Taylor, P., & Ramírez, J. (1984). Effect of histidine, cysteine, glutathione or beef on iron absorption in humans. *Journal of Nutrition*, *114*, 217–223.

Lee, K., & Clydesdale, F. M. (1979). Iron sources used in food fortification and their changes due to food processing. *Critical Reviews in Food Science and Nutrition*, *11*, 117–153.

Lee, K., & Clydesdale, F. M. (1980). Chemical changes of iron in food and drying process. *Journal of Food Science*, *45*, 711–715.

Lee, K., & Clydesdale, F. M. (1981). Effect of thermal processing on endogenous and added iron in canned spinach. *Journal of Food Science*, *46*, 1064–1068, 1073.

Lim, K. H., Riddell, L. J., Nowson, C. A., Booth, A. O., & Szymlek-Gay, E. A. (2013). Iron and zinc nutrition in the economically-developed world: A review. *Nutrients*, *5*(8), 3184–3211.

Liu, Y. M., Neal, P., Ernst, J., Weaver, C., Rickard, K., Smith, D. L., & Lemons, J. (1989). Absorption of calcium and magnesium from fortified human milk by very low birth weight infants. *Pediatric Research*, *25* (5), 496–502.

Lönnerdal, B. (1991) Concentrations, compartmentation, and bioavailability of trace elements in human milk and infant formula. In R. K. Chandra (Ed.), *Trace elements in nutrition of Children-II*. Nestle Nutrition Workshop Series (Vol. 23). New York: Nestle Ltd. Vevey/Raven Press, Ltd.

Lönnerdal, B. (2000). Dietary factors influencing zinc absorption. *Journal of Nutrition*, *130*, 1378S–1383S.

Lönnerdal, B., Cederblad, A., Davidsson, L., & Sandström, B. (1984). The effect of individual components of soy formula and cows' milk formula on zinc bioavailability. *American Journal of Clinical Nutrition*, *40*, 1064–1070.

Lönnerdal, B., Yuen, M., & Huang, S. (1994). Calcium, iron, zinc, copper and manganese bioavailability from infant formulas and weaning diets assessed in rat pups. *Nutrition Research*, *14*(10), 1535–1548.

Luten, J., Crews, H., Flynn, A., Van Dael, P., Kastenmayer, P., Hurrell, R., … Frohlich, W. (1996). Interlaboratory trial on the determination of the in vitro iron dialysability from food. *Journal of the Science of Food and Agriculture*, *72*, 415–424.

Lynch, S. R. (1997). Interaction of iron with other nutrients. *Nutrition Review*, *55*(4), 102–110.

Lynch, S. R., Dasenko, S. A., Cook, J. D., Juillerat, M. A., & Hurrell, R. F. (1994). Inhibitory effect of a soybean-protein-related moiety on iron absorption in humans. *American Journal of Clinical Nutrition*, *60*, 567−572.

Macfarlane, B. J., Van Der Riet, W. B., Bothwell, R. D., Baynes, R. D., & Siengenberg, D. (1990). Effect of traditional oriental soy products on iron absorption. *American Journal of Clinical Nutrition*, *51*, 873−880.

Martínez, C., Ros, G., Periago, M. J., & López, G. (1999). Biodisponibilidad del hierro de los alimentos. *Arch Latinoam Nutr*, *49*(2), 106−113.

Martínez-Torres, C., Leets, I., Taylor, P., Ramírez, J., Camacho, M. V., & Laryisse, M. (1986). Heme, ferritin and vegetable iron absorption in human from meals denatured of heme iron during the cooking of beef. *Journal of Nutrition*, *116*, 1720−1725.

Martinez-Torrez, C., Romano, E. L., & Layrisse, M. (1981). Effect of cysteine on iron absorption in man. *American Journal of Clinical Nutrition*, *34*, 322−327.

Mcphail, A. P., Patel, R. C., Bothwell, T. H., & Lamparelli, R. D. (1994). EDTA and the absorption of iron from food. *American Journal of Clinical Nutrition*, *59*, 644−648.

Miller, D., & Berner, L. A. (1989). Is solubility *in vitro* a reliable predictor of iron bioavailability?. *Biological Trace Element Research*, *19*, 11−24.

Miller, D. D., Schricker, B. R., Rasmussen, R. R., & Van Campen, D. (1981). An *in vitro* method for estimation of iron availability from meals. *American Journal of Clinical Nutrition*, *34*, 2248−2256.

Milner, J. A. (1990). Trace minerals in the nutrition of children. *Journal of Pediatrics*, *117*, S147−155.

Moore, C. V., & Dubach, R. (1951). Observations on the absorption of iron from foods tagged with radio-iron. *Transactions of the Association of American Physicians*, *64*, 245−256.

Muthayya, S., Rah, J. H., Sugimoto, J. D., Roos, F. F., Kraemer, K., & Robert, R. E. (2013). The global hidden hunger indices and maps: an advocacy tool for action. *PLoSOne*, *8*(6), e67860. Available from http://dx.doi.org/10.1371/journal.pone.0067860.

Nair, M. K., Augustine, L. F., & Konapur, A. (2016). Food-based interventions to modify diet quality and diversity to address multiple micronutrient deficiency. *Front Public Health*, *5*(3), 277.

Naito, H., Gunshin, H., & Noguchi, T. (1989). Bioavailability of calcium affected by luminal and mucosal factors in the small intestine. In D. Southgate, I. Johnson, & G. R. Fenwick (Eds.), *Nutrient availability: Chemical & biological aspects*. Cambridge: Royal Society of Chemistry.

O'dell, B. L. (1989). Mineral interactions relevant to nutrient requirements. *Journal of Nutrition*, *119*, 1832−1838.

Pabón, M. L., & Lönnerdal, B. (1993). Effect of citrate on zinc bioavailability from milk fractions and infant formulas. *Nutrition Research*, *13*, 103−111.

Pantako, T. O., & Amiot, J. (1994). Correlation between in vitro solubility and absorption in rat of calcium, magnesium and phosphorous from milk protein diets. *Science des Aliments*, *14*(2), 139−158.

Phillippy, B. Q., & Wyatt, C. J. (2001). Degradation of phytate in foods by phytases in fruit and vegetable extracts. *Journal of Food Science*, *66*(4), 535−539.

Pinto, M., Robine-León, S., Apia, M. D., Dedinger, M., Triadol, N., Dussaulx, E., ... Zweibaum, A. (1983). Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Biollogy of the Cell*, *47*, 323−330.

Portela, M.L.P.M. (2015) *Aspectos nutricionales de Vitaminas y Minerales en el siglo XXI*. Asociación Argentina de Tecnólogos Alimentarios, Buenos Aires, Argentina.

Pushpanjali, & Khokhar, S. (1996). In vitro availability of iron and zinc from some Indian vegetarian diets: correlations with dietary fibre and phytate. *Food Chemistry*, *56*(2), 111−114.

Rastogi, N. K., Raghavarao, K. S., Balasubramaniam, V. M., Niranjan, K., & Knorr, D. (2007). Opportunities and challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition*, *47*, 69−112.

Reddy, M., & Cook, J. (1991). Assessment of dietary determinants of nonheme iron absorption in humans and rats. *American Journal of Clinical Nutrition*, *54*, 723−728.

Reykdal, O., & Lee, K. (1991). Soluble, dialyzable and ionic calcium in raw and processed skim milk, whole milk and spinach. *Journal of Food Science*, *56*(3), 864−866.

Roig, M. J., Alegría, A., Barberá, R., Farré, R., & Lagarda, M. J. (1999). Calcium bioavailability in human milk, cow milk and infant formulas—comparison between dialysis and solubility methods. *Food Chemistry*, *65*, 353−357.

Sandström, B. (1997). Bioavailability of zinc. *European Journal of Clinical Nutrition*, *561*(S1), S17−S19.

Sandström, B., Arvidsson, B., Cederblad, A., & Björn-Rasmussen, E. (1980). Zinc absorption from composite meals. I. The significance of wheat extraction rate, zinc calcium and protein content in meals based on bread. *American Journal of Clinical Nutrition*, *33*, 739−745.

Sandström, B., & Cederblad, A. (1980). Zinc absorption from composite meals. II. Influence of the main protein source. *American Journal of Clinical Nutrition*, *33*, 1778−1783.

Sandström, B., Fairweather-Tait, S., Hurrell, R., & Van Dokkum, W. (1993). Methods for studying mineral and trace element absorption in human using stable isotopes. *Nutrition Research Reviews*, *6*, 71−95.

Sandström, B., Keen, C. L., & Lönnerdal, B. (1983). An experimental model for studies of zinc bioavailability from milk and infant formulas using extrinsic labeling. *American Journal of Clinical Nutrition*, *38*, 420−448.

Scheers, N., Rossander-Hulthen, L., Torsdottir, I., & Sandberg, A. S. (2016). Increased iron bioavailability from lactic-fermented vegetables is likely an effect of promoting the formation of ferric iron (Fe^{3+}). *European Journal of Nutrition*, *55*(1), 373−382.

Schricker, B. R., Miller, D. D., Rasmussen, R. R., & Van Campen, D. (1981). A comparison of *in vivo* and *in vitro* methods for determining availability of iron from meals. *American Journal of Clinical Nutrition*, *34*, 2257−2263.

Schwartz, R., Belko, A. Z., & Wien, E. M. (1982). An *in vitro* system for measuring intrinsic dietary mineral exchangeability: Alternative to intrinsic isotopic labeling. *Journal of Nutrition*, *112*, 497−504.

Serfass, R. E., Ziegler, E. E., Edwards, B. B., & Houk, R. S. (1989). Intrinsic and extrinsic stable isotopic zinc absorption by infants from formulas. *Journal of Nutrition*, *119*, 1661−1669.

Shears, G. E., Ledward, D. A., & Neale, R. I. (1987). Iron complexation to carboxyl groups in a bovine serum albumin digest. *International Journal of Food Science and Technology*, *22*, 265−272.

Sheikh, M. S., Santa Ana, C. A., Nicar, M. J., Schiller, L. R., & Fordtran, J. S. (1987). Gastrointestinal absorption of calcium from milk and calcium salts. *New England Journal of Medicine*, *27*, 532−536.

Shen, L., Luten, J., Robberecht, H., Bindels, J., & Deelstra, H. (1994). Modification of an *in-vitro* method for estimating the bioavailability of zinc and calcium from foods. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *199*, 442−445.

Shen, L., Robberecht, H., Van Dael, P., & Deelstra, H. (1995). Estimation of the bioavailability of zinc and calcium from human, cow's, goat, and sheep milk by an *in vitro* method. *Biological Trace Element Research*, *49*, 107−118.

Sila, D. N., Smout, C., Vu, T. S., & Hendrickx, M. E. (2004). Effects of highpressure pretreatment and calcium soaking on the texture degradation kinetics of carrots during thermal processing. *Journal of Food Science*, *69*(5), E205−E211.

Smith, K. T. (1983). Effects of chemical environment on iron bioavailability measurements. *Food Technology*, *37*(10), 115−120.

Solomons, N. W., & Ruz, M. (1997). Zinc and iron interaction: Concepts and perspectives in the developing world. *Nutrition Research*, *17*(1), 177−185.

Sørensen, H., Mortensen, K., Sørland, G. H., Hofmann Larsen, F., Paulsson, M., & Ipsen, R. (2015). Dynamic ultra-high pressure homogenisation of whey protein-depleted milk concentrate. *International Dairy Journal*, *46*, 12−21.

South, P. K., & Miller, D. D. (1998). Iron binding by tannic acid: Effects of selected ligands. *Food Chemistry*, *63*(2), 167−172.

Sparacino, G., Shames, D. M., Vicini, P., King, J. C., & Cobelli, C. (2002). Double isotope tracer method for measuring fractional zinc absorption: Theoretical analysis. *American Journal of Physiology - Endocrinology and Metabolism*, *282*(3), 679−687.

Speroni, F., Jung, S., & De Lamballerie, M. (2010). Effects of calcium and pressure treatment on thermal gelation of soybean protein. *Journal of Food Science*, *75*, 30−38.

Stekel, A., Olivares, M., Pizarro, F., Chadud, P., López, I., & Amar, M. (1986). Absorption of fortification iron from milk formulas in infants. *American Journal of Clinical Nutrition*, *43*, 917−922.

Svanberg, U., Lorri, W., & Sandberg, A. S. (1993). Lactic fermentation of nontannin and high-tannin cereals: Effects on *in vitro* estimation of iron availability and phytate hydrolysis. *Journal of Food Science*, *58*(2), 408−412.

Taylor, P. G., Martinez-Tórrez, C., Romano, E., & Layrisse, M. (1986). The effect of cysteine-containing peptides released during meat digestion on iron absorption in humans. *American Journal of Clinical Nutrition*, *43*, 68−71.

Teucher, B., Olivares, M., & Cori, H. (2004). Enhancers of iron absorption: Ascorbic acid and other organic acids. *International Journal of Vitamin and Nutrition Research*, *74*(6), 403−419.

Thankachan, P., Walczyk, T., Muthayya, S., Kurpad, A. V., & Hurrell, R. F. (2008). Iron absorption in young Indian women: The interaction of iron status with the influence of tea and ascorbic acid. *American Journal of Clinical Nutrition*, *87*, 881−886.

Tran, C. D., Gopalsamy, G. L., Mortimer, E. K., & Young, G. P. (2015). The potential for zinc stable isotope techniques and modelling to determine optimal zinc supplementation. *Nutrients*, *7*(6), 4271−4295.

Ummadi, P., Chenoweth, W. L., & Uebersax, M. A. (1995). The influence of extrusion processing on iron dialyzability, phytates and tannins in legumes. *Journal of Food Processing and Preservation*, *19*, 119−131.

Van Campen, D. (1983). Iron bioavailability techniques: An overview. *Food Technology*, *37*(10), 127−132.

Van Dokkum, W. (1992). Significance of iron bioavailability for iron recommendations. *Biological Trace Element Research*, *35*, 1−11.

Villa, I., Arizmendiarrieta, C., & Morán, J. (1990). Zinc y cobre: Importancia en nutrición infantil. *Acta Pediatrica Española*, *48*(6), 355−359.

Watzke, H. J. (1998). Impact of processing on bioavailability examples of minerals in foods. *Trends in Food Science & Technology*, *9*, 320−327.

Whittaker, P. (1998). Iron and zinc interactions in humans. *American Journal of Clinical Nutrition*, *68*(2 Suppl), 442−446.

Whittaker, P., & Vanderveen, J. E. (1990). Effect of EDTA on the bioavailability to rats of fortification iron used in Egyptian balady bread. *British Journal of Nutrition*, *63*, 587−595.

WHO (2008). *World Prevalence of Anaemia 1993−2005 Who Global Database on Anaemia*. Geneva: World Health Organization.

Wien, E. M., & Schwartz, R. (1983). Comparison of in vitro and in vivo measurements of dietary Ca exchangeability and bioavailability. *Journal of Nutrition*, *133*, 388−393.

Wienk, K. J. H., Marx, J. J. M., & Beynen, A. C. (1999). Review. The concept of iron bioavailability and its assessment. *European Journal of Nutrition*, *38*, 51−75.

Wise, A. (1995). Phytate and zinc bioavailability. *International Journal of Food Science and Nutrition*, *46*, 53−63.

Wolfgor, R., Drago, S. R., Rodríguez, V., Pellegrino, N., & Valencia, M. (2002). *In vitro* measurement of available iron in fortified foods. *Food Research International*, *35*, 85−90.

Wolfgor, R., Rodríguez, V., Pellegrino, N. & Valencia, M. (1995). *Biodisponibilidad de hierro in vitro: estudio de variables metodológicas y su aplicación a infusiones de yerba mate, te, sus mezclas con leche y cacao en leche*. Conferencia Internacional en Biodisponibilidad de Nutrientes. 1-3 de Marzo. Quito Ecuador.

Wolters, M. G. E., Schreuder, H. A. W., Van Den Heuvel, G., Van Lonkhuijsen, H. J., Hermus, R. J. J., & Voragen, A. G. J. (1993). A continuous *in vitro* meted for estimation of the bioavailability of minerals and trace elements in foods: application to breads varying in phytic acid content. *British Journal of Nutrition*, *69*, 849−861.

Yip, R., Reeves, J. D., Lönnerdal, B., Keen, C. L., & Dallman, P. R. (1985). Does iron supplementation compromise zinc nutrition in healthy infants? *American Journal of Clinical Nutrition*, *42*, 683−687.

Zamora, A., Trujillo, A. J., Armaforte, E., Waldron, D. S., & Kelly, A. L. (2012). Effect of fat content and homogenization under conventional or ultra-high-pressure conditions on interactions between proteins in rennet curds. *Journal of Dairy Science*, *95*(9), 4796−4803.

Zemel, M. B. (1984). *In vitro* evaluation of the effects of ortho-, tripoly-and hexameta- phosphate on zinc, iron and calcium bioavailability. *Journal of Food Science*, *49*, 1562−1565.

This page intentionally left blank

CHAPTER

VITAMINS

6

Amadeo Gironés-Vilaplana[1], Débora Villaño[2], Javier Marhuenda[2], Diego A. Moreno[3] and Cristina García-Viguera[3]

[1]University Miguel Hernández, Orihuela, Alicante, Spain [2]Universidad Católica San Antonio de Murcia (UCAM), Murcia, Spain [3]CEBAS-CSIC, Murcia, Spain

6.1 INTRODUCTION

Vitamins are wide a group of organic compounds, generally unable to be synthesized by the human body but necessary for the correct maintenance of its normal functions. Under normal conditions, we are able to obtain the different vitamins from food and correct nutrition, but often minimum nutritional requirements are not met, which means they have to be obtained through supplements. Vitamins are essential to metabolism as well as for the growth and the proper function of the body. Only vitamin D is produced by the body; all other vitamins are obtained from food.

Many diseases are related to the lack of a specific vitamin; e.g., scurvy (vitamin C) or beriberi (vitamin D) (García, 2009). In general, organic disorders related to vitamins may refer to:

- Avitaminosis: total lack of one or more vitamins.
- Hypovitaminosis: partial lack of vitamins.
- Hypervitaminosis: excess accumulation of one or more vitamins, especially those that are poorly soluble in water and therefore difficult to be excreted by urine.

Depending on their solubility, vitamins can be classified into two groups: water-soluble vitamins and fat-soluble vitamins. The first group comprises B group vitamins and vitamin C, while the second group is formed by vitamin A, vitamin D, vitamin E, and vitamin K (García, 2009).

Therefore the biosynthesis and physiological role of both soluble and insoluble vitamins will be discussed in this chapter including a summary of the well-known traditional role of vitamins as well as a more extensive overview of the latest evidence or discoveries of the health effects of vitamins.

Better knowledge of the effect of emerging technologies on food vitamins is also necessary. These new methods allow the processing of foods below temperatures used during thermal pasteurization, so flavors, essential nutrients, and vitamins undergo minimal or no changes. Foods can be nonthermally processed by ionizing radiation, cold plasma, irradiation, high hydrostatic pressure, antimicrobials, ultrasound, filtration, and electrical methods such as pulsed electric field, light pulse, and oscillating magnetic field. Thus we will also discuss how these emerging technologies affect food vitamins.

Nutraceutical and Functional Food Components. DOI: http://dx.doi.org/10.1016/B978-0-12-805257-0.00006-5

Demand for rapid, specific, and simple methodologies to determine vitamins is growing because of their importance for health. Several papers have been published concerning the separation and quantification of vitamins by simpler methodologies, which differ depending on whether water-soluble or fat-soluble vitamins are being analyzed.

The stability and bioaccessibility of vitamins into food systems is often compromised by factors such as low permeability and/or solubility within the gut, lack of stability (temperature and oxygen), and the technology used during food processing, as well as by factors in the gastrointestinal tract (pH, enzymes, presence of other nutrients). For this reason it is interesting to understand the emerging technologies that can improve the stability and bioaccessibility of water- and fat-soluble vitamins. The use of vitamins as preservatives will be also discussed in this chapter, as well as other mechanisms of action by which they can be used as additives to increase shelf-life of food products.

In addition to vitamin biosynthesis we discuss the physiological role and health effects, effects of emerging technologies, innovative techniques for extraction and analytical procedures, stability and bioaccessibility, and applications and impacts on shelf-life of food products. We also discuss the future challenges and opportunities for vitamins in today's healthier world.

6.2 BIOSYNTHESIS

The biosynthesis of vitamins has been widely studied by the research community (Bacher et al., 2001; Begley, Xi, Kinsland, Taylor, & Mclafferty, 1999; Dewick, 2002; Du, Wang, & Xie, 2011; Goodman, 1969; Halliwell, 1996; Hancock, Galpin, & Viola, 2000; Jäpelt & Jakobsen, 2013; Munné-Bosch & Alegre, 2002; Napoli, 1999; Velíšek & Cejpek, 2007). Nevertheless, a brief introduction is needed here to introduce the following more relevant sections.

6.2.1 WATER-SOLUBLE VITAMINS

6.2.1.1 Vitamin B Family

The family of vitamin B is wide and extensive and is comprised of vitamin B_1 (thiamin), vitamin B_2 (riboflavin), vitamin B_3 (nicotinamide), vitamin B_5 (pantothenic acid), vitamin B_6 (piridoxine), vitamin B_8 (biotin), vitamin B_9 (folacin), and vitamin B_{12} (cobalamins). Detailed information on the biosynthesis of each vitamin is available elsewhere (references included throughout) and therefore only the main characteristics of the synthesis of these compounds are discussed in this chapter. Most can be synthetized by microorganisms and by some plants, and to a lesser degree, by animals.

6.2.1.2 Vitamin B_1 (Thiamin)

The synthesis of thiamin by microorganisms occurs as a whole or in two parts: synthesis of only the pyrimidine or of only the thiazole part. Thus microorganisms need the part they cannot synthesize by themselves (Hohmann & Meacock, 1998). In the case of higher plants, thiamin is synthesized de novo. On the other hand, animals can only develop these phosphorylation reactions in the formation of thiamin. The pyrimidine half and the thiazole half of thiamin are formed along the thiamin formation route. However, they are synthesized in two different ways. A methylene group is

required to lead to the formation of thiamin phosphate and is transformed into thiamin diphosphate (Velíšek & Cejpek, 2007). A large number of enzymes are involved in the different steps of the thiamin synthesis route (Du et al., 2011).

6.2.1.3 Vitamin B_2 (Riboflavin)

The main substrate for the synthesis of riboflavin is guanosine 5′-triphosphate (Bacher et al., 2001), which suffers hydrolysis on the C-8, and is removed from the chain. The following reactions include some dephosphorization steps and condensation of dephosphorylated metabolites, which leads to both 5-amino-6-(1-deoxy-d-ribitol-1-yl)-amino-(1H,3H)-pyrimidine-2,4-dione (which will be recycled) and riboflavin as the final product (Bacher et al., 2001).

6.2.1.4 Vitamin B_3 (Nicotinamide)

Vitamin B_3 is the amine of nicotinic acid (also known as vitamin PP). As nicotinamide adenine dinucleotides (NAD +, NADH, NADP +, and NADPH), nicotinamide exerts coenzyme functions in redox reactions on the organism (Begley et al., 1999). Bacteria are able to form NADP + from L-aspartic acid and 1,3-dihydroxyacetone phosphate (Begley et al., 1999), which is catalyzed by L-aspartate oxidase. The resulting metabolite is iminosuccinic acid. The subsequent isomerization and condensation reactions lead to the synthesis of imine, which is transformed into NADP + before other chemical reactions (involving the formation of quinolinic acid as the previous metabolite). In many plants, the precursor of NADP + formation route is L-tryptophan (Begley et al., 1999). The beginning of the route is different than that in bacteria, and leads to the formation of quinolinic acid and the subsequent synthesis of NADP + by NAD + kinase.

6.2.1.5 Vitamin B_5 (Pantothenic Acid)

Vitamin B_5, pantothenic acid, is present in nature only as D- or (R)-enantiomer (Begley et al., 1999). In plants and microorganisms, the route of pantothenic acid synthesis begins from 3-methyl-2-oxobutanoic acid, a precursor of valine (Begley et al., 1999). Secondary metabolites of pantothenic acid formation are 2-oxopantoic acid and its reduced metabolite, (R)-pantoic acid (formed from pantoic acid and β-alanine), which lead to the final synthesis of pantothenic acid.

6.2.1.6 Vitamin B_6 (Piridoxine)

The family of vitamin B_6 is comprised of three 3-hydroxy-2-methylpyridine derivatives (pyridoxol, pyridoxal, and pyridoxamine) and their 5′-phosphates (Velíšek & Cejpek, 2007). The active forms of vitamin B_6 are pyridoxal-5′-phosphate and pyridoxamine-5′-phosphate, but other bioactive forms are pyridoxol-5′-phosphate, pyridoxal, pyridoxamine, and pyridoxol. Its latest form and its phosphate are mainly found in plants, whereas pyridoxamine and their phosphates are mainly found in animals. The main catabolite, pyridoxal-5′-phosphate, exerts its action on many reactions related to amino acids and metabolism of fats and sugars as decarboxylation, deamination, racemization, transamination, and transculturation. The initial form of vitamin B_6 in prokaryotes is pyridoxol-5′-phosphate (Drewke & Leistner, 2001). Apparently, it is synthesized from one molecule of 1-deoxy-D-xylulose 5-phosphate and one molecule of 2-amino-2-deoxy-D-threo-tetronic.

6.2.1.7 Vitamin B_8 (Biotin)

Vitamin B_8, specifically the bioactive isomer (3aS,4S,6aR) of biotin, is essential to all existing cells (Velíšek & Cejpek, 2007). In the majority of microorganisms, apart from plants and fungi, the biotin-synthesis ability has been kept throughout evolution (De Leenheer & Lambert, 2000). Pimelic acid is the starter metabolite, and is converted to pimeloyl-CoA, which reacts with L-alanine, leading to 8-amino-7-oxopelargonic acid. The final step to finish the synthesis of biotin is the formation of dethiobiotin, which will be transformed to biotin by biotin synthase.

6.2.1.8 Vitamin B_9 (Folic Acid)

Folic acid (folate, vitamin B9) is a common name for the biologically active folates (Hancock et al., 2000), a conjugate of 4-aminobenzoic acid and L-glutamic acid. The biologically active form of folic acid is tetrahydrofolic acid (TH_4-folate), with similar function as cobalamins. The 7,8-dihydrofolic acid can be synthesized from guanosine 5′-triphosphate by various microorganisms and plants (Hancock et al., 2000). Enough folates must be incorporated through the diet, because in mammals, tetrahydrofolic acid is obtained via dihydrofolic acid—by the dihydrofolate reductase (Velíšek & Cejpek, 2007).

6.2.1.9 Vitamin B_{12} (Cobalamins)

The synthesis of vitamin B_{12} and its constituents can be defined in two ways (Velíšek & Cejpek, 2007). As a whole, it could be defined as a group of cobalt-containing organometallic compounds (including 5,6-dimethylbenzimidazole), the so-called cobalamins. Biological forms of vitamin B_{12} include cyanocobalamin (vitamin B_{12}), aquacobalamin (vitamin B_{12a}), hydroxocobalamin (vitamin B_{12b}), and the two metabolically active coenzyme forms of vitamin B_{12}, methylcobalamin (methylvitamin B_{12}) and 5′-deoxyadenosylcobalamin (adenosylvitamin B_{12}). More specifically, the term vitamin B_{12} is used to refer to only one of these forms, cyanocobalamin, which is the principal vitamin B_{12} form used for foods and in nutritional supplements.

6.2.1.10 Vitamin C

Vitamin C is the common name of the structure of L-ascorbic acid (AA), which with a reversible reaction of oxidation-reduction results in its oxidized form, L-dehydroascorbic acid (DHAA). Both structures possess vitamin bioactivity and hence the addition (AA + DHAA) can be considered vitamin C as a whole in terms of biological activity. The latter also has vitamin bioactivity (Fig. 6.1) (Halliwell, 1996). The biosynthesis of L-ascorbic acid is different in yeasts, plants, and animals. Yeasts synthesize D-erythroascorbate from D-arabinose, whereas plants synthesize vitamin C from

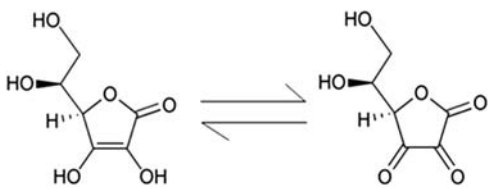


FIGURE 6.1

General structure of vitamin C: ascorbic acid (AA) and dehydroascorbic acid (DHAA).

GDP-D-mannose and animals synthesize vitamin C from UDP-D-glucouronate (Drouin, Godin, & Page, 2011). The synthesis proposed in plants is comprised of the conversion to carbonyl group, water removal and its readdition on the opposite side of the chain, and finally, to reduction mediated by GDP-mannose-3,5-isomerase of C-4 carbonyl group, leading to GDP-L-galactose. Later, the L-galactose dehydrogenase leads to L-galactono-1,4-lactone, which is converted to L-2-oxogalactono-1,4-lactone and isomerized to L-ascorbic acid (Velíšek & Cejpek, 2007).

6.2.2 FAT-SOLUBLE VITAMINS

Fat-soluble vitamins include many secondary bioactive compounds: Vitamins A and D, including their provitamins (provitamins A and D), lead to terpenoids and steroids, derived from the mevalonate and deoxyxylulose phosphate pathways. On the other hand, vitamins E and K are secondary metabolites of the shikimate pathway that further produce aromatic amino acids and phenylpropanoids.

6.2.2.1 Vitamin A

The vitamin A family includes vitamin A_1 (retinol) and vitamin A_2 (dehydroretinol). These vitamins and their biologically active metabolites are known as retinoids (Velíšek & Cejpek, 2007). Mammals do not synthesize retinoids de novo, so they depend on the dietary intake of retinol, derivatives, and precursors (provitamins A). Provitamins A are thoroughly distributed in plants and microorganisms, and they are able to boost the biosynthesis of carotenoids, which takes place in the plastids (Dewick, 2002). Retinoids are key metabolites of carotenoids, as carotenes (β-carotene, α-carotene, and γ-carotene) and xanthophyls (β-cryptoxanthin and echinenone), and have at least one nonhydroxylated β-ring (Velíšek & Cejpek, 2007). The biosynthesis of vitamin A takes place on the human intestine from provitamin A in two stages: First, β-carotene suffers a cleavage leading to two molecules of retinal and, after that, retinal is reduced to retinol. Then retinol is primarily esterified with long-chain fatty acids, and the subsequent retinyl esters are transported from the intestine via the intestinal lymphatics (Goodman, 1969).

There are different natural compounds thoroughly distributed in plants and microorganisms, as carotenoids, that are synthesized within the plastids in vegetal cells. They are considered as "provitamins A" because in the intestinal tract of humans their structures are transformed into metabolites readily to be absorbed that have a physiological function similar to vitamin A (Dewick, 2002).

6.2.2.2 Vitamin D

Vitamin D has been produced by plants and animals almost since the beginning of time. The two main forms of vitamin D are cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2). The main difference is in the chain, where vitamin D_2 has a C22−C23 double bound and an additional methyl group at C24 (Jäpelt & Jakobsen, 2013). Animals synthesize vitamin D from 7-dehydrocholesterol by exposure to sunlight in a photochemical reaction. In this way they can meet the daily requirements of this vitamin.

However, cholecalciferol cannot be considered the active form of vitamin D, since there are previous steps required for the activation of vitamin D (Velíšek & Cejpek, 2007). The first step is the transport of cholecalciferol from the skin to the liver by the vitamin D binding protein, which is

hydrolyzed to calcidiol (25-hydroxyvitamin D_3) by the cholecalciferol 25-hydroxylase. Calcidiol should be changed by calcifediol (25-hydroxycholecalciferol).

Finally, the formation of vitamin D_2 could be mainly due to the presence of ergosterol, a compound found in plants and fungi (including *Saccharomyces cerevisiae* yeasts and higher fungi), in a similar process as the formation of cholecalciferol from 7-dehydrocholesterol (Mattila, Piironen, Uusi-Rauva, & Koivistoinen, 1994).

6.2.2.3 Vitamin E

The main form of vitamin E in higher plants is α-tocopherol, which is generally synthesized in plastids, while γ-tocopherol is the predominant form in tissues without chloroplasts (Munné-Bosch & Alegre, 2002). The family of vitamin E includes bioactive lipid-soluble compounds commonly called tocochromanols. Tocochromanols are a group of four tocopherols and four tocotrienols produced at various levels and in different combinations by all plant tissues and some cyanobacteria. Although tocochromanols are only synthesized by photosynthetic organisms, most of our understanding of their chemistry and function comes from studies in artificial membranes and animal systems because of the vitamin E activity of tocochromanols in animal diets (DellaPenna & Pogson, 2006).

The biosynthesis of tocopherols is dependent on two main routes (Dewick, 2002): the nonmevalonate isoprenoid and the shikimate routes. The first route renders a hydrophobic side-chain, from the all-trans geranylgeranyl diphosphate molecule, in a reaction catalyzed by the enzyme geranylgeranyl reductase. The second route (shikimate route) leads to the chromanol ring via 4-hydroxyphenylpyruvic acid and homogentisic acid. Two enzymes, the 4-hydroxyphenylpyruvate decarboxylase and the phytyltransferase, catalyze reactions of hydroxylation, relocation of the side-chain, and decarboxylation. Then, the 2-methyl-6-phytylhydroquinone methyltransferase leads to the formation of 2,3-dimethyl-5-phytylhydroquinone, which leads to γ-tocopherol by phytylquinol cyclase. Finally, a second methylation leads to α-tocopherol due to γ-tocopherol methyltransferase. In the last place, the biosynthesis of tocotrienol is catalyzed by the condensation of homogentisic acid and geranylgeranyl diphosphate producing 2-methyl-6-geranylgeranylhydroquinone, leading in turn to γ-tocotrienol. Sequentially, methylation reaction primes the formation of α-tocotrienol or δ-tocotrienol and the final synthesis of β-tocotrienol.

6.2.2.4 Vitamin K

Vitamin K is comprised of vitamin K_1 (phylloquinone or phytylmenaquinone) originally formed in plants and vitamin K_2 (menaquinones) synthesized by microorganisms and green/brown algae. While phylloquinone possess a diterpenoid structure, menaquinone refers to a group of compounds with 1-13 isoprene units in the skeleton. The intake of vitamin K_1 can be achieved with the consumption of dark leafy vegetables. Vitamin K_2 can be produced by the intestinal microflora at small quantities (Velíšek & Cejpek, 2007).

Vitamin K_1 and vitamin K_2 are produced by isochorismic acid route (Dewick, 2002), derived from isomerization of chorismic acid by isochorismate synthase and followed by the incorporation of 2-oxoglutaric acid, forming naphthoquinone skeleton. The process continues as follows: The decarboxylation reaction of 2-oxoglutaric acid is catalyzed by thiamine diphosphate, due to the formation of thiamine diphosphate anion and its attack (Michael-type reaction) on isochorismic acid, which leads to o-succinylbenzoic acid (Dewick, 2002). The activation of this compound is carried

out by o-succinylbenzoate-CoA ligase, leading to a coenzyme A ester. The naphthoate synthase catalyze the following Dieckmann-like condensation leading to ring formation and the synthesis of 1,4-dihydroxy-2-naphthoic acid as a mayor compound. The final step is the formation of alkylated naphthoquinone by 1,4-dihydroxy-2-naphthoic acid, which includes alkylation with phytyl or polyprenyl diphosphate, decarboxylation, and oxidation to p-quinone, with the final methylation at C2.

6.3 HEALTH EFFECTS OF VITAMINS

Vitamins are essential molecules for humans, as they cannot be synthesized by the body in sufficient amounts, with the exception of vitamin D. Vitamins are essential nutrients from food sources that have health effects and play a physiological role in human body (Table 6.1). They are required for specific metabolic reactions within cells and are not catabolized for cell-energy requirements, nor do they have plastic or structural functions. Small doses of vitamins are needed and thus the recommendations are low (from micrograms to milligrams), but their absence can cause a specific deficiency syndrome. Most of them cannot be used in the way they are absorbed but have to be biotransformed to their active form.

6.3.1 WATER-SOLUBLE VITAMINS

6.3.1.1 Vitamin B_1 (Thiamin)

This vitamin forms part of the coenzyme thiamine pyrophosphate, which is involved in the energy metabolism pathways of cells, as the tricarboxylic acid cycle and the pentose synthesis cycle. Specifically, thiamine is involved in the oxidative decarboxylation of alpha-keto acids, which includes the oxidative conversion of pyruvate to acetyl-CoA. It also participates as a coenzyme of transketolase, the enzyme that catalyzes the exchange reactions of two carbon fragments in the oxidation of glucose via the hexose monophosphate (Kochetov & Solovjeva, 2014).

Deficiency of vitamin B_1 is observed in cases of malnourishment and chronic alcoholism. A deficit of this vitamin causes a condition called beriberi, which affects the heart and neurological system. Symptoms include peripheral neuropathy, peripheral paralysis of lower extremities, cyanosis, tachycardia, and ultimately death by heart failure (Spinazzi, Angelini, & Patrini, 2010). Without thiamine pyrophosphate, pyruvate cannot enter the tricarboxylic acid cycle and the lack of energy for heart muscle results in heart failure.

6.3.1.2 Vitamin B_2 (Riboflavin)

Riboflavin forms part of the coenzymes flavin adenine dinucleotide (FAD) and flavin adenine mononucleotide (FMN). These are redox cofactors that participate in redox reactions in cells as hydrogen acceptors and donors (Joosten & van Berkel, 2007). Flavin adenine dinucleotide acts as acceptor of two hydrogens into FADH2, and transports the hydrogens into the electron transport chain in the mitochondrial system. The two hydrogens react with oxygen, forming water and regenerating the FAD. Two molecules of ATP are generated in each transport cycle.

The implication of riboflavin in FAD-catalyzed reactions in a wide range of cell processes supports its consideration as an essential nutrient. According to the European Food Safety Authority

Table 6.1 Vitamins, Food Sources, and Human Body Effects

Vitamin	Food Sources	Human Body Effects	Reference
Vitamin B1 (Thiamin)	Watermelon, tomato, spinach, soy milk, ham, pork chops, sunflower seeds	Supports energy metabolism, nerve function	(Haas, 1988; Nichols & Basu, 1994; Depeint et al., 2006)
Vitamin B2 (Riboflavin)	Eggs, liver, fish, meat, spinach, broccoli, mushroom, milk	Supports energy metabolism, normal vision, skin health	(Cardoso, Libardi, & Skibsted, 2012; Alhamad, O'brart, O'brart, & Meek, 2012; Depeint et al., 2006)
Vitamin B3 (Niacin)	Grains, dairy products, nuts, poultry, spinach, potatoes, tomato, meat, fish	Promotes food digestion, nerve function, improves lipoprotein profiles, skin health	(Jungnickel, Maloley, Vander Tuin, Peddicord, & Campbell, 1997; Maccubbin et al., 2009)
Vitamin B5 (Pantothenic acid)	Mushrooms, avocados, whole grains, chicken, tomato, broccoli	Supports energy metabolism	(Depeint et al., 2006)
Vitamin B6 (Pyridoxine)	Beans, nuts, red meat, fish, eggs, spinach, legumes, potatoes, banana, watermelon	Plays role in amino acid and fatty acid metabolism and, red blood cell production, lowers homocysteine levels and risk of heart diseases	(Depeint et al., 2006; Refsum, Ueland, Nygård, & Vollset, 1998; Verhoef et al., 1996)
Vitamin B8 (Biotin)	Egg yolks, soybeans, whole grains, fish	Promotes healthy bones and hair, converts food into energy, fat and glycogen synthesis	(Ballard & Hanson, 1967)
Vitamin B9 (Folic acid)	Orange juice, tomato, broccoli, spinach, legumes, fortified grains, asparagus, green beans, black-eyed peas	Supports DNA synthesis and new cell formation, lowers homocysteine levels	(Refsum et al., 1998; Choi & Mason, 2000; Verhoef et al., 1996)
Vitamin B12	Meat, fish, milk, eggs, poultry, cheese, fortified soy milk	Lowers homocysteine levels, lowers risk of heart diseases, assists in new cell production, break down fatty and amino acids, supports nerve cell maintenance	(Verhoef et al., 1996; Refsum et al., 1998)
Vitamin C (Ascorbic acid)	Citrus fruits, strawberry, kiwis, broccoli, spinach, potatoes, tomatoes, snow peas, bell peppers	Participates in new cell synthesis, breaks down fatty and amino acids, supports nerve cell maintenance, act as antioxidant, promotes immune system	(Kim, Lee, Lee, & Lee, 2002; Buettner, 1993)
Vitamin A (Retinol)	Beef, liver, eggs, shrimp, fish, fortified milk, cheese, carrots, mangoes, squash, spinach, pumpkin, turnip greens, broccoli	Supports vision, skin, bone and tooth growth, immunity and reproduction, acts as antioxidant	(Quadro et al., 1999; Rao & Rao, 2007)
Vitamin D	Fortified milk and margarine, fatty fish, egg yolk, liver, self-synthesis via sun light	Promotes bones mineralization, maintain normal blood levels of calcium and phosphorus to strengthen bones	(Klibanski et al., 2001; Holick, 2007)
Vitamin E	Polyunsaturated plant oils, avocados, margarine, whole grains, nuts, wheat, sunflower seeds, shrimp, tofu	Antioxidant, regulation of oxidation reactions, supports cell membrane stabilization, protects vitamin A, certain lipids and antioxidant enzymes	(Buettner, 1993; Van Den Branden et al., 2002; Rivera, Duff, Galyean, Walker, & Nunnery, 2002)
Vitamin K	Cabbage, spinach, broccoli, liver, eggs, milk, brussels sprouts	It is involved in the synthesis of blood-clotting proteins and regulates blood calcium	(Josic, Hoffer, & Buchacher, 2003; Shea & Booth, 2007)

(EFSA) riboflavin "contributes to normal energy-yielding metabolism," "contributes to normal functioning of the nervous system," and "contributes to the maintenance of normal red blood cells," among functions (EFSA, 2009).

Deficiency of this vitamin is rare; when it manifests it is usually in conjunction with deficiency of other B vitamins. Clinical symptoms are evident after months of deprivation of this vitamin and include photophobia, tearing, pain and burning sensation on the lips, mouth, and tongue, and dermatitis. It also causes peripheral neuropathy.

6.3.1.3 Vitamin B_3 (Niacin)

Niacin forms part of two coenzymes, the nicotinamide adenine dinucleotide (NAD) and the phosphate nicotinamide adenine dinucleotide (NADP). Both coenzymes play a key role in most enzymatic reactions within cells, especially in metabolism of glucose, lipids, and alcohol. Nicotinamide adenine dinucleotide acts in a similar way as the coenzyme FAD, as it transports hydrogens (and electrons) in metabolic reactions, including transport from the tricarboxylic acid cycle to the electron transport chain.

Niacin deficiency is first manifested as muscle weakness, anorexia, indigestion, and cutaneous rash. Severe deficiency causes a disease called pellagra, which is known as the disease of the 3 Ds (diarrhea, dermatitis, and dementia) and occasionally can cause death. Dermatological manifestations include pigmented and desquamative dermatitis. Central nervous system disorders are manifested by confusion, disorientation, and neuritis (Prakash, Gandotra, Singh, Das, & Lakra, 2008). Digestive disorders cause irritation and inflammation of mucous membranes of the mouth and digestive tract.

Some of the most promising data have been reported for B vitamins supporting brain functions. According to the EFSA, niacin "contributes to the reduction of tiredness and fatigue" and "contributes to normal functioning of the nervous system" (EFSA, 2009).

6.3.1.4 Vitamin B_5 (Pantothenic Acid)

Pantothenic acid is involved in cell reactions related to the synthesis of lipids, neurotransmitters, steroid hormones, and hemoglobin. It forms part of two factors in acylation reactions: coenzyme A (CoA) and the acyl carrier protein (ACP).

Pantothenic acid is a vitamin widely distributed in foods and thus deficiency is rare. It has only been observed in conditions of malnutrition or in subjects treated with pantothenic acid antagonists. Symptoms of deficiency are fatigue, tiredness, depression, insomnia, weakness, and paresthesia in the fingers and in the soles of the feet (Mataix & Várela Moreiras, 2009).

6.3.1.5 Vitamin B_6 (Piridoxine)

The active form of the vitamin is pyridoxal phosphate, a coenzyme of multiple enzymes involved in the synthesis of nonessential amino acids, as well as in the metabolism of proteins and urea due to the ability of pyridoxal phosphate to transfer amino groups.

Pyridoxal phosphate is implicated in the conversion of the amino acid tryptophan to niacin or serotonin in the synthesis of heme group and nucleic acids (Mataix & Sánchez de Medina, 2009). Vitamin B_6 is also involved in the release of glucose from glycogen for the biosynthesis of sphingolipids of the myelin in nervous cells, as well as in the modulation of receptors of steroid hormones.

Deficiency cases are uncommon, since vitamin B_6 is widely distributed in nature. Unlike other water-soluble vitamins, it is stored in considerable quantities in muscle tissue. Deficiency is frequently related to its interaction with certain medicines. The clinical manifestations of deficiency, as with other B vitamins, are related to neurological impairment: weakness, insomnia, peripheral neuropathies, and irritability (Plecko & Stöckler, 2009).

6.3.1.6 Vitamin B_7 (Biotin)

Biotin plays an important role in metabolism as a coenzyme carrier of dioxide carbon. It is critical to the TCA cycle, where it transfers a carboxyl group to pyruvate. It also plays a crucial role in gluconeogenesis, synthesis of fatty acids, and the catabolism of fatty acids and amino acids.

Deficiencies are uncommon, as it is widely distributed and can be synthesized by the intestinal microbiota and absorbed in the colon. The clinical manifestations of deficiency are seborrheic dermatitis, alopecia, loss of appetite, nausea, depression, hallucinations, and fatigue (Schellack, Harirari, & Schellack, 2015).

6.3.1.7 Vitamin B_9 (Folic Acid)

Folic acid acts as a donor of methyl groups, and is involved in DNA synthesis, particularly in cells that require further growth. The active form is tetrahydrofolic acid.

The deficiency of folic acid manifests in megaloblastosis of intestinal cells and macrocytic anemia. In pregnant women, lack of this vitamin may produce neural tube defects in newborns, as spine bifida and anencephaly. Deficiency of folates is related to hiperhomocysteniemia or elevated homocysteine, which carries a higher incidence of coronary vascular disorders and strokes. High homocysteine (Hcy) plasma levels have been linked to Alzheimer's disease (Ellinson, Thomas, & Patterson, 2004). Supplementing these patients with high doses of vitamins B6 and B12 and folate lowers plasma Hcy levels (Aisen et al., 2008). In Western countries deficiencies occurs in older individuals, as shown in the National Health and Nutrition Examination Survey (NHANES) and the European Nutrition and Health Survey, which reported average intakes of vitamins C, D, E, and folates below the recommended levels (Elmadfa, Meyer & Nowak, 2009; Troesch, Hoeft, Mcburney, Eggersdorfer, & Weber, 2012).

6.3.1.8 Vitamin B_{12} (Cyanocobalamin)

The activity of vitamin B_{12} is highly correlated with the methylation cycle. Folates donate a methyl group to vitamin B_{12} to get the active form. The regeneration of the amino acid methionine for the synthesis of S-adenosylmethionine (SAM) and DNA synthesis requires folates and vitamin B_{12} (Mataix & Várela Moreiras, 2009).

Vitamin B_{12} deficiency causes alterations in cell division, especially in bone-marrow cells and in intestinal mucosa. Altered mitosis of erythrocytes originates abnormally large cells, immature erythrocytes and a characteristic anemia, called megaloblastic anemia. Another clinical syndrome is progressive neuropathy with neural demyelination. Symptoms include numbness, tingling, burning feet, stiffness, and general weakness.

The most common cause of vitamin B_{12} deficiency is the insufficient absorption of the vitamin due to the lack of hydrochloric acid or intrinsic factor. This vitamin requires the presence of hydrochloric acid in the stomach (to break free from the proteins to which it is attached) and intrinsic factor, a glycoprotein secreted by stomach cells (without which it cannot be absorbed).

Elderly people frequently suffer from atrophic gastritis, which causes a decrease in the synthesis of hydrochloric acid and intrinsic factor. This causes a disorder called pernicious anemia. Consumption of strict vegetarian diets also causes a deficit intake of vitamin B_{12}; however, clinical signs in these individuals are rare, and in some cases do not appear for years.

6.3.1.9 Vitamin C

Vitamin C is an essential nutrient for the synthesis of collagen, L-carnitine, and the conversion of dopamine to noradrenaline. Collagen is the main protein that maintains the integrity of fibrous tissue as connective tissue, cartilage, bone matrix, dentin, tendons, and skin. Synthesis of collagen is particularly important in the formation of arterial walls, which should expand and contract with blood circulation.

During collagen synthesis, the aminoacids proline and lysine must be hydroxylated by a hydroxylase enzyme to form hydroxyproline or hydroxylysine, respectively (Majamaa, Günzler, Hanauske-Abel, Myllylä, & Kivirikko, 1986). This enzyme requires vitamin C and iron. Iron acts as a cofactor in the reaction and vitamin C as cosubstrate, maintaining the iron in the active form. Without them, the hydroxylation does not occur. Vitamin C increases the absorption of iron and promotes the release of their deposits (Traber & Stevens, 2011).

Vitamin C is an important antioxidant for the organism, as it acts as an electron donor. It reacts with free radicals generated during oxygen burst and rapidly loses its electrons as a reducing agent, removing reactive oxygen species in cells and biological fluids. Vitamin C prevents oxidative damage in tissues by preventing oxidation of LDL (Retsky, Freeman, & Frei, 1993). It also attenuates the atherogenic process by inhibiting oxLDL-related ICAM-1 overexpression and monocyte adhesion (Son, Mo, Rhee, & Pyo, 2004).

There is a synergism between vitamin C and vitamin E. The reactive tocopherol radicals are formed by oxidation of α-tocopherols bound to lipids by free radicals and further react with unsaturated lipids of membranes, initiating a chain peroxidation reaction. Ascorbic acid has the ability to regenerate and restore vitamin E from the α-tocopherol radical (Nagaoka, Kakiuchi, Ohara, & Mukai, 2007).

In regards to the role of vitamin C as a cure for the common cold, evidence is conflicting. Vitamin C does not heal or act as prevent the common cold, but studies suggest that it could be helpful in people exposed to brief periods of severe physical exercise or cold environment (Hemila & Chalker, 2013). Possible explanations could be that, in situations of nasal congestion, vitamin C acts as an antihistamine, deactivating histamine, which is responsible for triggering this process, thereby modifying cold symptoms (Johnston, 1996).

Vitamin C, together with vitamins A and E, protects against pathologies with an oxidative stress component, such as atherosclerosis, due to their antioxidant properties against free radicals. Epidemiologic observational studies have shown that diets rich in fruit and vegetables are associated with a reduction of cardiovascular diseases, stroke, and cardiovascular mortality (Osganian et al., 2003; Rimm et al., 1993). In contrast, conflicting results have been obtained in randomized controlled trials with vitamin supplements (Ashor et al., 2015).

Potential explanations of this lack of consistency could be the short intervention period, physiological conditions of subjects, doses used (as vitamins may have opposite effects, antioxidant vs. prooxidant actions in lower or higher concentrations, respectively), timing of supplementation, and interindividual variation (Badimon, Vilahur, & Padro, 2010). The controversial results stress the

importance of understanding the mechanism behind the action of these vitamins as well as the interactions between them and with other biological components. Furthermore, fruits and vegetables are complex matrices rich in fiber and multiple bioactive compounds that can interact in a synergistic manner.

Acute lack of vitamin C leads to scurvy. The main symptom of vitamin C deficiency is manifested in the decrease in the integrity of blood vessels. The dental gums bleed easily and capillaries under the skin tend to break, producing small punctate hemorrhages (petechiae).

6.3.2 FAT-SOLUBLE VITAMINS

6.3.2.1 Vitamin A

Vitamin A plays essential roles in vision, bone growth, reproduction, cell division, and differentiation of epithelial tissues as well as in the regulation of the immune system (Hernandez, 2010). These functions can be ensured by ingesting both provitamin A carotenoids from plants, as well as retynil esters, retinol, or retinal, since each of them can be metabolized into the active forms retinol, retinal, and retinoic acid (Ochoa & Mataix, 2009).

Retinal is a component of the visual pigments of the retina and is essential for photoreception. Vitamin A also helps to maintain the integrity of epithelial cells and promote their differentiation. Retinol is involved in sperm production in men and contributes to fetal development during pregnancy (Ochoa & Mataix, 2009). Vitamin A deficiency depends on the stores in the liver. When a healthy adult stops eating foods rich in vitamin A, the deficiency symptoms will not appear until the deposits are exhausted, which can take between 1 and 2 years. However, this period is more limited in children during the growth phase. This vitamin deficiency is one of the biggest problems of underdeveloped countries. More than 100 million children around the world show signs of deficiency, which increases their vulnerability to infections, incidence of night or total blindness (xerophthalmia), and keratinization and can cause death (Rao & Rao, 2007).

6.3.2.2 Vitamin D

Vitamin D is not an essential nutrient if there is sufficient exposure to UV radiation, because its biologically active form, the 1,25-$(OH)_2$colecalciferol or vitamin D_3, is a hormone that can be synthesized in the body from cholesterol.

Vitamin D interacts with a vitamin D receptor (VDR) in cell membranes and generates rapid responses through second messengers. These receptors are also located in the nucleus, where the complex vitamin D_3−VDR regulates gene transcription by its union to VDREs (Vitamin D responsive elements) in DNA. Therefore it has been suggested that vitamin D is involved in a wide range of physiological functions (Battault et al., 2013). These genes are involved in regulatory processes such as cell proliferation and differentiation, apoptosis, oxidative stress, membrane transport, matrix homeostasis, tissue mineralization, and cell adhesion (Norman & Powell, 2014).

Vitamin D plays an essential role in the homeostasis of calcium and phosphorus as well as in cell differentiation. The active form of vitamin D is found in the small intestine increasing the active transport of calcium ion. At kidney, it increases renal tubular reabsorption of both calcium and phosphate ions. It regulates the mobilization and deposition of calcium and phosphorus in

bones. Hence, vitamin D restores plasma calcium levels by intensifying enteral absorption, renal retention, and bone mobilization (Lips & van Schoor, 2011).

Vitamin D also plays physiological roles in nonskeletal tissues. Its receptors have been found in most organs of the body, including colon, small intestine, breast, brain, pancreas, or muscles (Rosen et al., 2012). Evidence has shown that $1,25(OH)_2$-vit D3 helps to maintain inflammatory and immune responses within physiological limits. It decreases the production of proinflammatory cytokines such as IL-1, IL-6, or TNF-α, while it increases the expression of the antiinflammatory IL-10 and TGFβ (Ardizzone et al., 2009).

One year of vitamin D supplementation in overweight subjects reduced plasma concentrations of IL-6 (Beilfuss, Berg, Sneve, Jorde, & Kamycheva, 2012). A NHANES (National Health and Nutrition Examination Survey) study showed a strong inverse relationship between plasma levels of vitamin D and C-reactive protein, suggesting that vitamin D supplementation in deficiency conditions may reduce inflammation status (Amer & Qayyum, 2012). This vitamin is also involved in the regulation of the renin-angiotensin system, which regulates blood pressure. Renin gene expression is suppressed by $1,25(OH)_2$-vit D3 and renin plasma levels have been inversely correlated to cholecalciferol levels (Vaidya, Forman, Hopkins, Seely, & Williams, 2011; Yuan et al., 2007).

A meta-analysis on cardiovascular risk on obese adults showed that the optimization of vitamin D status can ameliorate the cardiometabolic profile of this population (Manousopoulou, Al-Daghri, Garbis, & Chrousos, 2015).

Vitamin D receptor has demonstrated a role in maintaining the integrity of the intestinal mucosa (Kong et al., 2008). Populations that consume more than 400 IU daily have shown a significantly lower incidence of intestinal bowel disease than those consuming less than 100 IU daily (Ananthakrishnan et al., 2012). Randomized-controlled trials showed a nonsignificant trend to lower relapse rate in patients treated with vitamin D (Jørgensen et al., 2010). Inverse associations for colon or rectal cancer and vitamin D supplementation have been observed (Giovannucci, 2010). Possible explanations are the inhibition of cell proliferation (Lamprecht & Lipkin, 2003) or the induction of cell apoptosis (Harris & Go, 2004). The data supporting the possible protective role of vitamin D against colorectal cancer need further confirmation by large interventional clinical trials to establish causal relationships.

The symptoms of vitamin D deficiency ($<$50 nmol/L) are the same as for calcium deficiency because even if there is sufficient calcium supply by foods, it cannot be absorbed due to vitamin D deficiency. Vitamin D deficiency in children manifests as rickets. It is characterized by structural abnormalities of bones that become too flexible and soft. Hence, they cannot withstand tension and bone deformities such as curved legs occur. This disease still affects the child population in South Asia and the Middle East.

Osteomalacia is a bone malformation comparable to rickets that occurs in adults. It is observed mostly in women who do not consume enough calcium, who have little exposure to sunlight, and who have had successive pregnancies with breastfeeding. It implies a decrease in bone density and prevention is usually possible by adequate intake of vitamin D, calcium, and phosphorus. Osteoporosis is often confused with osteomalacia, but here the bones have a normal histologic appearance, although they also have reduced bone density. Osteoporosis is associated with aging and affects postmenopausal women (Holick, 2007).

Vitamin D deficiency has also been related to diabetes mellitus (DM) in cross-sectional studies by the NHANES, in which the vitamin D level in the first quartile was associated with DM with an

odds ratio of 1.73 versus fourth quartile of intake (Martins et al., 2007). In β-pancreatic cells, vitamin D appears to modulate insulin synthesis through VDREs in the insulin-promoter genes (Maestro, Dávila, Carranza, & Calle, 2003).

Hence, the deficiency in vitamin D may compromise normal metabolism of carbohydrates. Nutritional interventions in prospective longitudinal studies have also shown that the consumption of high levels of vitamin D (700 IU/day) over 3 years reduced the incidence of diabetes mellitus in older adults with impaired fasting glucose (Pittas, Sun, Manson, Dawson-Hughes, & Hu, 2010). However, results from interventional studies are inconsistent.

A meta-analysis of prospective studies on the relationship between vitamin D levels and coronary heart disease has shown that the risk of any coronary event is 33% higher for the lowest quartile vs. the highest quartile of vitamin D status (Brøndum-Jacobsen, Benn, Jensen, & Nordestgaard, 2012). Trials such as the BEST-D (Biochemical Efficacy and Safety Trial of vitamin D) are currently being performed to establish the best dose of vitamin D to assess in future large outcome trials of vitamin D supplementation and osteoporosis, cardiovascular risk, and cancer (Clarke et al., 2015).

6.3.2.3 Vitamin E

Vitamin E is the main fat-soluble antioxidant in the body. It is located in biological membranes, where it protects the polyunsaturated fatty acids (PUFA) of membrane phospholipids from oxidative degradation by free radicals (Brigelius-Flohe & Traber, 1999). It acts synergistically with other cell-defense systems (such as the enzymes superoxide dismutase, glutathione peroxidase, or glutathione reductase) and its function is affected by the nutritional status of other nutrients, such as selenium. The antioxidant function of vitamin E against oxidative stress in cells helps with aging, arthritis, cancer, cardiovascular diseases, cataracts, diabetes, and infections.

A meta-analysis showed that increased vitamin E intake can reduce Alzheimer's disease risk in older populations (Li, Shen, & Ji, 2011) compared to other antioxidants such as vitamin C and β-carotene. Vitamin E exhibits the most significant protective effect. According to the EFSA vitamin E "contributes to the protection of cells from oxidative stress" (EFSA, 2009).

Vitamin E deficiencies are rare and are associated with states of fat malabsorption, such as in the case of cystic fibrosis. A prolonged deficiency may cause hemolysis of erythrocytes due to oxidation of PUFA, which can be observed in premature infants. Prolonged states of lack of vitamin E can also cause neuromuscular dysfunction, which affects the spinal cord and eye retina (Doerflinger et al., 1995).

6.3.2.4 Vitamin K

Vitamin K is essential in the activation of several of the proteins involved in the pathway of blood clotting. This vitamin is also involved in the synthesis of bone proteins that enable the bonding of minerals for bone formation (Bügel, 2003).

The deficiency is not common, and is mostly due to low absorption. There are some substances that antagonize the action of vitamin K (coumarins—acenocumarol, Sintrom®—, blood thinners and anticoagulants, salicylates, antibiotics) as they decrease the intestinal microbiota needed to synthesize the bioactive form. The main manifestation of vitamin K deficiency is bleeding, which can trigger lethal anemia.

6.4 EFFECT OF PROCESSING EMERGING TECHNOLOGIES ON FOOD VITAMINS

Consumer demand for high-quality foods with "fresh-like" characteristics, which require only a minimum amount of effort and time to prepare, has led to the introduction of convenience foods preserved by mild treatments. Today emerging technologies are investigated at the research level or sometimes applied in the food industry, as they promise to shorten processing and residence times, accelerate heat and mass transfer, control Maillard reactions, improve product quality, enhance functionality, protect from environmental stresses, and extend preservation (Deng et al., 2014; Galanakis, 2012; 2013; Galanakis, Barba, & Prasad, 2015; Galanakis & Schieber, 2014; Roselló-Soto, Barba, et al., 2015; Roselló-Soto, Galanakis, et al., 2015; Zinoviadou et al., 2015).

This new state-of-the-art requires better knowledge of the effect of emerging technologies on food vitamins. These new methods allow the processing of foods below temperatures used during thermal pasteurization, so flavors, essential nutrients, and vitamins undergo minimal or no changes. Foods can be nonthermally processed by ionizing radiation, cold plasma, irradiation, high hydrostatic pressures (HHP), antimicrobials, ultrasounds (US), filtration, and electrical methods such as pulsed electric field (PEF), light pulse, and oscillating magnetic field. It worth evaluating the effects of these emerging technologies on food vitamins. In this section we discuss these technologies and their effects on vitamins as reported in the literature.

6.4.1 IONIZING RADIATION

The sensibility of vitamins to irradiation is diverse, as described by Kilcast (1994) (Table 6.2). Vitamins D and K, carotene, and most B vitamins are moderately sensitive to irradiation. However, the effect on vitamins A and E does not present a problem because of the high consumption in the diet. Other vitamins such as B_1 and C are highly reactive to irradiation, but if the doses used for the treatment are low and are used in combination with other lighter treatments, the effects of irradiation on the organoleptic properties and reduction of vitamins could be minimized (Dionísio, Gomes, & Oetterer, 2009).

Vitamin C is highly modified by irradiation. However, the changes observed in vitamin C concentration of different foods depend widely on the type of food. For example, at early stages of storage, irradiated potatoes showed a loss in vitamin C content. However, after many months of

Table 6.2 Sensitivity of Vitamins Against Irradiation

High sensitivity	Vitamin A	
	Vitamin B_1	
	Vitamin C	
	Vitamin E	
Low sensitivity	Vitamin B_2	Vitamin B_7
	Vitamin B_3	Vitamin B_9
	Vitamin B_5	Vitamin B_{10}
	Vitamin B_6	Vitamin K

storage, vitamin C was higher in irradiated samples than in untreated controls (Diehl, 1999; Dionísio et al., 2009; Wang & Chao, 2003). In contrast, irradiating onion and garlic does not seem to affect the maintenance of vitamin C, since the vitamin C content remained at similar levels in the samples of irradiated onions and garlics and in the untreated controls (Croci, Banek, & Curzio, 1995; Kilcast, 1994).

In fruits and vegetables, the maintenance of vitamin C or ascorbic acid has been improved. For example, lettuce and potatoes are quite susceptible to radiation. Fresh-cut lettuce was evaluated after radiation during storage at 4°C and showed a significant drop in vitamin C content (Zhang, Lu, Lu, & Bie, 2006). As noted, in potatoes, vitamin C was reduced after irradiation (Chuanyao, Mengyue, Meixu, Shufen, & Shucheng, 1993; Lastarria-Tapia & Sequeiros, 1985; Saito & Igarashi, 1970), but its retention during shelf-life was quite effective (Saito & Igarashi, 1970). Other fruits and vegetables, including green and red peppers, cucumbers, custard apples, lemons, lychees, mandarins, nectarines, peaches, persimmons, tomatoes, and zucchini were also irradiated, suffering an initial drop in the content of vitamin C in storage. However, this drop was more minor than the effects of storage (Maxie & Sommer, 1968; Mitchell, Mclauchlan, Isaacs, Williams, & Nottingham, 1992). The stability of vitamin C after irradiation has also been measured in rio red grapefruit and showed different stability depending of the fruit's stage of growth (Patil, Vanamala, & Hallman, 2004).

Citrus fruits are characteristically vitamin C-rich food, and the maintenance of vitamin C after radiation could be of great interest. The two main citric fruits are oranges and lemons, but vitamin C preservation after radiation is quite different in both cases. While vitamin C in oranges was retained after irradiation and storage over 100 days (Fan & Mattheis, 2001), the vitamin C content in lemons was not as resistant after irradiation and storage for 30 days (Maxie, Eaks, & Sommer, 1964). In kiwi fruits, the use of radiation is valuable as a tool to maintain vitamin C in fruits during storage. The final content of vitamin C (ascorbic acid) and the antioxidant capacity significantly decreased with gamma irradiation treatment (Yook, 2009). Increasing irradiation doses reduced the initial content of vitamin C in passion fruit, orange, and tangerine juices (Muller & Riel, 1990). Ascorbic acid can be strongly reduced after irradiation of tomato juice and black and red currant sirups in comparison to pasteurization (Wilska-Jeszka & Skorupinska, 1975).

It is well known that meat and fish are great sources of water-soluble B vitamins. The integrity of B vitamins depends on the individual vitamin. However, thiamin is the most susceptible vitamin B to irradiation due to its oxidation (Kilcast, 1994). In fact, Fellows (2009) reported strong resistance of riboflavin and niacin to irradiation. However, thiamin was reduced by 47% after irradiation and by 10% with cooking, while with a cooking−irradiation combination it dropped 54%. The degradation of thiamin is actually dependent on the dose of irradiation used, and is diminished with lower temperatures (Kilcast, 1994). Thiamin is more sensitive to thermal treatments than to irradiation. In fact, irradiated-sterilized beef and pork retained more thiamin than thermosterilized products (Josephson, Thomas, & Calhoun, 1978). The effects of γ-radiation in thiamin, riboflavin, and niacin were studied in shrimp, after irradiation at different temperatures. The drop of thiamin was higher with increasing irradiation doses and temperature, but riboflavin and niacin did not suffer any significant change (Lee & Hau, 1996).

The stability of vitamin E is also uncertain when irradiation is applied. Free α-tocopherol was measured after low-dose ionizing radiation in pork, turkey, and lamb. Irradiation resulted in a significant decrease in tocopherol levels, especially in turkey (Lakritz et al., 1995). In addition to these meats, the effect of ionizing radiation on free tocopherols in chicken packaged aerobically and

irradiated at 4°C was studied. The results showed a reduction in α- and γ-tocopherol with increasing ionizing radiation dose levels, reaching losses up to 30% (Lakritz & Thayer, 1992). Finally, vitamin E was measured after ionizing radiation in the cereal *Tribolium confusum.* The vitamin E content decreased after radiation treatment, but remained higher than the specified level of food-quality standards (Kovács et al., 1986).

6.4.2 HIGH HYDROSTATIC PRESSURE

High hydrostatic pressure (HHP), also described as high-pressure processing (HPP), or ultrahigh pressure (UHP) processing, is a cold-pasteurization technique that consists of subjecting food, previously sealed in flexible and water-resistant packaging, to a high level of hydrostatic pressure (pressure transmitted by water), up to 600 MPa (87,000 psi) for a few seconds to a few minutes. High hydrostatic pressure is a safe, natural, and environmentally friendly process that helps maintain fresh food characteristics such as flavor and nutrients. It is a viable alternative to traditional thermal and chemical treatments (San Martin, Barbosa-Canovas, & Swanson, 2002). Process temperature during pressure treatment can be specified from below 0°C (to minimize any effects of adiabatic heat) to above 100°C. Vessels are uniquely designed to safely withstand these pressures over many cycles. Commercial exposure times at pressure can range from a millisecond pulse (obtained by oscillating pumps) to a treatment time of over 1200 s (20 min). In contrast to thermal processing, the costs of throughput may limit practical exposure times to less than 20 min. Pressures used in the HHP of food appear to have little effect on covalent bonds (FDA, 2000). Thus foods subjected to HHP treatment at or near room temperature do not undergo significant chemical transformations due to the pressure treatment itself. High hydrostatic pressure may be combined with heat to achieve an increased rate of inactivation of microbes and enzymes. Chemical changes in the food will generally be a result of the process temperature and time selected in conjunction with the pressure treatment (FDA, 2000).

The effect of ultrahigh hydrostatic pressure on selected hydrosoluble vitamins (B1, B6, and C) was studied (Sancho et al., 1999). Minor variations were found, with no significant losses of vitamins B1 and B6 after the treatments. Reduction of vitamin C levels, although significant, was not dependent on the intensity of the ultrahigh hydrostatic pressure process. High-pressure treatment of 500 MPa at 35°C for 5 min led to better retention of ascorbic acid during postprocessing storage of orange juice at 0−15°C compared to conventional thermal pasteurization (80°C, 30 s) (Polydera, Stoforos, & Taoukis, 2003). Recently, higher vitamin C content than untreated controls were observed for HHP-treated oranges (Escobedo-Avellaneda, Gutiérrez-Uribe, Valdez-Fragoso, Torres, & Welti-Chanes, 2015). Indeed, samples treated at 550 MPa showed significantly lower vitamin C losses than the those obtained for UHT-processed samples of prickly pear (*Opuntia ficus-indica*) beverages (Jiménez-Aguilar et al., 2015). On the other hand, after immediate application of HHP, a notable increase in B3 and B6 vitamins was observed in cape gooseberry (uchuva) pulp versus control samples, with treatment of 500 MPa/5 min as the maximum value of these vitamins (Vega-Gálvez et al., 2016). The ultrahigh hydrostatic pressure was the technological process that affected the tested hydrosoluble vitamins the least, thus helping to preserve their nutritional quality in food.

In the literature, information about the effect of HHP on the stability of fat-soluble vitamins is less abundant than that on water-soluble vitamins. Thus there is little information available on the effect of HHP on the stability of tocopherols in different food matrices. Nevertheless, controversial

reports do exist. For example, Barba, Esteve, and Frigola (2013) showed an increase of total tocopherol content in orange juice with milk treated at 100−400 MPa/15−30°C/9 min, mainly due to an increase in α-tocopherol content, in particular at 200−300 MPa. Moreover, Vega-Gálvez et al (Vega-Gálvez et al., 2012) observed that 400 MPa/5 min improved the content of vitamin E compared to the untreated sample of aloe vera gel. Tocopherols scavenge lipid peroxy radicals and yield tocopheroxyl radicals that can be recycled back to the corresponding tocopherol by reacting with ascorbate or other antioxidants through different chemical reactions. In this way, this recycling reaction explains the increase on tocopherol content, which is not lost in the oxidative reactions (Sattler, Gilliland, Magallanes-Lundback, Pollard, & Dellapenna, 2004). On the other hand, a significant decrease was detected in milk tocopherol content when applying 100 MPa, while an increase was observed when processing at 200−400 MPa. Moltó-Puigmartí et al. (Moltó-Puigmartí, Permanyer, Castellote, & López-Sabater, 2011) did not find significant changes in the total tocopherol content when applying 400−600 MPa/22−27°C/5 min in human milk.

It has been shown that β-carotene in ethanolic model solutions is reduced by more than 50% after 20 min treatment at 75°C (Tauscher, 1998). Protecting the sample from oxygen does not reduce the loss of β-carotene under pressure/temperature. In all cases, main degradation was 9-*cis*- and 13-*cis* β-carotene. In carrot puree, the carotenoids are well protected against pressure/temperature attack since they are buried in lipophilic environments. Even after 40 min at 600 MPa and 75°C, the initial amount of β-carotene was not significantly reduced. This demonstrates the importance of the food matrix and its beneficial protective action (Butz & Tauscher, 2002).

6.4.3 PULSED ELECTRIC FIELDS

High-intensity pulsed electric fields (PEF) processing involves the application of high-voltage pulses (typically 20−80 kV/cm) to foods placed between two electrodes. The PEF treatment is conducted at ambient, subambient, or slightly above ambient temperature for less than 1 s, and energy loss due to heating of foods is minimized. The PEF technology is considered superior to traditional heat treatment of foods because it avoids or greatly reduces the detrimental changes of the sensory and physical properties of foods (FDA, 2000). Although some studies have concluded that PEF preserves the nutritional components of food, the effects of PEF on the chemical and nutritional aspects of foods must be better understood before it is used in food processing (Qin, Pothakamury, Barbosa-Cánovas, & Swanson, 1996).

A PEF-treated orange juice retained greater amounts of vitamin C than a heat-pasteurized orange juice during storage at 4°C (Yeom, Streaker, Howard Zhang, & Min, 2000). Moreover, significantly higher vitamin C retention in orange juice processed by high-intensity PEF—fitting recommended daily intake standards over 56 days storage at 4°C—was observed, while heat-processed juice exhibited poor vitamin C retention beyond 14 days storage (Elez-Martínez, Soliva-Fortuny, & Martín-Belloso, 2006). The same authors also reported higher vitamin C retention of high-intensity PEF-treated orange juice and gazpacho than that of heat-pasteurized products (Elez-Martínez & Martín-Belloso, 2007). The vitamin C of fresh blueberry juice samples treated with PEF was also higher than those treated with heat sterilization (Chen et al., 2014). On the other hand, PEF technology was shown to affect water-soluble vitamin content (biotin, folic acid, pantothenic acid) of a mixed orange juice and milk beverage at least in the same way as mild heat treatments (84°C) and showed better results than high-intensity thermal treatments (such as 95°C and 45 s). In the case of riboflavin, PEF

treatments preserved its stability better than thermal treatments when stored at 4°C for 60 days (Rivas, Rodrigo, Company, Sampedro, & Rodrigo, 2007). Other work showed that PEF treatment had no effect on the levels of thiamin, riboflavin, retinol, and α-tocopherol in milk (Riener, Noci, Cronin, Morgan, & Lyng, 2009). Nonetheless, other effects have been reported for apple juice, where the content of vitamin C decreased significantly during PEF treatment (Bi et al., 2013).

It has also been demonstrated that pasteurization produced a greater decrease in carotenoid and vitamin A concentrations than the application of high-intensity PEF, and showed a greater tendency toward the yellow color and a lesser tendency toward the red with respect to untreated orange juice, while the luminance of the juice remained practically unchanged (Cortés, Esteve, Rodrigo, Torregrosa, & Frígola, 2006). The same results were also noted for orange-carrot juice carotenoids, where a high-intensity PEF treatment, at 25 and 30 kV/cm, provided higher vitamin A content than that found in the pasteurized juice (Torregrosa, Cortés, Esteve, & Frígola, 2005). Pulsed electric field-treated tomato juice also maintained higher lycopene and vitamin C content than the thermally treated juices during the storage time (Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2008). Another paper reported that the degradation kinetics of carotenoids, during storage, were similar for the pasteurized and PEF-treated orange juice-milk beverages, although they were less affected initially in the beverages treated by PEF technology and were therefore maintained in greater quantities throughout the storage period (Zulueta, Barba, Esteve, & Frígola, 2010). Other recent research has demonstrated that PEF treatment resulted in higher total carotenoid content of carrot purée than its untreated counterpart, leading to an improved bioprotective effect (Leong, Oey, & Burritt, 2016). No results were found for PEF effects on vitamin D and vitamin K in foods.

6.4.4 ULTRASOUND

According to Gunasekaran and Ay (1994) ultrasound (US) is "energy generated by sound waves of 20,000 or more vibrations per second." High frequencies in the range of 0.1−20 MHz, pulsed operation, and low power levels (100 mW) are used for nondestructive testing (Gunasekaran & Ay, 1994). Ultrasonic excitation was examined for nondestructive evaluation of the internal quality and latent defects of whole fruits and vegetables. Floros and Liang (Floros & Liang, 1994) noted that the use of low-intensity high-frequency US improved food product/process monitoring due to the acceleration of diffusion. These industrial applications include:

i. texture, viscosity, and concentration measurements of many solid or fluid foods;
ii. composition determination of eggs, meats, fruits and vegetables, dairy and other products;
iii. thickness, flow level, and temperature measurements for monitoring and control of several processes; and
iv. nondestructive inspection of egg shells and food packages.

These authors also noted direct process improvements such as cleaning surfaces, enhancement of dewatering, drying and filtration, inactivation of microorganisms and enzymes, disruption of cells, degassing of liquids, acceleration of heat transfer and extraction processes, and enhancement of any process dependent on diffusion. Although the heterogeneous and protective nature of food (e.g., inclusion of particulates) severely curtails the singular use of US for food preservation, combination with heat or pressure, for example, has potential (Butz & Tauscher, 2002). Therefore it is evident that US technology has a wide range of applications in the food industry.

The US processing of juices has been reported to have beneficial effects on the ascorbic acid content in some juices, showing higher levels of vitamin C in US-treated fruit juices such as orange juice and guava juice as well as in kasturi lime juice (Bhat, Kamaruddin, Min-Tze, & Karim, 2011; Cheng, Soh, Liew, & Teh, 2007; Tiwari, O'donnell, Muthukumarappan, & Cullen, 2009). Recent results for ascorbic acid content confirmed that US and UV treatment (US only retains it to a marginally higher extent but gives lower microbial disinfection as compared to the combined approach) retain the heat-sensitive ascorbic acid more than thermal treatment due to the absence or minimization of heat release in the US and UV treatments of different fruit and vegetable juices (Khandpur & Gogate, 2015). This positive effects of US are attributed to the effective removal of occluded oxygen from the juice and to the degassing action (Bhat et al., 2011; Cheng et al., 2007). High levels of vitamin C retention (65−84%) were recently found in dried strawberries at temperatures between 40 and 70°C, with 30 and 60 W obtaining the highest preservation of vitamin C after US treatments carried out at 40 and 50°C (Gamboa-Santos et al., 2014). Higher retention of vitamin C in a heat + US treatment (about 94%) was reported for watercress (*Nasturtium officinale*) as compared to heat blanching, which reduces the content to 29% (Cruz, Vieira, & Silva, 2008). Frias, Peñas, Ullate, and Vidal-Valverde (2010) reported retention of vitamin C ranging 82−92% in sliced carrots (4 × 24 mm) subjected to dehydration in a US system by contact (100 W) at temperatures of 20−60°C and drying times of 75−120 min. In the same work, great retention (96−98%) of β-carotene was achieved, which was higher than when a convective air drying system was used.

Literature on the effects on fat-soluble vitamins is limited. One publication of the US effects on vitamin E, where oils extracted from olive paste pretreated by high-power US, showed higher content of tocopherols and carotenoids than those untreated (Jiménez, Beltrán, & Uceda, 2007).

6.5 EXTRACTION AND ANALYTICAL PROCEDURES FOR WATER-SOLUBLE AND FAT-SOLUBLE VITAMINS

Standard (Strohecker et al, 1965) and official (United States Pharmacopeia) methods include spectrophotometric, polarographic, fluorimetric, enzymatic, and microbiological procedures Nevertheless, in the last 20 years, several papers have been published concerning the separation and quantification of vitamins by more simple methodologies, such as chromatography (Fotsing, Fillet, Bechet, Hubert, & Crommen, 1997), capillary electrophoresis (Dinelli & Bonetti, 1994), and high-performance liquid chromatographic-diode (HPLC) (Montesano, Gennari, Seccia, & Albrizio, 2012). These provide rapid, sensitive, and accurate methods for vitamin determination and have the advantages of solvent economy, easy coupling with other techniques, as well as the fact that small samples are required. These methods show small differences when analyzing water-soluble or fat-soluble vitamins.

6.5.1 WATER-SOLUBLE VITAMINS

Current international methods are mainly based on microbiological assays that have been established for more than 30 years. However, they are no longer considered to be the gold standard in vitamins analysis as many studies have shown their deficiencies.

All the B-group vitamins may be determined microbiologically. Ball (2005) provided a complete review of current microbiological assays. The principles of these techniques are:

(i) Extraction of the vitamins from the food matrix either by autoclaving in the presence of acids, or by digesting with suitable enzymes (e.g., protease, takadiastase) for labile vitamins;

i. incubation of the food extract at several dilution levels with the growth medium and microbiological culture; reading of the turbidity, related to the growth of the microorganisms, with a spectrophotometer at 540−660 nm;
ii. and calibration. Semilogarithmic plot of the absorbance against increasing concentrations of vitamins. Recent improvements in these microbiological assays have focused on folates, due to the mandatory fortification of folic acid in cereal products in various countries. A new microbiological procedure involving three-enzyme sample preparation was collaboratively studied (DeVries et al., 2005) and later adopted as an AOAC (Official Method of Analysis) official method (AOAC, 2006). Later, Chun et al. reported a differential assay of folic acid and total folate in foods containing enriched cereal-grain products to calculate dietary folate equivalents (Chun et al., 2006). This involved a modification of the existing AOAC folate method, where two digestion steps were removed from the three-enzyme digestion method enabling folic acid to be quantified. Total folate was then determined by the full method and "food folate" was calculated by the difference.

Other biospecific methods of analysis used for B-group vitamins are immunoassays and protein-binding assays. Immunoassays include radioimmunoassay and enzyme-linked immunosorbent assay (ELISA), while protein-binding methods utilize naturally occurring vitamin-binding proteins with either radiolabels or enzyme labels (Blake, 2007). New developments such as more specific binding proteins and smaller multichannel devices should help to scale up use.

However, faster, newer, and more specific methods to analyze water-soluble vitamins include chromatographic (HPLC, gas chromatography, paper chromatography, etc.) methods. A relatively large number of chromatographic systems for separation and determination of water-soluble vitamins can be found in the literature. They usually allow simultaneous analysis of several compounds. The speed of analysis is an important parameter, because the vitamins have low stability in solution and decompose fast. For larger numbers of analytes it was necessary to change the chromatographic conditions or significantly increase the duration of a single analysis. In determination of water-soluble vitamins the main components of the mobile phase are strong polar solvents such as water, buffers (usually phosphate), or methanol (Płonka, 2012).

Numerous HPLC methods have been validated in the literature. A HPLC array detector-fluorescence detector/mass spectrometric method for simultaneous determination of water-soluble vitamins in multivitamin dietary tablets was validated by AOAC (Chen, Atkinson, & Wolf, 2009). This method was developed to be as simple and as versatile as possible, requiring no expensive SPE and no ion-pairing reagent in the HPLC procedure. In addition to being used to measure vitamin C content in pharmaceutical tablets, this method can be easily adapted for the determination of water-soluble vitamins in fortified foods and other food matrices. Engel, Stefanovits-Bányai, and Abrankó (2010) developed and validated an HPLC−LC−UV screening method for the simultaneous determination of ascorbic acid (C), and the free forms of thiamine (B_1) riboflavin (B_2), niacin (B_3), and pyridoxine (B_6) in enriched food products. Besides extracting vitamin C, this procedure was extended to the extraction of the free forms of vitamins B_1, B_2, B_3, B_6, and B_9. The developed

analytical method was successfully applied for the simultaneous determination of vitamin C content along with the free vitamin B forms of three different enriched food products, without using ion-pairing reagent. Nonetheless, due to lack of any sample cleanup, the quantitative values should be revised individually for every particular matrix. Vitamin B_{12} was also extracted and determined in urine by an ionic liquid-based aqueous two-phase system prior to HPLC (Berton, Monasterio, & Wuilloud, 2012).

6.5.2 FAT-SOLUBLE VITAMINS

Many analytical methods including colorimetry, spectrophotometric, thin-layer chromatography, gas chromatography, and HPLC have been used for the study of these vitamins.

Among all these methods, the most widely applied are the chromatographic techniques, mainly HPLC (both normal and reversed-phase), which provides rapid, sensitive, and accurate tools for vitamin determination possessing the advantages of solvent economy, easy coupling with other techniques, and small sample size. A method for the simultaneous determination of different fat-soluble vitamins in animal-derived products using HPLC and after small-scale extraction was developed, being at that moment an effective and fast technique to enable the processing of a large number of samples (Salo-Väänänen et al., 2000). Later, a simple and rapid reversed-phase HPLC procedure was developed for the simultaneous determination of eight fat-soluble vitamins (retinol, menadione, menaquinone, δ-tocopherol, cholecalciferol, α-tocopherol, α-tocopherol acetate, and phylloquinone) in blood serum and urine (Chatzimichalakis, Samanidou, & Papadoyannis, 2004). The advantage of the proposed method was that in a single run, a screening of the fat-soluble vitamins (potentially present in biological samples) was completed in less than 15 min.

Recently, the feasibility of using reversed-phase liquid chromatography/diode array/tandem mass spectrometry (LC−DAD−MS/MS) for rapid and comprehensive profiling of fat-soluble vitamins and carotenoid pigments in some foods of plant origin (maize flour, green and golden kiwi) was developed (Gentili & Caretti, 2011). Moreover, Montesano et al. (2012) described a simple and selective analytical procedure for the extraction and quantification of lutein from tomato by-products by HPLC−DAD.

Milk products have been subjected to analysis and to extraction of their fat-soluble vitamins. An efficient and inexpensive semimicro liquid−liquid extraction followed by a HPLC−UV method was developed for simultaneous cleanup and extraction of vitamin A and vitamin E in infant milk formula samples (Yazdi & Yazdinezhad, 2014). Gentili et al. (2013) described a novel and efficient analytical method for evaluating the profile of carotenoids and fat-soluble vitamins in milk from different animal species by LC-hyphenated techniques such as HPLC−DAD−EIS−MS/MS. A rapid and accurate isocratic HPLC method and sample-extraction procedure for measuring carotenoid, retinoid, and tocopherol concentrations in human blood and breast milk were described by Turner and Burri (Turner & Burri, 2012). A simple and rapid method was also recently developed for the isolation and determination of vitamin D2 in fortified milk samples by HPLC (Kaushik, Sachdeva, Arora, & Wadhwa, 2014).

More recently, Jia, Chu, Chang, and Zhang (2015) developed and validated an advanced analytical method for the simultaneous analysis of fat- and water-soluble vitamins. The automated extraction procedure was achieved in a simple disposable pipet extraction, and ultrahigh performance liquid chromatography and electrospray ionization quadrupole−orbitrap high-resolution mass

spectrometry (UHPLC Q−Orbitrap MS) allowed the isolation and detection of all 52 analytes. This validated method was applied on products derived from green tea extract for validation using 136 different commercial samples.

6.6 STABILITY, BIOAVAILABILITY, AND BIOACCESSIBILITY OF DIFFERENT VITAMINS

Stability and bioaccessibility of vitamins in foods is often compromised by factors like food matrix, low permeability, and/or solubility within the gut, lack of stability (temperature and oxygen)_in the technology used during food processing, as well as in the gastrointestinal tract (pH, enzymes, presence of other nutrients). Before going into detail in this section it is necessary to define bioaccessibility and bioavailability. Bioaccessibility is a prerequisite for the function of vitamins in the human body, i.e., their liberation from the food matrix, incorporation into mixed micelles, and subsequent absorption by the enterocyte. Thus the term "bioaccessibility" refers to "the fraction of a compound that is released from its matrix in the gastrointestinal tract and thus becomes available for intestinal absorption" (Aschoff et al., 2015). Bioavailability is then defined as the proportion of an ingested nutrient that becomes available to the body for metabolic processes or storage.

Food fortification has been shown to be an effective strategy for overcoming vitamin deficiency, and micronutrient interactions strongly argue for multiple micronutrient fortification (Wegmüller, Zimmermann, Bühr, Windhab, & Hurrell, 2006). In this sense, food science and industry is trying to improve the stability and bioaccessibility of vitamins with emerging technologies, adding other components or changing the molecular structure of the vitamin (Diarrassouba et al., 2014; Puthusseri, Divya, Lokesh, & Neelwarne, 2013). Nonetheless, the bioaccessibility of vitamins depends largely on the nature and type of vitamin, so this section is divided into water-soluble vitamins and fat-soluble vitamins.

6.6.1 WATER-SOLUBLE VITAMINS

In the United States, folic acid is now added to enrich grain products and is included in the majority of commercial breakfast cereals. Recent data indicate that the folate status in the population of the United States has improved significantly, presumably due to the effects of this fortification. Folic acid (not food folate) intake in excess of the tolerable upper intake level may mask the diagnosis of a vitamin B_{12} deficiency, which is more prevalent in the elderly than in younger individuals. Deficiency of this vitamin was found to play a major role in several nutritional disorders, such as anemia, physiological discrepancies, psychological disorders, and neural tube defects in newborn infants (Puthusseri et al., 2013).

Natural food folates or pteroylpolyglutamates are hydrolyzed to pteroylmonoglutamate forms prior to absorption in the small intestine. The monoglutamate forms of folate, including folic acid, are transported through the proximal small intestine via a saturable pH-dependent process. Higher doses of folic acid are absorbed via a nonsaturable passive diffusion process. The bioaccessibility of the ingested folate monoglutamates is significantly higher than that of folate polyglutamates due to the hydrolysis requirement of the latter (Fitzpatrick, 2003). Dietary folate consists of monoglutamate and

polyglutamate folate species. In the small intestine, folate polyglutamate is deconjugated to the monoglutamate form before absorption takes place by the α-glutamyl hydrolase, also known as conjugase, present in the brush border of the mucosal cells in the small intestine, and then the monoglutamate may be absorbed and transported into the portal vein (McNulty & Pentieva, 2004).

Salicylic acid-induced elicitation of folates in coriander (*Coriandrum sativum* L.) improved their bioaccessibility and also imparted reduction of oxidative status and enhancement of antioxidant enzyme activities in coriander foliage (Puthusseri et al., 2013).

Oat-bran consumption did not affect the vitamin B_6 and folate status, but helped to improve the vitamin B_{12} bioavailability in the elderly, with multiple chronic diseases and living in nursing homes (Sturtzel, Dietrich, Wagner, Gisinger, & Elmadfa, 2010). Therefore nitrile glycoside "niaziridin" was also shown to promote and augment the intestinal absorption of vitamin B_{12} (Khanuja et al., 2005). A novel controlled-release gastroretentive unfolding dosage form was shown to increase the bioavailability of riboflavin in humans (Kagan et al., 2006). And more recently, the bioavailability of vitamin B_{12} increased with the use of intraintestinal administration of chitosan in vivo (Goto, Masuda, & Aiba, 2015).

On the other hand, the bioavailability of dietary vitamin C represents the proportion of the micronutrient that is absorbed by the intestines and is available for metabolic processes within the body. Maintaining adequate vitamin C levels in the body is essential for collagen, catecholamine, and carnitine biosynthesis; absorption of nonheme iron; and antioxidant protection. Hence, it plays a critical role in normal functioning of the body and optimum health. Numerous factors, both endogenous (vitamin C transporters that regulate the vitamin's bioavailability and plasma and tissue concentrations; metabolic oxidative-reductive reactions in which vitamin C participates with metal ions as cofactors) and exogenous (environmental conditions, oxidative stress, infections, and inflammation), contribute to the status of vitamin C in the body (Michels, Hagen, & Frei, 2013). Vitamin C is actively transported into the body via two sodium-dependent vitamin C transporters, SVCT1 and SVCT2 (Savini, Rossi, Pierro, Avigliano, & Catani, 2008). These transporters exhibit different tissue distributions and uptake kinetics. SVCT1 is expressed in epithelial tissues and is primarily responsible for intestinal uptake and renal reabsorption of vitamin C. The latter helps to maintain whole-body homeostasis (Savini et al., 2008). Although the bioavailability of vitamin C (comprising ascorbic acid and dehydroascorbic acid) ranges between 80% and 100% at doses normally ingested, its oxidation and further degradation during processing, storage, and digestion may limit the nutritional value of a dietary source (Aschoff et al., 2015).

As noted earlier, the bioaccessibility of vitamin C is high, so studies on the technology and chemicals or additives that can enhance this bioavailability are limited. This vitamin is also used to improve the bioavailability of other micronutrients, especially iron (Teucher, Olivares, & Cori, 2004). Nonetheless, aloe vera liquid preparations were particularly effective in maintaining high levels of circulating ascorbate with prolonged plasma concentrations even for 24 h (Vinson, Al Kharrat, & Andreoli, 2005), demonstrating the benefits of aloe vera preparations in increasing the bioavailability of vitamin C, among other vitamins (Yun et al., 2010).

6.6.2 FAT-SOLUBLE VITAMINS

Vitamin A and carotenoids are absorbed into the gastrointestinal mucosal cells and appear unchanged in circulation and tissues (Rao & Rao, 2007). In the intestine the carotenoids are absorbed by passive diffusion after being incorporated into the micelles that are formed by dietary fat and bile

acids. The micellular carotenoids are then incorporated into the chylomicrons and released into the lymphatic system. Thereafter, they are again incorporated into the lipoprotein at the site of the liver and released into the blood stream. Carotenoids are lipophilic molecules and, as such, their absorption in the different tissues of the body depends on the lipidic composition of the particular tissue. (Rao & Rao, 2007). Nevertheless, the influence of each digestive phase has not been completely explored and the stability of food fortificants (pure carotenoids, retinyl acetate, or palmitate) during digestion is unknown. Indeed, only carotenoids and retinoids (retinol and retinyl esters) that are stable throughout digestion may be absorbed and reach the target tissues via plasma carriers (such as lipoproteins and retinol-binding protein) (Courraud, Berger, Cristol, & Avallone, 2013).

The bioavailability of vitamin A depends on many dietary factors (Fig. 6.2) such as the food matrix; amount, type, and digestibility of dietary fat; and interactions between carotenoids (Van Het Hof, West, Weststrate, & Hautvast, 2000). In some studies an increase in the bioaccessibility of vitamin A was achieved. The bioaccessibility of vitamin A and iron, estimated through in vitro digestibility of milk fortified with both nutrients, was found to be slightly higher than that of milk fortified with iron alone (Sachdeva, Kaushik, Arora, & Indumathi, 2015). Various fats and oils and long-chain triacylglycerols enhanced the bioaccessibility of β-carotene present in spinach (Nagao, Kotake-Nara, & Hase, 2013). Moreover, the bioaccessibility of vitamin A was reported to increase 30% if combined with ginger extract (Ajazuddin et al., 2014).

Serum vitamin D and 25(OH)D levels are dependent on several factors: cutaneous production of vitamin D_3, dietary intake, and intestinal absorption of D_3 and D_2, as well as the metabolic activation and subsequent conversion of vitamin D to its metabolites for excretion. Vitamin D is a secosteroid hormone that is made in the skin from 7-dehydrocholesterol on exposure of the skin to UV−B radiation. Vitamin D is also obtained in the diet primarily from vitamin D-fortified foods or by the use of vitamin D supplements (Holick, 2007). However, it is difficult to obtain it from the diet because it is not naturally present in many foods. In the 1930s, food and beverage manufacturers began to fortify milk, breads, hot dogs, sodas, and even beer with vitamin D. Nevertheless, the outbreak of vitamin D intoxication in Europe in the 1950s and the strict regulations issued by the US Food and Drug Administration limited the fortification to only milk and cereals in the 1950s. These policies have persisted today. In most European countries, fortification of dairy products is forbidden (Tangpricha et al., 2003).

Several studies have reported differences in the bioavailability of vitamin D supplements in some populations (Lark et al., 2001). Decreased bioavailability may be due to altered absorption of vitamin D from the small intestine or it may be due to altered metabolism of vitamin D in the body. Intestinal malabsorption disorders may cause a decrease in vitamin D absorption because of decreased ability to absorb lipids. Obesity has also been found to be associated with decreased 25(OH)D levels and may reflect the larger body volume of obese individuals or the sequestration of vitamin D in excess adipose tissue (Wortsman, Matsuoka, Chen, Lu, & Holick, 2000). Other factors have recently shown to improve the bioaccessibility of vitamin D, like a β-lactoglobulin-based coagulum, which was not rapidly disrupted by the proteases in the intestines, leading to a slow release of D_3, increased uptake of D_3, and subsequent enhancement of the bioavailability of D_3 in rats (Diarrassouba et al., 2014).

With respect to vitamin E, its bioavailability may be highly variable (ranging from $<1\%$ to 100%) depending on its chemical form and the nature of the food matrix it is incorporated within (O'Callaghan & O'Brien, 2010). Normally, vitamin E must first be released from the food matrix, then incorporated into mixed micelles, and subsequently transported across the mucous layer before it is absorbed by epithelium cells in the small intestine (Rigotti, 2007). It has also been reported that the esterified form of vitamin E (α-tocopheryl acetate) is less bioaccessible than the free form

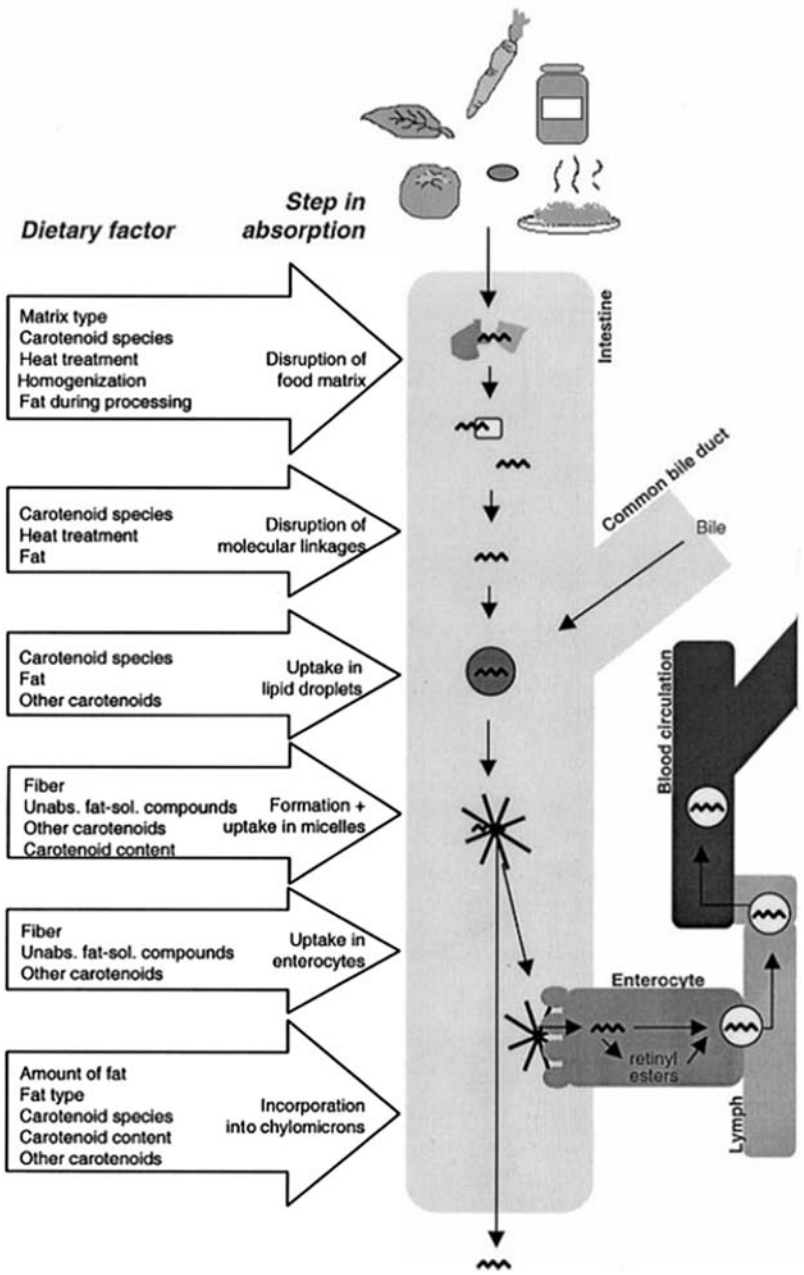


FIGURE 6.2

Steps of carotenoid absorption and dietary factors that affect carotenoid absorption (from Van Het Hof et al., 2000).

(α-tocopherol) (Eicher, Morrill, & Velazco, 1997), so it has to be converted into α-tocopherol by digestive enzymes (e.g., pancreatic esterases) within the gastrointestinal tract prior to absorption (Herrero-Barbudo, Granado-Lorencio, Blanco-Navarro, Pérez-Sacristán, & Olmedilla-Alonso, 2009). Some of the observed variations in vitamin E bioaccessibility may therefore also be due to differences in the chemical nature of the ingested form, e.g., free versus esterified. The wide variability reported in the bioavailability of vitamin E makes it difficult to interpret the results of clinical trials on the efficacy of vitamin E supplementation and to develop appropriate guidelines on the consumption levels of vitamin E (Rigotti, 2007).

One study reported some in vivo interactions between phenolic compounds and tocopherols that increased the bioavailability of vitamin E and decreased cholesterol in rats (Kamal-Eldin et al., 2000). Another paper, previously cited in this chapter, demonstrated that aloe vera preparations improve the absorption of both vitamins C and E (Vinson et al., 2005). It has also been shown that enhancement of intestinal permeability utilizing solid lipid nanoparticles increased γ-tocotrienol (a member of the vitamin E family) oral bioavailability (Abuasal et al., 2012). Long-chain triacylglycerols as carrier oils increased the conversion of α-tocopheryl acetate to α-tocopherol, which may impact the subsequent absorption of vitamin E, as previously described (Yang & McClements, 2013). A recent work evaluated the in vitro and in vivo performance of γ-tocotrienol incorporated in a self-emulsifying drug delivery system, resulting in an increase in its cellular uptake and oral bioavailability (Alqahtani, Alayoubi, Nazzal, Sylvester, & Kaddoumi, 2014).

The intestinal absorption of vitamin K follows a well-established pathway that applies to most dietary lipids, which includes bile salt and pancreatic-dependent soluble characteristics, uptake of mixed micelles into the enterocytes, the packaging of dietary lipids into chylomicrons, and their exocytosis into the lymphatic system (Shearer, Fu, & Booth, 2012). Chylomicrons are secreted from within the intestinal villi into the lymphatic capillaries (lacteals), which join larger lymphatic vessels and empty into the blood circulation via the thoracic duct. In the bloodstream, chylomicrons acquire apoC and apoE from HDL to enter the capillary beds of peripheral tissues. There, they lose much of their triglycerides through the action of lipoprotein lipase, at the same time losing apoA and C. The resulting chylomicron remnants that reenter the circulation are smaller and have a central lipid core with surface apoB-48 and apoE as you can see in Fig. 6.3 (Shearer et al., 2012).

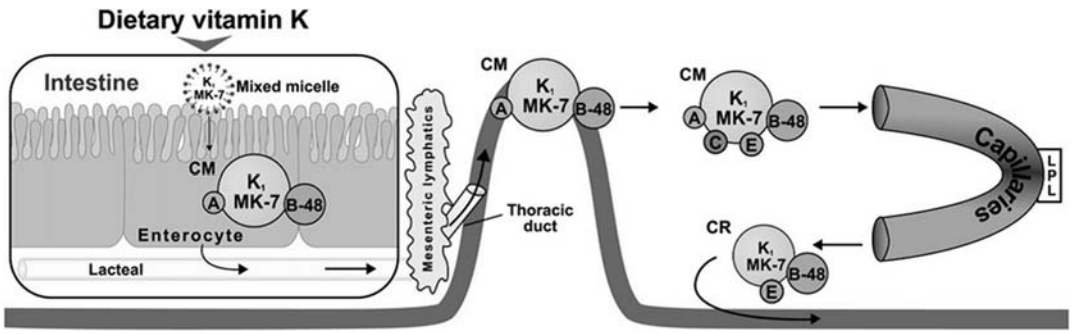


FIGURE 6.3

Intestinal absorption of dietary vitamin K: phylloquinone (K1) and MK-7 (from Shearer et al., 2012).

It has long been demonstrated that the oral bioavailability of lipid-soluble vitamin K is influenced by the fat content of a meal, although the increase in bioavailability seems to reach a peak when the lipid content of the meal is >35 g (Uematsu et al., 1996). There are some studies about warfarin and other vitamin K antagonists that are widely used as oral anticoagulants and blood thinners for treatment or prevention of thromboembolism, in particular, in cases of atrial fibrillation (Ageno et al., 2012; Vannacci et al., 2011). However, there is not much research on mechanisms that increase bioaccessibiliy of vitamin K, and only few studies exist with the aim of enhancing its skin penetration and absorption (Lopes, Speretta, & Bentley, 2007).

6.7 APPLICATION AND IMPACT ON SHELF-LIFE OF FOOD PRODUCTS

Vitamins can be used as food preservatives to increase the shelf-life of food products mainly due to their antioxidant activity. EU regulation 1129/2011 includes a list of food additives approved for use in foods and the conditions of use (Commission, 2011). In the case of vitamins, the use of ascorbic acid (E300) and its salts sodium ascorbate (E 301) and calcium ascorbate (E 302) is approved. Vitamin E has been approved in the forms of tocopherol-rich extract (E 306), alpha-tocopherol (E 307), gamma-tocopherol (E 308), delta-tocopherol (E 309), and fatty esters of ascorbic acid (E 304).

Meat is a good candidate for antioxidant addition due to its high content of fatty acids, metals, heme group, and low presence of antioxidants, which makes it more prone to oxidation by free radicals (Falowo, Fayemi, & Muchenje, 2014). High levels of reactive oxygen radicals cause a loss of amino acids such as phenylalanine and tryptophan (Ganhão, Morcuende, & Estévez, 2010) and reduce sensory properties.

Synthetic antioxidants such as butylated hydroxyl anisole or butylated hydroxy toluene have been used to retard lipid peroxidation. Today consumers demand safer food products with high nutritional quality and preferably using natural additives from different sources, such as fruit and vegetable extracts. Their intended uses are both to improve the nutrient profile as well as antioxidant and antimicrobial activities to avoid spoilage (Hygreeva, Pandey, & Radhakrishna, 2014). Natural products are often preferred by consumers as they are perceived as safer and to have more nutritional value.

The addition of vitamins to the final product is a strategy to avoid oxidation of meat and to increase the nutritional value. Another strategy is diet fortification in the animal. The animal can incorporate the nutrient in specific locations of different tissues (as cell membranes). This approach might be safer as the risk of overloading levels and potential toxic effects is minor (Bou, Codony, Tres, Decker, & Guardiola, 2009). In fact, addition of α-tocopherol in animal feeds is more effective at decreasing lipid peroxidation than the addition to processed meat, as the α-tocopherol ingested is able to incorporate into cell membranes where lipid peroxidation occurs (Bou et al., 2009). Attempts have also been made with PUFA-rich oils to improve the lipid profile of food products (Barroeta, 2007). However, concerns exist about the possible negative on the sensory qualities of meat products.

A current application of vitamins as food additives is in the production of edible coatings. These are thin layers of material that cover the surface of food that can be eaten. They act as gas

barriers and control moisture transfer, respiration rate, and oxidation processes and can incorporate preservatives to extend the commercial shelf-life of food (Vargas, Pastor, Chiralt, Mcclements, & González-Martínez, 2008).

Vitamin E has been used in conjunction with chitosan in edible films to improve physicochemical and antioxidant properties (Bonilla, Talón, Atarés, Vargas, & Chiralt, 2013; Lin & Pascall, 2014). Chitosan is a polymer used in food technology due to its antimicrobial properties; these large molecules have multiple cationic charges in their functional groups, which interact with anionic charges of the microbial membranes. As a result of these interactions, the membranes are disorganized and disrupted, which fact causes leakage of intracellular components (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001). Chitosan-based coatings have been enriched with vitamin E and incorporated in strawberries and raspberries (Han, Zhao, Leonard, & Traber, 2004).

Films made of polysaccharides are widely used for coating of fruits. Ascorbic acid has also been used as coating to maintain the nutritional quality of strawberries, as well as to reduce microbial populations (Sogvar, Saba, & Emamifar, 2016). Model fruit juices have been made using a combination of vitamins as additives to improve the stability of probiotic bacteria (Shah, Ding, Fallourd, & Leyer, 2010). The results showed that the model juice containing vitamin C was the most effective at preserving probiotic stability, while the one containing vitamin E did not have a favorable effect compared to the control juice (Shah et al., 2010). Vitamin C seemed to create a more favorable environment for probiotic bacteria.

6.8 CHALLENGES AND OPPORTUNITIES: VITAMINS FOR A HEALTHY POPULATION IN 2050

It is important to acknowledge the future challenges and opportunities for vitamins. The profitability of individual companies depends on product innovation, effective merchandising, and competitive pricing. Some of the major products sold in health supplement stores include vitamins, minerals, dietary supplements (about 80% of industry sales in the United States), and grocery items. This global market for vitamins is projected to reach US$9.3 billion by 2020, driven by rising health consciousness among the aging population and adoption of preventive healthcare practices.

Consumption of vitamins in the human healthcare and personal-care markets is driven by urbanization, and the resulting rise in stress caused by hectic lifestyles and pollution. Magnesium, B-complex vitamins, vitamin E, and multivitamins are some of the most widely consumed vitamins. Increase in life expectancy and the world's rapidly aging population are also spurring growth in the market. Emphasis on maintaining active lifestyles and the growing popularity of preventive healthcare are also creating a lucrative market for health supplements capable of preventing age-related diseases. Also poised to witness growth are vitamin-fortified foods and cosmetics among gen-X consumers.

As noted elsewhere in market research reports on vitamins, Asia–Pacific represents the largest market worldwide, followed closely by Europe. Increasing popularity of water-soluble vitamins, growing vitamin C demand, particularly in China, expanding middle-class population, aging population, higher disposable incomes, and changing personal health perceptions are key growth drivers in the region, and China represents the largest producer and consumer of vitamins worldwide.

Concomitant with the aging trend is the increase in the number of older adults with mental and physical disabilities. These facts necessitate new solutions to address primary and secondary prevention care and geriatric research. According to a report by the United Nations (United Nations Population Facts, 2012), the major cause of global deaths (36 million or 63%) by 2050 will be from noncommunicable diseases (NCD) instead of infections. Four categories of NCDs account for 80% (20 million) of global mortality causes: cardiovascular disease, cancer, diabetes, and chronic respiratory diseases. The World Health Organization (WHO) estimates that over 20 million deaths can be prevented by reducing the exposure level to key modifier risks such as unhealthy diets, physical inactivity, tobacco, and alcohol use. Thus far, obesity, hypertension, cardiovascular diseases, and diabetes continue to pose significant risks at pandemic proportion. Of further concern is the growing number of children who are overweight and at risk of obesity, sedentary behavior, and early onset of type 2 diabetes. To address these global challenges in food and health, countries have issued national food, nutrition, and health guidelines and regulations, and in all these programs, appropriate vitamin intake is needed for a balanced diet and reduced risk of disease (e.g., vitamin D and Alzheimer's disease).

Vitamins are complex molecular structures and even today, many areas of vitamin biochemistry are not yet fully understood. Novel effects and functions of vitamins remain and continue to be discovered. The relationship between vitamins and some health effects are clear today including:

1. Vitamin B_1 and energy metabolism and nerve function (Kochetov & Solovjeva, 2014);
2. Vitamin B_2 and energy metabolism, normal vision, and skin health (Joosten & van Berkel, 2007);
3. Vitamin B_3 and food digestion, nerve function, improves lipoprotein profiles, and skin health (Maccubbin et al., 2009);
4. Vitamin B_5 and energy metabolism (Depeint, Bruce, Shangari, Mehta, & O'brien, 2006);
5. Vitamin B_6 and amino acid and fatty acid metabolism, red blood cell production, and lower homocysteine levels (Depeint et al., 2006);
6. Vitamin B_8 and metabolism, food-energy conversion, and fat and glycogen synthesis (Schellack et al., 2015);
7. Vitamin B_9 and DNA synthesis and new cell formation (Choi & Mason, 2000);
8. Vitamin B_{12} and the reduction of homocysteine levels, reduction of risk of heart diseases, DNA synthesis, and support of nerve-cell maintenance (Mataix & Várela Moreiras, 2009);
9. Vitamin C and their antioxidant function, synthesis of collagen, L-carnitine, the conversion of dopamine to noradrenaline, and the absorption of iron and the release of their deposits (Traber & Stevens, 2011);
10. Vitamin A and vision, bone growth, reproduction, cell division, and differentiation of epithelial tissues as well as in the regulation of the immune system (Hernandez, 2010);
11. Vitamin D and the homeostasis of calcium and phosphorus in bones (Lips & van Schoor, 2011);
12. Vitamin E and its function against oxidative stress and protection of other antioxidants in cells (Li et al., 2011);
13. Vitamin K and the synthesis of bone proteins that enable the bonding of minerals for bone formation (Bügel, 2003).

Moreover, it is well known that deficiencies of any vitamin can cause serious health problems, so food fortification and health supplements capable of preventing age-related diseases or to be incorporated as coadjutants in nutrition programs is a growing trend today, which is expected to continue in the coming years.

ACKNOWLEDGMENTS

This work was partially supported by the Project MINECO AGL2013-466247-P in Spain, and the Grant for *Research Group of Excellence—Fundación Seneca*, Murcia Regional Agency for Science and Technology, Project 19900/GERM/15. The authors would also like to thank the CYTED Programme, Action 112RT0460 CORNUCOPIA Thematic Network.

REFERENCES

Abuasal, B. S., Lucas, C., Peyton, B., Alayoubi, A., Nazzal, S., Sylvester, P. W., & Kaddoumi, A. (2012). Enhancement of intestinal permeability utilizing solid lipid nanoparticles increases γ-tocotrienol oral bioavailability. *Lipids*, *47*(5), 461−469.

Ageno, W., Gallus, A. S., Wittkowsky, A., Crowther, M., Hylek, E. M., & Palareti, G. (2012). Oral anticoagulant therapy − Antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*, *141*(2 Suppl.), e44S−e88S.

Aisen, P. S., Schneider, L. S., Sano, M., Diaz-Arrastia, R., Van Dyck, C. H., Weiner, M. F., … Thomas, R. G. (2008). High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: A randomized controlled trial. *JAMA: The Journal of the American Medical Association*, *300*(15), 1774−1783.

Ajazuddin, Alexander, A., Qureshi, A., Kumari, L., Vaishnav, P., Sharma, M., … Saraf, S. (2014). Role of herbal bioactives as a potential bioavailability enhancer for Active Pharmaceutical Ingredients. *Fitoterapia*, *97*, 1−14.

Alhamad, T. A., O'brart, D. P. S., O'brart, N. A. L., & Meek, K. M. (2012). Evaluation of transepithelial stromal riboflavin absorption with enhanced riboflavin solution using spectrophotometry. *Journal of Cataract and Refractive Surgery*, *38*(5), 884−889.

Alqahtani, S., Alayoubi, A., Nazzal, S., Sylvester, P. W., & Kaddoumi, A. (2014). Enhanced solubility and oral bioavailability of γ-tocotrienol using a self-emulsifying drug delivery system (SEDDS). *Lipids*, *49*(8), 819−829.

Amer, M., & Qayyum, R. (2012). Relation between serum 25-hydroxyvitamin D and C-reactive protein in asymptomatic adults (from the continuous National Health and Nutrition Examination Survey 2001 to 2006). *The American journal of Cardiology*, *109*(2), 226−230.

Ananthakrishnan, A. N., Khalili, H., Higuchi, L. M., Bao, Y., Korzenik, J. R., Giovannucci, E. L., … Chan, A. T. (2012). Higher predicted vitamin D status is associated with reduced risk of Crohn's disease. *Gastroenterology*, *142*(3), 482−489.

Aoac, I. (2006). *Official methods of AOAC international*, Gaithersburg.

Ardizzone, S., Cassinotti, A., Trabattoni, D., Manzionna, G., Rainone, V., Bevilacqua, M., … Clerici, M. (2009). Immunomodulatory effects of 1, 25-dihydroxyvitamin D3 on TH1/TH2 cytokines in inflammatory bowel disease: An in vitro study. *International Journal of Immunopathology and Pharmacology*, *22*(1), 63−71.

Aschoff, J. K., Kaufmann, S., Kalkan, O., Neidhart, S., Carle, R., & Schweiggert, R. M. (2015). In vitro bioaccessibility of carotenoids, flavonoids, and vitamin C from differently processed oranges and orange juices [*Citrus sinensis* (L.) osbeck]. *Journal of Agricultural and Food Chemistry*, *63*(2), 578−587.

Ashor, A. W., Siervo, M., Lara, J., Oggioni, C., Afshar, S., & Mathers, J. C. (2015). Effect of vitamin C and vitamin E supplementation on endothelial function: A systematic review and meta-analysis of randomised controlled trials. *British Journal of Nutrition*, *113*(08), 1182−1194.

Bacher, A., Eberhardt, S., Eisenreich, W., Fischer, M., Herz, S., Illarionov, B., … Richter, G. (2001). Biosynthesis of riboflavin. *Vitamins & Hormones*, *61*, 1−49.

Badimon, L., Vilahur, G., & Padro, T. (2010). Nutraceuticals and atherosclerosis: Human trials. *Cardiovascular Therapeutics*, *28*(4), 202−215.

Ball, G. F. M. (2005). *Vitamins in foods, analysis, bioavailability and stability*. Boca Ratón: CRC Press.
Ballard, F. J., & Hanson, R. W. (1967). The citrate cleavage pathway and lipogenesis in rat adipose tissue: Replenishment of oxaloacetate. *Journal of Lipid Research*, *8*(2), 73−79.
Barba, F. J., Esteve, M. J., & Frigola, A. (2013). Physicochemical and nutritional characteristics of blueberry juice after high pressure processing. *Food Research International*, *50*(2), 545−549.
Barroeta, A. (2007). Nutritive value of poultry meat: Relationship between vitamin E and PUFA. *World's Poultry Science Journal*, *63*(02), 277−284.
Battault, S., Whiting, S., Peltier, S., Sadrin, S., Gerber, G., & Maixent, J. (2013). Vitamin D metabolism, functions and needs: From science to health claims. *European Journal of Nutrition*, *52*(2), 429−441.
Begley, T. P., Xi, J., Kinsland, C., Taylor, S., & Mclafferty, F. (1999). The enzymology of sulfur activation during thiamin and biotin biosynthesis. *Current Opinion in Chemical Biology*, *3*(5), 623−629.
Beilfuss, J., Berg, V., Sneve, M., Jorde, R., & Kamycheva, E. (2012). Effects of a 1-year supplementation with cholecalciferol on interleukin-6, tumor necrosis factor-alpha and insulin resistance in overweight and obese subjects. *Cytokine*, *60*(3), 870−874.
Berton, P., Monasterio, R. P., & Wuilloud, R. G. (2012). Selective extraction and determination of vitamin B12 in urine by ionic liquid-based aqueous two-phase system prior to high-performance liquid chromatography. *Talanta*, *97*, 521−526.
Bhat, R., Kamaruddin, N. S. B. C., Min-Tze, L., & Karim, A. A. (2011). Sonication improves kasturi lime (*Citrus microcarpa*) juice quality. *Ultrasonics Sonochemistry*, *18*(6), 1295−1300.
Bi, X., Liu, F., Rao, L., Li, J., Liu, B., Liao, X., & Wu, J. (2013). Effects of electric field strength and pulse rise time on physicochemical and sensory properties of apple juice by pulsed electric field. *Innovative Food Science and Emerging Technologies*, *17*, 85−92.
Blake, C. J. (2007). Analytical procedures for water-soluble vitamins in foods and dietary supplements: A review. *Analytical and Bioanalytical Chemistry*, *389*(1), 63−76.
Bonilla, J., Talón, E., Atarés, L., Vargas, M., & Chiralt, A. (2013). Effect of the incorporation of antioxidants on physicochemical and antioxidant properties of wheat starch−chitosan films. *Journal of Food Engineering*, *118*(3), 271−278.
Bou, R., Codony, R., Tres, A., Decker, E. A., & Guardiola, F. (2009). Dietary strategies to improve nutritional value, oxidative stability, and sensory properties of poultry products. *Critical Reviews in Food Science and Nutrition*, *49*(9), 800−822.
Brigelius-Flohe, R., & Traber, M. G. (1999). Vitamin E: Function and metabolism. *The FASEB Journal*, *13* (10), 1145−1155.
Brøndum-Jacobsen, P., Benn, M., Jensen, G. B., & Nordestgaard, B. G. (2012). 25-Hydroxyvitamin D levels and risk of ischemic heart disease, myocardial infarction, and early death population-based study and meta-analyses of 18 and 17 Studies. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *32*(11), 2794−2802.
Buettner, G. R. (1993). The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol, and ascorbate. *Archives of Biochemistry and Biophysics*, *300*(2), 535−543.
Bügel, S. (2003). Vitamin K and bone health. *Proceedings of the Nutrition Society*, *62*(04), 839−843.
Butz, P., & Tauscher, B. (2002). Emerging technologies: Chemical aspects. *Food Research International*, *35* (2−3), 279−284.
Cardoso, D. R., Libardi, S. H., & Skibsted, L. H. (2012). Riboflavin as a photosensitizer. Effects on human health and food quality. *Food and Function*, *3*(5), 487−502.
Chatzimichalakis, P. F., Samanidou, V. F., & Papadoyannis, I. N. (2004). Development of a validated liquid chromatography method for the simultaneous determination of eight fat-soluble vitamins in biological fluids after solid-phase extraction. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *805*(2), 289−296.

Chen, P., Atkinson, R., & Wolf, W. R. (2009). Single-laboratory validation of a high-performance liquid chromatographic-diode array detector-fluorescence detector/mass spectrometric method for simultaneous determination of water-soluble vitamins in multivitamin dietary tablets. *Journal of AOAC International*, *92* (2), 680−687.

Chen, J., Tao, X. Y., Sun, A. D., Wang, Y., Liao, X. J., Li, L. N., & Zhang, S. (2014). Influence of pulsed electric field and thermal treatments on the quality of blueberry juice. *International Journal of Food Properties*, *17*(7), 1419−1427.

Cheng, L. H., Soh, C. Y., Liew, S. C., & Teh, F. F. (2007). Effects of sonication and carbonation on guava juice quality. *Food Chemistry*, *104*(4), 1396−1401.

Choi, S. W., & Mason, J. B. (2000). Folate and carcinogenesis: An integrated scheme. *Journal of Nutrition*, *130*(2), 129−132.

Chuanyao, W., Mengyue, J., Meixu, G., Shufen, Z., & Shucheng, L. (1993). A study of the physiological changes and the nutritional qualities of irradiated apples and the effect of irradiation on apples stored at room temperature. *Radiation Physics and Chemistry*, *42*(1), 347−350.

Chun, J., Martin, J. A., Chen, L., Lee, J., Ye, L., & Eitenmiller, R. R. (2006). A differential assay of folic acid and total folate in foods containing enriched cereal-grain products to calculate μg dietary folate equivalents (μg DFE). *Journal of Food Composition and Analysis*, *19*(2−3), 182−187.

Clarke, R., Newman, C., Tomson, J., Hin, H., Kurien, R., Cox, J., … Emberson, J. (2015). Estimation of the optimum dose of vitamin D for disease prevention in older people: Rationale, design and baseline characteristics of the BEST-D trial. *Maturitas*, *80*(4), 426−431.

Commission, E. (2011). Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. *Official Journal of the European Union L*, *295*(4), p. 12.11.

Cortés, C., Esteve, M. J., Rodrigo, D., Torregrosa, F., & Frígola, A. (2006). Changes of colour and carotenoids contents during high intensity pulsed electric field treatment in orange juices. *Food and Chemical Toxicology*, *44*(11), 1932−1939.

Courraud, J., Berger, J., Cristol, J. P., & Avallone, S. (2013). Stability and bioaccessibility of different forms of carotenoids and vitamin A during in vitro digestion. *Food Chemistry*, *136*(2), 871−877.

Croci, C., Banek, S., & Curzio, O. (1995). Effect of gamma-irradiation and extended storage on chemical quality in onion (*Allium cepa* L.). *Food Chemistry*, *54*(2), 151−154.

Cruz, R. M. S., Vieira, M. C., & Silva, C. L. M. (2008). Effect of heat and thermosonication treatments on watercress (*Nasturtium officinale*) vitamin C degradation kinetics. *Innovative Food Science and Emerging Technologies*, *9*(4), 483−488.

De Leenheer, A. P., & Lambert, W. (2000). *Modern Chromatographic Analysis Of Vitamins: Revised And Expanded*. CRC Press.

Dellapenna, D., & Pogson, B. J. (2006). Vitamin synthesis in plants: Tocopherols and carotenoids. *Annual Review of Plant Biology*, *57*, 711−738.

Deng, Q., Zinoviadou, K. G., Galanakis, C. M., Orlien, V., Grimi, N., Vorobiev, E., … Barba, F. J. (2014). The effects of conventional and nonconventional processing on glucosinolates and its derived forms, isothiocyanates: Extraction, degradation and applications. *Food Engineering Reviews*, *7*, 357−381.

Depeint, F., Bruce, W. R., Shangari, N., Mehta, R., & O'brien, P. J. (2006). Mitochondrial function and toxicity: Role of the B vitamin family on mitochondrial energy metabolism. *Chemico-Biological Interactions*, *163*(1−2), 94−112.

Devries, J. W., Rader, J. I., Keagy, P. M., Hudson, C. A., Angyal, G., Arcot, J., … Wurz, C. (2005). Microbiological assay-trienzyme procedure for total folates in cereals and cereal foods: Collaborative study. *Journal of AOAC International*, *88*(1), 5−15.

Dewick, P. M. (2002). *Medicinal natural products: A biosynthetic approach*. John Wiley & Sons.

Diarrassouba, F., Garrait, G., Remondetto, G., Alvarez, P., Beyssac, E., & Subirade, M. (2014). Improved bioavailability of vitamin D3 using a β-lactoglobulin-based coagulum. *Food Chemistry*, *172*, 361−367.

Diehl, J. F. (1999). *Safety of irradiated foods*. CRC Press.

Dinelli, G., & Bonetti, A. (1994). Micellar electrokinetic capillary chromatography analysis of water-soluble vitamins and multi-vitamin integrators. *Electrophoresis*, *15*(1), 1147−1150.

Dionísio, A. P., Gomes, R. T., & Oetterer, M. (2009). Ionizing radiation effects on food vitamins: A review. *Brazilian Archives of Biology and Technology*, *52*(5), 1267−1278.

Doerflinger, N., Linder, C., Ouahchi, K., Gyapay, G., Weissenbach, J., Le Paslier, D., ... Hentati, F. (1995). Ataxia with vitamin E deficiency: Refinement of genetic localization and analysis of linkage disequilibrium by using new markers in 14 families. *American Journal of Human Genetics*, *56*(5), 1116.

Drewke, C., & Leistner, E. (2001). Biosynthesis of vitamin B 6 and structurally related derivatives. *Vitamins & Hormones*, *61*, 121−155.

Drouin, G., Godin, J. R., & Page, B. (2011). The genetics of vitamin C loss in vertebrates. *Current Genomics*, *12*(5), 371−378.

Du, Q., Wang, H., & Xie, J. (2011). Thiamin (vitamin B1) biosynthesis and regulation: A rich source of antimicrobial drug targets? *International Journal of Biological Sciences*, *7*(1), 41−52.

EFSA Panel on Dietetic Products, & Nutrition and Allergies (NDA) (2009). Scientific opinion on the substantiation of health claims related to biotin and energy-yielding metabolism (ID 114, 117), macronutrient metabolism (ID 113, 114, 117), maintenance of skin and mucous membranes (ID 115), maintenance of hair (ID 118, 2876) and function of the nervous system (ID 116) pursuant to Article 13(1) of Regulation (EC) No 1924/2006 on request from the European Commission. *EFSA Journal*, *7*(9), 1209 . [17 pp.]. http://dx.doi.org/10.2903/j.efsa.2009.1209. Available online: www.efsa.europa.eu.

Eicher, S. D., Morrill, J. L., & Velazco, J. (1997). Bioavailability of α-tocopherol fed with retinol and relative bioavailability of D-α-tocopherol or DL-α-tocopherol acetate1. *Journal of Dairy Science*, *80*(2), 393−399.

Elez-Martínez, P., & Martín-Belloso, O. (2007). Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, *102*(1), 201−209.

Elez-Martínez, P., Soliva-Fortuny, R. C., & Martín-Belloso, O. (2006). Comparative study on shelf life of orange juice processed by high intensity pulsed electric fields or heat treatment. *European Food Research and Technology*, *222*(3−4), 321−329.

Ellinson, M., Thomas, J., & Patterson, A. (2004). A critical evaluation of the relationship between serum vitamin B12, folate and total homocysteine with cognitive impairment in the elderly. *Journal of Human Nutrition and Dietetics*, *17*(4), 371−383.

Elmadfa, I., Meyer, A., & Nowak, V. (2009). European Nutrition and Health Report 2009 (Title Page). *Annals of Nutrition and Metabolism*, *55*(Suppl. 2), I−V.

Engel, R., Stefanovits-Bányai, É., & Abrankó, L. (2010). LC simultaneous determination of the free forms of B group vitamins and vitamin C in various fortified food products. *Chromatographia*, *71*(11−12), 1069−1074.

Escobedo-Avellaneda, Z., Gutiérrez-Uribe, J., Valdez-Fragoso, A., Torres, J. A., & Welti-Chanes, J. (2015). High Hydrostatic Pressure Combined with Mild Temperature for the Preservation of Comminuted Orange: Effects on Functional Compounds and Antioxidant Activity. *Food and Bioprocess Technology*, *8*(5), 1032−1044.

Falowo, A. B., Fayemi, P. O., & Muchenje, V. (2014). Natural antioxidants against lipid−protein oxidative deterioration in meat and meat products: A review. *Food Research International*, *64*, 171−181.

Fan, X., & Mattheis, J. P. (2001). 1-Methylcyclopropene and storage temperature influence responses of 'Gala'apple fruit to gamma irradiation. *Postharvest Biology and Technology*, *23*(2), 143−151.

FDA (2000). Kinetics of microbial inactivation for alternative food processing technologies.

Fellows, P. J. (2009). *Food processing technology: Principles and practice*. Elsevier.
Fitzpatrick, A. (2003). Folate (folic acid): Implications for health and disease. *Agro Food Industry Hi-Tech*, *14* (3), 45–48.
Floros, J. D., & Liang, H. (1994). Acoustically assisted diffusion through membranes and biomaterials. *Food Technology*, *48*(12), 79–84.
Fotsing, L., Fillet, M., Bechet, I., Hubert, P., & Crommen, J. (1997). Determination of six water-soluble vitamins in a pharmaceutical formulation by capillary electrophoresis. *Journal of Pharmaceutical and Biomedical Analysis*, *15*(8), 1113–1123.
Frias, J., Peñas, E., Ullate, M., & Vidal-Valverde, C. (2010). Influence of drying by convective air dryer or power ultrasound on the Vitamin C and β-carotene content of carrots. *Journal of Agricultural and Food Chemistry*, *58*(19), 10539–10544.
Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology*, *26*, 68–87.
Galanakis, C. M. (2013). Emerging technologies for the production of nutraceuticals from agricultural by-products: A viewpoint of opportunities and challenges. *Food and Bioproducts Processing*, *91*, 575–579.
Galanakis, C.M., Barba, F.J., Prasad, K.N., 2015. Cost and safety issues of emerging technologies against conventional techniques, In Galanakis, C.M. (Eds.), Food Waste Recovery: Processing Technologies and Industrial Techniques.
Galanakis, C. M., & Schieber, A. (2014). Editorial. Special issue on recovery and utilization of valuable compounds from food processing by-products. *Food Research International*, *65*, 299-230.
Gamboa-Santos, J., Montilla, A., Soria, A. C., Cárcel, J. A., García-Pérez, J. V., & Villamiel, M. (2014). Impact of power ultrasound on chemical and physicochemical quality indicators of strawberries dried by convection. *Food Chemistry*, *161*, 40–46.
Ganhão, R., Morcuende, D., & Estévez, M. (2010). Protein oxidation in emulsified cooked burger patties with added fruit extracts: Influence on colour and texture deterioration during chill storage. *Meat Science*, *85* (3), 402–409.
García, E. V. (2009). *Manual práctico de nutricion y dietoterapia*. Instituto Monsa de Ediciones, S.A.
Gentili, A., & Caretti, F. (2011). Evaluation of a method based on liquid chromatography-diode array detector-tandem mass spectrometry for a rapid and comprehensive characterization of the fat-soluble vitamin and carotenoid profile of selected plant foods. *Journal of Chromatography A*, *1218*(5), 684–697.
Gentili, A., Caretti, F., Bellante, S., Ventura, S., Canepari, S., & Curini, R. (2013). Comprehensive profiling of carotenoids and fat-soluble vitamins in milk from different animal species by LC-DAD-MS/MS hyphenation. *Journal of Agricultural and Food Chemistry*, *61*(8), 1628–1639.
Giovannucci, E. (2010). Epidemiology of vitamin D and colorectal cancer: Casual or causal link? *The Journal of Steroid Biochemistry and Molecular Biology*, *121*(1), 349–354.
Goodman, D. S. (1969). Biosynthesis of vitamin A from beta-carotene. *American Journal of Clinical Nutrition*, *22*(7), 963–965.
Goto, Y., Masuda, A., & Aiba, T. (2015). In vivo application of chitosan to improve bioavailability of cyanocobalamin, a form of vitamin B12, following intraintestinal administration in rats. *International Journal of Pharmaceutics*, *483*(1–2), 250–255.
Gunasekaran, S., & Ay, C. (1994). Evaluating milk coagulation with ultrasonics. *Food Technology*, *48*(12), 74–78.
Haas, R. H. (1988). Thiamin and the brain. *Annual Review of Nutrition*, *8*, 483–515.
Halliwell, B. (1996). Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radical Research*, *25*(1), 57–74.

Han, C., Zhao, Y., Leonard, S., & Traber, M. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (Fragaria × ananassa) and raspberries (Rubus ideaus). *Postharvest Biology and Technology*, *33*(1), 67–78.

Hancock, R. D., Galpin, J. R., & Viola, R. (2000). Biosynthesis of L-ascorbic acid (vitamin C) by *Saccharomyces cerevisiae*. *FEMS Microbiology Letters*, *186*(2), 245–250.

Harris, D. M., & Go, V. L. W. (2004). Vitamin D and colon carcinogenesis. *The Journal of Nutrition*, *134*(12), 3463S–3471S.

Helander, I., Nurmiaho-Lassila, E.-L., Ahvenainen, R., Rhoades, J., & Roller, S. (2001). Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. *International Journal of Food Microbiology*, *71*(2), 235–244.

Hemila, H., & Chalker, E. (2013). Vitamin C for preventing and treating the common cold. *Cochrane Database of Systematic Reviews (Online)*, *1*.

Hernandez, A. G. D. (2010). *Tratado de nutrición: Bases fisiológicas y bioquímicas de la nutrición*. Ed. Médica Panamericana.

Herrero-Barbudo, M. C., Granado-Lorencio, F., Blanco-Navarro, I., Pérez-Sacristán, B., & Olmedilla-Alonso, B. (2009). Applicability of an in vitro model to assess the bioaccessibility of vitamins A and E from fortified commercial milk. *International Dairy Journal*, *19*(1), 64–67.

Hohmann, S., & Meacock, P. A. (1998). Thiamin metabolism and thiamin diphosphate-dependent enzymes in the yeast *Saccharomyces cerevisiae*: Genetic regulation. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, *1385*(2), 201–219.

Holick, M. F. (2007). Vitamin D deficiency. *New England Journal of Medicine*, *357*(3), 266–281.

Hygreeva, D., Pandey, M., & Radhakrishna, K. (2014). Potential applications of plant based derivatives as fat replacers, antioxidants and antimicrobials in fresh and processed meat products. *Meat Science*, *98*(1), 47–57.

Jäpelt, R. B., & Jakobsen, J. (2013). Vitamin D in plants: A review of occurrence, analysis, and biosynthesis. *Frontiers in Plant Science*, *4*(May).

Jia, W., Chu, X., Chang, J., & Zhang, F. (2015). High-throughput screening of vitamins and natural antioxidants in nutraceuticals from green tea extracts by liquid chromatography coupled to quadrupole orbitrap mass spectrometry. *Journal of Chromatography A*, *1406*, 337–341.

Jiménez, A., Beltrán, G., & Uceda, M. (2007). High-power ultrasound in olive paste pretreatment. Effect on process yield and virgin olive oil characteristics. *Ultrasonics Sonochemistry*, *14*(6), 725–731.

Jiménez-Aguilar, D. M., Escobedo-Avellaneda, Z., Martín-Belloso, O., Gutiérrez-Uribe, J., Valdez-Fragoso, A., García-García, R., … Welti-Chanes, J. (2015). Effect of high hydrostatic pressure on the content of phytochemical compounds and antioxidant activity of prickly pears (*Opuntia ficus-indica*) beverages. *Food Engineering Reviews*, *7*(2), 198–208.

Johnston, C. S. (1996). *The antihistamine action of ascorbic acid. Subcellular Biochemistry*. Springer.

Joosten, V., & Van Berkel, W. J. (2007). Flavoenzymes. *Current Opinion in Chemical Biology*, *11*(2), 195–202.

Jørgensen, S. P., Agnholt, J., Glerup, H., Lyhne, S., Villadsen, G. E., Hvas, C. L., … Dahlerup, J. F. (2010). Clinical trial: Vitamin D3 treatment in Crohn's disease–a randomized double-blind placebo-controlled study. *Alimentary Pharmacology & Therapeutics*, *32*(3), 377–383.

Josephson, E. S., Thomas, M., & Calhoun, W. (1978). Nutritional aspects of food irradiation: An overview. *Journal of Food Processing and Preservation*, *2*(4), 299–313.

Josic, D., Hoffer, L., & Buchacher, A. (2003). Preparation of vitamin K-dependent proteins, such as clotting factors II, VII, IX and X and clotting inhibitor protein C. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *790*(1–2), 183–197.

Jungnickel, P. W., Maloley, P. A., Vander Tuin, E. L., Peddicord, T. E., & Campbell, J. R. (1997). Effect of two aspirin pretreatment regimens on niacin-induced cutaneous reactions. *Journal of General Internal Medicine*, *12*(10), 591–596.

Kagan, L., Lapidot, N., Afargan, M., Kirmayer, D., Moor, E., Mardor, Y., … Hoffman, A. (2006). Gastroretentive Accordion Pill: Enhancement of riboflavin bioavailability in humans. *Journal of Controlled Release*, *113*(3), 208−215.

Kamal-Eldin, A., Frank, J., Razdan, A., Tengblad, S., Basu, S., & Vessby, B. (2000). Effects of dietary phenolic compounds on tocopherol, cholesterol, and fatty acids in rats. *Lipids*, *35*(4), 427−435.

Kaushik, R., Sachdeva, B., Arora, S., & Wadhwa, B. K. (2014). Development of an analytical protocol for the estimation of vitamin D2 in fortified toned milk. *Food Chemistry*, *151*, 225−230.

Khandpur, P., & Gogate, P. R. (2015). Effect of novel ultrasound based processing on the nutrition quality of different fruit and vegetable juices. *Ultrasonics Sonochemistry*, *27*, 125−136.

Khanuja, S.P.S., Arya, J.S., Tiruppadiripuliyur, R.S.K., Saikia, D., Kaur, H., Singh, M., … Srivastava, S.K. (2005). Nitrile glycoside useful as a bioenhancer of drugs and nutrients, process of its isolation from moringa oleifera. Google Patents.

Kilcast, D. (1994). Effect of irradiation on vitamins. *Food Chemistry*, *49*(2), 157−164.

Kim, D. O., Lee, K. W., Lee, H. J., & Lee, C. Y. (2002). Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *Journal of Agricultural and Food Chemistry*, *50*(13), 3713−3717.

Klibanski, A., Adams-Campbell, L., Bassford, T., Blair, S. N., Boden, S. D., Dickersin, K., … Russell, W. E. (2001). Osteoporosis prevention, diagnosis, and therapy. *Journal of the American Medical Association*, *285*(6), 785−795.

Kochetov, G. A., & Solovjeva, O. N. (2014). Structure and functioning mechanism of transketolase. *Biochimica et Biophysica Acta (BBA)−Proteins and Proteomics*, *1844*(9), 1608−1618.

Kong, J., Zhang, Z., Musch, M. W., Ning, G., Sun, J., Hart, J., … Li, Y. C. (2008). Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *294*(1), G208−G216.

Kovács, E., Kiss, I., Boros, A., Horváth, N., Tóth, J., Gyulai, P., & Szalma, A. (1986). Disinfestation of different cereal products by irradiation. *International Journal of Radiation Applications and Instrumentation. Part C. Radiation Physics and Chemistry*, *28*(5), 545−548.

Lakritz, L., Fox, J., Hampson, J., Richardson, R., Kohout, K., & Thayer, D. (1995). Effect of gamma radiation on levels of α-tocopherol in red meats and turkey. *Meat Science*, *41*(3), 261−271.

Lakritz, L., & Thayer, D. (1992). Effect of ionizing radiation on unesterified tocopherols in fresh chicken breast muscle. *Meat Science*, *32*(3), 257−265.

Lamprecht, S. A., & Lipkin, M. (2003). Chemoprevention of colon cancer by calcium, vitamin D and folate: Molecular mechanisms. *Nature Reviews. Cancer*, *3*(8), 601−614.

Lark, R. K., Lestet, G. E., Ontjes, D. A., Blackwood, A. D., Hollis, B. W., Henslet, M. M., & Aris, R. M. (2001). Diminished and erratic absorption of ergocalciferol in adult cystic fibrosis patients. *American Journal of Clinical Nutrition*, *73*(3), 602−606.

Lastarria-Tapia, H. & Sequeiros, N. (1985). Efecto de la radiacion gamma em manzanas "delicias" almacenadas al médio ambiente y em refrigeracion. *International Atomic Energy Agency. Food Irradiation Processing. Vienna*. 55−60.

Lee, K.-F., & Hau, L.-B. (1996). Effect of γ-irradiation and post-irradiation cooking on thiamine, riboflavin and niacin contents of grass prawns (Penaeus monodon). *Food Chemistry*, *55*(4), 379−382.

Leong, S. Y., Oey, I., & Burritt, D. J. (2016). Pulsed electric field improves the bioprotective capacity of purées for different coloured carrot cultivars against H_2O_2-induced oxidative damage. *Food Chemistry*, *196*, 654−664.

Li, F.-J., Shen, L., & Ji, H.-F. (2011). Dietary intakes of vitamin E, vitamin C, and β-carotene and risk of Alzheimer's disease: A meta-analysis. *Journal of Alzheimer's Disease*, *31*(2), 253−258.

Lin, S.-J., & Pascall, M. A. (2014). Incorporation of vitamin E into chitosan and its effect on the film forming solution (viscosity and drying rate) and the solubility and thermal properties of the dried film. *Food Hydrocolloids*, *35*, 78−84.

Lips, P., & Van Schoor, N. M. (2011). The effect of vitamin D on bone and osteoporosis. *Best Practice & Research. Clinical Endocrinology & Metabolism*, *25*(4), 585–591.

Lopes, L. B., Speretta, F. F. F., & Bentley, M. V. L. B. (2007). Enhancement of skin penetration of vitamin K using monoolein-based liquid crystalline systems. *European Journal of Pharmaceutical Sciences*, *32*(3), 209–215.

Maccubbin, D., Koren, M. J., Davidson, M., Gavish, D., Pasternak, R. C., Macdonell, G., … Mitchel, Y. (2009). Flushing profile of extended-release niacin/laropiprant versus gradually titrated niacin extended-release in patients with dyslipidemia with and without ischemic cardiovascular disease. *American Journal of Cardiology*, *104*(1), 74–81.

Maestro, B., Dávila, N., Carranza, M. C., & Calle, C. (2003). Identification of a vitamin D response element in the human insulin receptor gene promoter. *The Journal of Steroid Biochemistry and Molecular Biology*, *84*(2), 223–230.

Majamaa, K., Günzler, V., Hanauske-Abel, H., Myllylä, R., & Kivirikko, K. (1986). Partial identity of the 2-oxoglutarate and ascorbate binding sites of prolyl 4-hydroxylase. *Journal of Biological Chemistry*, *261*(17), 7819–7823.

Manousopoulou, A., Al-Daghri, N. M., Garbis, S. D., & Chrousos, G. P. (2015). Vitamin D and cardiovascular risk among adults with obesity: A systematic review and meta-analysis. *European Journal of Clinical Investigation*, *45*(10), 1113–1126.

Martins, D., Wolf, M., Pan, D., Zadshir, A., Tareen, N., Thadhani, R., … Norris, K. (2007). Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: Data from the Third National Health and Nutrition Examination Survey. *Archives of Internal Medicine*, *167*(11), 1159–1165.

Mataix, J., Sánchez de Medina, F. (2009). *Vitaminas con funciones coenzimáticas en el metabolismo intermediario*. Madrid, Ergon.

Mataix, J., Várela Moreiras, G. (2009). *Vitaminas y proliferación celular: ácido fólico y vitamina B12*. Madrid, Ergon.

Mattila, P. H., Piironen, V. I., Uusi-Rauva, E. J., & Koivistoinen, P. E. (1994). Vitamin D contents in edible mushrooms. *Journal of Agricultural and Food Chemistry*, *42*(11), 2449–2453.

Maxie, E., Eaks, I., & Sommer, N. (1964). Some physiological effects of gamma irradiation on lemon fruit. *Radiation Botany*, *4*(4), 405–411.

Maxie, E., & Sommer, N. (1968). *Changes in some chemical constituents in irradiated fruits and vegetables*. Davis: Univ. of California.

Mcnulty, H., & Pentieva, K. (2004). Folate bioavailability. *Proceedings of the Nutrition Society*, *63*(4), 529–536.

Michels, A. J., Hagen, T. M., & Frei, B. (2013). Human genetic variation influences vitamin C homeostasis by altering vitamin C transport and antioxidant enzyme function. *Annual Review of Nutrition*, *33*, 45–70.

Mitchell, G., Mclauchlan, R., Isaacs, A., Williams, D., & Nottingham, S. (1992). Effect of low dose irradiation on composition of tropical fruits and vegetables. *Journal of Food Composition and Analysis*, *5*(4), 291–311.

Moltó-Puigmartí, C., Permanyer, M., Castellote, A. I., & López-Sabater, M. C. (2011). Effects of pasteurisation and high-pressure processing on vitamin C, tocopherols and fatty acids in mature human milk. *Food Chemistry*, *124*(3), 697–702.

Montesano, D., Gennari, O., Seccia, S., & Albrizio, S. (2012). A simple and selective analytical procedure for the extraction and quantification of lutein from tomato by-products by HPLC-DAD. *Food Analytical Methods*, *5*(4), 710–715.

Muller, P.G. & Riel, R. (1990). *Tecnologias de America del Norte para el procesamiento de alimentos*, IICA Biblioteca Venezuela.

Munné-Bosch, S., & Alegre, L. (2002). The function of tocopherols and tocotrienols in plants. *Critical Reviews in Plant Sciences*, *21*(1), 31−57.

Nagao, A., Kotake-Nara, E., & Hase, M. (2013). Effects of fats and oils on the bioaccessibility of carotenoids and vitamin e in vegetables. *Bioscience, Biotechnology, and Biochemistry*, *77*(5), 1055−1060.

Nagaoka, S.-I., Kakiuchi, T., Ohara, K., & Mukai, K. (2007). Kinetics of the reaction by which natural vitamin E is regenerated by vitamin C. *Chemistry and Physics of Lipids*, *146*(1), 26−32.

Napoli, J. L. (1999). Interactions of retinoid binding proteins and enzymes in retinoid metabolism. *Biochimica et Biophysica Acta (BBA)−Molecular and Cell Biology of Lipids*, *1440*(2), 139−162.

Nichols, H. K., & Basu, T. K. (1994). Thiamin status of the elderly: Dietary intake and thiamin pyrophosphate response. *Journal of the American College of Nutrition*, *13*(1), 57−61.

Norman, P., & Powell, J. (2014). Vitamin D and cardiovascular disease. *Circulation Research*, *114*(2), 379−393.

O'callaghan, Y., & O'brien, N. (2010). Bioaccessibility, cellular uptake and transepithelial transport of α-tocopherol and retinol from a range of supplemented foodstuffs assessed using the caco-2 cell model. *International Journal of Food Science and Technology*, *45*(7), 1436−1442.

Ochoa J.J., Mataix B. (2009). *Vitamina A y carotenoides: Función visual y expresión génica*, Madrid, Ergon.

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments. *Innovative Food Science and Emerging Technologies*, *9*(3), 272−279.

Osganian, S. K., Stampfer, M. J., Rimm, E., Spiegelman, D., Hu, F. B., Manson, J. E., & Willett, W. C. (2003). Vitamin C and risk of coronary heart disease in women. *Journal of the American College of Cardiology*, *42*(2), 246−252.

Patil, B. S., Vanamala, J., & Hallman, G. (2004). Irradiation and storage influence on bioactive components and quality of early and late season 'Rio Red'grapefruit (*Citrus paradisi* Macf.). *Postharvest Biology and Technology*, *34*(1), 53−64.

Pittas, A. G., Sun, Q., Manson, J. E., Dawson-Hughes, B., & Hu, F. B. (2010). Plasma 25-hydroxyvitamin D concentration and risk of incident type 2 diabetes in women. *Diabetes care*, *33*(9), 2021−2023.

Plecko, B., & Stöckler, S. (2009). Vitamin B6 dependent seizures. *The Canadian Journal of Neurological Sciences. Le Journal Canadien des Sciences Neurologiques*, *36*, S73−S77.

Płonka, J. (2012). Food analysis − Samples preparation and chromatographic methods in determination of selected biogenic amines, methylxanthines and water-soluble vitamins. *Analytical Methods*, *4*(10), 3071−3094.

Polydera, A. C., Stoforos, N. G., & Taoukis, P. S. (2003). Comparative shelf life study and vitamin C loss kinetics in pasteurised and high pressure processed reconstituted orange juice. *Journal of Food Engineering*, *60*(1), 21−29.

Prakash, R., Gandotra, S., Singh, L. K., Das, B., & Lakra, A. (2008). Rapid resolution of delusional parasitosis in pellagra with niacin augmentation therapy. *General Hospital Psychiatry*, *30*(6), 581−584.

Puthusseri, B., Divya, P., Lokesh, V., & Neelwarne, B. (2013). Salicylic acid-induced elicitation of folates in coriander (*Coriandrum sativum* L.) improves bioaccessibility and reduces pro-oxidant status. *Food Chemistry*, *136*(2), 569−575.

Qin, B. L., Pothakamury, U. R., Barbosa-Cánovas, G. V., & Swanson, B. G. (1996). Nonthermal pasteurization of liquid foods using high-intensity pulsed electric fields. *Critical Reviews in Food Science and Nutrition*, *36*(6), 603−627.

Quadro, L., Blaner, W. S., Salchow, D. J., Vogel, S., Piantedosi, R., Gouras, P., ... Gottesman, M. E. (1999). Impaired retinal function and vitamin A availability in mice lacking retinol-binding protein. *EMBO Journal*, *18*(17), 4633−4644.

Rao, A. V., & Rao, L. G. (2007). Carotenoids and human health. *Pharmacological Research : The Official Journal of the Italian Pharmacological Society*, *55*(3), 207−216.

Refsum, H., Ueland, P.M., Nygård, O. & Vollset, S.E. (1998). Homocysteine and cardiovascular disease. Annual Review of Medicine.

Retsky, K., Freeman, M., & Frei, B. (1993). Ascorbic acid oxidation product (s) protect human low density lipoprotein against atherogenic modification. Anti-rather than prooxidant activity of vitamin C in the presence of transition metal ions. *Journal of Biological Chemistry*, *268*(2), 1304–1309.

Riener, J., Noci, F., Cronin, D. A., Morgan, D. J., & Lyng, J. G. (2009). Effect of high intensity pulsed electric fields on enzymes and vitamins in bovine raw milk. *International Journal of Dairy Technology*, *62*(1), 1–6.

Rigotti, A. (2007). Absorption, transport, and tissue delivery of vitamin E. *Molecular Aspects of Medicine*, *28* (5–6), 423–436.

Rimm, E. B., Stampfer, M. J., Ascherio, A., Giovannucci, E., Colditz, G. A., & Willett, W. C. (1993). Vitamin E consumption and the risk of coronary heart disease in men. *New England Journal of Medicine*, *328*(20), 1450–1456.

Rivas, A., Rodrigo, D., Company, B., Sampedro, F., & Rodrigo, M. (2007). Effects of pulsed electric fields on water-soluble vitamins and ACE inhibitory peptides added to a mixed orange juice and milk beverage. *Food Chemistry*, *104*(4), 1550–1559.

Rivera, J. D., Duff, G. C., Galyean, M. L., Walker, D. A., & Nunnery, G. A. (2002). Effects of supplemental vitamin E on performance, health, and humoral immune response of beef cattle. *Journal of Animal Science*, *80*(4), 933–941.

Roselló-Soto, E., Barba, F. J., Parniakov, O., Galanakis, C. M., Grimi, N., Lebovka, N., & Vorobiev, E. (2015). High voltage electrical discharges, pulsed electric field and ultrasounds assisted extraction of protein and phenolic compounds from olive kernel. *Food & Bioprocess Technology*, *8*, 885–894.

Roselló-Soto, E., Galanakis, C. M., Brncic, M., Orlien, V., Trujillo, F. J., Mawson, R., … Barba, F. J. (2015). Clean recovery of antioxidant compounds from plant foods, byproducts and algae assisted by ultrasounds processing. Modeling approaches to optimize processing conditions. *Trends in Food Science & Technology*, *42*, 134–149.

Rosen, C. J., Adams, J. S., Bikle, D. D., Black, D. M., Demay, M. B., Manson, J. E., … Kovacs, C. S. (2012). The nonskeletal effects of vitamin D: An Endocrine Society scientific statement. *Endocrine Reviews*, *33* (3), 456–492.

Sachdeva, B., Kaushik, R., Arora, S., & Indumathi, K. P. (2015). Impact of fortification with iron salts and vitamin A on the physicochemical properties of laboratory-pasteurised toned milk and bioaccessibility of the added nutrients. *International Journal of Dairy Technology*, *68*(2), 253–260.

Saito, Z., & Igarashi, Y. (1970). *Effects of gamma irradiation on changes in acidity, vitamin C, and nonprotein nitrogen of apples*. Aomori, Japan: Hirosaki Univ.

Salo-Väänänen, P., Ollilainen, V., Mattila, P., Lehikoinen, K., Salmela-Mölsä, E., & Piironen, V. (2000). Simultaneous HPLC analysis of fat-soluble vitamins in selected animal products after small-scale extraction. *Food Chemistry*, *71*(4), 535–543.

Sancho, F., Lambert, Y., Demazeau, G., Largeteau, A., Bouvier, J. M., & Narbonne, J. F. (1999). Effect of ultra-high hydrostatic pressure on hydrosoluble vitamins. *Journal of Food Engineering*, *39*(3), 247–253.

San Martin, M. F., Barbosa-Canovas, G. V., & Swanson, B. G. (2002). Food processing by high hydrostatic pressure. *Critical Reviews in Food Science and Nutrition*, *42*(6), 627–645.

Sattler, S. E., Gilliland, L. U., Magallanes-Lundback, M., Pollard, M., & Dellapenna, D. (2004). Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination. *The Plant Cell*, *16* (6), 1419–1432.

Savini, I., Rossi, A., Pierro, C., Avigliano, L., & Catani, M. V. (2008). SVCT1 and SVCT2: Key proteins for vitamin C uptake. *Amino Acids*, *34*(3), 347–355.

Schellack, G., Harirari, P., & Schellack, N. (2015). B-complex vitamin deficiency and supplementation: Review. *SA Pharmaceutical Journal*, *82*(4), 28–32.

Shah, N., Ding, W., Fallourd, M., & Leyer, G. (2010). Improving the stability of probiotic bacteria in model fruit juices using vitamins and antioxidants. *Journal of Food Science*, *75*(5), M278–M282.

Shea, M.K. & Booth, S.L. (2007). Role of vitamin K in the regulation of calcification. International Congress Series.

Shearer, M. J., Fu, X., & Booth, S. L. (2012). Vitamin K nutrition, metabolism, and requirements: Current concepts and future research. *Advances in Nutrition*, *3*(2), 182–195.

Sogvar, O. B., Saba, M. K., & Emamifar, A. (2016). Aloe vera and ascorbic acid coatings maintain postharvest quality and reduce microbial load of strawberry fruit. *Postharvest Biology and Technology*, *114*, 29–35.

Son, E. W., Mo, S. J., Rhee, D. K., & Pyo, S. (2004). Vitamin C blocks TNF-alpha-induced NF-kappaB activation and ICAM-1 expression in human neuroblastoma cells. *Archives of Pharmacal Research*, *27*(10), 1073–1079.

Spinazzi, M., Angelini, C., & Patrini, C. (2010). Subacute sensory ataxia and optic neuropathy with thiamine deficiency. *Nature Reviews Neurology*, *6*(5), 288–293.

Strohecker, R. & Henning, H.M. (1965). *Vitamin assay tested methods*. Limburg.

Sturtzel, B., Dietrich, A., Wagner, K. H., Gisinger, C., & Elmadfa, I. (2010). The status of vitamins B6, B12, folate, and of homocysteine in geriatric home residents receiving laxatives or dietary fiber. *Journal of Nutrition, Health and Aging*, *14*(3), 219–223.

Tangpricha, V., Koutkia, P., Rieke, S. M., Chen, T. C., Perez, A. A., & Holick, M. F. (2003). Fortification of orange juice with vitamin D: A novel approach for enhancing vitamin D nutritional health. *American Journal of Clinical Nutrition*, *77*(6), 1478–1483.

Tauscher, B. (1998). Effect of high pressure treatment to nutritive substances and natural pigments. In K. Audio (Ed.), *Fresh novel foods by high pressure*. Espoo: VTT Technical Research Centre of Finland.

Teucher, B., Olivares, M., & Cori, H. (2004). Enhancers of iron absorption: Ascorbic acid and other organic acids. *International Journal for Vitamin and Nutrition Research*, *74*(6), 403–419.

Tiwari, B. K., O'donnell, C. P., Muthukumarappan, K., & Cullen, P. J. (2009). Effect of sonication on orange juice quality parameters during storage. *International Journal of Food Science and Technology*, *44*(3), 586–595.

Torregrosa, F., Cortés, C., Esteve, M. J., & Frígola, A. (2005). Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids. *Journal of Agricultural and Food Chemistry*, *53*(24), 9519–9525.

Traber, M. G., & Stevens, J. F. (2011). Vitamins C and E: Beneficial effects from a mechanistic perspective. *Free Radical Biology and Medicine*, *51*(5), 1000–1013.

Troesch, B., Hoeft, B., Mcburney, M., Eggersdorfer, M., & Weber, P. (2012). Dietary surveys indicate vitamin intakes below recommendations are common in representative Western countries. *British Journal of Nutrition*, *108*(04), 692–698.

Turner, T., & Burri, B. J. (2012). Rapid isocratic HPLC method and sample extraction procedures for measuring carotenoid, retinoid, and tocopherol concentrations in human blood and breast milk for intervention studies. *Chromatographia*, *75*(5–6), 241–252.

Uematsu, T., Nagashima, S., Niwa, M., Kohno, K. I., Sassa, T., Ishii, M., ... Kanamaru, M. (1996). Effect of dietary fat content on oral bioavailability of menatetrenone in humans. *Journal of Pharmaceutical Sciences*, *85*(9), 1012.

United Nations Population Facts. (2012). Available from: http://www.un.org/en/development/desa/population/publications/pdf/popfacts/popfacts_2012-1.1.pdf.

United States Pharmacopeia XXIIth revision. (1990). US Pharmacopeial Convention. Rockville, MD.

Vaidya, A., Forman, J. P., Hopkins, P. N., Seely, E. W., & Williams, J. S. (2011). 25-Hydroxyvitamin D is associated with plasma renin activity and the pressor response to dietary sodium intake in Caucasians. *Journal of Renin-Angiotensin-Aldosterone System*, , 1470320310391922.

Van Den Branden, C., Deman, A., Ceyssens, B., Pauwels, M., Empsen, C., & Verbeelen, D. (2002). Vitamin E protects renal antioxidant enzymes and attenuates glomerulosclerosis in Adriamycin-treated rats. *Nephron*, *91*(1), 129–133.

Van Het Hof, K. H., West, C. E., Weststrate, J. A., & Hautvast, J. G. A. J. (2000). Dietary factors that affect the bioavailability of carotenoids. *Journal of Nutrition*, *130*(3), 503–506.

Vannacci, A., Paoletti, A., Gallo, E., Benemei, S., Vietri, M., Lapi, F., … Firenzuoli, F. (2011). Interactions between natural health products and oral anticoagulants: Spontaneous reports in the italian surveillance system of natural health products. *Evidence-based Complementary and Alternative Medicine*, 2011

Vargas, M., Pastor, C., Chiralt, A., Mcclements, D. J., & González-Martínez, C. (2008). Recent advances in edible coatings for fresh and minimally processed fruits. *Critical Reviews in Food Science and Nutrition*, *48*(6), 496–511.

Vega-Gálvez, A., Díaz, R., López, J., Galotto, M. J., Reyes, J. E., Perez-Won, M., … Di Scala, K. (2016). Assessment of quality parameters and microbial characteristics of Cape gooseberry pulp (Physalis peruviana L.) subjected to high hydrostatic pressure treatment. *Food and Bioproducts Processing*, *97*, 30–40.

Vega-Gálvez, A., Giovagnoli, C., Pérez-Won, M., Reyes, J. E., Vergara, J., Miranda, M., … Di Scala, K. (2012). Application of high hydrostatic pressure to aloe vera (Aloe barbadensis Miller) gel: Microbial inactivation and evaluation of quality parameters. *Innovative Food Science and Emerging Technologies*, *13*, 57–63 , (JANUARY).

Velíšek, J., & Cejpek, K. (2007). Biosynthesis of food constituents: Vitamins. 1. Fat-soluble vitamins - A review. *Czech Journal of Food Sciences*, *25*(1), 1–16.

Verhoef, P., Stampfer, M. J., Buring, J. E., Gaziano, J. M., Allen, R. H., Stabler, S. P., … Willett, W. C. (1996). Homocysteine metabolism and risk of myocardial infarction: Relation with vitamins B6, B12, and folate. *American Journal of Epidemiology*, *143*(9), 845–859.

Vinson, J. A., Al Kharrat, H., & Andreoli, L. (2005). Effect of Aloe vera preparations on the human bioavailability of vitamins C and E. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, *12*(10), 760–765.

Wang, J., & Chao, Y. (2003). Effect of 60 Co irradiation on drying characteristics of apple. *Journal of Food Engineering*, *56*(4), 347–351.

Wegmüller, R., Zimmermann, M. B., Bühr, V. G., Windhab, E. J., & Hurrell, R. F. (2006). Development, stability, and sensory testing of microcapsules containing iron, iodine, and vitamin A for use in food fortification. *Journal of Food Science*, *71*(2), S181–S187.

Wilska-Jeszka, J. & Skorupinska, A. (1975). Evaluation of radurizited and pasteurized fruits juices during 12 months of storage.

Wortsman, J., Matsuoka, L. Y., Chen, T. C., Lu, Z., & Holick, M. F. (2000). Decreased bioavailability of vitamin D in obesity. *The American Journal of Clinical Nutrition*, *72*(3), 690–693.

Yang, Y., & Mcclements, D. J. (2013). Vitamin E bioaccessibility: Influence of carrier oil type on digestion and release of emulsified α-tocopherol acetate. *Food Chemistry*, *141*(1), 473–481.

Yazdi, A. S., & Yazdinezhad, S. R. (2014). Simultaneous determination of vitamin A and E in infant milk formulas using semimicro liquid–liquid extraction followed by HPLC-UV. *Journal of Liquid Chromatography and Related Technologies*, *37*(3), 391–403.

Yeom, H. W., Streaker, C. B., Howard Zhang, Q., & Min, D. B. (2000). Effects of pulsed electric fields on the quality of orange juice and comparison with heat pasteurization. *Journal of Agricultural and Food Chemistry*, *48*(10), 4597–4605.

Yook, H.-S. (2009). Effect of gamma irradiation on quality of kiwifruit (*Actinidia deliciosa* var. deliciosa cv. Hayward). *Radiation Physics and Chemistry*, *78*(6), 414–421.

Yuan, W., Pan, W., Kong, J., Zheng, W., Szeto, F. L., Wong, K. E., … Li, Y. C. (2007). 1, 25-dihydroxyvitamin D3 suppresses renin gene transcription by blocking the activity of the cyclic AMP response element in the renin gene promoter. *Journal of Biological Chemistry*, *282*(41), 29821–29830.

Yun, J. M., Singh, S., Jialal, R., Rockwood, J., Jialal, I., & Devaraj, S. (2010). A randomized placebo-controlled crossover trial of aloe vera on bioavailability of Vitamins C and B 12, blood glucose, and lipid profile in healthy human subjects. *Journal of Dietary Supplements*, *7*(2), 145–153.

Zinoviadou, K. G., Barba, F. J., Galanakis, C. M., Brnčić, M., Trujillo, F., Mawson, R., & Knoerzer, K. (2015). Fruit juice sonication: Implications on food safety and physicochemical and nutritional properties. *Food Research International*, *77*(4), 743–752.

Zhang, L., Lu, Z., Lu, F., & Bie, X. (2006). Effect of γ irradiation on quality-maintaining of fresh-cut lettuce. *Food Control*, *17*(3), 225–228.

Zulueta, A., Barba, F. J., Esteve, M. J., & Frígola, A. (2010). Effects on the carotenoid pattern and vitamin A of a pulsed electric field-treated orange juice-milk beverage and behavior during storage. *European Food Research and Technology*, *231*(4), 525–534.

This page intentionally left blank

CHAPTER

POLYPHENOLS

7

Jelena Hogervorst Cvejić[1], Milica Atanacković Krstonošić[1], Mira Bursać[1] and Uroš Miljić[2]
[1]University of Novi Sad, Novi Sad, Serbia [2]University of Novi Sad, Novi Sad, Serbia

7.1 FOOD SOURCES AND PROPERTIES OF POLYPHENOLS

It is universally recognized that diet plays a key role in prevention of some serious pathologies such as cardiovascular conditions, atherosclerosis, and cancer. Polyphenols are widely present in the human diet and many health benefits have been attributed to the consumption of phenol-rich foods, such as fruits and vegetables. In addition to the vitamins and minerals present in fruits and vegetables, phytochemicals such as flavonoids and other phenolics strongly contribute to these beneficially protective effects (Chun et al., 2005). Indeed, numerous studies suggest that the consumption of dietary phenolics may improve human health. These food components are not essential for body metabolism, but contribute to overall health due to their enhanced physiological activity.

Dietary intake of polyphenols has been associated with reduced risk of chronic diseases, such as heart disease and cancer, most likely due to their pronounced antioxidant properties (Del Rio et al., 2013). Polyphenols also play an important role in plant resistance and defense against microbial infections that are related to oxidative reactions. Naturally occurring plant polyphenols include several groups of compounds that have health-promoting properties.

7.1.1 CLASSIFICATION OF PHENOLIC COMPOUNDS

Phenolics are ubiquitous compounds found in all plants as their secondary metabolites, which are synthesized by plants during normal development, as well as in response to stress conditions (UV radiation, infection, wounding, etc.) (Beckman, 2000; Harborne, 1982; Malencic et al., 2013). In their structure, at least one aromatic ring with one or more hydroxyl groups attached is present. Phenolics can be classified into two main groups: flavonoids and nonflavonoids (Galanakis, Markouli, & Gekas, 2013; Galanakis, Kotanidis, Dianellou, & Gekas, 2015). Flavonoids are polyphenolic compounds containing two aromatic rings connected by a three-carbon bridge. These compounds are the most common and the largest plant polyphenolics present in the human plant-source diet (Valant-Vetschera & Wollenweber, 2006). This large group of flavonoids is additionally divided into six different subclasses: flavonols, flavones, flavanones, flavan-3-ols, isoflavones, and anthocyanidins. In the nonflavonoid class, the main groups are phenolic acids, hydrixycinnamates, and stilbenes.

Nutraceutical and Functional Food Components. DOI: http://dx.doi.org/10.1016/B978-0-12-805257-0.00007-7

Table 7.1 Content of Total Phenolics (mg/100 g) in Selected Foods

Source	Total Phenolics	Source	Total Phenolics
Fruits		*Cereals and legumes*	
Orange, sweet	1343	Soyabean	414
Grapefruit	893	Oat	352
Lemon	843	Wheat flour	184
Kiwi	791	*Herbs and spices*	
Lime	751	Basil	4425
Blueberry	362	Shallot	1718
Strawberry	199	Pepper, black	1600
Apple, red	125	Mint	400
Vegetables		*Beverages*	
Cabbage, red	186	Red wine	242
Potato	150	Coffee	188
Spinach	112	Tea, green	83
Lettuce	107	Tea, black	62

7.1.2 PHENOLICS IN FOOD

In general, the average daily intake of dietary polyphenols in humans is approximately 1 g per person. It has been noted that the main sources of these compounds are beverages and fruits, but vegetables and legumes are also sources in smaller amounts (Scalbert & Williamson, 2000). Consumption of phenolics-rich food, and consequently, their dietary intake, is greatly affected by the eating habits of different regions as well as by individual preferences (Shahidi & Naczk, 2004). The contents of total phenolics (mg per 100 g) in some foods are given in Table 7.1 (Reis Giada, 2013; and references therein).

Phenolics may also affect the organoleptic properties of food; they can contribute to the bitterness, astringency, color, flavor, odor, and oxidative stability of foodstuff. These properties as well as the health beneficial capacity of plant phenolics are of great importance not only for consumers, but also for food producers and product developers (Shahidi & Naczk, 2004). The addition of polyphenols to foods can improve the general quality of products by extending the shelf-life, slowing down toxic oxidation product formation, and increasing their nutritional value. Various studies performed in the last two decades clearly indicate that a diet with high plant phenolic content can improve the health status of individual and quality of life in general.

7.2 OXIDATIVE STRESS AND THE PROTECTIVE ROLE OF POLYPHENOLS

7.2.1 OXIDATIVE STRESS

Oxidative stress can be defined as disturbance of the balance between prooxidants and antioxidants, or formation of an excessive amount of prooxidants (Poljsak, Šuput, & Milisav, 2013).

Prooxidants, often referred to as reactive oxygen species (ROS), can be classified as radicals that contain at least one unpaired electron in outer orbit, or highly reactive nonradicals. They can cause damage to lipids, DNA, and proteins in biological systems, as well as to the protective mechanisms of the cell (enzymes, reducing equivalents) (Kohen & Nyska, 2002). Antioxidants are considered to be compounds that can prevent the prooxidation process or biological oxidative damage (Prior & Cao, 1999). Oxidative stress caused by ROS plays an important role in the development of different diseases including cardiovascular and neurodegenerative diseases, cancer, and diabetes. The human body has its own mechanisms of defense from oxidative stress, but polyphenols present in diet can also potentially contribute to prevention of biological damage.

Polyphenols are the most abundant antioxidant compounds found in the human diet. It has been reported that their daily intake (up to 1 g) is higher than that of all other types of dietary antioxidants (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; Scalbert & Wiliamson, 2000). Their antioxidative properties are due to the presence of phenolic hydroxyl groups as well as of the conjugated aromatic system (Dai & Mumper, 2010). In addition to antioxidant capacity, polyphenols can act also as metal-ion chelators (Brown, Khodr, Hider, & Rice-Evans, 1998; Engelmann, Hutcheson, & Cheng, 2005).

7.2.2 IN VIVO AND IN VITRO STUDIES

Different in vivo and in vitro studies of antioxidative properties of polyphenols have been performed using polyphenolic extracts or individual compounds. A study by Jefremov, Zilmer, Zilmer, Bogdanovic, and Karelson (2007) showed that resveratrol, curcumin, and genistein significantly delayed Cu^{2+}-induced oxidation of plasma low density lipoprotein (LDL) particles, with resveratrol being the most effective. It was suggested that resveratrol can also improve glucose tolerance, attenuate β-cell loss, and reduce oxidative stress in pancreatic islet and thus is beneficial in the prevention of diabetes (Szkudelski & Szkudelska, 2011). Similar antidiabetic effects were observed for phenol-rich chestnut extract (Yin et al., 2011). Several polyphenolic compounds including myricetin, procatechuic acid, and epigallocatechin-3-gallate inhibited copper-mediated DNA damage, most likely through a radical scavenging mechanism (Perron, García, Pinzón, Chaur, & Brumaghim, 2011).

It has also been shown that consumption of grape-seed extract (rich in proanthocyanidins) decreased the oxidized LDL cholesterol in human plasma (Sano et al., 2007). Consumption of a single dose of phenol-rich grape juice also reduced oxidative stress in human serum (Garcia-Alonso, Ros, Vidal-Guevara, & Periago, 2006). Meta-analysis of 10 different human studies demonstrated that consumption of black tea, with teaflavins and catechins as the main phenolic compounds, was beneficial for subjects with higher cardiovascular risk, lowering LDL cholesterol, but with no significant effect on total or HDL cholesterol (Zhao, Asimi, Wu, Zheng, & Li, 2015). Additionally, polyphenols from green tea play a protective role and decrease damage in oxidative stress-induced spinal cord neurons from rat embryos (Zhao et al., 2014). It is also well known that isoflavoves present in soy and its derivatives are related to estrogen-like effects (Cvejić, Bursać, & Atanackovic, 2012). In order to elucidate the biological effects of polyphenol extracts, the possible synergistic effects of individual compounds present in dietary sources should be considered.

7.3 OTHER HEALTH EFFECTS OF POLYPHENOLS

Polyphenols and their health effects have been extensively studied in the past several decades. Their biological benefits are particularly interesting due to their role in the potential prevention of major chronic noninfectious diseases such as cardiovascular and cancer diabetes. These ailments are among the leading causes of death today, which indicates that phenolic compounds will continue to be object of scientific interest (Scalbert, Johnson, & Saltmarsh, 2005). Apart from the well-known antioxidative properties of polyphenols, it is clear that their biological activity is a result of various other complex mechanisms. Some of the recent in vitro and in vivo studies on the different biological effects of phenolic compounds and/or extracts are presented in Table 7.2.

As Table 7.2 shows, polyphenolic compounds have great potential for exerting various beneficial biological effects. Nevertheless, the biological efficacy of polyphenols should also consider metabolism as well as their real concentration in target tissues and cells in human body. Future experiments should focus on elucidating these topics.

7.4 INTERACTION OF POLYPHENOLS WITH THE INTESTINAL MICROBIOTA

The role of the large intestine in the metabolism of many compounds was, until recently, often neglected. Newer studies show that intestinal microbiota play an important role in the metabolism of many ingredients present in food, and have a significant impact on nutritional and health status of the host.

7.4.1 INTESTINAL MICROBIOME

Numerous bacteria inhabiting the large intestine form a special ecosystem called the "intestinal microbiome," defining the collective genomes of the microbiota (Hooper & Gordon, 2001). The number of genes of the collective genome (microbiome) of the microbiota exceeds by far those of the human genome, giving additional metabolic features (Gill et al., 2006; Turnbaugh et al., 2007). Estimations are that the human microbiota contains 10^{14} bacterial cells, which have the metabolic capacity 100-fold greater than that of the human liver (Zhu, & Wang, Li, 2010). The composition of different bacteria present in the human gut is very diverse within the population, and is influenced by many factors (age, health, origin, diet, environment, etc.) and thus it can be said that each individual has its own unique profile of microbial species (Del Rio et al., 2013; Saarela, Lahteenmaki, Crittenden, Salminen, & Mattila-Sandholm, 2002). Despite the great diversity of bacterial species (more than 800 different bacterial species), the majority belong to four bacterial phyla: *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria* (8%), and *Actinobacteria* (3%) (Frank et al., 2007; Matsuki, Watanabe, Fujimoto, Takada, & Tanaka, 2004; Zhu et al., 2010).

Since bacterial processes occur under anaerobic conditions using specific enzymes, the metabolites can be different from those formed by human enzymes. In most cases, bacterial metabolism reduces the activity of dietary compounds, but sometimes it can enhance certain properties. It is

Table 7.2 Biological Effects of Polyphenols

Ailment	Polyphenol Source/ Compound	Study Results	Reference
Cardiovascular disease	Grape-seed extract (proanthocyanidins)	Decrease of systolic and diastolic blood pressure by approx. 5 mm Hg in hypertensive women	Terauchi et al. (2014)
	Cocoa powder in skimmed milk	Increased HDL and decreased oxLDL in subjects with high coronary heart disease risk	Khan et al. (2012)
	Dark chocolate (epicatechin and catechin)	Increased HDL and decreased total cholesterol/HDL ratio and LDL/HDL ratio in normal weight obese women	Di Renzo et al. (2013)
	Olive oil	Decreased systolic and diastolic blood pressure by approx. 7 mm Hg and lower oxLDL in pre- and mild hypertensive women	Moreno-Luna et al. (2012)
	Green-tea extract (EGCG)	Reduction of blood pressure, inflammatory biomarkers, and oxidative stress	Bogdanski et al. (2012)
		Improvement of insulin resistance in obese hypertensive patients	
Cancer	Citrus polyphenols	Cytotoxic to oral and cervical cancer cells	Tsai, Li, Hsu, Young, and Chen (2015)
	Tea polyphenols	Lower risk of development of colon cancer in humans	Yuan et al. (2007)
	Polyphenol-rich extract of *Salvia chinensis*	Cytotoxic to the human breast cancer cells, human lung cancer cells, human colon cancer cells, and human pancreatic cancer cells	Zhao, Huo, Sun, and Dong (2015)
	Soy isoflavone daidzein	Induces breast cancer cell apoptosis by generation of ROS	Jin, Zhang, Kang, Wang, and Zhao (2010)
	Curcumin	Induces gastric cancer cell apoptosis by generation of ROS	Liang et al. (2014)
Diabetes	Anthocyanin-rich bilberry extract	Improvement of hyperglycemia and insulin sensitivity in type II diabetic mice	Takikawa, Inoue, Horio, and Tsuda (2010)
	Chlorogenic and ferulic acid	Enhance glucose uptake and show synergistic effect with antidiabetic drugs	Prabhakar and Doble (2009)
	Phenolic extract of *Castanea mollissima*	Decrease in serum glucose, triglyceride, total cholesterol and LDL in diabetic rats; significant body weight loss	Yin et al. (2011)

(*Continued*)

Table 7.2 Biological Effects of Polyphenols *Continued*

Ailment	Polyphenol Source/ Compound	Study Results	Reference
Obesity	Mulberry leaf polyphenol extract (quercetin, caffeic acid, hydroxyflavin, hesperetin)	Inhibition of differentiation preadipocytes	Chang, Yang, Chen, and Wang (2016)
	EGCG	Dose-dependent reduction of body weight of New Zealand black mice	Klaus, Pultz, Thone-Reineke, and Wolfram (2005)
	Polypheno-rich peach and plum juice	Protection from obesity-induced metabolic disorders (hyperglycemia, insulin, and leptin resistance, dyslipidemia) Only plum juice prevented body weight gain in Zucker rats	Noratto, Martino, Simbo, Byrne, and Mertens-Talcott (2015)
	Black-tea polyphenol extract	Inhibition of pancreatic lipase activity—inhibition of lipid absorption	Uchiyama, Taniguchi, Saka, Yoshida, and Yajima (2011)
Other	Anthocyanin-enriched bilberry extract	Improves visual function by antioxidant activity in retinal pigment epithelium	Milbury, Graf, Curran-Celentano, and Blumberg (2007)
	Green-tea polyphenols	Protection of retinal pigment epithelial cells from UVB damage	Xu et al. (2010)
	Polyphenol-rich blueberry extract	Cognitive enhancement in adult mice (improvement in learning and memory); higher brain antioxidant properties	Papandreou et al. (2009)
	Blueberry juice	Improvement of memory performance in older adults	Krikorian et al. (2010)
	Polyphenol-rich extract from tea (*Camellia sinensis*)	Antiinflammatory effects on acute and immunological inflammation in mice	Chen, Li, et al. (2012)
	Grape seed extract	Antiallergic activity through inhibition of degranulation of inflammatory mediators, reduction of expression of IgE receptors, and reduction of calcium influx in mast cells	Chen, Hung, et al. (2012)

also known that some nutraceuticals, like polyphenols, can modulate the composition and activity of the gut microbial community (Duda-Chodak, Tarko, Satora, & Sroka, 2015). The "polyphenols−microbiota" two-way relationship is still poorly understood, but some recent studies have focused on revealing this complex interaction (Cardona, Andres-Lacueva, Tulipani, Tinahones, & Queipo-Ortuno, 2013).

7.4.2 IMPACT OF POLYPHENOLS ON THE MICROBIOTA

There is evidence that selected polyphenols may modify the gut microbial composition, inhibiting certain bacterial groups, while the growth of other groups can be intact or even stimulated in some cases. These effects have mainly been investigated concerning pathogens, but it is also known that the activity and composition of nonpathogenic bacteria can be altered (Duda-Chodak et al., 2015).

Polyphenolic compounds found in green and black tea are potent inhibitors of growth of many pathogen bacteria such as *Helicobacter pylori* (Ankolekar et al., 2011), *Staphylococcus aureus*, meticilin-resistant *S. aureus*, *Escherichia coli* (Nakayama et al., 2012), and *Pseudomonas aeruginosa* (Bancirova, 2010). They can even influence viruses like hepatitis C virus, Epstein-Barr virus (Chen, Li, et al., 2012), or influenza virus (Nakayama et al., 1993). Theaflavin derivatives in black tea, catechin derivatives in green tea, and epigallocatechin gallate (EGCG) have the potential to prevent HIV infection (Hamza & Zhan, 2006; Liu et al., 2005; Williamson, Mccormick, Nance, & Shearer, 2006). Tea phenolics like (+) cathechin leave the commensal anaerobes such as *Bifidobacterium* and *Lactobacillus* spp. relatively unaffected, but have been shown to inhibit the growth of *Clostridium histolyticum* (Tzounis et al., 2008).

Hesperetin, naringenin, poncirin, and diosmetin—polyphenols present in citrus fruits—can also have an inhibitory effect on *H. pylori* growth (Bae, Han, & Kim, 1999). Parkar, Stevenson, and Skinner (2008) found that naringenin and quercetin effectively inhibited *Lactobacillus rhamnosus*, *E. coli*, *S. aureus*, and *Staphylococcus typhimurium*. Naringenin and phloridzin inhibited adherence of *S. typhimurium* to enterocytes, and phloridzin and rutin enhanced the adherence of the probiotic *L. rhamnosus*. In the study by Duda-Chodak (2012) it was demonstrated that only flavonoid aglycones had an inhibiting effect on intestinal bacteria growth, but not on their glycoside forms. For example, naringenin and hesperetin inhibited growth of almost all analyzed bacteria, while their glycosides naringin and hesperidin had no effect.

Consumption of red wine polyphenols in a human study was shown to significantly increase growth of *Enterococcus*, *Prevotella*, *Bacteroides*, *Bifidobacterium*, *Bacteroides uniformis*, *Eggerthella lenta*, and *Blautia coccoides*, while *Lactobacillus* was unaffected (Queipo-Ortuño et al., 2012). Resveratrol, stilbene found in grapes and red wine, promoted growth of *Bifidobacterium* spp. and *Lactobacillus* in a rat model (Larrosa et al., 2009). Proanthocyanidin-rich extract from grape seed significantly promoted growth of bifidobacteria (Yamakoshi, Tokutake, & Kikuchi, 2001). Wild blueberry drink consumption also had a positive effect on human intestinal microbiota by increasing the number of *Bifidobacterium* (Vendrame et al., 2011).

Flavan-3-ols from sources like chocolate, green tea, blackcurrant, and grapes can be beneficial to the composition of intestinal microbiota promoting growth of *Lactobacillus* spp. and inhibiting *Clostridium* spp. in vivo and in vitro (Molan, Liu, & Kruger, 2011; Tzounis, Rodriguez-Mateos, Vulevic, & Gibson, 2011; Tzounis et al., 2008; Viveros et al., 2011). Cocoa-fed animals showed a decrease in the proportion of *Bacteroides*, *Clostridium*, and *Staphylococcus* genera in feces (Massot-Cladera, Pérez-Berezo, Franch, Castell, & Pérez-Cano, 2012).

Polyphenols individually, or as dietary constituents, can contribute to the maintenance of microbial balance in the human gut, inhibiting pathogens or promoting growth of beneficial bacteria. Mechanisms of these actions are still not completely understood, but it is certain that their dietary intake can be beneficial for human health and immunity.

7.4.3 POLYPHENOLS AND BIOACTIVE METABOLITES PRODUCED BY INTESTINAL MICROBIOTA

Many dietary polyphenols are modified by intestinal microbiota, which can play a crucial role in their metabolism. Commensal bacteria can also generate different metabolites of certain polyphenols, with different bioavailability, activity, or functional effect compared to parent molecule.

One of the best known examples of production of bioactive metabolite by gut microbiota is the change of soy isoflavone daidzein to equol and/or O-desmethylangolensin (Fig. 7.1). Intestinal bacteria such as *Eggerthella* spp., *Enterococcus faecium*, *Adlercreutzia equolifaciens*, *Slackia equolifaciens*, *Lactobacillus mucosae*, *Bifidobacterium* spp., *Slackia isoflavoniconvertens*, and *Bacteroides ovatus* are capable of this modification (Maruo, Sakamoto, Ito, Toda, & Benno, 2008; Matthies, Blaut, & Braune, 2009; Raimondi et al., 2009; Wang, Kim, Kang, Kim, & Hur, 2007; Yokoyama, Niwa, Osawa, & Suzuki, 2010; Yokoyama & Suzuki, 2008). Not all humans are capable of producing equol as a bioactive metabolite of soy isoflavones. This conversion depends on many factors, including composition of intestinal microflora (Setchell & Clerici, 2010). It is considered that only 30–50% of the population is capable of metabolizing daidzein to *S*-(-)equol, which significantly alters the beneficial health effect of a soy-based diet (Yuan, Wang, & Liu, 2007). Equol has higher binding affinity to estrogen receptors (type β) than daidzein and its glucosides (Hwang et al., 2006; Muthyala et al., 2004) and thus equol producers could benefit more from isoflavone intake.

Different gut bacteria (e.g., *Bacteroides* sp., *Clostridium* sp., *Eubacterium* sp., *E. lenta*) can metabolite lignans such as pinoresinol present in sesame seed, linseed, flaxseed, or vegetables to enterolactone and enterodiol (Clavel et al., 2005; Wang, Meselhy, Li, Qin, & Hattori, 2000). These compounds are able to modulate estrogen activity (similar to equol) (Damdimopoulou et al., 2011), but they also showed anticancer activity in some in vivo studies (Lindahl, Saarinen, Abrahamsson, & Dabrosin, 2011; Miura, Saarinen, Miura, Santti, & Yagasaki, 2007). One of the bioactive metabolites with estrogenic activity is 8-prenylnaringenin, which is produced by *Eubacterium limosum* from isoxanthohumol from hops (Possemiers et al., 2006, 2008). Urolithins are active metabolites of ellagtannins and ellagic acid found in strawberries, raspberries, and walnuts (Clifford & Scalbert, 2000). They exhibit estrogenic effects (Larrosa, Gonzalez-Sarrias, Garcia Conesa, Tomas-Barberan, & Espin, 2006), but they are antiinflammatory and antioxidant agents in vivo (Ishimoto et al., 2011). Additionally, the polyphenols quercetin and rutin can be converted by various gut bacteria to 3,4-dihydroxyphenyl acetic acid (DOPAC/DHPAA). This compound is a metabolite of the neurotransmitter dopamine in the central nervous system (Kopin, 1985). With the exception of neuroprotective effect (Pavlica & Gebhardt, 2010), DOPAC in certain concentrations can lead to

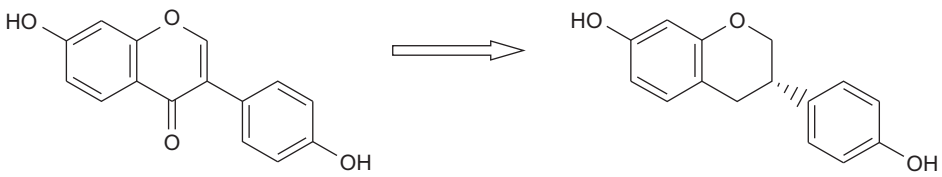


FIGURE 7.1

Conversion of daidzein to equol.

induction of mitochondrial dysfunction, causing potential neurodegeneration (Nunes, Almeida, & Laranjinha, 2005; Nunes, Almeida, & Laranjinha, 2008).

The mechanisms of action of many polyphenol metabolites are not fully understood, but there is strong evidence that the health of gut microbiota highly influences the bioactivity of certain compounds present in human diet. It is still to be discovered if bacteria-produced metabolites are further modified in target cells (Hogger, 2013).

7.5 BIOAVAILABILITY AND BIOEFFICACY OF POLYPHENOLS IN HUMANS

Bioavailability can be described as the degree to which a substance becomes available to the target tissue and can be used by the human body after administration.

For polyphenols, it can depend on various digestive processes from release of polyphenols from the food matrix, different changes in small intestine, enterocytes, or colon, to excretion by urine or bile (Bohn, 2014). Generally, the aim of bioavailability studies is to analyze absorption of dietary polyphenols and their role in the human organism including formation of metabolites with various biological activities.

Bioavailability varies greatly among different polyphenols. Sometimes, polyphenols present in high concentration in food do not necessarily have a good bioavailability profile, which lowers their bioefficacy and health effects. Consequently, the amount of polyphenols present in certain food is not crucial, but the part that is bioavailable in the human organism. Therefore to better understand the connection between the dietary intake and final biological activity of specific compound, it is important to consider some general aspects of metabolism of major polyphenol groups.

The human body recognizes polyphenols as xenobiotics, so their bioavailability is generally low compared to nutrients (Cardona et al., 2013). In most cases, the chemical structure of polyphenol determines the rate and extent of absorption, and the form of metabolites present in human plasma (D'Archivio et al., 2007). It is known that a very small percentage (5−10%) of dietary polyphenols (mainly low molecular ones) can be absorbed in the small intestine (Manach, Williamson, Morand, Scalbert, & Remesy, 2005). The remaining compounds (oligomeric and polymeric polyphenols) go to the large intestine where bacteria act with their enzymes and produce metabolites, which can have altered physiological activity compared to parent polyphenol (Bowey, Adlercreutz, & Rowland, 2003).

Many dietary polyphenols are present in food as glycosides, and they are transferred to aglycones by commensal bacteria called glycohydrolases (Gee & Johnson, 2001). They can also be present in the form of esters or polymers, which cannot be absorbed in this native form, and have to be hydrolyzed by intestinal enzymes or bacteria microflora in the gut (Day et al., 1998; Nemeth et al., 2003). After absorption, polyphenols undergo extensive modification during metabolic Phase I (oxidation, reduction, hydrolysis) and Phase II (conjugation) in enterocytes and liver hepatocytes, biliary excretion, absorption in systematic circulation, interaction with organs, and excretion in urine (Cardona et al., 2013). Largely conjugated metabolites are mostly eliminated by bile, while small conjugates (monosulfates) are usually excreted in urine. Considering that there are great differences in the structures of polyphenols, there are various metabolic pathways, and new studies are still trying to determine all of the important metabolites produced in the human organism.

7.5.1 ANTHOCYANINS

Anthocyanins are widely spread in different fruits and vegetables, and their average intake is estimated to be 180−215 mg/day in the United States (Kuhnau, 1976). Although their daily intake can be relatively high compared to other polyphenols, the bioavailability and bioefficacy of anthocyanins have not been found.

They are more specific than other polyphenols, because anthocyanins glycoside forms are the most common circulating forms in humans. This may be due to instability of aglycones or a specific metabolic pathway of these compounds (Passamonti, Vrhovsek, & Mattivi, 2002; Wu, Cao, & Prior, 2002). During digestion, anthocyanins are exposed to different environments and pH values, which have a major influence on their form (they can exist in four different molecular species) (Mcghie, Ainge, Barnett, Cooney, & Jensen, 2003). Some recent studies show that anthocyanins are absorbed predominantly in the stomach (Matuschek, Hendriks, Mcghie, & Reynolds, 2006; Passamonti, Vrhovsek, Vanzo, & Mattivi, 2005; Talavera et al., 2003). This could be explained by higher stability of anthocyanins at lower pH value (Matuschek et al., 2006). It appears that they are rapidly absorbed and eliminated, but they have low bioefficiency. The reason for this could be the instability of anthocyanin forms and metabolites, and the methods used for their determination in plasma or urine (Manach et al., 2005). The extension degradation of anthocyanins by gut microbiota is relatively low, compared to other flavonoids (Scheline, 1991). The important metabolites of anthocyanins still need to be investigated with new analytical methods to reveal the specific metabolic paths of certain compounds from this group of polyphenols.

7.5.2 PROANTHOCYANIDINS

Proanthocyanidins have polymeric or oligomeric structure and high molecular weight, which limits their absorption in the gut. It is considered that oligomers (larger than trimers) are not absorbed in the small intestine in their unchanged form, but absorption of some dimmers is possible in humans (Manach et al., 2004). Although proanthocyanidins are very abundant polyphenols in the human diet (Gu et al., 2003), their low absorption limits their potential biological effects. Despite this fact, the possibility of their beneficial local activity in the gastrointestinal tract is often discussed, since they can express antioxidant and/or antiinflammatory effect in the intestine, which could be useful for prevention of colon cancer (Halliwell, Zhao, & Whiteman, 2000).

An in vitro study suggested that polymers could be degraded into monomers or dimmers during their transit in the stomach (Spencer et al., 2000), but this does not appear to happen in humans. The reason is probably the milder acidic environment than in the in vitro study, which is not optimal for proanthocyanidin hydrolysis. Proanthocyanidins are very stable in the stomach environment, and they actually reach the small intestine intact (Rios et al., 2002). Some health effects of procyanidins could be attributed to biological activities of their metabolites formatted by the microflora in the colon. An in vitro study showed that the formation of different low-molecular weight aromatic compounds from proanthocyanidins is possible (Déprez et al., 2000), but the biological effects of these metabolites have not yet been investigated. However, further in vivo analysis should be conducted for better understanding of the metabolism of these oligomers and polymers and the importance of gut microbiota in this process.

7.5.3 CATECHINS

Procyanidins (oligomeric catechins) often occur together with monomeric (+)-catechin and (−)-epicatechin, so it is difficult to differentiate whether health effects should be attributed to oligomers or their constitutive parts. Catechin and epicatechin are widely spread in different fruits, beans, and chocolate, while gallates (galloyted catechins) are mainly found in green tea, making the intake assessment easier (Williamson & Manach, 2005).

Generally, methylation, glucuronidation, and sulfatation are the main metabolic biotransformations of catechins, with small intestine and liver as important organs for absorption and metabolism (Donovan et al., 2001). But bioavailability can differ greatly among catechins. Meng et al. (2002) studied the metabolism of EGCG and detected its methylated form in plasma and urine. Another study showed that catechin was present almost exclusively as methylated metabolite in plasma after consumption of red wine. Glucuronide and sulfate conjugates were also determined after enzymatic hydrolysis (Donovan et al., 1999). Epigallocatechin and epicatechin seem to have higher bioavailability than EGCG, but these compounds also appear in the plasma in low concentrations. Elimination of catechins is rapid, and galloylated forms were not found in urine (Chow et al., 2001; Lee et al., 2002).

7.5.4 FLAVONOLS

Quercetin is one of the most studied flavonols concerning bioavailability. It was shown that quercetin is absorbed by humans and that absorption in the small intestine is enhanced by conjugation with glucose (Hollman, Devries, Vanleeuwen, Mengelers, & Katan, 1995). However, some studies indicate that glucosides could be hydrolyzed by β-glucosidases, enzymes present in the small intestine, before absorption (Day, Canada, et al., 2000; Day et al., 1998). Absorption of quercetin is efficient in some subjects, while in others it is very slow, indicating high interindividual variability (Erlund et al., 2000). After ingestion of glucoside quercetin forms from apples and onions, long half-life (23−28 h) was determined. It was concluded that this slow elimination could lead to accumulation of quercetin in blood if consumption was repeated (Hollman et al., 1997). Quercetin is not found in the plasma as the free form or its glucoside, but usually as methyl, sulfate, or glucuronic acid conjugates (Day et al., 2001). These conjugates probably have lower biological activity than aglycones, although the position of conjugation can be of great importance for determining the potential activity of these compounds (Day, Bao, Morgan, & Williamson, 2000).

7.5.5 FLAVANONES

Flavanones are a small group of polyphenols specific for citrus fruits. The most studied are hesperetin and naringenin along with their glucosides (Manach et al., 2005). In this case, aglycones are absorbed more rapidly than glucosides (Bugianesi, Catasta, Spigno, D'uva, & Maiani, 2002), although aglycone forms are rarely found in natural sources. Their absorption takes place in the colon. Circulating forms of hesperetin are mainly glucuronides (87%) and sulfoglucuronides (13%). Urinary excretion for hesperetin and naringenin is usually finished 24 h after ingestion. The elimination of flavanones in urine is slower than for catechins, but it is faster than the excretion of quercetin (Felgines et al., 2000; Manach, Morand, Gil-Izquierdo, Bouteloup-Demange, & Remesy, 2003).

7.5.6 ISOFLAVONES

Isoflavones and their bioavailability have been extensively studied because of their numerous biological effects. They are mainly found in soybean as glucosides, but a higher percentage of aglycones can occur in different fermented soy products (Cvejić, Tepavčević, Bursać, Miladinović, & Malenčić, 2011; Kishida, Ataki, Takebe, & Ebihara, 2000).

Different results concerning the rate of absorption of aglycones and glucosides have been obtained (Izumi et al., 2000; Setchell et al., 2001; Zubik & Meydani, 2003) but their bioavailability seems to be similar. It is generally considered that glucoside forms are converted to corresponding aglycones by intestinal microbiota or gut glucosidases, and that the aglycones are then actually absorbed in the small intestine (Day et al., 1998). On the other hand, there is some evidence of the greater bioavailability of glucosides (Setchell et al., 2001), thus this topic should be further investigated. Compared to previously mentioned flavonols and flavanones, isoflavones are better absorbed by humans (Felgines et al., 2000). Izumi et al. (2000) also found that genistein, which is biologically the most active isoflavone, is more efficiently absorbed than daidzein.

In human plasma glucosides are not present, but mainly conjugated forms like glucuronides and sulfates and a small percentage of free aglycones are found (Setchell et al., 2001). Biochanin A and formononetin, isoflavones present in red clover or in dietary supplements containing phytoestrogens, are absorbed and then undergo demethylation, which gives high concentrations of the corresponding compounds genistein and daidzein (Setchell et al., 2001). The pharmacokinetics of glycitein is similar to that of daidzein (Richelle, Pridmore-Merten, Bodenstab, Enslen, & Offord, 2002).

Isoflavones were detected in urine and excreted relatively slowly, usually during the 24 h after intake. The recovery in urine was 50% for daidzein, 38% for glycitein, and 20% for genistein (Richelle et al., 2002). The low recovery of genistein could be explained by the fact that greater fraction is eliminated in bile (Sfakianos, Coward, Kirk, & Barnes, 1997). It is interesting to note that high concentrations of isoflavones can be detected in breast tissue and in prostate (Hong, Kim, Kwon, Lee, & Chung, 2002; Maubach et al., 2003), probably because of the numerous estrogen receptors that are sites of actions for isoflavones as phytoestrogen compounds. It was also shown that bacteria microflora play a great role in the metabolism of isoflavones, because of the production of equol, an important metabolite with high biological potential, as previously discussed.

7.5.7 PHENOLIC ACIDS

Hydroxycinnamic acids like chlorogenic (found in coffee) and ferulic acids (e.g., in cereals, tomatoes) are very abundant in the human diet (Bourne & Rice-Evans, 1998; Clifford, 2000). Chlorogenic acids present in green coffee are highly absorbed and metabolized in humans (Farah, Monteiro, Donangelo, & Lafay, 2008). The absorption of chlorogenic acids occurs mainly in the colon (Manach et al., 2005). Over 30% of ingested compounds were recovered in plasma. Is it also considered that urine is not the major elimination pathway of chlorogenic acids and their metabolites. Generally, the bioavailability of these compounds varies greatly among humans, requiring further investigation (Farah et al., 2008).

On the other hand, the bioavailability of ferulic acid is mainly determined by the percentage of free compounds in the dietary source. The release in the small intestine is limited when it is bound to arabinoxylans or other indigestible polysaccharides in cereals (Anson, Van Den Berg, Havenaar,

Bast, & Haenen, 2009). An animal study has shown that free ferrulic acid is easily absorbed and 50% of the ingested dose can be detected in urine. It is found in plasma only in conjugated form, but extensive elimination by the kidneys limits its accumulation (Adam et al., 2002).

Gallic acid, a derivative of benzoic acid, has high bioavailability and is well absorbed compared to other polyphenolic compounds, but it has a short half-life in plasma. Its main detected metabolite is metylated derivate (Cartron et al., 2003; Shahrzad, Aoyagi, Winter, Koyama, & Bitsch, 2001; Shahrzad & Bitsch, 1998). There is still much to be investigated about gallic metabolism and the activity of metabolite, as well as the disposition in different tissues.

7.5.8 EMERGING TECHNOLOGIES FOR IMPROVEMENT OF POLYPHENOL BIOAVAILABILITY

Limiting factors for the absorption of polyphenols are generally their molecular size and their low lipid solubility. Thus they usually cannot be absorbed by simple diffusion processes and their ability to pass the lipid-rich outer membrane of the small intestine is typically poor (Awasthi, Kulkarni, & Pawar, 2011).

The recently introduced phytosome technology is one way of enhancing the bioavailability of herbal extracts or certain natural compounds. Phytosomes, also known as herbosomes, are cell-like phospholipid structures ("phyto" means plant, "some" means cell-like) (Bhattacharya & Ghosh, 2008). The phospholipid mainly used in the formation of phytosomes is phosphatidylcholine, derived from soybean (Citernesi & Sciacchitano, 1995). For the preparation of phytosomes, the usual ratios of the phosphatidylcholine molecule and plant component are 1:1 or 2:1 (Sharma & Roy, 2010). Together they form a molecular complex involving hydrogen bonds (Bhattacharya & Ghosh, 2008), where the polar compound is anchored to the polar head of phospholipid and acts as an integral part of the membrane. Because of its dual solubility, phospholipid is an emulsifier that provides enhanced absorption and bioavailability in the intestine (Chauhan, Gowtham, & Gopalakrishna, 2009). Another advantage of phytosomes is the fact that they protect active ingredient from destruction from gastric juices and gut bacteria (Murray, 2016). Besides acting as a carrier for polar compounds, phosphatidylcholine also has an hepatoprotective effect (Semalty, Semalty, & Rawat, 2007). Several commercial phytosome preparations, which contain terpens, ginkgoflavonoids, green tea catechines, procyanidines, flavonoids, olive oil polyphenols, and sylibin (Awasthi et al., 2011; Maffei et al., 1994; Naik, 2009; Tedesco et al., 2004), have shown to be very effective in improving bioavailability. Additionally, phytosomes containing curcumin and naringenin also showed favorable health characteristics (Maiti, Mukherjee, Gantait, Saha, & Mukherjee, 2006; Maiti, Mukherjee, Gantait, Saha, & Mukherjee, 2007).

In addition, there is a growing number of studies concerning incorporation of phenolics into nanocarriers (Bonifácio et al., 2014; Li, Jiang, Xu, & Gu, 2015; Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001). Nanoparticles or nanocarriers are defined as submicron colloidal systems with particle size $1-100\,\mu m$. Nano-based formulations have many advantages such as physical and chemical stability, better bioavailability, and increased concentration in target tissues. They can also protect active substances from oxidation or other degradation reactions in the gastrointestinal tract (De Souza, Casanova, & Costa, 2015). Nanocarrier technology was recently used for the encapsulation of catechin (Curcio et al., 2012; Li et al., 2015) and coating of resveratrol (Singh & Pai, 2014).

One of the strategies for enhancing the bioavailability and bioefficacy of phenolic substances is changing their basic structure (prodrug strategy), obtaining a form that has favorable kinetics and can be transformed into active form in an organism (de Souza et al., 2015). An additional way of solving the solubility problem of polyphenols is by formation of inclusion complexes with cyclodextrins, or coadministration with absorption enhancers (Lewandowska, Szewczyk, Hrabec, Janecka, & Gorlach, 2013). Finally, new techniques are still developed in order to use polyphenols in the best possible way, obtaining the most health potential.

7.6 EFFECT OF EMERGING TECHNOLOGIES ON THE FUNCTIONAL PROPERTIES OF POLYPHENOLS

Awareness of the role diet plays in maintaining and promoting health as well as in preventing diseases has increased significantly among consumers in the last two decades. Today high-quality functional food products ensuring health benefits, providing nutrients beyond basic nutrition without negative impact on sensory properties, are usually expected. Loss of phytonutrients responsible for the functional properties of products becomes more significant as foods are processed, stored, and transported. As the demand for functional food increases, intense research and development of new processing technologies is taking place in order to ensure maximal nutritional and functional properties.

Classical processing technologies such as wet milling, mechanical pressing, microfiltration, ultrafiltration, spray drying, alcohol precipitation, isoelectric solubilization, etc., are well described and established. It is generally assumed that these technologies are safe and economically viable, since they have been used in various fields of the food industry for many decades. However, despite good applicability of conventional processing techniques in laboratory conditions, their efficacy and commercial implementation on an industrial scale is considerably poorer due to certain technological limitations (Galanakis, 2013). The major drawbacks of conventional processing (recovery) technologies are usually related to the overheating of the food matrix, loss of functionality and poor stability of the final product, fulfillment of increasingly strict legal requirements on materials safety, and high energy consumption and general costs of application (Galanakis, 2012). Due to their insufficiently examined toxicological effects, the safety of conventional extraction-solvent utilization inside the food chain is still questionable (Galanakis, 2013; Mujumdar & Law, 2010).

A possible solution to the reported deficiencies of conventional technologies, as an answer to consumer demand for high-quality food and in accordance with increased economic and ecological concerns, could be the development and application of novel methodologies, the so-called emerging technologies (Galanakis, 2013). A large number of new, emerging technologies such as ultrasound-assisted extraction (UAE), laser ablation, radio-frequency (RF) drying, electro-osmotic dewatering, high-hydrostatic pressure, low-temperature plasma treatment, pulsed electric field (PEF), high-voltage electrical discharge (HVED), and nanotechnology has been developed and more commonly applied in food science.

Processing of food has an impact on the chemical constituents as well as on the physical and sensory properties of the final product. Applied technologies may influence the content of bioactive compounds leading to changes in their functional properties (i.e., antioxidant activity) and potential

health benefits. The phenolic compounds, as one of the most important bioactive compounds in plant-based foods, are sensitive to the effect of environmental conditions during processing. Application of high temperature and/or pressure can lead to their degradation or modification of their biological activities. For instance, food containing anthocyanins are often thermally processed (heating to temperatures from 50 to 150°C) prior to consumption. This fact can greatly influence the content of anthocyanins in the final product, as well as its antioxidant activity, which is connected with the prevention of cancer and neuronal and cardiovascular diseases (Giusti & Wrolstad, 2003). It has been shown that anthocyanin stability is not only a function of the final processing temperature but is also influenced by the characteristics of the product and factors such as pH, chemical structure, and concentration of anthocyaninsthe presence of enzymes, proteins, metallic ions, light, and oxygen (Patras, Brunton, O'donnell, & Tiwari, 2010).

Generally, the different operations applied during food production can influence (increase/decrease) changes in the content of phenolics and their activity (Atanackovic et al., 2012; Cvejić & Atanacković, 2015; Duodu, 2011). A decrease in phenolic compounds may be caused by a processing method that leads to the transfer of these components out of the matrix into the specific fluid used in certain phases of processing (e.g., water used in soaking). Chemical degradation or transformation of polyphenols into other forms and induction of interactions between phenolics and other food components is also common during processing and can lead to reduction of their functional properties (Cvejić, Puškaš, Miljić, Torović, & Rakić, 2016). On the other hand, an increase in the content of polyphenols may be due to a processing method that releases bound forms of these compounds, making them more extractable and biologically active. Finally, technological operations to which these phytonutrients could be subjected within the food matrix sometimes do not cause changes in their content, which is due to the protection effects of other food components.

7.6.1 NONTHERMAL TECHNIQUES

Techniques such as high-hydrostatic pressure, PEFs, high-intensity ultrasound, ultraviolet light, and ionizing radiation are classified as nonthermal since they avoid the use of high temperatures, in contrast to thermal-processing technologies. However, these treatments may involve heat as a result of internal energy generation (e.g., adiabatic heating and resistive heating during high-hydrostatic pressure and PEFs, respectively), which avoids the harmful effects of heat on the functional, nutritive and on the sensory value of foods (Corbo, Bevilacqua, Petruzzi, Casanova, & Sinigaglia, 2014; Pereira & Vicente, 2010).

7.6.1.1 High-pressure processing

High-pressure processing (HPP) is an efficient alternative to thermal processing, which often has an adverse impact on food quality. It can be used for both preservation and preparation of food, since it ensures sterilization and enzyme deactivation after processing.

The high-pressure treatment of food involves the application of pressures from 100 to 1000 MPa, typically applied and maintained for a short time and combined with moderate heat. During this procedure, the product is heated to much lower extent than usually used during thermal processing. This process ensures color retention and prevents formation of darker, polymerized colors that sometimes occur as a result of classical thermal processing (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2006).

During the HPP process, high pressure is uniformly and instantaneously applied through a food material, independent of its mass, shape, and composition, causing reactions responsible for the reduction in volume of biomolecules. Unlike large molecules whose structure is significantly affected by such reactions, small molecules with simple structures, such as vitamins, flavor, and aroma components contributing to the sensory and nutritional quality of food, remain unaffected (Pereira & Vicente, 2010).

Systems for HPP use hydrostatic or throttling pressure, with the first one being more commonly applied. Treatment of food with high-hydrostatic pressure usually requires prepacking of material in flexible or semirigid bags or plastic containers and their placement into a sealed chamber where they are pressurized. Another possibility is the use of a continuous-flow high-pressure throttling device, in which a continuous stream of liquid (commonly used in the beverage industry) is compressed through a throttling valve. Unlike more conventional hydrostatic pressure treatment and its limitations, this method allows easy adjustment to a semicontinuous process. Furthermore, it does not require prepackaging of material intended for processing, and the unit is more adaptable for installation of connection fittings for pumping product (Corey, 2009).

High-hydrostatic pressure methodology has been used in the production of beer, milk, emulsions, salmon, cheese, and hot dogs (Corey, 2009). Since HPP allows production of nondamaged, safer and longer life products, it could be efficient as a methodology for recovering bioactive compounds from by-products, as these compounds have already been overprocessed and further thermal treatment could cause excessive loss of their functionality. The main disadvantage of HPP applications is the high capital cost, e.g., industrial scale vessels can cost from 0.5 up to 4 million euros.

Patras, Brunton, Da Pieve, and Butler (2009) compared the impacts of HPP and conventional thermal treatment on the antioxidant activity and content of bioactive compounds (polyphenols and ascorbic acid) of strawberry and blackberry purées. There was no significant difference in the anthocyanins content of pressure-treated and unprocessed purées, but conventional thermal treatment resulted in significant reduction in the levels. Furthermore, the antioxidant activity of pressure-treated purées was significantly higher than in thermally processed samples. Color changes were negligible for pressurized purées. HPP was shown to be an efficient technology to preserve product quality. Similar results obtained by Ferrari, Maresca, and Ciccarone (2011) showed the potential of high-hydrostatic pressure treatment at moderate temperatures for the sanitation of products rich in bioactive compounds (i.e., red-fruit derivatives since enhanced extractability of colored pigments and increase of the polyphenol content). The application of high-hydrostatic pressure for gooseberry pulp treatment showed an increase of total phenolic content and antioxidant capacity with an increase in time and pressure (Vega-Gálvez et al., 2014). Buzrul (2012) reported that HPP treatment of beer and wine not only inactivates the undesirable microorganisms but also improves the organoleptic properties. It was emphasized that this technology has huge potential to eliminate the negative effect of heat on beer's aroma and flavor and also to reduce the SO_2 levels used in wine.

7.6.1.2 Cold plasma

Low-temperature (cold) plasma is defined as a quasi-neutral fluid-gas system consisting of charged and neutral high-energy particles (electrons, ions, or molecules) characterized by collective behavior (Wan, Coventry, Swiergon, Sanguansri, & Versteeg, 2009). This technique also allows effective

UV light generation for disinfection treatments. It is applied at both atmospheric and low pressures to heat-sensitive surfaces. The tendency to minimize external electric and magnetic fields inside the matrix (i.e., food), contrary to its behavior in the surrounding cover, is one of the most essential properties of plasma state and this technique (Galanakis, 2012). Low-temperature plasma is mainly applied for nonthermal pasteurization (as a surface-cleaning technology) and inactivation of enzymatic processes. In particular, it is suitable for dry particulate food products with low lipid and fat content (e.g., horticultural products such as dried herbs and spices) in which UV and radical exposure would have minimal influence on oxidation or other chemical changes (Wan et al., 2009). Grzegorzewski, Zietz, Rohn, Kroh, and Schlueter (2011) examined the effects of a nonthermal oxygen plasma on the stability and functionality of phenolic compounds in kale (*Brassica oleracea* convar. *sabellica*). The treatment significantly affected the flavonoid content in leaf tissue through the action of existing ROS and radicals in the plasma effluent. This led to the formation of characteristic low-molecular-weight degradation products.

In general, this processing method is known to be an effective, but expensive emerging technology. For instance, this technique is characterized by low-energy consumption, but also by extremely high operating costs due to feed-gas consumption (Niemira, 2012).

7.6.1.3 Pulsed electric field

PEF technology is a nonthermal food-processing technology that ensures microbial and enzymatic inactivation and increase of juice-extraction yield, with, due to the absence of heat effects, minimal effects on the nutritional, sensory, and functional characteristics of foods. Induced electrical potential across the cell membrane leads to pore development, breakdown, and increased permeability of cell membrane (Barba, Galanakis, Esteve, Frigola, & Vorobiev, 2015; Galanakis, 2012; Galanakis & Schieber, 2014). This method is based on treatment with high-voltage pulses delivered to the product, which is placed between a set pair of electrodes that confine the treatment gap of the PEF chamber. A large amount of energy is stored in a series of capacitors, which is then almost instantaneously discharged in the form of high-voltage pulses (Deeth, Datta, Ross, & Dam, 2007). PEF can be conducted at low or moderate temperatures ($<60^{\circ}$C) and obtained pulses flow through the food matrix in an extremely short period of time (1−100 μs) (Pereira & Vicente, 2010). Typical PEF treatment parameters are pulsed field intensity of 15−50 kV/cm, pulse width of 1−5 μs, and pulse frequency of 200−400 Hz (pulses/s) (Wan et al., 2009). Taking into account all the properties of this emerging technology and the possibility of its use in more than one stage of the production process, the development of a cost-effective impulse-generation system with sufficient electrical field strength, power, and repetition rate is considered necessary (Galanakis, 2013).

Compared to conventional liquid extraction, grape pomace treatment with PEF (energy input of 10 KJ/kg) can lead to 30−35% higher yield of anthocyanin monoglucosides recovery (Töpfl, 2006). Donsì, Ferrari, Fruilo, and Pataro (2011) applied PEF treatments during the vinification process for the permeabilization of the grape skins in order to enhance the polyphenolic composition of four Italian red wines. The PEF treatment of Aglianico grapes was responsible for a significant increase in polyphenols (100%) and anthocyanins (30%) and thus for the improvement of the color intensity (20%) and antioxidant activity of the wine (40%), as well as for preserving other organoleptic characteristics. However, there was a minor impact on the polyphenolic-release kinetics of the other three grape varieties, despite the significant degree of cell-membrane permeabilization, which was attributed to the predominance of mass transfer limitations through the vacuole

membrane. PEF treatments can cause the depolymerization of grape-skin tannins, improving the diffusion of formed smaller decondensed tannins. Longer-duration PEF treatment with higher energy has more influence on the parietal tannins and the cell walls of the skins while higher strength treatment modifies the vacuolar tannins more (Delsart et al., 2014).

Apart from being an enhanced extraction process, this nonthermal technique was shown to be very effective at preservation of *Opuntia macrorhiza* juice, since its application, in comparison to traditional pasteurization, prevents changes in the content and structures of sensitive bioactive compounds (vitamin C, betacyanins, and flavonols) and consequently maintains the antioxidant activity of the product (Moussa-Ayoub et al., 2011).

7.6.1.4 Electro-osmotic dewatering

Electro-osmotic dewatering is an alternative drying process that merges conventional pressure integration with electrostatic effects. Compared to traditional thermal processes, this method allows the reduction of energy consumption up to two-thirds. The principles of osmotic dehydration and enhanced mass transfer determine electro-osmotic dewatering. Enhanced mass transfer is induced by electrochemical double layers formed at the particle−water interface of colloidal aqueous suspensions. Electro-osmotic dewatering is mostly applied for substrates that are hard to dehydrate such as high-sugar, gelatinous matrixes containing susceptible antioxidants and colorants since it does not overdry the product's surface. The processing of fluids containing colloidal components and possessing high viscosity with this technique can be rather difficult (Galanakis, 2012, 2013). A direct current electro-osmotic dewatering technique was used to concentrate tomato paste suspension (Al.-Asheh, Jumah, Banat, & Al.-Zou'bi, 2004).

7.6.1.5 Ultrasound waves

The application of ultrasound waves is another emerging methodology that has become very popular in food processing. The physical effects of ultrasound on the improvement of food technological properties such as emulsification ability, solubility, and texture, as well as its application for homogenization, viscosity alteration, extraction, drying, crystallization, and defoaming have been reported by Soria and Villamiel (2010). Ultrasonic irradiation generates cavitation in the liquid medium, leading to local supersaturation and later to spontaneous cell disruption and therefore enhanced heat and mass transfer in the substrate, facilitating the extraction of nutraceuticals and bioactive compounds (e.g., polyphenols; Galanakis, 2012). Ultrasound is propagated *via* a series of compression and rarefaction waves induced on the molecules of the medium. Cavitation bubbles are formed from gas nuclei existing in the fluid and then distributed throughout the liquid. The bubbles grow over the period of a few cycles to a critical size at which they become unstable. This causes their implosion, which leads to energy accumulations in hotspots, generating extreme temperatures (5000 K) and pressures (1000 atm) (Soria & Villamiel, 2010).

Apart from UAE, as may be the most usual application of this methodology, it is also important to emphasize it as an alternative drying technique based on several effects such as microagitation, creation of microscopic channels, and water cavitation.

The synergic effect of ultrasound and temperature in convective drying assisted by high-power ultrasound allowed the treatment of substrate at lower temperatures and increased process efficiency, while preserving the bioactivity of heat-sensitive food components (García-Pérez, Rosselló, Cárcel, De La Fuente, & Mulet, 2006).

Sonochemically generated hydroxyl radicals can be formed due to cavitation phenomenon. The effect of these radicals on easily oxidable food compounds may be either beneficial or detrimental, depending on the process and the food substrate. Ashokkumar et al. (2008) observed the potential of sonochemical hydroxylation of phenolic compounds as an efficient way of enhancing the antioxidant properties of certain food materials. The increase in the antioxidant yield by 24% and reduction of the extraction time by 90% was achieved with continuous UAE of phenolics from pomegranate peels, in comparison to conventional liquid extraction (Pan, Qu, Ma, Atungulu, & Mchugh, 2011). On the other hand, the application of ultrasound for emulsification and processing of sunflower, olive, and soybean oils had a significant negative impact on their composition, due to the oxidation produced during the ultrasound treatment (Chemat, Grondin, Shum Cheong Sing, & Smadja, 2004).

7.6.1.6 Nanotechnology

Nanotechnology presents a challenging and effective way to deliver nanosized or nanoencapsulated nutrients and bioactive compounds to targeted sites within the body. Its application ensures enhanced stability and bioavailability of the components, and allows their release at controlled rates (e.g., moisture- and pH-triggered controlled release) (Ofori & Peggy, 2013; Wang & Bohn, 2012). The physical stability and increased bioavailability of the food can be achieved through the production of big, multiphase colloidal droplets (nanoemulsions) (Choi, Kim, Cho, Hwang, & Kim, 2011). Nanoemulsions are a class of extremely small droplets that appear to be transparent or translucent with a bluish color. The range of their appearance is 10−200 nm, which is much smaller than the range for conventional emulsions (from 1 to 100 μm). For instance, dibenzoylmethane (DBM) nanoemulsion shows about a threefold increase in oral bioavailability compared to conventional DBM emulsion. Biopolymer micelles demonstrated considerably improved water solubility/dispersibility and in vitro anticancer activity of phytochemicals. Curcumin (principal curcuminoid phenol of *Curcuma longa*) nanoemulsions expressed 85% inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-induced mouse ear inflammation (Huang, Yu, & Ru, 2010). The study by Amendola et al. (2011) reported the efficiency of nanoemulsion formulation in improving the solubility of a grape marc extract into a high-lipid-content food such as hazelnut paste. Addition of the extract significantly improved the paste shelf-life by inhibiting its oxidation. The production of nanoemulsions at industrial scale is still very expensive due to the necessity of ensuring the high pressures (high energy demand) needed for processing larger volumes (Galanakis, 2013; Galanakis, Barba, & Prasad, 2015).

7.6.1.7 UV irradiation

The application of UV irradiation in disinfection has several advantages over other, more conventional methods (Roselló-Soto, Barba, et al., 2015; Roselló-Soto, Galanakis, et al., 2015; Zinoviadou et al., 2015). First of all, no chemicals are used and no residuals are left in the fluid stream. Furthermore, it is a nonthermal method with an insignificant impact on substrate sensory properties and pH. The effect of ultraviolet (UV-C) treatment on total phenol, flavonoid, and vitamin C content of fresh-cut honey pineapple, banana, mangoes, and guava was evaluated (Alothman, Bhat, & Karim, 2009; González-Aguilar, Villegas-Ochoa, Martínez-Téllez, Gardea, & Ayala-Zavala, 2007). The total phenol and flavonoid content of the fruits increased significantly with increased treatment time.

This novel technology has the potential to enhance the health-promoting compounds in foods. The main concern regarding the application of this technique in scaled up processes is a potential problem of using short-wave UV light, which can cause human eye damage, as well as severe burns and skin cancer (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). Another important, emerging, nonthermal technology capable of inactivating the microbial population on the surface of foods and food-processing equipment is pulsed light. This methodology uses short and intense pulses of light in the ultraviolet–near infrared range, has relatively low operation costs, and no negative environmental impact.

7.6.2 THERMIC TECHNIQUES

Due to the numerous drawbacks of conventional and well-established (mostly thermal) preservation processes, electromagnetic technologies in food processing were recently suggested as a potential and effective replacement in both scientific and industrial environments. Some of the most promising alternatives are ohmic heating and dielectric heating, which includes RF and microwave (MW) heating. These novel thermal techniques are based on volumetric forms of heating since the thermal energy is generated directly inside the food, allowing shortening of heating times and increase of energetic and heating efficiency (Pereira & Vicente, 2010). For comparison, Sadilova, Stintzing, and Carle (2006) observed that elderberry anthocyanin contents were very sensitive to thermal treatment and that only 50% of elderberry pigments were retained at 95°C after 3 h of heating.

7.6.2.1 RF drying

RF drying is a novel thermal technology with optimized heat transfer unlike classical drying with hot air. In particular, the material is heated uniformly while the water evaporates in situ at relatively low temperatures (<80°C), allowing reduction of processing time. This technique is based on the interaction mechanisms between an electromagnetic alternating field and the ionic charges of a food product (volumetric heating) (Galanakis, 2013; Pereira & Vicente, 2010). Its main drawback is the energy transfer from the generator to the product, whose efficiency does not exceed a maximum of 60% yield (Orsat & Raghavan, 2005). In addition, its application in multicomponent matrixes is rather difficult since emitted energy must be absorbed uniformly.

Vető-Kiszter (2011) investigated the production of mustard seed flour with the application of radio-frequency heating, aiming at myrosinase enzyme inactivation, which is responsible for the unfavorable pungent flavor. Optimal conditions were determined to be 112°C and 16% moisture content. RF heat treatment carried out under the properly adjusted parameters inactivated myrosinase enzyme, and had no harmful effect on nutritional compounds, or water- and fat-binding capacity of the mustard seed flour. Furthermore, total polyphenol content and free radical scavenging activity increased.

7.6.2.2 MW heating

Similar to RF systems, MW heating systems work through constant realignment of polar molecules (i.e., water) and ionic species, by reversing an electric field around the food matrix. Due to the high frequency of the field (300–3000 MHz), this molecular movement is extremely fast (Marra, Lyng, Romano, & Mckenna, 2007; Pereira & Vicente, 2010). The use of MWs has been largely applied in many food processes, such as cooking, thawing, blanching, dehydration, pasteurization, sterilization,

and tempering. Enhanced extraction of biologically active compounds in MW-assisted systems is probably the most common application of this technology. Indeed, Gallo, Ferracane, Graziani, Ritieni, and Fogliano (2010) found that the extraction efficiency of bioactive compounds using MW irradiation is about fourfold higher than that obtained for sonication extraction. Furthermore, the antioxidant activity of extracts obtained using MWs was higher than those obtained by ultrasonic extraction (Gallo et al., 2010).

7.6.2.3 Ohmic heating

Ohmic heating (also called Joule heating, electrical resistance heating, or electroconductive heating) is an alternative heating technology that can ensure uniform, rapid heating, compact design, and minimal heating of the product surroundings (Somavat, 2011). This technology is based on the electrical resistance of food. Heating occurs when an alternating electrical current passes through the food, which results in the internal generation of heat (Pereira & Vicente, 2010). The specifics distinguishing this technology from other electrical heating methods are the presence of electrodes in contact with the matrix, the application of unrestricted frequency, and the type of waveform (typically sinusoidal; Vicente & Castro, 2007). It is particularly suitable for the processing of viscous, liquid foods and foods containing particles. Furthermore, differences in the electrical conductivities of various ingredients of a multicomponent food system can be overcome by selective pretreatment during the formulation phase. According to Pereira and Vicente (2010), ohmic heating technology has numerous advantages compared to classical thermal methods including quick achievement of the temperature required for "high temperature-short time" processes, continuous processing without heat transfer surfaces, the absence of problems with substrate overheating, low heat losses, no residual heat transfer after the current shut off, better impact on sensory properties of food, and low maintenance costs.

Ohmic heating has been recommended as an alternative fast-heating method for fruit juices (Yildiz, Bozkurt, & Icier, 2009). The authors treated pomegranate juice by changing the voltage gradient (10−40 V/cm) at 50 Hz. The samples were heated from 20 to 90°C, and different retention times at 90°C (0, 3, 6, 9, or 12 min) were studied. It was observed that rheological properties, color, and total phenolic content values changed at the initial heating-up period, but there were no significant changes during the holding period ($p < 0.05$). Another study investigated the stabilization effect of ohmic heating application on lipase activity, bioactive compounds, and antioxidant activity of rice bran. Alternative current electricity (50 Hz), with three levels of electrical field strengths (75, 150, and 225 V/cm) were applied, while the moisture content of rice-bran samples was adjusted to 20%, 30%, and 40%. The applied treatment yielded the highest levels of phenolic compound, α-tocopherol, γ-oryzanol, and antioxidant activity (Loypimai, Moonggarm, & Chottanom, 2009). The nonthermal effects of electric field on bacterial spores, enzymes, carotenoids, flavonoids, and quality parameters in food were also investigated in a batch-type container, considering that industrial applications of ohmic heating are mainly limited to continuous thermal-processing systems. The carotenoids and phenolic compounds of fresh tomato juice were unaffected by the treatment. Inherent amounts of β-carotene, lycopene, phenolic acids, and quercetin indicated no or minimal changes at a temperature range from 90 to 110°C at a pH of 3.9 and 4.4. Chalconaringenin converted to naringenin in greater proportions under ohmic heating. No changes in color parameters were observed over the studied pH and temperature ranges (Somavat, 2011).

It is important to emphasize that emerging technologies are intended to overcome technological, environmental, and economic challenges due to long-term use of traditional food-processing

technologies. These technologies represent a rapid, efficient, and reliable alternative to improving the quality of food, but also have the potential for development of new enhanced functional products. However, most of these new technologies are still being investigated on a smaller scale and currently, only in a certain cases, are they fully implemented within the food industry.

7.7 INNOVATIVE EXTRACTION TECHNIQUES FOR THE RECOVERY OF POLYPHENOLS FROM FOOD SOURCES

Recovery of phenolic compounds from food sources is a multistage process, which includes macroscopic treatment, separation of macro- and micromolecules, extraction, purification, and product formation (Galanakis, 2012, 2015; Galanakis, Martínez-Saez, Del Castillo, Barba, & Mitropoulou, 2015). Extraction is one of the most important steps for the recovery of polyphenolic compounds from natural products. Over the years, several methods have been developed for the extraction of phenols with respect to their physico-chemical properties. Nevertheless, there is no standard method proposed, which indicates a persistent need for further development of diverse procedures for this purpose (Fontana, Antoniolli, & Bottini, 2013).

7.7.1 SOLID–LIQUID AND SOXHLET EXTRACTION

The most common methods for extraction of phenols are standard solid–liquid and Soxhlet extraction. Soxhlet extraction is time consuming and a large quantity of organic solvent is used, which is not ecologically sound (Wang & Weller, 2006). Due to the application of elevated temperature for long periods, thermal degradation can also occur (Wang & Weller, 2006). On the other hand, solid–liquid extraction allows the use of different solvents with the possibility of modifying temperature, pH, etc. Because of the polar properties of polyphenols, polar protic media like hydroalcoholic solutions are usually used for their extraction (Fontana et al., 2013; Galanakis, Goulas, Tsakona, Manganaris, & Gekas, 2013; Tsakona, Galanakis, & Gekas, 2012). The most desirable alcohol is ethanol due to its "GRAS" status (Generally-Recognized-As-Safe according to the Food and Drug Administration) as well as its relatively low price (Galanakis, 2012). For example, maximal extraction of total phenols and proantocyanidins from cranberry pomace was obtained after 2 h agitation at 80°C with 50% ethanol adjusted to pH 2. The resulting extract was successfully used for the production of cranberry polyphenol soy protein isolate complex, which preserved the stability of these compounds at 37°C (Roopchand et al., 2013).

7.7.2 MW-ASSISTED EXTRACTION

Microwave-assisted extraction (MAE) is one of the procedures that can be used to increase extraction efficiency. MWs can heat the sample via two mechanisms—ionic conduction of electromagnetic waves and dipole rotation (Tatke & Jaiswal, 2011). Due to the high intensity of MWs as well as specific heating procedure, satisfactory extraction yields can be obtained in shorter extraction time. In addition, since consumption of solvent and energy is decreased this type of extraction is considered to be a green extraction technique (Li et al., 2010; Pasrija, Ezhilarasi, Indrani, &

Anandharamakrishnan, 2015). MAE has been successfully applied for the extraction of polyphenols from green tea (Nkhili et al., 2009), spent espresso coffee (Ranic et al., 2014), rice grains (Setyaningsih, Saputro, Palma, & Barroso, 2015), and *Myrtus communis* leaves (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015). In a recent study, the extraction of total phenols and anthocyanins from citrus peel was shown to be more efficient than conventional, ultrasound-assisted and accelerated solvent extraction (Nayak et al., 2015). These kinds of experiments usually demand optimization of different extraction parameters (temperature, extraction time, liquid-to-solid ratio, MW power) in order to define the conditions for the highest phenolic content in the final extract. Optimization of microwave-assisted enzymatic extraction (MAEE) was conducted in order to increase the yield of polyphenols from wasted peanut shells. The obtained results showed that optimized MAEE yield was higher than that obtained from other extraction methods including heat refluxing and enzyme-assisted extraction (Zhang et al., 2013).

7.7.3 ULTRASOUND RADIATION

Use of ultrasound radiation is a preferred treatment when compared to classical shaking or stirring due to the fact that cell disruption and efficient mass transfer can increase the extraction efficiency (Mason, Paniwnyk, & Lorimer, 1996). It is also a convenient method for the extraction of thermolabile components like polyphenols because it does not require high operating temperature (Romdhane & Gourdon, 2002). A study conducted by Drosou, Kyriakopoulou, Bimpilas, Tsimogiannis, and Krokida (2015) showed that UAE of dried red-grape pomace with mixture ethanol-water (1:1) resulted in extracts with the highest total phenolic content when compared to Soxhlet and MAE. Water ethanol mixture recovered the highest amount of phenols in regard to pure water and pure ethanol extracts. In addition, air drying and grinding of the fresh red-grape pomace sample had a positive effect on total phenol extraction as well as flavonol content. UAE resulted in extracts from apple pomace with 30% higher total phenolic content than that obtained with conventional extraction (Pingret, Fabiano-Tixier, Le Bourvellec, Renard, & Chemat, 2012). Another experiment conducted on orange peels showed the same 30% increase of total phenols yield compared to conventional extraction. Interestingly, this procedure was carried out using water as a solvent, which was formed in situ by the plant after extraction of essential oils (Boukroufa, Boutekedjiret, Petigny, Rakotomanomana, & Chemat, 2015). Another example of green UAE of onion peels with water and glycerol as extraction solvent was described by Katsampa, Valsamedou, Grigorakis, and Makris (2015). The optimal conditions for extraction of polyphenols and pigments from onion peels were found to be 90% aqueous glycerol and 80°C temperature. Further chromatographic analysis showed that the dominant phenols in this extract were quercetin and quercetin 4-*O*-glucoside.

7.7.4 SUPERCRITICAL FLUID EXTRACTION

Supercritical fluid extraction is a form of extraction where fluid is in a supercritical state, having characteristics of both a gas and a liquid. The temperature and pressure of supercritical fluid (usually CO_2) is above its critical value, which induces good solvating power (like liquid), high diffusivity, low viscosity, and increased mass transfer. Supercritical fluids have dissolving

properties that depend on their density and can be controlled with temperature and pressure (Fontana et al., 2013; Wang & Weller, 2006). This method is an environmental friendly alternative to standard extraction procedures requiring large quantities of organic solvents. In order to increase the polarity of fluid and enable extraction of polyphenols a cosolvent (e.g., ethanol) is usually necessary (Wijngaard, Hossain, Rai, & Brunton, 2012). For example, addition of 5−7.5% of ethanol has been applied for the supercritical fluid extraction of resveratrol and polyphenols from pomace (Casas et al., 2010) and grape skins (Pascual-Martí, Salvador, Chafer, & Berna, 2001). Other applications include the recovery of polyphenols and flavonoids from brewer's spent grain, which is the major by-product of the brewing industry. Optimal conditions included 35 MPa of pressure, 40°C of temperature, and CO_2 + 60% ethanol (v/v) (Spinelli, Conte, Lecce, Padalino, & Del Nobile, 2016).

Another procedure used for the extraction of polyphenolic compounds allowing the use of environmentally friendly solvents is pressurized liquid extraction. This method uses a solvent at elevated pressure and temperature above boiling point. The pressure value is usually between 4 and 20 MPa, allowing the solvent to remain in a liquid state. It is similar to supercritical fluid extraction, except that the solvent is below its critical state. Described conditions increase mass transfer and extraction rate, force penetration of liquid solvent into solid matrix, and consequently enhance analyte solubility (Ramos, Kristenson, & Brinkman, 2002; Wang & Weller, 2006; Wijngard et al., 2012). This method can be applied with organic solvents as well as pure water (subcritical water extraction) (Eskilsson, Hartonen, Mathiasson, & Riekkola, 2004). Due to the fact that dielectric constant of water varies with variation of temperature, water becomes less polar (Duba, Casazza, Mohamed, Perego, & Fiori, 2015; Herrero, Cifuentes, & Ibanez, 2006). Subcritical water extraction has been applied for the extraction of polyphenols from winery by-products (García-Marino, Rivas-Gonzalo, Ibáñez, & García-Moreno, 2006), pomegranate peel (Çam & HisIl, 2010), onion skin (Ko, Cheigh, Cho, & Chung, 2011), and potato peel (Singh & Saldaña, 2011). Total phenols yield from defatted grape skin and seeds using subcritical water increased by increasing operating temperature from 80 to 120°C (Duba et al., 2015). However, elevation of temperature in order to increase the amount of extracted polyphenol compounds should be conducted carefully, because of the degradation of anthocyanins above 100°C (Ju & Howard, 2005) and phenolic acids above 180°C (Singh & Saldaña, 2011). Ko, Cheigh, and Chung (2014) established optimum conditions for subcritical extraction of 13 flavonoids from different food sources (onion skins, *Saururus chinensis*, seabuckthorn leaves, parsleys, carrots, lemons peels, orange peels, and grapefruit peels) based on their chemical structure. It was shown that flavonoids having an OH side chain were optimally extracted at lower temperatures than OCH_3 and H side chains. The extraction temperatures of the glycosides were also lower than those of the less-polar aglycones. Álvarez-Casas, García-Jares, Llompart, and Lores (2014) analyzed different operating conditions for the extraction of main polyphenolic compounds from white-grape marc. Optimized parameters included a temperature of 105°C, hydromethanolic solvent (63% methanol), and 5 min extraction time. Gallic acid, catechin, and epicatechin were the dominant phenolic compounds in the extracts. Application of pressurized liquid extraction on brown alga *Sargassum muticum* without any pretreatment gave the highest yield in terms of total phenolic content when compared to samples treated with proteases or carbohydrases (Sánchez-Camargo et al., 2016).

7.7.5 PULSE ELECTRIC FIELD

Another approach for the extraction of polyphenols from food material includes application of PEF (Donsi, Ferrari, & Pataro, 2010). Its application on vegetable tissue was carried out for the first time on potato, causing release of intracellular liquid due to the cellular damage induced by the electrical pulses (Angersbach, Heinz, & Knorr, 1997). PEF extraction can increase the yield of different compounds from food sources, and is a convenient method for thermo-sensitive substances (Wijngaard et al., 2012). In a recent study conducted by Corrales, Toepfl, Butz, Knorr, and Tauscher (2008), the application of PEF increased twofold the extraction of total phenolics from grape by-products compared to classic solid−liquid extraction. There was no significant difference between results for total phenolic content after PEF and ultrasonic extraction, but antioxidant activity of PEF extract was significantly higher. This could be due to the fact that PEF treatment released some other nonphenolic substances with antioxidant activity (e.g., vitamins, amino acids) Additionally, PEF extraction increased extraction of anthocyanin monoglucosides when compared to other methods. In another study, PEF treatment improved the extraction polyphenols from orange peel and increased the antioxidant capacity of the extract as well as the naringin and hesperidin content (Luengo, Álvarez, and Raso, 2013). In this case, no organic solvent was used. Finally, PEF pretreatment followed by aqueous extraction of mango peels produced stable and clear extracts with increased antioxidant potential and total phenolic recovery (Parniakov, Barba, Grimi, Lebovka, & Vorobiev, 2016).

7.7.6 HIGH-VOLTAGE ELECTRICAL DISCHARGE

HVED is a method in which aqueous solutions are exposed to electrical and mechanical effects caused by shockwaves (Boussetta, Lanoisellé, Bedel-Cloutour, & Vorobiev, 2009). In a study conducted on grape pomace HVED induced the highest cell disintegration with the lowest energy input when compared to ultrasonic and PEF extraction. This method provided the highest total phenolic recovery, but was not very efficient for the extraction of anthocyanins (Barba et al., 2015). Boussetta et al. (2009) optimized HVED extraction of grape pomace followed by diffusion in different mixtures of water and ethanol. Energy input, electrode distance, solid−liquid ratio, and temperature were varied in order to achieve the highest yield. Optimal conditions included an HVED pretreatment at 80 kJ/kg, electrode distance of 5 mm, liquid-to-solid ratio of 5, and following diffusion with 30% ethanol in water at 60°C for 30 min. Furthermore, HVED can effectively extract polyphenols from grape seeds. HVED can also effectively extract polyphenols from grape seeds. An experiment by Liu, Vorobiev, Savoire, and Lanoisellé (2011) showed that the maximal concentration of extracted polyphenols from grape seeds was achieved after application of 300 40 kV−10 kA discharges. In a study by Sarkis et al. (2015), both HVED and PEF as pretreatments improved extraction yields of polyphenols from sesame cake during diffusion in different ethanol water mixtures. Application of these methods can reduce the quantity of organic solvents and compensate for the need for high temperatures to improve diffusion.

Overall, innovative extraction techniques for the recovery of beneficial phenolic compounds are constantly developing. Current studies are often focused on optimization of experimental parameters in order to achieve the highest possible yields. There is an obvious need for the development

of environmentally friendly approaches that include wastewater formation, use of green solvents, and lower energy consumption.

7.8 ENCAPSULATION

As already noted, phenolic compounds have attracted great interest due to their antioxidant properties and potential beneficial effects on human health. However, the efficiency of these compounds depends upon their bioavailability in the human body. The polyphenols that are usually present in the human diet are not always the most active form within the body, because of different factors including low solubility, poor absorption, or intensive metabolism and elimination. Other limiting factors include physic-chemical properties (e.g., light and heat sensitivity and instability in the presence of oxygen; Munin & Edvards-Levy, 2011), as well as sensory properties (e.g., bitterness and astringency; Pasrija et al., 2015). In order to overcome these limitations, it is necessary to develop formulation able to maintain stability of the active ingredient and to deliver it in suitable form to the target (Chen, Remondetto, & Subirade, 2006). Encapsulation may be one of the most promising methods for this purpose.

Encapsulation is a technology that packages small solid particles, liquid droplets, or gas molecules into a form that allows releasing the content in certain conditions or upon the influence of adequate stimulus (Desai & Park, 2005; Picot & Lacroix, 2003). It is a process that includes entrapping one substance (active agent) into the wall material of another substance and the production of nanometer, micrometer, or millimeter sized particles. Molecules of active agent are enclosed within a layer of coating material or incorporated into a homogenous or heterogenous matrix forming particles called microcapsules or microspheres, respectively. Their size range is usually between 1 μm and 1 mm; particles with smaller size (from 1 nm to 1 μm) are called nanoparticles (e.g., nanocapsules and nanospheres; Burgain, Gaiani, Linder, & Scher, 2011; Parisi et al., 2014). Several methods have been proposed for the encapsulation of polyphenols including spray drying, freeze drying, coacervation, emulsions, liposomes, nanoparticles, micelles, and inclusion encapsulation with cyclodextrins.

7.8.1 SPRAY AND FREEZE DRYING

These methods include the preparation of a liquid formulation containing an active agent and the wall material. During spray drying, the mixture is atomized with a nozzle or a spinning wheel in the spray dryer. Solvent (water) is evaporated by the contact of atomized material with hot air. Finally, the obtained particles are collected after they fall to the bottom of the dryer (Desai & Park, 2005; Gibbs, Kermasha, Alli, & Mulligan, 1999). Various wall materials can be used for the encapsulation of polyphenol such as gum, maltodextrin, chitosan, and starch. Another drying technique, called freeze drying (also known as lyophilization), involves dehydration at low temperature (i.e., elimination of water by sublimation of the frozen product; Munin & Edwards-Lewy, 2011). It is a convenient technique for thermosensitive molecules, but demands high energy input (Ray, Raychaudhuri, & Chakraborty, 2016).

Spray drying was successfully applied for the encapsulation of phenolic compounds extracted from olive pomace (Paini et al., 2015) as well as for pure phenolic substances such as curcumin (Liu, Chen, Cheng, & Selomulya, 2016). The latter was combined with whey protein isolate in order to form complexes with increased solubility. The obtained solutions were spray dried and uniform microparticles with high (over 95%) retention rates were produced (Liu et al., 2016).

Cabernet sauvignon red wine with the addition of maltodextrin was freeze dried and "wine powder" with negligible polyphenol loss was produced. The powder contained 3.7-fold higher concentration of polyphenols while containing less than 1% of ethanol (Sanchez, Baeza, Galmarini, Zamora, & Chirife, 2013). Maltodextrin has also been used for the preparation of freeze-dried polyphenol extract from raspberry (Laine, Kylli, Heinonen, & Jouppila, 2008).

Grape-skin extract of Bordo variety was microencapsulated by spray and freeze drying by applying different encapsulation agents (i.e., arabic gum, partially hydrolyzed guar gum, and polydextrose). The retention percentage of total phenolics and anthocyanins were all above 80. Spray-drying treatments had lower moisture, water activity, and particle size, while possessing higher solubility and more spherical shape than freeze-drying treatments. Freeze-dried samples were less hygroscopic. The best method for the production of these particles was shown to be spray-drying treatment with the use of 5% of partially hydrolyzed guar gum and 5% of polydextrose (Kuck & Norena, 2016).

7.8.2 EMULSIONS

Emulsions are mixtures of at least two immiscible liquids, usually oil and water, in which one of them is dispersed in small droplets into the other (Friberg, Larsson, & Sjoblom, 2003). They can be divided based on the nature of dispersed phase: oil-in-water (O/W) emulsions where oil droplets are dispersed in aqueous continuous phase and water-in-oil (W/O) emulsions where water droplets are dispersed in oil phase. In order to obtain stable emulsion systems it is necessary to add emulsifiers and texture modifiers. Polyphenols can be encapsulated in emulsion systems and the final formulation can be used in the liquid form or dried to powders (Parisi et al., 2014).

Resveratrol has been encapsulated in emulsions with triacylglycerols as the oil phase and a mixture (Nemen & Lemos-Senna, 2011). Formulation of a topical application containing this compound was also developed with Tween 80 as an emulsifying agent and isopropyl myristate as a carrier oil (Yutani, Morita, Teraoka, & Kitagawa, 2012). Tea polyphenols were encapsulated in sunflower O/W emulsion with the addition of bovine albumin serum. This formulation enhanced phenols stability and showed strong antioxidant activity (Almajano, Carbo, Jimenez, & Gordon, 2008).

7.8.3 NANOPRECIPITATION

Homogenization/emulsion-solvent removal methods are generally based on the evaporation or extraction of the internal phase of an emulsion, leading to precipitation of the polymer coating and formation of particles with active component. After the removal of the solvent, particles are washed, filtered, and dried or freeze dried (Munin & Edwards-Levy, 2011). The emulsion-solvent extraction method, also known as nanoprecipitation, includes injection of the polymer solution with active compound into aqueous phase containing emulsifier. Polymer solvent is miscible with water, but due to the fact that polymer is not soluble in mixtures of water and solvent, precipitation occurs

and nanoparticles with entrapped compound are formed (Lu, Kelly, & Miao, 2016). Spherical microparticles of curcumin were prepared with an emulsion solvent evaporation method with biocompatible poly(D,L,-lactideco-glycolide) (Shahani et al., 2010). The nanoprecipitation method was applied for the encapsulation of resveratrol with over 90% encapsulation efficiency (Shao et al., 2009). Finally, this method was employed for the preparation of quercetin-loaded biodegradable poly (ε caprolactone) nanoparticles with the potential for controlled release (Dinesh Kumar, Prasad Verma, & Kumar Singh, 2015).

7.8.4 COACERVATION

Coacervation is another method proposed for the encapsulation of polyphenols. It includes phase separation of the hydrocolloid present in initial solution and the deposition of the newly developed coacervate around suspended or emulsified active compound (Gouin, 2004). Although considered an expensive method that results in particles with nonspherical shape, it can be useful for the development of formulations with polyphenols, some of which are propolis extract with soy protein and pectin (Nori et al., 2011) and Yerba mate extract with calcium alginate and calcium alginate-chitosan coacervates (Deladino, Anbinder, Navarro, & Martino, 2008).

7.8.5 LIPOSOMES AND MICELLES

Vesicles such as liposomes and micelles can be useful carriers of polyphenols. Liposomes consist of one or more concentric lipid bilayers separating water compartments, which makes them adequate carriers for lipid- and water-soluble molecules (Munin & Edvards-Levy, 2011). The encapsulation efficiency of polyphenols in liposomes depends on different factors including the preparation method and the properties of the encapsulated compounds (Fang & Bhandaria, 2010). Polyphenolic grape seed extract was encapsulated in soy lecithin liposomes. Coating of these liposomes with chitosan or citrus pectin decreased the possibility of reaction of incorporated compounds with other surrounding components, making them highly capable carrier systems for polyphenols (Gibis, Vogta, & Weiss, 2012). Black-mulberry waste extract was incorporated in lecithin-chitosan coated liposomes and spray dried after mixing with maltodextrin. The resulting liposomal powder provided better protection of anthocyanins than spray-dried pure extract. Additionally, this formulation improved the in vitro bioaccessibility of anthocyanins and can be used for the fortification of chocolate (Gültekin-Özgüven, Karadag, Duman, Özkal, & Özçelik, 2016). Liposomal carriers are also proposed for the enhancement of the bioavailability of individual polyphenols such as resveratrol (Wenche Jøraholmen, Škalko-Basnet, Acharya, & Basnet, 2015) and curcumin for oral delivery (Takahashi, Uechi, Takara, Asikin, & Wada, 2009). Resveratrol has been solubilized in bile acid (Atanacković, Poša, Heinle, Gojković-Bukarica, & Cvejić, 2009; Cvejić, Poša, Sebenji, & Atanacković, 2014) as well as polycaprolactone/poly(ethylene glycol) micelle (Cvejic et al., 2014; Lu et al., 2009).

7.8.6 CYCLODEXTRINS

Another proposed technology for the entrapment of polyphenols is inclusion in cyclodextrins. Cyclodextrins are a group of cylinder-shaped cyclic oligosaccharides derived from starch, with six,

seven, or eight glucose residues linked by (1−4) glycosidic bonds (Pagington, 1986). In nature, they exist as α-, β-, and γ-cyclodextrins, but β-cyclodextrin is the most commonly applied form for encapsulation purposes. The external zone of cyclodextrins is hydrophilic, while the internal, hydrophobic part enables the formation of inclusion complexes with poorly water-soluble molecules like polyphenols (Loftsson & Duchêne, 2007; Singh, Sharma, & Banerjee, 2002). Encapsulation of many different individual polyphenols with cyclodextrins has been reported including daidzein and genistein (Yatsu et al., 2013), catechin (Dias, Nikolaou, & Giovani, 2011), and epigallocatechingallate (Folch-Cano, Guerrero, Speisky, Jullian, & Olea-Azar, 2013). A study by Kalogeropoulos, Yannakopoulou, Gioxari, Chiou, and Makris (2010) showed that the inclusion complex for a flavonoid-rich extract of St. John's wort and β-cyclodextrin improved the thermal stability of the nutraceuticals present in the extract. It has also been reported that aqueous cyclodextrins can be successfully applied for the recovery of phenolics from grape and grape by-products (Ratnasooriya & Vasantha Rupasinghe, 2012). Considering the fact that cyclodextrins are inexpensive and friendly to humans, they are a promising option for improving the biological, chemical, and physical properties of bioactive molecules.

7.9 NATURAL PIGMENTS AND COLORANTS, FOOD, BEVERAGE, AND OTHER INNOVATIVE APPLICATIONS

7.9.1 COLORANTS

Color is one of the most important qualities of foods and beverages. Inappropriate color is often associated with lower food quality, spoilage, bad processing, storage, or transportation. Hence, producers devote a significant attention to preserving or improving of foods' color (Delgado-Vargas & Paredes-López, 2002b). The color-production industry provides a full range of colors for applications in various fields, within current legislation. The stability and handling properties of colors are continuously being improved using formulation technology, new processing methods, and emerging technologies (Downham & Collins, 2000). Different definitions of pigments, dyes, and colorants are available in literature and are often used interchangeably. According to the most widely used definitions, a pigment is insoluble in the given medium, whereas a dye is soluble (Delgado-Vargas & Paredes-López, 2002c; Mapari, Thrane, & Meyer, 2010). Pigments, dyes, and colorants are used in this text to represent colored substances that modify the perceived color of objects.

The safety of synthetic dyes is questionable, since the use of a number of them has been associated with adverse impacts on the environment as well as with allergic, toxic, carcinogenic, and harmful responses (Downham & Collins, 2000). Interest in natural colorants has significantly increased in the last several decades. However, the higher stability of synthetic colorants with respect to light, oxygen, temperature, and pH, among other factors, makes the replacement of synthetic dyes with natural colorants challenging (Bakowska-Barczak, 2005). A few of the patented processes for improving pigment stability are realized by chemical modification, but such processes are often considered unacceptable since natural colorants must be stabilized using natural methods (Wissgott & Bortlik, 1996).

Natural colorants are mostly extracted from plant materials, but other sources such as insects, algae, cyanobacteria, and fungi are becoming more popular. Food wastes are considered as a cheap

source of valuable nutraceuticals since an enormous amount of food-related materials are discharged worldwide. The recovery of target compounds and their recycling inside the food chain as functional additives is possible using available technologies (Galanakis, 2012). In general, natural colorants can be derived from the following sources: primary products from agriculture, waste, and by-products from farming and forestry, and wastes from the food and beverage industries. Huge amounts of colored plant wastes such as pressed berries and distillation residues, pomace, peels, shells, and other residual by-products are being obtained from industrial food and beverage production processes (Shahid, Ul Islam, & Mohammad, 2013). Additionally, plant-tissue culture is used as an alternative for the production of natural colorants (primarily anthocyanins). Plant-tissue culture is an expensive methodology, often characterized by low yield. However, its application for anthocyanin production is noteworthy because it assures a continuous supply of uniform-quality anthocyanin pigments (Delgado-Vargas & Paredes-López, 2002a).

Natural food colorants can be grouped into a few major classes. The most important natural colorants belong to tetrapyrrols, tetraterpenoids, and flavonoids. Chlorophyll (green color) is the most significant tetrapyrrol, while the most important tetraterpenoids are carotenoids (yellow−orange−red color). They are ubiquitous pigments found in all higher plants as part of the photosynthetic apparatus. Anthocyanins are a group of flavonoids responsible for the red−purple hue of many fruits and vegetables (e.g., grapes, strawberries, elderberries, purple sweet potato, red-fleshed potato, red cabbage; Mortensen, 2006). Anthocyanins, highly reactive species, are the major polyphenol pigments in plants, followed by the yellow flavonols and, to a lesser extent, flavones and flavanonols. Tannins are also yellow and their color varies according to the oxidation level of the medium (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006b). Phenolic acids are colorless in a dilute alcohol solution, but as a result of oxidation they may become yellow (El Gharras, 2009).

7.9.2 ANTHOCYANINS

The high potential of anthocyanins to be used as natural colorants has been emphasized due to their characteristic orange, red, and purple color hues and water solubility, which enables their implementation into various food systems (Bakowska-Barczak, 2005). Anthocyanins occurring in nature contain several anthocyanidins (aglycones), but only six are common in foods—cyanidin, peonidin, pelargonidin, malvidin, delphinidin, and petunidin. However, only their corresponding glycosides (anthocyanins) are found in plants. More than 540 pigments based on anthocyanidins and anthocyanin structures have been identified in nature. This is a result of the structural variation of the six common anthocyanidins coming from glycosidic substitution at the three and five positions and possible acylation of sugar residues with organic acids (Andersen & Francis, 2004). The sugar residues (mainly glucose) may be further acylated with organic acids such as cinnamic acids (caffeic, p-coumaric, ferulic and sinapic acid) and a range of aliphatic acids (acetic, malic, malonic, oxalic, succinic acids, etc.) (Tanaka, Sasaki, & Ohmiya, 2008). The color is affected by the number of hydroxyl and methoxyl groups. M hydroxyl groups directs color toward more bluish hues, while the presence of methoxyl groups increase the red color intensity (Delgado-Vargas & Paredes-López, 2002a). For example, malvidin monoglucoside (malvine) may be considered to be the main pigment responsible for the color of red grapes and, consequently, red wine (Ribéreau-Gayon et al., 2006b). Furthermore, anthocyanin properties are influenced by the copigmentation phenomenon. This molecular association between the pigments and their copigmentation cofactors (mostly

colorless phenolics) involves the anthocyanin glucosides, certain phenolic acids, and flavonoids (in particular, derivatives of the flavonols and flavones). As a result a more stable class of color molecules is formed. During aging, a small fraction of the anthocyanins is decomposed by the effects of external factors (temperature, light, oxygen, etc.) or precipitated in colloidal coloring matter, leading to color loss (Boulton, 2001).

The molecular structure of anthocyanins affects their stability. The color of food based on pelargonidin, cyanidin, or delphinidin derivatives is less stable than that of food containing petunidin or malvidin derivatives. Bakowska-Barczak (2005) emphasized the importance of anthocyanin pigments containing acyl substituent due to their significant stability with pH changes and increased heat and light exposure. The stable acylated anthocyanins are present in large amounts in vegetables such as red cabbage, black carrot, red radish, red potatoes, or red corn.

7.9.3 INFLUENCE OF PROCESSING CONDITIONS ON COLORANT STABILITY

The exposure of polyphenolic pigments to harsh conditions (pH, temperature, light) during food processing often leads to their degradation (Francis & Markakis, 1989). Conversions of genuine anthocyanins to other molecules leads either to loss or stabilization of color and increases the range of available hues (El Gharras, 2009). Optimization of processing conditions and application of emerging technologies are important to ensure minimal degradation of natural pigments and to obtain better-colored products (Delgado-Vargas, Jiménez, & Paredes-López, 2000).

Color fading with increase of pH is much more pronounced in unacylated anthocyanins than in their acylated analogs. Degradation of anthocyanin colorants can be caused by exposure of food to high temperatures. Interestingly, the color can be regained after a cooling stage of several hours when the heating applied is not excessive (Delgado-Vargas et al., 2000). Acylated anthocyanins from red cabbage showed greater stability to the heating effect than the unacylated anthocyanins obtained from red grape, blackcurrant, and elderberry (Bakowska-Barczak, 2005; Dyrby, Westergaard, & Stapelfeldt, 2001). Light has deleterious effects on anthocyanin stability in naturally colored foods and beverages (Francis & Markakis, 1989). Coating of fruits and vegetables is another strategy used to preserve good coloration. Improved stability is connected with the inhibition of degradative enzymatic activities of polyphenol oxidase and peroxidase since plastic coating is a barrier that reduces the supply of oxygen required for the enzyme activity (Francis & Markakis, 1989). In order to inhibit degradative enzymatic activity on pomace anthocyanins, Ayed, Yu, and Lacroix (1999) suggested and evaluated the application of methodologies such as gamma irradiation, vacuum packaging, and SO_2. Gamma irradiation in the processing of vegetables (usually 2 kGy) ensured color stability and improvement of product shelf-life. Additionally, packaging in a mixture of air with SO_2 improved the color of the product expressed through 12% more anthocyanins than in the case of packaging at vacuum without addition of SO_2.

7.9.4 APPLICATION OF NATURAL COLORANTS

A large number of plant and other sources has been used for the extraction of colorants. These compounds have been applied in textile dyeing and functional finishing, for food coloration,

cosmetics, dye-sensitized solar cells, as well as for histological staining, pH indicator, and several other application disciplines (Shahid et al., 2013).

There are several reasons for adding colorants to foods and beverages. The most important are to enhance the color of a product (e.g., soft drinks), standardize its color and appearance (like margarine, confectioneries, and desserts), restore the color lost during processes (e.g., extruded food), add health-promoting properties of the natural colorant, and finally increase acceptability of the food as an appetizing item.

From the beginning of the 21st century there has been increased interest in effective natural substitutes for FD&C red no. 40, the certified dye with the highest per capita consumption in the United States. The use of red radish extract, which has similar coloring characteristics as the synthetic colorant, was suggested. The anthocyanins of red radish have good stability associated with the presence of acylated pelargonidin derivatives. For instance, maraschino cherries colored with radish anthocyanins have a shelf-life of at least 6 months at 25°C (Delgado-Vargas & Paredes-López, 2002a; Rodriguez-Saona, Giusti, Durst, & Wrolstad, 2001).

Today apart from the widely used commercial preparations of grape extracts (e.g., enocyanin), preparations based on anthocyanins are obtained from the by-products of red cabbage, red radish, purple sweet potato, black carrot, aronia, cherry, elderberry, and blackberry. Vegetable sources such as radish, purple sweet potato, red-fleshed potato, or red cabbage have generally been shown to provide a high yield of stable acylated anthocyanins. Extracts containing acylated anthocyanins are suitable for foods with a low pH level, including soft drinks, confectioneries, table jellies, conserves, and sauces, as well as in certain neutral or slightly alkaline products, such as ice creams, panned products, and milk drinks (Bakowska-Barczak, 2005; Giusti & Wrolstad, 2003).

Bakowska-Barczak (2005) reported that red cabbage (*B. oleracea* L), due to its low prices, year-round availability, high production yields, and high content of anthocyanins, is the most important source of stable anthocyanins for the Polish food industry. These extracts are water soluble and available in a liquid or sprayed-dried form, and are mostly derivatives of cyanidin-3-diglucoside-5-glucoside acylated with ferulic, sinapic, and/or p-coumaric acids (Giusti, Rodríguez-Saona, & Wrolstad, 1999). They exhibit bright pink/mauve shades at low pH and mauve/blue shades at more neutral pH values. Their use does not produce unpleasant taste or odor. They are naturally low in polyphenols, unlike grape-skin anthocyanins, which reduces the risk of haze formation (due to the reaction with proteins) and precipitation problems. Furthermore, natural colorants derived from exotic fruit by-products can also be effectively applied in food production (Ayala-Zavala et al., 2011).

Finally, it is important to emphasize the importance of the formulation, the process in which the extracted colorant is mixed with other components. The purpose of colorant formulation is to mix two or more colorants to get a different color or shade, as well as to obtain the same color shade as an existing color (e.g., to replace a synthetic or nature-identical colorant with natural colors). Formulation of colorants may be performed to enhance their handling by diluting highly viscous liquids or semisolids (e.g., with vegetable oils) or by their spray drying using maltodextrin as a carrier if powder is preferred over liquid. Colorant formulations are often supplemented with antioxidants (e.g., α-tocopherol and ascorbic acid) to increase stability and to inhibit color fading. Various hydrocolloids such as gelatin, gum arabic, pectin, and others can be used for pigment coating (microencapsulation) in order to make oil-soluble colors dispersible in water and to create a physical barrier that protects them from degradation (Mortensen, 2006).

7.10 EFFECTS ON THE SENSORY QUALITY OF FOOD PRODUCTS AND BEVERAGE PREFERENCES

In addition to expressing valuable biological properties, phenolic compounds may affect the sensory characteristics of food. These compounds can have a positive or negative impact on the important organoleptic properties of foods and beverages such as color, aroma, bitterness, and astringency. Interest in nutraceuticals and functional foods was expressed through the new products development, and the attention was more dedicated to ingredient functionality than to sensory acceptability. Initially, balanced flavor was anticipated and accepted by consumers, as far as the product provides a positive impact on health. Due to rapid expansion of the "healthy aliments" market and the availability of a large number of these products, development of functional foods and beverages has increasingly become influenced by the importance of flavor to consumers. Products having proven health benefits and, at the same time good taste as well as appealing organoleptic characteristics in general, are the most widely expected.

7.10.1 IMPACT OF PHENOLICS ON FOOD TASTE

Polyphenols exhibit widely known health benefits, such as high antioxidant activity, and are suitable as functional ingredients. However, these compounds can lack palatability, mainly due to pronounced taste of bitterness. This sensory characteristic is usually associated with diminished food acceptability (de Graaf, 2007; Jaeger, Axten, Wohlers, & Sun-Waterhouse, 2009; Miljić, Puškaš, Vučurović, & Razmovski, 2014).

There are more than 8000 structures among phenolic compounds, from the simplest (phenolic acids) to the most complex ones (proanthocyanidins) (Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2014). There is a lack of available data concerning the individual sensory profile of these compounds as well as the mechanisms that determine the different mouthfeel characteristics. Flavanols and flavonols (flavonoids) and phenolic acids are primarily responsible for the tactile sensation of astringency and the bitter taste of food and beverages such as fruits, nuts, chocolate, tea, cider, wines, beer.

Higher-molecular-weight (>500) polymers (tannins) tend to be astringent, and phenolic compounds of lower molecular weight are more likely to be bitter. Bitter phenolics, such as quercetin, are the most common bitter compounds in immature apples and other fruit (Bravo, 1998; Drewnowski & Gomez-Carneros, 2000; Noble, 1994). Structure, molecular size, and concentration of tannins are thought to define the sensory properties of food. Astringency depends on the number of protein interaction sites in the molecule while bitterness is restricted to small molecules with particular structural features that enable them to enter the receptor and activate the signal-transduction process (Cheynier et al., 2006). As the molecular weight increases to approximately ten units, tannins are becoming gradually more astringent and less bitter. It has been reported that the sensation of astringency increase significantly with the increase of tannin concentration, together with a tendency to mask perceived bitterness (Villamor, 2012).

The astringent perception can be elicited by different kinds of compounds including metal salts (particularly aluminum salts), phenolic compounds, acids, and dehydrating agents such as alcohols (Villamor, 2012). Individuals perceive astringency due to the complex reactions between dietary

polyphenols and saliva proteins. Saliva is a highly diluted aqueous fluid that contains proteins, glycoproteins, glycolipids, carbohydrates, serum transudates, and inorganic ions (El Gharras, 2009). Each class of compound usually contributes to the many biological activities of saliva. Polyphenols make complexes with salivary proteins. This results in formation of a "layer" that acts as a water barrier and produces a mouth-drying sensation. These interactions play a role in the understanding of the sensation of astringency, which is often described as a drying, roughing, and puckering feeling (Condelli, Dinnella, Cerone, Monteleone, & Bertuccioli, 2006). Variations in salivary flow rates and in their preferences cause different perceptions of astringency among consumers.

Unlike astringency, a large range of molecules, including organic molecules, inorganic ions, and salts, are responsible for bitter taste (Drewnowski & Gomez-Carneros, 2000; Lesschaeve & Noble, 2005). Fontoin, Saucier, Teissedre, and Glories (2008) suggested that bitter receptors could operate as heteromeric receptors in order to accommodate diversity. Bitterness of fruits, vegetables, and derived products could be induced by certain polyphenols even if they are present in very low concentrations (Soares et al., 2013).

Ferrer-Gallego et al. (2014) investigated the sensory profile of flavan-3-ol monomers and reported that epicatechin was more bitter and astringent than catechin, and showed longer persistence. In general, epicatechin was more "unpleasant" than catechin. The astringent intensity of the tested phenolic acids (coumaric, caffeic, protocatechuic, and gallic acids) and the epicatechin was very similar. The difference between individual phenolic acids and epicatechin was mainly due to its bitterness. A synergistic astringent effect was reported since the significant increase in the astringency was observed when combination of different tannic substances were tasted (Ferrer-Gallego et al., 2014).

Different phenolic compounds determine the flavor of various foods and beverages. Phenolic compounds in wine range from low-molecular-weight phenolic acids and catechins to high-molecular-weight tannins. Flavan-3-ol monomers such as catechin, epicatechin, epigallocatechin, epicatechin gallate, and EGCG and their oligomers and polymers, which are called proanthocyanidins or condensed tannins, are the most abundant compounds in wine and tea (Lesschaeve & Noble, 2005; Oliveira, Carvalho, & Melo, 2014). Perceived bitterness and astringency increase linearly with the concentration of catechins and grape-seed tannins. Catechin polymers are gradually becoming more astringent at higher molecular weights (Drewnowski & Gomez-Carneros, 2000). Catechol is the predominant volatile phenolic compound found in coffee aroma, followed by 4-ethylguaiacol, 4-ethylcatechol, pyrogallol, quinol, and 4-vinylcatechol (Drewnowski & Gomez-Carneros, 2000). EGCG is one of the most important green-tea polyphenols. Bitterness of tea is generally ascribed to the combination of catechins, saponin, caffeine, and amino acids (Ahmad & Mukhtar, 1999). Cocoa bean and its products (cocoa powder, chocolate, and cocoa liquor) are rich in phenolic compounds, mainly flavan-3-ols (monomeric epicatechin and catechin, as well as their oligomers and polymers) and to a lesser extent anthocyanins (mainly cyanidin glycosides) and flavonols (quercetin glycosides). The bitter taste of chocolate probably comes from catechins, which are present in higher amounts in dark than in milk chocolate (Shahidi & Ambigaipalan, 2015).

Phenolic acids, flavonoids, tannins, and amino-phenolics are phenolic compounds found in beer. However, beer bitterness mainly comes from α-acids (humulones) that are present in the hop cone. Six iso-α-acids, which impart about 80% of the bitter taste in beer, are formed during wort boil, while the remaining bitterness is caused by polyphenols (Tanimura & Mattes, 1993).

Content of ethanol, pH value, sweetness, and viscosity are all the factors that have an impact on the sensations of bitterness and astringency in beverages. Reported that increase in ethanol

concentration of wine from 8% to 14% (v/v) approximately doubled the bitterness intensity, but without any effect on astringency. Sweetness and bitterness generally show suppressed effect in mixtures. Increase of sweetness or viscosity result with decrease in the intensity of bitter sensation in vermouths. Despite the increased sourness, lowering of the wine pH had no effect on bitterness. On the other hand, lowering the pH was reported to increase the astringency of cranberry juice and wines. Furthermore, consuming the same food with different beverages can significantly change the perception of astringency. For instance, eating milk chocolate increases the astringency of red wine far less than does dark chocolate. Preferences and acceptance of a product may vary significantly among individual consumers.

7.10.2 DEBITTERING OF FOODS AND BEVERAGES

The degree of bitterness in plant foods depends on the characteristics of the raw material (cultivar, changes during ripening) and production and storage conditions. As the taste of bitterness is often connected with a major sensory deficiency, debittering can be an important step toward creating an acceptable product (Drewnowski & Gomez-Carneros, 2000). Responding to taste-driven consumer demand, food scientists have investigated different possibilities for lowering bitterness in various sources.

Bitter phenolic compounds are effectively adsorbed to resins, trapped on polymers, precipitated, extracted with solvents, or converted to nonbitter compounds (Oliveira et al., 2014). A variety of procedures can be applied for the removal of phenolic compounds from wine. The latter is achieved through fining with protein-based preparations such as casein, gelatin, egg white, or isinglass. Polyvinyl polypyrrolidone, a synthetic fining agent, is also used for adsorption of wine tannins (Puškaš & Miljić, 2012; Ribéreau-Gayon et al., 2006b). Wine aging reduces both bitterness and astringency due to polymerization and, eventually, precipitation of phenolics with time (El Gharras, 2009; Ferrer-Gallego et al., 2014). Young red wines sold without being aged sometimes have high residual sugar concentrations. Reduction of bitter taste and astringency is sometimes achieved by stopping the fermentation and leaving higher concentrations of residual sugar in wine or just by adding various sugar sources (sucrose, fructose, or rectified concentrated must) in dry wine.

In order to obtain a product with acceptable organoleptic properties and a suitable level of nutraceuticals, it is important to control and optimize food-processing conditions. For instance, the use of pectinolytic and hemi-cellulolytic enzymes blackcurrant juice production was found to increase juice yield and concentration of phenolic compounds, especially anthocyanins (Laaksonen et al., 2012). However, maximizing the phenolic content of the juice may have a negative impact on sensory characteristics, such as enhanced bitterness. The application of new technologies in orange-juice processing (PEFs as an alternative to heat pasteurization) allows better preservation of phenolic compounds and at the same time of their sensory properties (Agcam, Akyildiz, & Akdemir Evrendilek, 2014).

7.10.3 IMPACT OF PHENOLICS ON FOOD COLOR

Some structures of polyphenols (anthocyanins, flavonols, and flavones) are also responsible for food color, representing yellow, orange, red, and blue pigments. Phenolic acids are colorless in a dilute alcohol solution such as wine, but due to oxidation they may become yellow. Proanthocyanidins in

wine may play a role in copigmentation reactions with anthocyanins to form new, more stable, red pigments (El Gharras, 2009; Puškaš & Miljić, 2012; Ribéreau-Gayon et al., 2006b).

7.10.4 IMPACT OF PHENOLICS ON FOOD AROMA

The content and composition of phenolic compounds have an impact on the expression of aroma substances in plant foods. A significant loss of aroma compounds through intermolecular interactions may be possible due to changes in the concentration of polyphenols (Dufour & Bayonove, 1999). Goldner, Lira, Van Baren, and Bandoni (2011) reported that the fruity and floral aroma intensity in wine seems to decrease when the level of polyphenols increases. Furthermore, the addition of grape-seed extracts to wine, apart from changing the astringency, reduces the fruity aroma and enhances the woody/earthy aroma (Cliff, Stanich, Edwards, & Saucier, 2012). On the other hand, the astringency and bitterness of wines were reduced with the addition of volatile fruity extracts (Sáenz-Navajas, Campo, Fernández-Zurbano, Valentin, & Ferreira, 2010).

Certain volatile polyphenols (e.g., vanillin, syringaldehyde, and eugenol) are strong odorants (El Gharras, 2009). Phenolic acids do not exhibit particular aroma or odor, but they are precursors of the volatile phenols produced by the activity of certain microorganisms (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006a). These transformations are considered to be olfactory defects and can be common for wines. Among volatile phenols vinyl- and ethyl-phenols are the most unpleasant odorants. Vinyl-4-phenol has an odor reminiscent of pharmaceuticals, gouache paint, and "band aids," while stable and sweaty saddle odor is characteristic of ethyl-4-phenol. It has been confirmed that these compounds result from the breakdown of p-coumaric acid and ferulic acid. The preference thresholds for vinyl- and ethyl-phenols have been estimated at 720 and 420 μg/L, respectively. The vinyl-phenols, more characteristic of white wines, are formed due to enzymic decarboxylation (cinnamate decarboxylase) by the *Saccharomyces cerevisiae* yeast of two cinnamic acids (p-coumaric acid and ferulic acid) in must, producing vinyl-4-phenol and vinyl-4-guaiacol, respectively. It was demonstrated that *Brettanomyces/Dekkera* yeasts were the only microorganisms capable of producing several milligrams of ethyl-phenols per liter of wine. Ethyl-phenol concentrations may, in some cases, reach several mg/L, but even at lower concentrations, such as 600−700 mg/L, these volatile phenols alter aroma. Biosynthesis of ethyl-phenols by *Brettanomyces* is performed through the sequential action of two enzymes. The first is a cinnamate decarboxylase that transforms cinnamic acids into vinyl-phenols. The second is a vinyl-phenol reductase, the enzyme totally absent in *S. cerevisiae*. These properties explain why *S. cerevisiae* is incapable of producing large quantities of volatile phenols in red wines.

ACKNOWLEDGMENTS

This work is a part of a projects TP 31020, TP 31022 financed by the Ministry of Science and Technological Development, Republic of Serbia and MEDLEM, id 690876 - HORIZON 2020, H2020-MSCA-RISE-2015.

REFERENCES

Adam, A., Crespy, V., Levrat-Verny, M., Leenhardt, F., Leuillet, M., Demigne, C., & Remesy, C. (2002). The bioavailability of ferulic acid is governed primarily by the food matrix rather than its metabolism in intestine and liver in rats. *Journal of Nutrition*, *132*, 1962−1968.

Agcam, E., Akyildiz, A., & Akdemir Evrendilek, G. (2014). Comparison of phenolic compounds of orange juice processed by pulsed electric fields (PEF) and conventional thermal pasteurisation. *Food Chemistry*, *143*, 354−361.

Ahmad, N., & Mukhtar, H. (1999). Green tea polyphenols and cancer: Biologic mechanisms and practical implications. *Nutrition Reviews*, *57*(3), 78−83.

Al.-Asheh, S., Jumah, R., Banat, F., & Al.-Zou'bi, K. (2004). Direct current electroosmosis dewatering of tomato paste suspension. *Food and Bioproducts Processing*, *82*(3), 193−200.

Almajano, M. P., Carbo, R., Jimenez, J. A. L., & Gordon, M. H. (2008). Antioxidant and antimicrobial activities of tea infusions. *Food Chemistry*, *108*(1), 55−63.

Alothman, M., Bhat, R., & Karim, A. A. (2009). UV radiation-induced changes of antioxidant capacity of fresh-cut tropical fruits. *Innovative Food Science & Emerging Technologies*, *10*(4), 512−516.

Álvarez-Casas, M., García-Jares, C., Llompart, M., & Lores, M. (2014). Effect of experimental parameters in the pressurized solvent extraction of polyphenolic compounds from white grape marc. *Food Chemistry*, *157*, 524−532.

Amendola, D., Donsì, F., Sessa, M., Ferrari, G., De Faveri, D.M. & Spigno, G. (2011) *Nanoemulsions of grape marc extract as natural additives to improve hazelnut paste shelf-lif*, The 11th International Congress on Engineering and Food, Athens, Greece.

Andersen, Ø. M., & Francis, G. W. (2004). Techniques of pigment identification. In K. Davies (Ed.), *Annual plant reviews, plant pigments and their manipulation*. Oxford: Blackwell Publishing.

Angersbach, A., Heinz, V., & Knorr, D. (1997). Effects of pulsed electric fields on cell membranes in real food systems. *Innovative Food Science and Emerging Technologies*, *1*, 135−149.

Ankolekar, C., Johnson, D., Pinto Mda, S., Johnson, K., Labbe, R., & Shetty, K. (2011). Inhibitory potential of tea polyphenolics and influence of extraction time against *Helicobacter pylori* and lack of inhibition of beneficial lactic acid bacteria. *Journal of Medicinal Food*, *14*(11), 1321−1329.

Anson, N. M., Van Den Berg, R., Havenaar, R., Bast, A., & Haenen, G. R. M. M. (2009). Bioavailability of ferulic acid is determined by its bioaccessibility. *Journal of Cereal Science*, *49*(2), 296−300.

Ashokkumar, M., Sunartio, D., Kentish, S., Mawson, R., Simons, L., Vilkhu, K., & Versteeg, C. (2008). Modification of food ingredients by ultrasound to improve functionality: A preliminary study on a model system. *Innovative Food Science & Emerging Technologies*, *9*(2), 155−160.

Atanacković, M., Petrović, A., Jović, S., Gojkovic-Bukarica, Lj, Bursać, M., & Cvejić, J. (2012). Influence of winemaking techniques on the resveratrol content, total phenolic content and antioxidant potential of red wines. *Food Chemistry*, *131*, 513−518.

Atanacković, M., Poša, M., Heinle, H., Gojković-Bukarica, L. J., & Cvejić, J. (2009). Solubilization of resveratrol in micellar solutions of different bile acids. *Colloids and Surfaces B: Biointerfaces*, *72*, 148−154.

Awasthi, R., Kulkarni, G. T., & Pawar, V. K. (2011). Phytosomes: An approach to increase the bioavailability of plant extracts. *International Journal of Pharmacy and Pharmaceutical Sciences*, *3*(2), 1−3.

Ayala-Zavala, J. F., Vega-Vega, V., Rosas-Domínguez, C., Palafox-Carlos, H., Villa-Rodriguez, J. A., Siddiqui, M. W., … González-Aguilar, G. A. (2011). Agro-industrial potential of exotic fruit byproducts as a source of food additives. *Food Research International*, *44*(7), 1866−1874.

Ayed, N., Yu, H. L., & Lacroix, M. (1999). Improvement of anthocyanin yield and shelf-life extension of grape pomace by gamma irradiation. *Food Research International*, *32*(8), 539−543.

Bae, E. A., Han, M. J., & Kim, D. H. (1999). In vitro anti-*Helicobacter pylori* activity of some flavonoids and their metabolites. *Planta Medica*, *65*(5), 442−443.

Bakowska-Barczak, A. (2005). Acylated anthocyanins as stable, natural food colorants—A review. *Polish Journal of Food and Nutrition Sciences*, *14*(2), 107−116.

Bancirova, M. (2010). Comparison of the antioxidant capacity and the antimicrobial activity of black and green tea. *Food Research International*, *43*, 1379−1382.

Barba, F. J., Galanakis, C. M., Esteve, M. J., Frigola, A., & Vorobiev, E. (2015). Potential use of pulsed electric technologies and ultrasounds to improve the recovery of high-added value compounds from blackberries. *Journal of Food Engineering*, *167*, 38–44.

Beckman, C. H. (2000). Phenolic-storing cells: Keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? *Physiological and Molecular Plant Pathology*, *57*, 101.

Bhattacharya, S., & Ghosh, A. (2008). Phytosomes: The emerging technology for enhancement of bioavailability of botanicals and nutraceuticals. *The Internet Journal of Aesthetic and Antiaging Medicine*, *2*(S1), 141–153.

Bintsis, T., Litopoulou-Tzanetaki, E., & Robinson, R. K. (2000). Existing and potential applications of ultraviolet light in the food industry—A critical review. *Journal of the Science of Food and Agriculture*, *80*(6), 637–645.

Bogdanski, P., Suliburska, J., Szulinska, M., Stepien, M., Pupek-Musialik, D., & Jablecka, A. (2012). Green tea extract reduces blood pressure inflammatory biomarkers and oxidative stress and improves parameters associated with insulin resistance in obese hypertensive patients. *Nutrition Research*, *32*, 421–427.

Bohn, T. (2014). Dietary factors affecting polyphenol bioavailability. *Nutrition Reviews*, *72*(7), 429–452.

Bonifácio, B. V., Silva, P. B. D., Ramos, M. A. D. S., Negri, K. M. S., Bauab, T. M., & Chorilli, M. (2014). Nanotechnology-based drug delivery systems and herbal medicines: A review. *International Journal of Nanomedicine*, *9*, 1–15.

Boukroufa, M., Boutekedjiret, C., Petigny, L., Rakotomanomana, N., & Chemat, F. (2015). Bio-refinery of orange peels waste: A new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. *Ultrasonics Sonochemistry*, *24*, 72–79.

Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American Journal of Enology and Viticulture*, *52*(2), 67–87.

Bourne, L. C., & Rice-Evans, C. (1998). Bioavailability of ferulic acid. *Biochemical and Biophysical Research Communications*, *253*, 222–227.

Boussetta, N., Lanoisellé, J. L., Bedel-Cloutour, C., & Vorobiev, E. (2009). Extraction of soluble matter from grape pomace by high voltage electrical discharges for polyphenol recovery: Effect of sulphur dioxide and thermal treatments. *Journal of Food Engineering*, *95*, 192–198.

Bowey, E., Adlercreutz, H., & Rowland, I. (2003). Metabolism of isoflavones and lignans by the gut microflora: A study in germ-free and human flora associated rats. *Food and Chemical Toxicology*, *41*, 631–636.

Bravo, L. (1998). Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, *56*(11), 317–333.

Brown, J. E., Khodr, H., Hider, R. C., & Rice-Evans, C. A. (1998). Structural dependence of flavonoid interactions with Cu^{2+} ions: Implications for their antioxidant properties. *Biochemistry Journal*, *330*, 1173–1178.

Bugianesi, R., Catasta, G., Spigno, P., D'uva, A., & Maiani, G. (2002). Naringenin from cooked tomato paste is bioavailable in men. *Journal of Nutrition*, *132*, 3349–3352.

Burgain, C., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering*, *104*, 467–483.

Buzrul, S. (2012). High hydrostatic pressure treatment of beer and wine: A review. *Innovative Food Science & Emerging Technologies*, *13*, 1–12.

Çam, M., & Hisil, Y. (2010). Pressurised water extraction of polyphenols from pome-granate peels. *Food Chemistry*, *123*, 878–885.

Cardona, F., Andres-Lacueva, C., Tulipani, S., Tinahones, F. J., & Queipo-Ortuno, M. I. (2013). Benefits of polyphenols on gut microbiota and implications in human health. *The Journal of Nutritional Biochemistry*, *24*, 1415–1422.

Cartron, E., Fouret, G., Carbonneau, M. A., Lauret, C., Michel, F., Monnier, L., . . . Leger, C. L. (2003). Red-wine beneficial longterm effect on lipids but not on antioxidant characteristics in plasma in a study comparing three types of wine: Description of two O-methylated derivatives of gallic acid in humans. *Free Radical Research*, *37*(9), 1021–1035.

Casas, L., Mantell, C., Rodríguez, M., Ossa, E. J. M. D. L., Roldán, A., Ory, I. D., et al. (2010). Extraction of resveratrol from the pomace of Palomino fino grapes by supercritical carbon dioxide. *Journal of Food Engineering*, *96*, 304–308.

Chang, Y.-C., Yang, M.-Y., Chen, S.-C., & Wang, C. J. (2016). Mulberry leaf polyphenol extract improves obesity by inducing adipocyte apoptosis and inhibiting preadipocyte differentiation and hepatic lipogenesis. *Journal of Functional Foods*, *21*, 249–262.

Chauhan, N. S., Gowtham, R., & Gopalakrishna, B. (2009). Phytosomes: A potential phyto-phospholipid carriers for herbal drug delivery. *Journal of Pharmaceutical Research*, *2*(S7), 1267–1270.

Chemat, F., Grondin, I., Shum Cheong Sing, A., & Smadja, J. (2004). Deterioration of edible oils during food processing by ultrasound. *Ultrasonics Sonochemistry*, *11*(1), 13–15.

Chen, B.-H., Hung, M.-H., Chen, J. Y.-F., Chang, H.-W., Yu, M.-L., Wan, L., et al. (2012). Anti-allergic activity of grapeseed extract (GSE) on RBL-2H3 mast cells. *Food Chemistry*, *132*(2), 968–974.

Chen, B.-T., Li, W.-X., He, R.-R., Li, Y.-F., Tsoi, B., Zhai, Y.-J., & Kurihara, H. (2012). Anti-inflammatory effects of a polyphenols-rich extract from tea (*Camellia sinensis*) flowers in acute and chronic mice models. *Oxidative Medicine and Cellular Longevity*, 7, Article ID 537923.

Chen, L., Remondetto, G. E., & Subirade, M. (2006). Food protein-based materials as nutraceutical delivery systems. *Trends in Food Science & Technology*, *17*, 272–283.

Cheynier, V., Dueñas-Paton, M., Salas, E., Maury, C., Souquet, J.-M., Sarni-Manchado, P., & Fulcrand, H. (2006). Structure and properties of wine pigments and tannins. *American Journal of Enology and Viticulture*, *57*(3), 298–305.

Choi, A.-J., Kim, C.-J., Cho, Y.-J., Hwang, J.-K., & Kim, C.-T. (2011). Characterization of capsaicin-loaded nanoemulsions stabilized with alginate and chitosan by self-assembly. *Food and Bioprocess Technology*, *4*(6), 1119–1126.

Chow, H. H. S., Cai, Y., Alberts, D. S., Hakim, I., Dorr, R., Shahi, F., et al. (2001). Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. *Cancer Epidemiology Biomarkers and Prevention*, *10*, 53–58.

Chun, O. K., Kim, D.-O., Smith, N. L., Schroeder, D., Han, J. T., & Lee, C. Y. (2005). Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of the Science of Food and Agriculture*, *85*, 1715–1724.

Citernesi, U., & Sciacchitano, M. (1995). Phospholipids/active ingredient complexes. *Cosmetics & Toiletries*, *110*(11), 57–68.

Clavel, T., Henderson, G., Alpert, C. A., Philippe, C., Rigottier-Gois, L., Dore, J., et al. (2005). Intestinal bacterial communities that produce active estrogen-like compounds enterodiol and enterolactone in humans. *Applied and Environmental Microbiology*, *71*, 6077–6085.

Cliff, M. A., Stanich, K., Edwards, J. E., & Saucier, C. T. (2012). Adding grape seed extract to wine affects astringency and other sensory attributes. *Journal of Food Quality*, *35*(4), 263–271.

Clifford, M. N. (2000). Chlorogenic acids and other cinnamates: Nature, occurrence, dietary burden, absorption and metabolism. *Journal of the Science of Food and Agriculture*, *80*, 1033–1043.

Clifford, M. N., & Scalbert, A. (2000). Ellagtannins—Nature, occurrence and dietary burden. *Journal of the Science of Food and Agricuture*, *80*, 1118–1125.

Condelli, N., Dinnella, C., Cerone, A., Monteleone, E., & Bertuccioli, M. (2006). Prediction of perceived astringency induced by phenolic compounds II: Criteria for panel selection and preliminary application on wine samples. *Food Quality and Preference*, *17*(1–2), 96–107.

Corbo, M. R., Bevilacqua, A., Petruzzi, L., Casanova, F. P., & Sinigaglia, M. (2014). Functional beverages: The emerging side of functional foods. *Comprehensive Reviews in Food Science and Food Safety*, *13*(6), 1192–1206.

Corey, M. (2009). *Developing functional food products through novel processing, ingredient, and shelf stability evaluation (PhD thesis)*. Athens, GA: Graduate Faculty of The University of Georgia.

Corrales, M., Toepfl, S., Butz, P., Knorr, D., & Tauscher, B. (2008). Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A comparison. *Innovative Food Science & Emerging Technologies*, *9*(1), 85–91.

Curcio, M., Cirillo, G., Parisi, O. I., Iemma, F., Picci, N., & Puoci, F. (2012). Quercetin-imprinted nanospheres as novel drug delivery devices. *Journal of Functional Biomaterials*, *3*, 269–282.

Cvejić, J., & Atanacković, M. (2015). *Effect of wine production techniques on wine resveratrol and total phenolics. Processing and impact on active components in food, part 8: Beverages, Chapter 60* (pp. 501–508). London: Academic Press, Elsevier.

Cvejić, J., Bursać, M., & Atanackovic, M. (2012). Phytoestrogens: "Estrogene-like" phytochemicals. In Atta-ur-Rahmann (Ed.), *Studies in natural products chemistry, bioactive natural products, Vol. 38, Chapter 1* (pp. 1–35). Oxford: Elsevier.

Cvejić, J., Poša, M., Sebenji, A., & Atanacković, M. (2014). Comparison of solubilization capacity of resveratrol in sodium 3α,12α-dihydroxy-7-oxo-5β-cholanoate and sodium dodecyl sulfate. *The Scientific World Journal*, Article ID 265953, 7 pages.

Cvejić, J., Puškaš, V., Miljić, U., Torović, L. J., & Rakić, D. (2016). Varietal phenolic composition of Probus, Rumenika and Frankovka red wines from Fruška Gora (Serbia) and changes in main compounds during maceration. *European Food Research and Technology*, <http://dx.doi.org/10.1007/s00217-016-2635-y>, 11 pages.

Cvejić, J., Tepavčević, V., Bursać, M., Miladinović, J., & Malenčić, D. J. (2011). Isoflavone composition in F1 soybean progenie. *Food Research International*, *44*, 2698–2702.

Dahmoune, F., Nayak, B., Moussi, K., Remini, H., & Madani, K. (2015). Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. *Food Chemistry*, *166*, 585–595.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules (Basel, Switzerland)*, *15*, 7313–7352.

Damdimopoulou, P., Nurmi, T., Salminen, A., Damdimopoulos, A. E., Kotka, M., & Van Der Saag, P. (2011). A single dose of enterolactone activates estrogen signaling and regulates expression of circadian clock genes in mice. *Journal of Nutrition*, *141*, 1583–1589.

D'archivio, M., Filesi, C., Di Benedetto, R., Gargiulo, R., Giovannini, C., & Masella, R. (2007). Polyphenols, dietary sources and bioavailability. *Annali dell'Istituto Superiore di Sanità*, *43*(4), 348–361.

Day, A. J., Bao, Y., Morgan, M. R. A., & Williamson, G. (2000). Conjugation position of quercetin glucuronides and effect on biological activity. *Free Radical Biology and Medicine*, *29*, 1234–1243.

Day, A. J., Canada, F. J., Diaz, J. C., Kroon, P. A., Mclauchlan, R., Faulds, C. B., et al. (2000). Dietary flavonoid and isoflavone glucosides are hydrolyzed by the lactase site of lactase phlorizin hydrolase. *FEBS Letters*, *468*, 166–170.

Day, A. J., Dupont, M. S., Ridley, S., Rhodes, M., Rhodes, M. J., Morgan, M. R., & Williamson, G. (1998). Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. *FEBS Letters*, *436*, 71–75.

Day, A. J., Mellon, F. A., Barron, D., Sarrrazin, G., Morgan, M. R. A., & Williamson, G. (2001). Human metabolism of dietary flavonoids: Identification of plasma metabolites of quercetin. *Free Radical Research*, *212*, 941–952.

Deeth, H. C., Datta, N., Ross, A. I. V., & Dam, X. T. (2007). *Pulsed electric field technology: Effect on milk and fruit juices*. *Advances in Thermal and Non-Thermal Food Preservation*. Ames, IA: Blackwell Publishing.

De Graaf, C. (2007). 2-Sensory influences on food choice and food intake. In L. Frewer, & H. V. Trijp (Eds.), *Understanding consumers of food products*. Cambridge: Woodhead Publishing.

Deladino, L., Anbinder, P. S., Navarro, A. S., & Martino, M. N. (2008). Encapsulation of natural antioxidants extracted from Ilex paraguariensis. *Carbohydrate Polymers*, *71*, 126–134.

Delgado-Vargas, F., Jiménez, A. R., & Paredes-López, O. (2000). Natural pigments: Carotenoids, anthocyanins, and betalains—Characteristics, biosynthesis, processing, and stability. *Critical Reviews in Food Science and Nutrition*, *40*(3), 173–289.

Delgado-Vargas, F., & Paredes-López, O. (2002a). *Anthocyanins and betalains*. *Natural Colorants for Food and Nutraceutical Uses*. Boca Raton, FL: CRC Press.

Delgado-Vargas, F., & Paredes-López, O. (2002b). *Colorants*. *Natural Colorants for Food and Nutraceutical Uses*. Boca Raton, FL: CRC Press.

Delgado-Vargas, F., & Paredes-López, O. (2002c). *Pigments*. *Natural Colorants for Food and Nutraceutical Uses*. Boca Raton, FL: CRC Press.

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly) phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants and Redox Signaling*, *18*(14), 1818–1892.

Delsart, C., Cholet, C., Ghidossi, R., Grimi, N., Gontier, E., Gény, L., et al. (2014). Effects of pulsed electric fields on cabernet sauvignon grape berries and on the characteristics of wines. *Food and Bioprocess Technology*, *7*(2), 424–436.

Déprez, S., Brézillon, C., Rabot, S., Philippe, C., Mila, I., Lapierre, C., & Scalbert, A. (2000). Polymeric proanthocyanidins are catabolized by a human colonic microflora into low molecular weight phenolic acids. *Journal of Nutrition*, *130*, 2733–2738.

Desai, K. G. H., & Park, H. J. (2005). Recent developments in microencapsulation of food ingredients. *Drying Technology*, *23*(7), 1361–1394.

De Souza, J. E., Casanova, L. M., & Costa, S. S. (2015). Bioavailability of phenolic compounds: A major challenge for drug development? *Revista Fitos, Rio de Janeiro*, *9*(1), 1–72.

Dias, K., Nikolaou, S., & Giovani, W. F. (2011). The in vitro antioxidant properties of the Al-quercetin/βCD and Al-catechin/βCD inclusion compounds, rationalized in terms of their electrochemical behaviour. *Medicinal Chemistry Research*, *21*(10), 2920–2925.

Dinesh Kumar, V., Prasad Verma, P. R., & Kumar Singh, S. (2015). Development and evaluation of biodegradable polymeric nanoparticles for the effective delivery of quercetin using a quality by design approach. *LWT - Food Science and Technology*, *61*, 330–338.

Di Renzo, L., Rizzo, M., Sarlo, F., Colica, C., Iacopino, L., Domino, E., et al. (2013). Effects of dark chocolate in a population of normal weight obese women: A pilot study. *European Review for Medical and Pharmacological Sciences*, *17*, 2257–2266.

Donovan, J. L., Bell, J. R., Kasim-Karakas, S., German, J. B., Walzem, R. L., Hansen, R. J., & Waterhouse, A. L. (1999). Catechin is present as metabolites in human plasma after consumption of red wine. *Journal of Nutrition*, *129*, 1662–1668.

Donovan, J. L., Crespy, V., Manach, C., Morand, C., Besson, C., Scalbert, A., & Remesy, C. (2001). Catechin is metabolized by both the small intestine and liver of rats. *Journal of Nutrition*, *131*(6), 1753–1757.

Donsì, F., Ferrari, G., Fruilo, M., & Pataro, G. (2011). Pulsed electric fields-assisted vinification. *Procedia Food Science*, *1*, 780–785.

Donsi, F., Ferrari, G., & Pataro, G. (2010). Applications of pulsed electric field treatments for the enhancement of mass transfer from vegetable tissue. *Food Engineering Reviews*, *2*(2), 109–130.

Downham, A., & Collins, P. (2000). Colouring our foods in the last and next millennium. *International Journal of Food Science & Technology*, *35*(1), 5–22.

Drewnowski, A., & Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: A review. *The American Journal of Clinical Nutrition*, *72*(6), 1424–1435.

Drosou, C., Kyriakopoulou, K., Bimpilas, A., Tsimogiannis, D., & Krokida, M. (2015). A comparative study on different extraction techniques to recover red grape pomace polyphenols from vinification byproducts. *Industrial Crops and Products*, *75*, 141–149.

Duba, K. S., Casazza, A. A., Mohamed, H. B., Perego, P., & Fiori, L. (2015). Extraction of polyphenols from grape skins and defatted grape seeds using subcritical water: Experiments and modeling. *Food and Bioproducts Processing*, *94*, 29–38.

Duda-Chodak, A. (2012). The inhibitory effect of polyphenols on human gut microbiota. *Journal of Physiology and Pharmacology*, *63*(5), 497–503.

Duda-Chodak, A., Tarko, T., Satora, P., & Sroka, P. (2015). Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: A review. *European Journal of Nutrition*, *54*, 325–341.

Dufour, C., & Bayonove, C. L. (1999). Interactions between wine polyphenols and aroma substances. An insight at the molecular level. *Journal of Agricultural and Food Chemistry*, *47*(2), 678–684.

Duodu, K. G. (2011). *Effects of processing on antioxidant phenolics of cereal and legume grains*. *Advances in cereal science: Implications to food processing and health promotion*. Washington, DC: American Chemical Society.

Dyrby, M., Westergaard, N., & Stapelfeldt, H. (2001). Light and heat sensitivity of red cabbage extract in soft drink model systems. *Food Chemistry*, *72*(4), 431–437.

El Gharras, H. (2009). Polyphenols: Food sources, properties and applications—A review. *International Journal of Food Science & Technology*, *44*(12), 2512–2518.

Engelmann, M. D., Hutcheson, R., & Cheng, I. F. (2005). Stability of ferric complexes with 3-hydroxyflavone (flavonol), 5,7-dihydroxyflavone (Chrysin), and 3',4'-dixydroxyflavone. *Journal of Agricultural and Food Chemistry*, *53*, 2953–2960.

Erlund, I., Kosonen, T., Alfthan, G., Perttunen, M. K., Kenraali, J., Parantainen, J., & Aro, A. (2000). Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. *European Journal of Clinical Pharmacology*, *56*, 545–553.

Eskilsson, C. S., Hartonen, K., Mathiasson, L., & Riekkola, M. L. (2004). Pressurized hot water extraction of insecticides from process dust—Comparison with supercritical fluid extraction. *Journal of Separation Science*, *27*, 59–64.

Fang, Z., & Bhandaria, B. (2010). Encapsulation of polyphenols—A review. *Trends in Food Science & Technology*, *21*, 510–523.

Farah, A., Monteiro, M., Donangelo, C. M., & Lafay, S. (2008). Chlorogenic acids from green coffee extract are highly bioavailable in humans. *Journal of Nutrition*, *138*(12), 2309–2315.

Felgines, C., Texier, O., Morand, C., Manach, C., Scalbert, A., Regerat, F., et al. (2000). Bioavailability of the flavanone naringenin and its glycosides in rats. *American Journal of Physiology: Gastrointestinal and Liver Physiology*, *279*, 1148–1154.

Ferrari, G., Maresca, P., & Ciccarone, R. (2011). The effects of high hydrostatic pressure on the polyphenols and anthocyanins in red fruit products. *Procedia Food Science*, *1*, 847–853.

Ferrer-Gallego, R., Hernández-Hierro, J. M., Rivas-Gonzalo, J. C., & Escribano-Bailón, M. T. (2014). Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: Synergistic effect and modulation by aromas. *Food Research International*, *62*, 1100–1107.

Folch-Cano, C., Guerrero, J., Speisky, H., Jullian, C., & Olea-Azar, C. (2013). NMR and molecular fluorescence spectroscopic study of the structure and thermodynamic parameters of EGCG/β-cyclodextrin inclusion complexes with potential antioxidant activity. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *78*, 287–298.

Fontana, A. R., Antoniolli, A., & Bottini, R. (2013). Grape pomace as a sustainable source of bioactive compounds: Extraction, characterization, and biotechnological applications of phenolics. *Journal of Agricultural and Food Chemistry*, *61*, 8987–9003.

Fontoin, H., Saucier, C., Teissedre, P.-L., & Glories, Y. (2008). Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. *Food Quality and Preference*, *19*(3), 286–291.

Francis, F. J., & Markakis, P. C. (1989). Food colorants: Anthocyanins. *Critical Reviews in Food Science and Nutrition*, *28*(4), 273–314.

Frank, D. N., St Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N., & Pace, N. R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences USA*, *104*, 13780–13785.

Friberg, S., Larsson, K., & Sjoblom, J. (2003). *Food emulsions*. New York: CRC Press.

Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology*, *26*(2), 68–87.

Galanakis, C. M. (2013). Emerging technologies for the production of nutraceuticals from agricultural by-products: A viewpoint of opportunities and challenges. *Food and Bioproducts Processing*, *91*(4), 575–579.

Galanakis, C.M., 2015. The universal recovery. In C. M. Galanakis (Ed.), Food waste recovery: Processing technologies and industrial techniques. Academic Press, ISBN 978-012-80-0351-0.

Galanakis, C.M., Barba, F.J., Prasad, K.N., 2015. Cost and safety issues of emerging technologies against conventional techniques. In C. M. Galanakis (Ed.), Food waste recovery: Processing technologies and industrial techniques. Academic Press, ISBN 978-012-80-0351-0.

Galanakis, C. M., Goulas, V., Tsakona, S., Manganaris, G. A., & Gekas, V. (2013). A knowledge base for the recovery of natural phenols with different solvents. *International Journal of Food Properties*, *16*, 382–396.

Galanakis, C. M., Kotanidis, A., Dianellou, M., & Gekas, V. (2015). Phenolic content and antioxidant capacity of Cypriot Wines. *Czech Journal of Food Sciences*, *33*, 126–136.

Galanakis, C. M., Markouli, E., & Gekas, V. (2013). Fractionation and recovery of different phenolic classes from winery sludge via membrane filtration. *Separation and Purification Technology*, *107*, 245–251.

Galanakis, C. M., Martínez-Saez, N., Del Castillo, M. D., Barba, F. J., & Mitropoulou, V. S. (2015). Chapter 15: Patented and commercialized applications. In C. M. Galanakis (Ed.), *Food waste recovery: processing technologies and industrial techniques*. Oxford: Elsevier-Academic Press.

Galanakis, C. M., & Schieber, A. (2014). Editorial. Special issue on recovery and utilization of valuable compounds from food processing by-products. *Food Research International*, *65*, 299–300.

Gallo, M., Ferracane, R., Graziani, G., Ritieni, A., & Fogliano, V. (2010). Microwave assisted extraction of phenolic compounds from four different spices. *Molecules (Basel, Switzerland)*, *15*(9), 6365.

Garcia-Alonso, J., Ros, G., Vidal-Guevara, M. L., & Periago, M. J. (2006). Acute intake of phenolic-rich juice improves antioxidant status in healthy subjects. *Nutrition Research*, *26*, 330–339.

García-Marino, M., Rivas-Gonzalo, J. C., Ibáñez, E., & García-Moreno, C. (2006). Recovery of catechins and proanthocyanidins from winery by-products using subcritical water extraction. *Analytica Chimica Acta*, *563*, 44–50.

García-Pérez, J. V., Rosselló, C., Cárcel, J. A., De La Fuente, S., & Mulet, A. (2006). Effect of air temperature on convective drying assisted by high power ultrasound. *Defect and Diffusion Forum*, *258-260*, 563–574.

Gee, J. M., & Johnson, I. T. (2001). Polyphenolic compounds: Interactions with the gut and implications for human health. *Current Medicinal Chemistry*, *8*, 1245–1255.

Gibbs, B. F., Kermasha, S., Alli, I., & Mulligan, C. N. (1999). Encapsulation in the food industry: A review. *International Journal of Food Science and Nutrition*, *50*, 213–224.

Gibis, M., Vogta, E., & Weiss, J. (2012). Encapsulation of polyphenolic grape seed extract in polymer-coated liposomes. *Food & Function*, *3*, 246–254.

Gill, S. R., Pop, M., Deboy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., et al. (2006). Metagenomic analysis of the human distal gut microbiome. *Science (New York, N.Y.)*, *312*, 1355–1359.

Giusti, M. M., Rodríguez-Saona, L. E., & Wrolstad, R. E. (1999). Molar absorptivity and color characteristics of acylated and non-acylated pelargonidin-based anthocyanins. *Journal of Agricultural and Food Chemistry*, *47*(11), 4631–4637.

Giusti, M. M., & Wrolstad, R. E. (2003). Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal*, *14*(3), 217–225.

Goldner, M. C., Lira, P. D., Van Baren, C., & Bandoni, A. (2011). Influence of polyphenol levels on the perception of aroma in *Vitis vinifera* cv. Malbec wine. *South African Journal of Enology and Viticulture*, *32* (1), 21–27.

González-Aguilar, G. A., Villegas-Ochoa, M. A., Martínez-Téllez, M. A., Gardea, A. A., & Ayala-Zavala, J. F. (2007). Improving antioxidant capacity of fresh-cut mangoes treated with UV-C. *Journal of Food Science*, *72*(3), S197–S202.

Gouin, S. (2004). Microencapsulation: Industrial appraisal of existing technologies and trends. *Trends in Food Science & Technology*, *15*, 330–347.

Grzegorzewski, F., Zietz, M., Rohn, S., Kroh, L.W. & Schlueter, O. (2011) *Modification of polyphenols and cuticular surface lipids of Kale (B. oleracea convar. sabellica) with non-thermal oxygen plasma gaseous species*, The 11th International Congress on Engineering and Food, Athens, Greece.

Gu, L., Kelm, M. A., Hammerstone, J. F., Beecher, G., Holden, J., Haytowitz, D., & Prior, R. L. (2003). Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *Journal of Agricultural and Food Chemistry*, *51*, 7513–7521.

Gültekin-Özgüven, M., Karadag, A., Duman, S., Özkal, B., & Özçelik, B. (2016). Fortification of dark chocolate with spray dried black mulberry (*Morus nigra*) waste extract encapsulated in chitosan-coated liposomes and bioaccessability studies. *Food Chemistry*, *201*, 205–212.

Halliwell, B., Zhao, K., & Whiteman, M. (2000). The gastrointestinal tract: A major site of antioxidant action? *Free Radical Research*, *33*, 819–830.

Hamza, A., & Zhan, C. G. (2006). How can (-)-epigallocatechin gallate from green tea prevent HIV-1 infection? Mechanistic insights from computational modeling and the implication for rational design of antiHIV-1 entry inhibitors. *The Journal of Physical Chemistry B*, *110*(6), 2910–2917.

Harborne, J. B. (1982). *Introduction to ecological biochemistry* (second ed.New York: Academic Press.

Herrero, M., Cifuentes, A., & Ibanez, E. (2006). Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae. A review. *Food Chemistry*, *98*, 136–148.

Hogger, P. (2013). Nutrition-derived bioactive metabolites produced by gut microbiota and their potential impact on human health. *Nutrition and Medicine*, *1*(1), 1.

Hollman, P. C., Van Trijp, J. M., Buysman, M. N., Van Der Gaag, M. S., Mengelers, M. J., De Vries, J. H., & Katan, M. B. (1997). Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Letters*, *418*, 152–156.

Hollman, P. C. H., Devries, J. H. M., Vanleeuwen, S. D., Mengelers, M. J. B., & Katan, M. B. (1995). Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *American Journal of Clinical Nutrition*, *62*, 1276–1282.

Hong, S. J., Kim, S. I., Kwon, S. M., Lee, J. R., & Chung, B. C. (2002). Comparative study of concentration of isoflavones and lignans in plasma and prostatic tissues of normal control and benign prostatic hyperplasia. *Yonsei Medical Journal*, *43*, 236–241.

Hooper, L. V., & Gordon, J. I. (2001). Commensal host-bacterial relationships in the gut. *Science (New York, N.Y.)*, *292*, 1115–1118.

Huang, Q., Yu, H., & Ru, Q. (2010). Bioavailability and delivery of nutraceuticals using nanotechnology. *Journal of Food Science*, *75*(1), R50–R57.

Hwang, C. S., Kwak, H. S., Lim, H. J., Lee, S. H., Kang, Y. S., Choe, T. B., et al. (2006). Isoflavone metabolites and their in vitro dual functions: They can act as an estrogenic agonist or antagonist depending on the estrogen concentration. *Journal of Steroid Biochemistry*, *101*, 246–253.

Ishimoto, H., Shibata, M., Myojin, Y., Ito, H., Sugimoto, Y., Tai, A., et al. (2011). In vivo anti-inflammatory and antioxidant properties of ellagitannin metabolite urolithin A. *Bioorganic & Medicinal Chemistry Letters*, *21*, 5901–5904.

Izumi, T., Piskula, M. K., Osawa, S., Obata, A., Tobe, K., Saito, M., et al. (2000). Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *Journal of Nutrition*, *130*, 1695–1699.

Jaeger, S. R., Axten, L. G., Wohlers, M. W., & Sun-Waterhouse, D. (2009). Polyphenol-rich beverages: Insights from sensory and consumer science. *Journal of the Science of Food and Agriculture*, *89*(14), 2356–2363.

Jefremov, V., Zilmer, M., Zilmer, K., Bogdanovic, N., & Karelson, E. (2007). Antioxidative effects of plant polyphenols: From protection of G protein signaling to prevention of age-related pathologies. *Annals of the New York Academy of Sciences*, *1095*, 449–457.

Jin, S., Zhang, Q. Y., Kang, X. M., Wang, J. X., & Zhao, W. H. (2010). Daidzein induces MCF-7 breast cancer cell apoptosis via the mitochondrial pathway. *Annals of Oncology*, *21*(2), 263–268.

Ju, Z. Y., & Howard, L. R. (2005). Subcritical water and sulfured water extraction of anthocyanins and other phenolics from dried red grape skin. *Journal of Food Science*, *70*, 270–276.

Kalogeropoulos, N., Yannakopoulou, K., Gioxari, A., Chiou, A., & Makris, D. P. (2010). Polyphenol characterization and encapsulation in β-cyclodextrin of a flavonoid-rich *Hypericum perforatum* (St John's wort) extract. *LWT - Food Science and Technology*, *43*, 882–889.

Katsampa, P., Valsamedou, E., Grigorakis, S., & Makris, D. P. (2015). A green ultrasound-assisted extraction process for the recovery of antioxidant polyphenols and pigments from onion solid wastes using Box–Behnken experimental design and kinetics. *Industrial Crops and Products*, *77*, 535–543.

Khan, N., Monagas, M., Andres-Lacueva, C., Casas, R., Urpi-Sarda, M., Lamuela-Raventos, R. M., & Estruch, R. (2012). Regular consumption of cocoa powder with milk increases HDL cholesterol and reduces oxidized LDL levels in subjects at high-risk of cardiovascular disease. *Nutrition, Metabolism and Cardiovascular Diseases*, *22*, 1046–1053.

Kishida, T., Ataki, H., Takebe, M., & Ebihara, K. (2000). Soybean meal fermented by *Aspergillus awamori* increases the cytochrome P-450 content of the liver microsomes of mice. *Journal of Agricultural and Food Chemistry*, *48*, 1367–1372.

Klaus, S., Pultz, S., Thone-Reineke, C., & Wolfram, S. (2005). Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. *International Journal of Obesity*, *29*(6), 615–623.

Ko, M. J., Cheigh, C. I., Cho, S. W., & Chung, M. S. (2011). Subcritical water extraction of flavonol quercetin from onion skin. *Journal of Food Engineering*, *102*, 327–333.

Ko, M. J., Cheigh, C. I., & Chung, M. S. (2014). Relationship analysis between flavonoids structure and subcritical water extraction (SWE). *Food Chemistry*, *143*, 147–155.

Kohen, R., & Nyska, A. (2002). Oxidation of biological systems: Oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicologic Pathology*, *30*(6), 620–650.

Kopin, I. J. (1985). Catecholamine metabolism: Basic aspects and clinical significance. *Pharmacological Reviews*, *37*, 333–364.

Krikorian, R., Shidler, M. D., Nash, T. A., Kalt, W., Vinqvist-Tymchuk, M. R., Shukitt-Hale, B., & Joseph, J. A. (2010). Blueberry supplementation improves memory in older adults. *Journal of Agricultural and Food Chemistry*, *58*, 3996−4000.

Kuck, L. S., & Norena, C. P. Z. (2016). Microencapsulation of grape (*Vitis labrusca* var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents. *Food Chemistry*, *194*, 569−576.

Kuhnau, J. (1976). The flavonoids. A class of semi-essential food components: Their role in human nutrition. *World Review of Nutrition and Dietetics*, *24*, 117−191.

Laaksonen, O., Sandell, M., Nordlund, E., Heiniö, R.-L., Malinen, H.-L., Jaakkola, M., & Kallio, H. (2012). The effect of enzymatic treatment on blackcurrant (*Ribes nigrum*) juice flavour and its stability. *Food Chemistry*, *130*(1), 31−41.

Laine, P., Kylli, P., Heinonen, M., & Jouppila, K. (2008). Storage stability of microencapsulated cloudberry (*Rubus chamaemorus*) phenolics. *Journal of Agricultural and Food Chemistry*, *56*(23), 11251−11261.

Larrosa, M., Gonzalez-Sarrias, A., Garcia Conesa, M. T., Tomas-Barberan, F. A., & Espin, J. C. (2006). Urolithins, ellagic acid-derived metabolites produced by human colonic microflora, exhibit estrogenic and antiestrogenic activities. *Journal of Agricultural and Food Chemistry*, *54*, 1611−1620.

Larrosa, M., Yáñez-Gascón, M. J., Selma, M. A., González-Sarrías, A., Toti, S., Cerón, J. J., et al. (2009). Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model. *Journal of Agricultural and Food Chemistry*, *57*, 2211−2220.

Lee, M. J., Maliakal, P., Chen, L., Meng, X., Bondoc, F. Y., Prabhu, S., et al. (2002). Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: Formation of different metabolites and individual variability. *Cancer Epidemiology Biomarkers and Prevention*, *11*, 1025−1032.

Lesschaeve, I., & Noble, A. C. (2005). Polyphenols: Factors influencing their sensory properties and their effects on food and beverage preferences. *The American Journal of Clinical Nutrition*, *81*(1), 330S−335S.

Lewandowska, U., Szewczyk, K., Hrabec, E., Janecka, A., & Gorlach, S. (2013). Overview of metabolism and bioavailability enhancement of polyphenols. *Journal of Agricultural and Food Chemistry*, *61*, 12183−12199.

Li, J., Zu, Y. G., Fu, Y. J., Yang, Y. C., Li, S. M., Li, Z. N., & Wink, M. (2010). Optimization of microwave-assisted extraction of triterpene saponins from defatted residue of yellow horn (*Xanthoceras sorbifolia* Bunge.) kernel and evaluation of its antioxidant activity. *Innovative Food Science & Emerging Technologies*, *11*, 637−643.

Li, Z., Jiang, H., Xu, C., & Gu, L. (2015). A review: Using nanoparticles to enhance absorption and bioavailability of phenolic phytochemicals. *Food Hydrocolloids*, *43*, 153−164.

Liang, T., Zhang, X., Xue, W., Zhao, S., Zhang, X., & Pei, J. (2014). Curcumin induced human gastric cancer BGC-823 s apoptosis by ROS-mediated ASK1-MKK4-JNK stress signaling pathway. *International Journal of Molecular Science*, *15*, 15754−15765.

Lindahl, G., Saarinen, N., Abrahamsson, A., & Dabrosin, C. (2011). Tamoxifen, flaxseed, and the lignan enterolactone increase stroma- and cancer cell-derived IL-1Ra and decrease tumor angiogenesis in estrogen-dependent breast cancer. *Cancer Research*, *71*, 51−60.

Liu, D., Vorobiev, E., Savoire, R., & Lanoisellé, J. L. (2011). Intensification of polyphenols extraction from grape seeds by high voltage electrical discharges and extract concentration by dead-end ultrafiltration. *Separation and Purification Technology*, *81*, 134−140.

Liu, S., Lu, H., Zhao, Q., He, Y., Niu, J., Debnath, A. K., et al. (2005). Theaflavin derivatives in black tea and catechin derivatives in green tea inhibit HIV-1 entry by targeting gp41. *Biochimica et Biophysica Acta*, *1723*(1−3), 270−281.

Liu, W., Chen, X. D., Cheng, Z., & Selomulya, C. (2016). On enhancing the solubility of curcumin by microencapsulation in whey protein isolate via spray drying. *Journal of Food Engineering*, *169*, 189−195.

Loftsson, T., & Duchêne, D. (2007). Cyclodextrins and their pharmaceutical applications. *International Journal of Pharmaceutics*, *329*(1/2), 1−11.

Loypimai, P., Moonggarm, A., & Chottanom, P. (2009). Effects of ohmic heating on lipase activity, bioactive compounds and antioxidant activity of rice bran. *Australian Journal of Basic and Applied Sciences*, *3*(4), 3642−3652.

Lu, W., Kelly, A. L., & Miao, S. (2016). Emulsion-based encapsulation and delivery systems for polyphenols. *Trends in Food Science & Technology*, *47*, 1−9.

Lu, X., Ji, C., Xu, H., Li, X., Ding, H., Ye, M., et al. (2009). Resveratrol-loaded polymeric micelles protect cells from Aβ-induced oxidative stress. *International Journal of Pharmaceutics*, *375*(1−2), 89−96.

Luengo, E., Álvarez, I., & Raso, J. (2013). Improving the pressing extraction of polyphenols of orange peel by pulsed electric fields. *Innovative Food Science and Emerging Technologies*, *17*, 79−84.

Maffei, F. R., Carini, M., Aldini, G., Bombardelli, E., Morazzoni, P., & Morelli, R. (1994). Free radicals scavenging action and anti-enzyme activities of procyanidins from Vitis vinifera. A mechanism for their capillary protective action. *Arzneimittel-Forschung*, *44*, 592−601.

Maiti, K., Mukherjee, K., Gantait, A., Saha, B. P., & Mukherjee, P. K. (2006). Enhanced therapeutic potential of naringenin phospholipid complex in rats. *Journal of Pharmaceutical Pharmacology*, *58*(9), 1227−1233.

Maiti, K., Mukherjee, K., Gantait, A., Saha, B. P., & Mukherjee, P. K. (2007). Curcumin-phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats. *International Journal of Pharmacy*, *330*(1−2), 155−163.

Malenčić, D. J., Cvejic, J., Tepavčević, V., Bursać, M., Kiprovski, B., & Rajkovic, M. (2013). Changes in L-phenylalanine ammonia-lyase activity and isoflavone phytoalexins accumulation in soybean seedlings infected with *Sclerotinia sclerotiorum*. *Central European Journal of Biology*, *8*(9), 921−929.

Manach, C., Morand, C., Gil-Izquierdo, A., Bouteloup-Demange, C., & Remesy, C. (2003). Bioavailability in humans of the flavanones hesperidin and narirutin after the ingestion of two doses of orange juice. *European Journal of Clinical Nutrition*, *57*, 235−242.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition*, *79*, 727−747.

Manach, C., Williamson, G., Morand, C., Scalbert, A., & Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition*, *81* (1), 230−242.

Mapari, S. A. S., Thrane, U., & Meyer, A. S. (2010). Fungal polyketide azaphilone pigments as future natural food colorants? *Trends in Biotechnology*, *28*(6), 300−307.

Marra, F., Lyng, J., Romano, V., & Mckenna, B. (2007). Radio-frequency heating of foodstuff: Solution and validation of a mathematical model. *Journal of Food Engineering*, *79*(3), 998−1006.

Maruo, T., Sakamoto, M., Ito, C., Toda, T., & Benno, Y. (2008). *Adlercreutzia equolifaciens* gen. nov., sp. nov., an equol-producing bacterium isolated from human faeces, and emended description of the genus *Eggerthella*. *International Journal of Systematic and Evolutionary Microbiology*, *58*, 1221−1227.

Mason, T. J., Paniwnyk, L., & Lorimer, J. P. (1996). The uses of ultrasound in food technology. *Ultrasonics Sonochemistry*, *3*, 253−260.

Massot-Cladera, M., Pérez-Berezo, T., Franch, A., Castell, M., & Pérez-Cano, F. J. (2012). Cocoa modulatory effect on rat faecal microbiota and colonic crosstalk. *Archives of Biochemistry and Biophysics*, *527*(2), 105−112.

Matsuki, T., Watanabe, K., Fujimoto, J., Takada, T., & Tanaka, R. (2004). Use of 16S rRNA gene-targeted group-specific primers for Real-Time PCR analysis of predominant bacteria in human feces. *Applied and Environmental Microbiology*, *70*(12), 7220−7228.

Matthies, A., Blaut, M., & Braune, A. (2009). Isolation of a human intestinal bacterium capable of daidzein and genistein conversion. *Applied and Environmental Microbiology*, *75*(6), 1740−1744.

Matuschek, M. C., Hendriks, W. H., Mcghie, T. K., & Reynolds, G. W. (2006). The jejunum is the main site of absorption for anthocyanins in mice. *Journal of Nutritional Biochemistry*, *17*, 31–36.

Maubach, J., Bracke, M. E., Heyerick, A., Depypere, H. T., Serreyn, R. F., Mareel, M. M., & De Keukeleire, D. (2003). Quantitation of soy-derived phytoestrogens in human breast tissue and biological fluids by high performance liquid chromatography. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *784*, 137–144.

Mcghie, T. K., Ainge, G. D., Barnett, L. E., Cooney, J. M., & Jensen, D. J. (2003). Anthocyanin glycosides from berry fruit are absorbed and excreted unmetabolized by both humans and rats. *Journal of Agricultural and Food Chemistry*, *51*, 4539–4548.

Meng, X., Sang, S., Zhu, N., Lu, H., Sheng, S., Lee, M., … Yang, C. S. (2002). Identification and characterization of methylated and ring-fission metabolites of tea catechins formed in humans, mice, and rats. *Chemical Research in Toxicology*, *15*, 1042–1050.

Milbury, P. E., Graf, B., Curran-Celentano, J. M., & Blumberg, J. B. (2007). Bilberry (*Vaccinium myrtillus*) anthocyanins modulate heme oxygenase-1 and glutathione S-transferase-pi expression in ARPE-19 cells. *Investigative Ophthalmology & Visual Science*, *48*(5), 2343–2349.

Miljić, U., Puškaš, V., Vučurović, V., & Razmovski, R. (2014). Acceptability of wine produced with an increased content of grape seeds and stems as a functional food. *Journal of the Institute of Brewing*, *120* (2), 149–154.

Miura, D., Saarinen, N. M., Miura, Y., Santti, R., & Yagasaki, K. (2007). Hydroxymatairesinol and its mammalian metabolite enterolactone reduce the growth and metastasis of subcutaneous AH109A hepatomas in rats. *Nutrition and Cancer*, *58*, 49–59.

Molan, A. L., Liu, Z., & Kruger, M. (2011). The ability of blackcurrant extracts to positively modulate key markers of gastrointestinal function in rats. *World Journal of Microbiology and Biotechnology*, *26*, 1735–1743.

Moreno-Luna, R., Munoz-Hernandez, R., Miranda, M. I., Costa, A. F., Jimenez-Jimenez, L., Vallejo-Vaz, A. J., et al. (2012). Olive oil polyphenols decrease blood pressure and improve endothelial function in young women with mild hypertension. *American Journal of Hypertension*, *25*, 1299–1304.

Mortensen, A. (2006). Carotenoids and other pigments as natural colorants. *Pure and Applied Chemistry*, *78* (8), 1477–1491.

Moussa-Ayoub, T. E., Jaeger, H., Knorr, D., El-Samahy, S., Rohn, S., & Kroh, L. W. (2011). Impact of traditional and innovative technologies on some characteristics and bioactive compounds of *Opuntia macrorhiza* juice. *Procedia Food Science*, *1*, 1410–1416.

Mujumdar, A. S., & Law, C. L. (2010). Drying technology: Trends and applications in postharvest processing. *Food and Bioprocess Technology*, *3*(6), 843–852.

Munin, A., & Edwards-Lewy, F. (2011). Encapsulation of natural polyphenolic compounds; a review. *Pharmaceutics*, *3*(4), 330–347.

Murray, D. *Phytosomes—Increase the absorption of herbal extract*. [online] Available at: <http://doctormurray.com/phytosomes-herbal-support-increase-the-absortion-of-herbal-extracts/> Accessed 01.02.16.

Muthyala, R. S., Ju, Y. H., Sheng, S., Williams, L. D., Doerge, D. R., Katzenellenbogen, B. S., et al. (2004). Equol, a natural estrogenic metabolite from soy isoflavones: Convenient preparation and resolution of R- and S-equols and their differing binding and biological activity through estrogen receptors alpha and beta. *Bioorganic & Medicinal Chemistry*, *12*, 1559–1567.

Naik, S. R. (2009). Hepatoprotective effect of ginkgoselect phytosome in rifampicin induced liver injury in rats: Evidence of antioxidant activity. *Fitoterapia*, *6*, 439–445.

Nakayama, M., Shigemune, N., Tsugukuni, T., Jun, H., Matsushita, T., Mekada, Y., et al. (2012). Mechanism of the combined anti-bacterial effect of green tea extract and NaCl against *Staphylococcus aureus* and *Escherichia coli* O157:H7. *Food Control*, *25*(1), 225–232.

Nakayama, M., Suzuki, K., Toda, M., Okubo, S., Hara, Y., & Shimamura, T. (1993). Inhibition of the infectivity of influenza virus by tea polyphenols. *Antiviral Research*, *21*(4), 289–299.

Nayak, B., Dahmoune, F., Moussi, K., Remini, H., Dairi, S., Aoun, O., & Khodir, M. (2015). Comparison of microwave, ultrasound and accelerated-assisted solvent extraction for recovery of polyphenols from *Citrus sinensis* peels. *Food Chemistry*, *187*, 507–516.

Nemen, D., & Lemos-Senna, E. (2011). Preparation and characterization of resveratrol-loaded lipid-based nanocarriers for cutaneous administration. *Quimica Nova*, *34*, 408–413.

Nemeth, K., Plumb, G. W., Berrin, J. G., Juge, N., Jacob, R., Naim, H. Y., et al. (2003). Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *European Journal of Nutrition*, *42*, 29–42.

Niemira, B. A. (2012). Cold plasma decontamination of foods. *Annual Review of Food Science and Technology*, *3*(1), 125–142.

Nkhili, E., Tomao, V., Hajji, H. E., Boustani, E. S. E., Chemat, F., & Dangles, O. (2009). Microwave-assisted water extraction of green tea polyphenols. *Phytochemical Analysis*, *20*, 408–415.

Noble, A. C. (1994). Bitterness in wine. *Physiology and Behavior*, *56*(6), 1251–1255.

Noratto, G., Martino, H. S. D., Simbo, S., Byrne, D., & Mertens-Talcott, S. U. (2015). Consumption of polyphenol-rich peach and plum juice prevents risk factors for obesity-related metabolic disorders and cardiovascular disease in Zucker rats. *The Journal of Nutritional Biochemistry*, *26*(6), 633–641.

Nori, M. P., Favaro-Trindade, C. S., Matias De Alencar, S., Thomazini, M., De Camargo Balieiro, J., & Contreras Castillo, C. J. (2011). Microencapsulation of propolis extract by complex coacervation. *LWT - Food Science and Technology*, *44*(2), 429–435.

Nunes, C., Almeida, L., & Laranjinha, J. (2005). Synergistic inhibition of respiration in brain mitochondria by nitric oxide and dihydroxyphenylacetic acid (DOPAC). Implications for Parkinson's disease. *Neurochemistry International*, *47*, 173–182.

Nunes, C., Almeida, L., & Laranjinha, J. (2008). 3,4-Dihydroxyphenylacetic acid (DOPAC) modulates the toxicity induced by nitric oxide in PC-12 cells via mitochondrial dysfunctioning. *Neurotoxicology*, *29*, 998–1007.

Ofori, J. A., & Peggy, Y.-H. (2013). *Novel technologies for the production of functional foods, bionanotechnology*. Oxford: Blackwell Publishing Ltd.

Oliveira, L. D. L. D., Carvalho, M. V. D., & Melo, L. (2014). Health promoting and sensory properties of phenolic compounds in food. *Revista Ceres*, *61*, 764–779.

Orsat, V., & Raghavan, G. S. V. (2005). Radio-frequency processing. In S. Da-Wen (Ed.), *Emerging technologies for food processing*. Amsterdam: Elsevier.

Pagington, J. S. (1986). α-Cyclodextrin and its uses in the flavour industry. In G. G. Birch, & M. G. Lindley (Eds.), *Developments in food flavours*. London: Elsevier Applied Science.

Paini, M., Aliakbarian, B., Casazza, A. A., Lagazzo, A., Botter, R., & Perego, P. (2015). Microencapsulation of phenolic compounds from olive pomace using spray drying: A study of operative parameters. *LWT - Food Science and Technology*, *62*, 177–186.

Pan, Z., Qu, W., Ma, H., Atungulu, G. G., & Mchugh, T. H. (2011). Continuous and pulsed ultrasound-assisted extractions of antioxidants from pomegranate peel. *Ultrasonics Sonochemistry*, *18*(5), 1249–1257.

Papandreou, M. A., Dimakopoulou, A., Linardaki, Z. I., Cordopatis, P., Klimis-Zacas, D., Margarity, M., & Lamari, F. N. (2009). Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. *Behavioural Brain Research*, *198*(2), 352–358.

Parisi, O. I., Puoci, F., Restuccia, D., Farina, G., Iemma, F., & Picci, N. (2014). Polyphenols and their formulations: Different strategies to overcome the drawbacks associated with their poor stability and bioavailabilityIn R. R. Watson, V. R. Preedy, & S. Zibadi (Eds.), *Polyphenols in human health and disease* (Vol. 1London: Academic Press.

Parkar, S. G., Stevenson, D. E., & Skinner, M. A. (2008). The potential influence of fruit polyphenols on colonic microflora and human gut health. *International Journal of Food Microbiology*, *124*(3), 295–298.

Parniakov, O., Barba, F. J., Grimi, N., Lebovka, N., & Vorobiev, E. (2016). Extraction assisted by pulsed electric energy as a potential tool for green and sustainable recovery of nutritionally valuable compounds from mango peels. *Food Chemistry*, *192*, 842–848.

Pascual-Martí, M. C., Salvador, A., Chafer, A., & Berna, A. (2001). Supercritical fluid extraction of resveratrol from grape skin of *Vitis vinifera* and determination by HPLC. *Talanta*, *54*, 735–740.

Pasrija, D., Ezhilarasi, P. N., Indrani, D., & Anandharamakrishnan, C. (2015). Microencapsulation of green tea polyphenols and its effect on incorporated bread quality. *LWT - Food Science and Technology*, *64*, 289–296.

Passamonti, S., Vrhovsek, U., & Mattivi, F. (2002). The interaction of anthocyanins with bilitranslocase. *Biochemical and Biophysical Research Communications*, *296*, 631–636.

Passamonti, S., Vrhovsek, U., Vanzo, A., & Mattivi, F. (2005). Fast access of some grape pigments to the brain. *Journal of Agricultural and Food Chemistry*, *53*, 7029–7034.

Patras, A., Brunton, N. P., Da Pieve, S., & Butler, F. (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science & Emerging Technologies*, *10*(3), 308–313.

Patras, A., Brunton, N. P., O'donnell, C., & Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology*, *21*(1), 3–11.

Pavlica, S., & Gebhardt, R. (2010). Protective effects of flavonoids and two metabolites against oxidative stress in neuronal PC12 cells. *Life Sciences*, *86*, 79–86.

Pereira, R. N., & Vicente, A. A. (2010). Environmental impact of novel thermal and non-thermal technologies in food processing. *Food Research International*, *43*(7), 1936–1943.

Perron, N. R., García, C. R., Pinzón, J. R., Chaur, M. N., & Brumaghim, J. L. (2011). Antioxidant and prooxidant effects of polyphenol compounds on copper-mediated DNA damage. *Journal of Inorganic Biochemistry*, *105*, 745–753.

Picot, A., & Lacroix, C. (2003). Production of multiphase water-insoluble microcapsules for cell microencapsulation using an emulsification/spray-drying technology. *Journal of Food Science*, *68*(9), 2693–2700.

Pingret, D., Fabiano-Tixier, A. S., Le Bourvellec, C., Renard, C. M. G. C., & Chemat, F. (2012). Lab and pilot-scale ultrasound-assisted water extraction of polyphenols from apple pomace. *Journal of Food Engineering*, *111*, 73–81.

Poljsak, B., Šuput, D., & Milisav, I. (2013). Achieving the balance between ROS and antioxidants: When to use the synthetic antioxidants? *Oxidative Medicine and Cellular Longevity*, 11 , 2013, Article ID 956792.

Possemiers, S., Bolca, S., Grootaert, C., Heyerick, A., Decroos, K., Dhooge, W., et al. (2006). The prenylflavonoid isoxanthohumol from hops (*Humulus lupulus* L.) is activated into the potent phytoestrogen 8-prenylnaringenin in vitro and in the human intestine. *Journal of Nutrition*, *136*, 1862–1867.

Possemiers, S., Rabot, S., Espin, J. C., Bruneau, A., Philippe, C., Gonzalez-Sarrias, A., et al. (2008). *Eubacterium limosum* activates isoxanthohumol from hops (*Humulus lupulus* L.) into the potent phytoestrogen 8-prenylnaringenin in vitro and in rat intestine. *Journal of Nutrition*, *138*, 1310–1316.

Prabhakar, P. K., & Doble, M. (2009). Synergistic effect of phytochemicals in combination with hypoglycemic drugs on glucose uptake in myotubes. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, *16*(12), 1119–1126.

Prior, R. L., & Cao, G. (1999). In vivo total antioxidant capacity: Comparison of different analytical methods. *Free Radical Biological & Medicine*, *27*, 1173–1181.

Puškaš, V., & Miljić, U. (2012). Effects of fining on phenolic compounds and colour of red wine obtained with addition of increased amounts of grape solid phase in pomace. *Hemijska Industrija*, *66*(5), 727–734.

Queipo-Ortuño, M. I., Boto-Ordóñez, M., Murri, M., Gomez-Zumaquero, J. M., Clemente-Postigo, M., Estruch, R., et al. (2012). Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *The American Journal of Clinical Nutrition*, *95*(6), 1323–1334.

Raimondi, S., Roncaglia, L., De Lucia, M., Amaretti, A., Leonardi, A., Pagnoni, U. M., & Rossi, M. (2009). Bioconversion of soy isoflavones daidzin and daidzein by Bifidobacterium strains. *Applied Microbiology and Biotechnology*, *81*(5), 943–950.

Ramos, L., Kristenson, E. M., & Brinkman, U. A. T. (2002). Current use of pressurised liquid extraction and subcritical water extraction in environmental analysis. *Journal of Chromatography. A*, *975*, 3–29.

Ranic, M., Nikolic, M., Pavlovic, M., Buntic, A., Siler-Marinkovic, S., & Dimitrijevic-Brankovic, S. (2014). Optimization of microwave-assisted extraction of natural antioxidants from spent espresso coffee grounds by response surface methodology. *Journal of Cleaner Production*, *80*, 69–79.

Ratnasooriya, C. C., & Vasantha Rupasinghe, H. P. (2012). Extraction of phenolic compounds from grapes and their pomace using β-cyclodextrin. *Food Chemistry*, *134*, 625–631.

Ray, S., Raychaudhuri, U., & Chakraborty, R. (2016). An overview of encapsulation of active compounds used in food products by drying technology. *Food Bioscience*, *13*, 76–83.

Reis Giada, M.L. (2013) *Food phenolic compounds: Main classes, sources and their antioxidant power*. In Oxidative stress and chronic degenerative diseases—A role for antioxidants, InTechOpen. p. 87–112.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006a). *Chemical nature, origins and consequences of the main organoleptic defects*. *Handbook of Enology*. Chichester: John Wiley & Sons, Ltd.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006b). *Phenolic compounds*. *Handbook of Enology*. Chichester: John Wiley & Sons, Ltd.

Richelle, M., Pridmore-Merten, S., Bodenstab, S., Enslen, M., & Offord, E. A. (2002). Hydrolysis of isoflavone glycosides to aglycones by glycosidase does not alter plasma and urine isoflavone pharmacokinetics in postmenopausal women. *Journal of Nutrition*, *132*, 2587–2592.

Rios, L. Y., Bennett, R. N., Lazarus, S. A., Remesy, C., Scalbert, A., & Williamson, G. (2002). Cocoa procyanidins are stable during gastric transit in humans. *American Journal of Clinical Nutrition*, *76*, 1106–1110.

Rodriguez-Saona, L. E., Giusti, M. M., Durst, R. W., & Wrolstad, R. E. (2001). Development and process optimization of red radish concentrate extract as potential natural red colorant. *Journal of Food Processing and Preservation*, *25*(3), 165–182.

Romdhane, M., & Gourdon, C. (2002). Investigation in solid–liquid extraction: Influence of ultrasound. *Chemical Engineering Journal*, *87*, 11–19.

Roopchand, D. E., Krueger, C. G., Moskal, K., Fridlender, B., Lila, M. A., & Raskin, I. (2013). Food-compatible method for the efficient extraction and stabilization of cranberry pomace polyphenols. *Food Chemistry*, *141*, 3664–3669.

Roselló-Soto, E., Barba, F. J., Parniakov, O., Galanakis, C. M., Grimi, N., Lebovka, N., & Vorobiev, E. (2015). High voltage electrical discharges, pulsed electric field and ultrasounds assisted extraction of protein and phenolic compounds from olive kernel. *Food & Bioprocess Technology*, *8*, 885–894.

Roselló-Soto, E., Galanakis, C. M., Brncic, M., Orlien, V., Trujillo, F. J., Mawson, R., … Barba, F. J. (2015). Clean recovery of antioxidant compounds from plant foods, byproducts and algae assisted by ultrasounds processing. Modeling approaches to optimize processing conditions. *Trends in Food Science & Technology*, *42*, 134–149.

Saarela, M., Lahteenmaki, L., Crittenden, R., Salminen, S., & Mattila-Sandholm, T. (2002). Gut bacteria and health foods—The European perspective. *International Journal of Food Microbiology*, *78*, 99–117.

Sadilova, E., Stintzing, F. C., & Carle, R. (2006). Thermal degradation of acylated and nonacylated anthocyanins. *Journal of Food Science*, *71*(8), C504–C512.

Sáenz-Navajas, M.-P., Campo, E., Fernández-Zurbano, P., Valentin, D., & Ferreira, V. (2010). An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. *Food Chemistry*, *121*(4), 1139–1149.

Sanchez, V., Baeza, R., Galmarini, M. V., Zamora, M. C., & Chirife, J. (2013). Freeze-drying encapsulation of red wine polyphenols in an amorphous matrix of maltodextrin. *Food and Bioprocess Technology*, *6*, 1350–1354.

Sánchez-Camargo, A. P., Montero, L., Stiger-Povreau, V., Tanniou, A., Cifuentes, A., Herrero, M., & Ibáñez, E. (2016). Considerations on the use of enzyme-assisted extraction in combination with pressurized liquids to recover bioactive compounds from algae. *Food Chemistry*, *192*, 67–74.

Sánchez-Moreno, C., Plaza, L., De Ancos, B., & Cano, M. P. (2006). Impact of high-pressure and traditional thermal processing of tomato purée on carotenoids, vitamin C and antioxidant activity. *Journal of the Science of Food and Agriculture*, *86*(2), 171–179.

Sano, A., Uchida, R., Saito, M., Shioya, N., Komori, Y., Tho, Y., & Hashizume, N. (2007). Beneficial effects of grape seed extract on malondialdehyde-Modified LDL. *Journal of Nutritional Science and Vitaminology*, *53*, 174–182.

Sarkis, J. R., Boussetta, N., Blouet, C., Tessaro, I. C., Marczak, L. D. F., & Vorobiev, E. (2015). Effect of pulsed electric fields and high voltage electrical discharges on polyphenol and protein extraction from sesame cake. *Innovative Food Science and Emerging Technologies*, *29*, 170–177.

Scalbert, A., Johnson, I. T., & Saltmarsh, M. (2005). Polyphenols: Antioxidants and beyond. *American Journal of Clinical Nutrition*, *81*(1), 215–217.

Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition*, *130*, 2073–2085.

Scheline, R. R. (1991). *CRC handbook of mammalian metabolism of plant compounds*. Boca Raton, FL: CRC Press.

Semalty, A., Semalty, M., & Rawat, M. S. M. (2007). The phyto-phospholipid complexes-phytosomes: A potential therapeutic approach for herbal hepatoprotective drug delivery. *Pharmacognosy Reviews*, *1*(S2), 369–374.

Setchell, K. D., Brown, N. M., Desai, P., Zimmer-Nechemias, L., Wolfe, B. E., Brashear, W. T., et al. (2001). Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *Journal of Nutrition*, *131*, 1362–1375.

Setchell, K. D., & Clerici, C. (2010). Equol: History, chemistry, and formation. *Journal of Nutrition*, *140*(7), 1355–1362.

Setyaningsih, W., Saputro, I. E., Palma, M., & Barroso, C. G. (2015). Optimisation and validation of the microwave-assisted extraction of phenolic compounds from rice grains. *Food Chemistry*, *169*, 141–149.

Sfakianos, J., Coward, L., Kirk, M., & Barnes, S. (1997). Intestinal uptake and biliary excretion of the isoflavone genistein in rats. *Journal of Nutrition*, *127*, 1260–1268.

Shahani, K., Swaminathan, S. K., Freeman, D., Blum, A., Ma, L., & Panyam, J. (2010). Injectable sustained release microparticles of curcumin: A new concept for cancer chemoprevention. *Cancer Research*, *70*(11), 4443–4452.

Shahid, M., Ul Islam, S., & Mohammad, F. (2013). Recent advancements in natural dye applications: A review. *Journal of Cleaner Production*, *53*, 310–331.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects—A review. *Journal of Functional Foods*, *18*(Part B), 820–897.

Shahidi, F., & Naczk, M. (2004). *Phenolics in food and nutraceuticals*. Boca Raton, FL: CRC Press.

Shahrzad, S., Aoyagi, K., Winter, A., Koyama, A., & Bitsch, I. (2001). Pharmacokinetics of gallic acid and its relative bioavailability from tea in healthy humans. *Journal of Nutrition*, *131*, 1207–1210.

Shahrzad, S., & Bitsch, I. (1998). Determination of gallic acid and its metabolites in human plasma and urine by high-performance liquid chromatography. *Journal of Chromatography B, Biomedical Sciences and Applications*, *705*, 87–95.

Shao, J., Li, X., Lu, X., Jiang, C., Hu, Y., Li, Q., et al. (2009). Enhanced growth inhibition effect of resveratrol incorporated into biodegradable nanoparticles against glioma cells is mediated by the induction of intracellular reactive oxygen species levels. *Colloids and Surfaces B: Biointerfaces*, *72*, 40–47.

Sharma, S., & Roy, R. K. (2010). Phytosomes: An emerging technology. *International Journal of Pharmaceutical Research and Development*, *2*, 1–5.

Singh, G., & Pai, R. S. (2014). Recent advances of resveratrol in nanostructured based delivery systems and in the management of HIV/AIDS. *Journal of Controlled Release*, *194*, 178–188.

Singh, M., Sharma, R., & Banerjee, U. C. (2002). Biotechnological applications of cyclodextrins. *Biotechnology Advances*, *20*(5/6), 341–359.

Singh, P. P., & Saldaña, M. D. A. (2011). Subcritical water extraction of phenolic compounds from potato peel. *Food Research International*, *44*, 2452–2458.

Soares, S., Kohl, S., Thalmann, S., Mateus, N., Meyerhof, W., & De Freitas, V. (2013). Different phenolic compounds activate distinct human bitter taste receptors. *Journal of Agricultural and Food Chemistry*, *61* (7), 1525–1533.

Somavat, R. (2011). *Applications and effects of ohmic heating: Sterilization, influence on bacterial spores, enzymes, bioactive components and quality factors in food (PhD thesis)*. Columbus: Graduate School of The Ohio State University.

Soppimath, K. S., Aminabhavi, T. M., Kulkarni, A. R., & Rudzinski, W. E. (2001). Biodegradable polymeric nanoparticles as drug delivery devices. *Journal of Controlled Release*, *70*, 1–20.

Soria, A. C., & Villamiel, M. (2010). Effect of ultrasound on the technological properties and bioactivity of food: A review. *Trends in Food Science & Technology*, *21*(7), 323–331.

Spencer, J. P., Chaudry, F., Pannala, A. S., Srai, S. K., Debnam, E., & Rice-Evans, C. (2000). Decomposition of cocoa procyanidins in the gastric milieu. *Biochemical and Biophysical Research Communications*, *272*, 236–241.

Spinelli, S., Conte, A., Lecce, L., Padalino, L., & Del Nobile, M. A. (2016). Supercritical carbon dioxide extraction of brewer's spent grain. *Journal of Supercritical Fluids*, *107*, 69–74.

Szkudelski, T., & Szkudelska, K. (2011). Anti-diabetic effects of resveratrol. *Annals of the New York Academy of Sciences*, *1215*, 34–39.

Takahashi, M., Uechi, S., Takara, K., Asikin, Y., & Wada, K. (2009). Evaluation of an oral carrier system in rats: Bioavailability and antioxidant properties of liposome-capsulated curcumin. *Journal of Agricultural and Food Chemistry*, *57*, 9141–9146.

Takikawa, M., Inoue, S., Horio, F., & Tsuda, T. (2010). Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. *Journal of Nutrition*, *140*, 527–533.

Talavera, S., Felgines, C., Texier, O., Besson, C., Lamaison, J. L., & Remesy, C. (2003). Anthocyanins are efficiently absorbed from the stomach in anesthetized rats. *Journal of Nutrition*, *133*, 4178–4182.

Tanaka, Y., Sasaki, N., & Ohmiya, A. (2008). Biosynthesis of plant pigments: Anthocyanins, betalains and carotenoids. *The Plant Journal*, *54*(4), 733–749.

Tanimura, S., & Mattes, R. D. (1993). Relationships between bitter taste sensitivity and consumption of bitter substances. *Journal of Sensory Studies*, *8*(1), 31–41.

Tatke, P., & Jaiswal, Y. (2011). An overview of microwave assisted extraction and its applications in herbal drug research. *Research Journal of Medicinal Plant*, *5*, 21–31.

Tedesco, D., Steidler, S., Galletti, S., Tameni, M., Sonzogni, O., & Ravarotto, L. (2004). Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B1 in broiler chicks. *Poultry Science*, *83*(11), 1839–1843.

Terauchi, M., Horiguchi, N., Kaiyama, A., Akiyoshi, M., Owa, Y., Kato, K., et al. (2014). Effects of grape seed proanthocyanidin extract on menopausal symptoms, body composition and cardiovascular parameters in middle-aged women: A randomized, double-blind, placebo controlled pilot study. *Menopause (New York, N.Y.)*, *21*, 990–996.

Töpfl, S. (2006). *Pulsed electric fields (PEF) for permeabilization of cell membranes in food and bioprocessing—Applications, process and equipment design and cost analysis (Ph.D. thesis)*. Berlin: University of Technology.

Tsai, H.-C., Li, Y.-C., Hsu, S.-H., Young, T.-H., & Chen, M.-H. (2015). Inhibition of growth and migration of oral and cervical cancer cells by citrus polyphenol. *Journal of the Formosan Medical Association*, in press http://dx.doi.org/10.1016/j.jfma.2015.01.020.

Tsakona, S., Galanakis, C. M., & Gekas, V. (2012). Hydro-ethanolic mixtures for the recovery of phenols from Mediterranean plant materials. *Food & Bioprocess Technology*, *5*, 1384–1393.

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. *Nature*, *449*, 804–810.

Tzounis, X., Rodriguez-Mateos, A., Vulevic, J., Gibson, G. R., et al. (2011). Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. *American Journal of Clinical Nutrition*, *93*(1), 62–72.

Tzounis, X., Vulevic, J., Kuhnle, G. G., George, T., Leonczak, J., Gibson, G. R., … Spencer, J. P. (2008). Flavanol monomerinduced changes to the human faecal microflora. *British Journal of Nutrition*, *99*(4), 782–792.

Uchiyama, S., Taniguchi, Y., Saka, A., Yoshida, A., & Yajima, H. (2011). Prevention of diet-induced obesity by dietary black tea polyphenols extract in vitro and in vivo. *Nutrition (Burbank, Los Angeles County, Calif.)*, *27*(3), 287–292.

Valant-Vetschera, K. M., & Wollenweber, E. (2006). Flavonoids. In O. M. Andersen, & K. R. Markham (Eds.), *Chemistry, biochemistry and applications.* (pp. 617–748). Boca Raton, FL: CRC Press.

Vega-Gálvez, A., López, J., Torres-Ossandón, M. J., Galotto, M. J., Puente-Díaz, L., Quispe-Fuentes, I., & Di Scala, K. (2014). High hydrostatic pressure effect on chemical composition, color, phenolic acids and antioxidant capacity of Cape gooseberry pulp (*Physalis peruviana* L.). *LWT - Food Science and Technology*, *58*(2), 519–526.

Vendrame, S., Guglielmetti, S., Riso, P., Arioli, S., Klimis-Zacas, D., & Porrini, M. (2011). Six-week consumption of a wild blueberry powder drink increases bifidobacteria in the human gut. *Journal of Agricultural and Food Chemistry*, *59*(24), 12815–12820.

Vető-Kiszter, A. K. (2011). Development of radio frequency heat treatment technology for the production of non-pungent mustard (Sinapis alba L.) seed flour *(Ph.D. thesis)*. Budapest, Hungary: Corvinus University of Budapest, Central Food Research Institute.

Vicente, A., & Castro, I. A. (2007). *Novel thermal processing technologies. Advances in Thermal and Non-Thermal Food Preservation*. Oxford: Blackwell Publishing.

Villamor, R. R. (2012). The impact of wine components on the chemical and sensory properties of wines *(PhD thesis)*. Pullman, WA: Washington State University.

Viveros, A., Chamorro, S., Pizarro, M., Arija, I., Centeno, C., & Brenes, A. (2011). Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. *Poultry Science*, *90*, 566–578.

Wan, J., Coventry, J., Swiergon, P., Sanguansri, P., & Versteeg, C. (2009). Advances in innovative processing technologies for microbial inactivation and enhancement of food safety-pulsed electric field and low-temperature plasma. *Trends in Food Science & Technology*, *20*(9), 414–424.

Wang, L. & Bohn, T. (2012) Health-promoting food ingredients and functional food processing. In J. Bouayed (Ed.), *Nutrition, well-being and health, InTech.* ISBN 978-953-51-0125-3.

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology*, *17*, 300–312.

Wang, L. Q., Meselhy, M. R., Li, Y., Qin, G. W., & Hattori, M. (2000). Human intestinal bacteria capable of transforming secoisolariciresinol diglucoside to mammalian lignans, enterodiol and enterolactone. *Chemical and Pharmacetucal Bulletin (Tokyo)*, *48*, 1606–1610.

Wang, X. L., Kim, H. J., Kang, S. I., Kim, S. I., & Hur, H. G. (2007). Production of phytoestrogen S-equol from daidzein in mixed culture of two anaerobic bacteria. *Archives of Microbiology*, *187*(2), 155–160.

Wenche Jøraholmen, M., Škalko-Basnet, N., Acharya, G., & Basnet, P. (2015). Resveratrol-loaded liposomes for topical treatment of the vaginal inflammation and infections. *European Journal of Pharmaceutical Sciences*, *79*, 112–121.

Wijngaard, H., Hossain, M. B., Rai, D. K., & Brunton, N. (2012). Techniques to extract bioactive compounds from food by-products of plant origin. *Food Research International*, *46*, 505–513.

Williamson, G., & Manach, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *American Journal of Clinical Nutrition*, *81*(suppl), 243S–255S.

Williamson, M. P., Mccormick, T. G., Nance, C. L., & Shearer, W. T. (2006). Epigallocatechin gallate, the main polyphenol in green tea, binds to the T-cell receptor, CD4: Potential for HIV-1 therapy. *Journal of Allergy and Clinical Immunology*, *118*(6), 1369–1374.

Wissgott, U., & Bortlik, K. (1996). Prospects for new natural food colorants. *Trends in Food Science & Technology*, *7*(9), 298–302.

Wu, X., Cao, G., & Prior, R. L. (2002). Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. *Journal of Nutrition*, *132*, 1865–1871.

Xu, J.-Y., Wu, L.-Y., Zheng, X.-O., Lu, J.-L., Wu, M.-Y., & Liang, Y.-R. (2010). Green tea polyphenols attenuating ultraviolet B-induced damage to human retinal pigment epithelial cells in vitro. *Investigative Ophthalmology & Visual Science*, *51*, 6665–6670.

Yamakoshi, J., Tokutake, S., & Kikuchi, M. (2001). Effect of proanthocyanidin- rich extract from grape seeds on human fecal flora and fecal odor. *Microbial Ecology in Health and Disease*, *13*, 25–31.

Yatsu, F. K. J., Koester, L. S., Lula, I., Passos, J. J., Sinisterra, R., & Bassani, V. L. (2013). Multiple complexation of cyclodextrin with soy isoflavones present in an enriched fraction. *Carbohydrate Polymers*, *98*(1), 726–735.

Yildiz, H., Bozkurt, H., & Icier, F. (2009). Ohmic and conventional heating of pomegranate juice: Effects on rheology, color, and total phenolics. *Food Science and Technology International*, *15*(5), 503–512.

Yin, P., Zhao, S., Chen, S., Liu, J., Shi, L., Wang, X., … Ma, C. (2011). Hypoglycemic and hypolipidemic effects of polyphenols from burs of *Castanea mollissima* blume. *Molecules (Basel, Switzerland)*, *16*, 9764–9774.

Yokoyama, S., Niwa, T., Osawa, T., & Suzuki, T. (2010). Characterization of an O-desmethylangolensin-producing bacterium isolated from human feces. *Archives of Microbiology*, *192*(1), 15–22.

Yokoyama, S., & Suzuki, T. (2008). Isolation and characterization of a novel equol-producing bacterium from human feces. *Bioscience, Biotechnology, and Biochemistry*, *72*(10), 2660–2666.

Yuan, J. P., Wang, J. H., & Liu, X. (2007). Metabolism of dietary soy isoflavones to equol by human intestinal microflora—implications for health. *Molecular Nutrition and Food Research*, *51*(7), 765–781.

Yutani, R., Morita, S., Teraoka, R., & Kitagawa, S. (2012). Distribution of polyphenols and a surfactant component in skin during aerosol OT microemulsion-enhanced intradermal delivery. *Chemical & Pharmaceutical Bulletin*, *60*, 989–994.

Zhang, G., Hu, M., He, L., Fu, P., Wang, L., & Zhou, J. (2013). Optimization of microwave-assisted enzymatic extraction of polyphenols from waste peanut shells and evaluation of its antioxidant and antibacterial activities in vitro. *Food and Bioproducts Processing*, *91*, 158–168.

Zhao, J., Fang, S., Yuan, Y., Guo, Z., Zeng, J., Guo, Y., et al. (2014). Green tea polyphenols protect spinal cord neurons against hydrogen peroxide-induced oxidative stress. *Neural Regeneration Research*, *9*(14), 1379–1385.

Zhao, Q., Huo, X. C., Sun, F. D., & Dong, R. Q. (2015). Polyphenol-rich extract of *Salvia chinensis* exhibits anticancer activity in different cancer cell lines, and induces cell cycle arrest at the G_0/G_1-phase, apoptosis and loss of mitochondrial membrane potential in pancreatic cancer cells. *Molecular Medicine Reports*, *12*(4), 4843–4850.

Zhao, Y., Asimi, S., Wu, K., Zheng, J., & Li, D. (2015). Black tea consumption and serum cholesterol concentration: Systematic review and meta-analysis of randomized controlled trials. *Clinical Nutrition*, *34*, 612–619.

Zhu, B., Wang, X., & Li, L. (2010). Human gut microbiome: The second genome of human body. *Protein Cell*, *1*(8), 718–725.

Zinoviadou, K. G., Barba, F. J., Galanakis, C. M., Brnčić, M., Trujillo, F., Mawson, R., & Knoerzer, K. (2015). Fruit juice sonication: Implications on food safety and physicochemical and nutritional properties. *Food Research International*, *77*(4), 743–752.

Zubik, L., & Meydani, M. (2003). Bioavailability of soybean isoflavones from aglycone and glucoside forms in American women. *American Journal of Clinical Nutrition*, *77*, 1459–1465.

CHAPTER

CAROTENOIDS

8

Jayesree Nagarajan[1], Ramakrishnan Nagasundara Ramanan[1], Mavinakere Eshwaraiah Raghunandan[1], Charis M. Galanakis[2] and Nagendra Prasad Krishnamurthy[1]

[1]Monash University Malaysia, Selangor, Malaysia [2]Galanakis Laboratories, Chania, Greece

8.1 INTRODUCTION

The human body is often regarded as a complex system made up of millions of individual systems. As a complex living organism, there is no single functional food that is capable of equipping the human immune system against chronic degenerative diseases. Therefore nutritionists have introduced the ideology of the so-called "rainbow diet" to emphasize and guide the regulatory uptake of various fruits and vegetables. The dazzling rainbow colors reflected by foods are due to the presence of colorful pigments known as "carotenoids." The striking yellow, orange, or red colors are not only attractants, but they are the prerequisite components for photosynthesis and all living creatures in oxygen atmosphere. Upon consumption, these molecules are found to possess high therapeutic effects on a wide range of diseases, which has been proven in many scientific studies. The healing functions and actions of carotenoids are dependent on their molecular structure, which determines their physical and chemical properties (Britton, 1995). To date, approximately 700 types of natural carotenoids have been identified of which 50 can be absorbed, transported, and deposited by the human body. Among them, 95% of human diets consist of a few pronounced carotenoids such as β-carotene, α-carotene, lycopene, β-cryptoxanthin, lutein, and zeaxanthin (Carilho et al., 2014; Rao & Rao, 2007).

Due to their versatile health-promoting properties, the global market demand for carotenoids is estimated to increase from US$1.5 billion in 2014 to US$1.8 billion in 2019 (Strati & Oreopoulou, 2014). Therefore various scientific reports and patent rights are being devoted to carotenoids and their medicinal values throughout the world. These scenarios show that carotenoids are a noteworthy phytonutrient necessary for human health. In terms of applications, carotenoid-rich products are widely distributed in the form of food and feed additives, supplements, and natural colorants. Commercially available carotenoids are largely produced using chemically synthesized products, but some are made by carotenoids from natural sources (Amaya Delia, 2016). The molecular structure of synthetic carotenoids is identical to that of natural carotenoids. In the 1950s, the first synthetic carotenoid introduced was β-carotene using β-ionine-derived precursors such as acetone and butadiene (Rutz, Borges, Zambiazi, Rosa, & Silva, 2016). There are some appealing qualities of synthetic carotenoids compared to natural carotenoids. First, synthetic carotenoids are specially formulated to minimize oxidation or isomerization, and hence are more stable. In order to make carotenoid application in food easier, synthetic carotenoids are prepared in

Nutraceutical and Functional Food Components. DOI: http://dx.doi.org/10.1016/B978-0-12-805257-0.00008-9

the form of colloidal suspension, emulsification, and dispersion colloids. They are generally distributed as water-soluble and stable emulsions in the market. Despite these advantages, chemically synthesized carotenoids are known to exhibit high toxicity, carcinogenicity, and teratogenicity properties and thus create great hesitation among health-conscious consumers. As a result, carotenoids extracted from natural resources are in high demand among today's consumers (Kirthi, Amita, Priti, Kumar, & Jyoti, 2014).

This growing shift in consumer interest has created a number of challenges to the industrialists creating effectual natural carotenoid formulations for the market. Food matrices contain a diverse range of pool of molecules and hence selective recovery of carotenoids requires many precaution steps and appropriate analysis protocols. Adequate extraction methodology and effective optimization processes are crucial to ensuring the recovery of high-purity carotenoids without interfering compounds. Unlike carotenoids from chemical synthesis, carotenoids from food sources require complicated extraction stages; they also possess higher hydrophobicity, are less stable, experience seasonal fluctuations, and are limited in supply. Motivated to overcome these limitations, studies to improvise carotenoid-extraction methods through technology advancements are common. In general, studies on carotenoids are aimed at (1) the search for carotenoid-rich natural resources, (2) composition and influencing factors of carotenoids occurrence, (3) improving stability of carotenoids, (iv) extraction for higher yield, and (4) health applications (Martinez & Heredia, 2007). The enhancement of carotenoid bioavailability is also of interest among researchers. Active, natural formulas commercialized as "ready-to-eat carotenoid-rich products" in the market are the result of the food-processing and carotenoid-extraction technologies practiced by the industry.

This chapter discusses the nature and properties of carotenoids and highlights recent developments implemented by food industrialists including:

- nature of carotenoids
- functional and technological properties
- nutrition values, health benefits, and clinical evidence
- stability, bioavailability, and bioaccessibility
- food processing technology for carotenoid bioaccessibility
- recovery of carotenoid from agroindustrial waste
- extraction and quantification of carotenoids.

8.2 NATURE OF CAROTENOIDS

Carotenoids are found in molds, yeast, bacteria, and more universally in the plant kingdom. Although they cannot be synthesized by animals or humans naturally, carotenoids are often present in animal and human body tissue in standard concentration. This is because they are absorbed, transported, and deposited in the body through food intake (Singh, Ahmad, & Ahmad, 2015).

8.2.1 GENERAL FEATURES AND PHYSIOLOGICAL PROPERTIES

Being an attractive pigment, carotenoids were originally synthesized in plastid through stepwise addition of isopentenyl pyrophosphate units to form a 20-carbon precursor known as

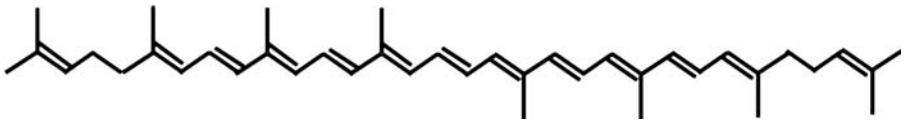


FIGURE 8.1

Basic structure of carotenoid.

Adapted and redrawn from Choksi, P.M. & Joshi, V.Y. (2007). A review on lycopene—Extraction, purification, stability and applications. International Journal of Food Properties, 10(2), 289–298.

geranylgeranyl diphosphate (GGPP). When two molecules of GGPP are combined, they form phytoene, the first carotenoid in biosynthetic pathway. Upon desaturation, phytoene forms 11 conjugated double bonds, which turn into all-trans lycopene, the red pigment. Lycopene then becomes the fundamental structure in which other carotenoids are derived through cyclization, hydroxylation, epoxidation, and rearrangement. The basic structure of carotenoids are C40 tetraterpenoid carbons built from eight isoprene units, joined together in a reversed isoprenoid manner at the center of the molecule (Fig. 8.1).

They are also known as polyunsaturated molecules due to the presence of 11 conjugated double bonds in their structure (alternate single and double bonds). As the number of double bonds increases in the polyene chain, the molecule becomes a reactive electron-rich system (from ground state π-electrons to delocalized π^*-electrons). Therefore the long-chain carotenoids are much more prone to oxidization and isomerization. The arrangement of single and double bonds in the structure determines the absorption of particular wavelength in the visible spectrum. While the reflected wavelength determine the colour of pigment itself (Choksi & Joshi, 2007). Carotenoids exhibit three distinct maximum absorption spectra in the visible spectrum (ranging from 430 to 480 nm). Minimally, seven conjugated double bonds are required to achieve the yellow characteristic of carotenoids. Notoriously, almost all the carotenoids are insoluble in water and possess high solubility in a hydrophobic environment. Hence, corrosive solvents like hexane, acetone, and chloroform are commonly used to solubilize and analyze the presence of carotenoids. When analyzing carotenoids, different organic solvents cause spectral shifts due to differences in the solvent nature such as polarity. In terms of structure, carotenoids can be differentiated by several features as reported by Esteban et al. (2015):

i. presence of oxygen molecules in the structure;
ii. hydrogenation of the carbon polyene chain;
iii. cyclize at one or both end of the structure with β-ionine rings;
iv. length of the polyene chain.

By nature, this pigment exists in all-trans forms expect under certain environmental conditions, where it undergoes structural modifications due to oxidation and isomerization (Strati & Oreopoulou, 2014). When carotenoids are isomerized, they are pronounced with the annotation of alpha (α), beta (β), gamma (γ), delta (δ), epsilon (ε), and zeta (ζ) (Khoo et al., 2011). These isomeric forms of carotenoids determine the shape and changes in chemical properties of the entire molecules including solubility and absorbability. The trans-isomeric form is known as the most thermodynamically stable form of carotenoid. All trans-carotenoids exhibit a greater tendency to

undergo cyclization and aggregation than cis forms. Hence, cis-isomers are more readily solubilized, absorbed, and transported in the body than their all-trans counterparts (Kirti et al., 2014).

8.2.2 CLASSIFICATION OF CAROTENOIDS

Carotenoids are classified either by chemical structure or by functionality.

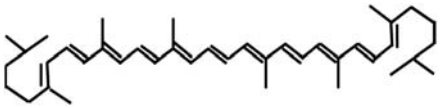
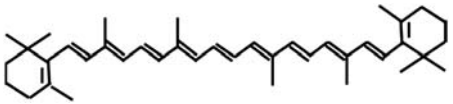
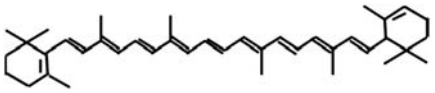
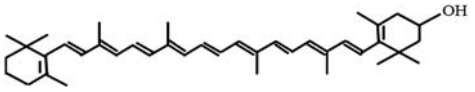
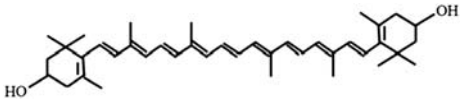
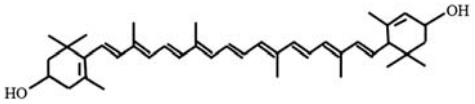
1. Chemical structure: based on the chemical structure, carotenoids that exist as pure hydrocarbons are referred to as carotenes (α-carotene, β-carotene, and lycopene). Furthermore, carotenoids that contain oxygen as a functional group in its structure (β-cryptoxanthin, lutein, and zeaxanthin) are referred to as xanthophylls. The presence of polar group in the structure (e.g., epoxy, hydroxyl and keto) affects the polarity and biological function of the compounds (Matea et al., 2009).
2. Functionality: carotenoids can also be classified as primary or secondary carotenoids. Primary carotenoids are known as photosynthetic pigments, which play a key role in photosynthesis. Carotenes that fall in the region of orange to red wavelength are responsible for transmitting light energy from the sunlight absorbed by chlorophyll. They are also known to act as antioxidants to the plant by absorbing energy from singlet oxygen formed during the photosynthesis process. On the other hand, the xanthophyll molecules are found abundantly in the leaves of plants but do not function directly during photosynthesis. Xanthophylls absorb the wavelength of sunlight, which is not absorbed by chlorophyll. They serve as accessory pigments or secondary carotenoids to plants (Carilho et al., 2014).

Table 8.1 highlights the structural, chemical, and electronic properties of the main carotenoids in human diets.

8.2.3 PLANTS AS UNIVERSAL SOURCE FOR CAROTENOIDS

Carotenoid distribution in plants is associated with the de novo synthesis that occurs in the differentiated plastids of roots, flowers, fruits, and seeds. Their accumulation can be subdivided as chloroplasts (green plastids), chromoplasts (yellow, orange, and red plastids), amyloplasts (plastids containing starch), elaioplasts (lipid containing plastids), leucoplasts (colorless plastids), and etioplasts (dark-matured precursors of the chloroplast) (Cazzonelli, 2011). Leucoplast is an interesting compartment that mainly accommodates colorless carotenoids known as phytoene and phytofluone. In the carotenoid kingdom, these two carotenoids are the key precursors of all others and are widely distributed in plants. However, due to their colorless nature, few researchers study them (Martínez-Hernández et al., 2015). The popularized carotenoids contain color and are well distributed in the chromoplasts, either in the form of free or esterified fatty acids. The esterification process aids the carotenoid storage and facilitates integration within the lipid-rich plastoglobules during storage (Saini, Nile, & Park, 2015). Higher plant usually contains similar carotenoids, however their distribution differs quantitatively. Table 8.2 lists a few examples of carotenoids distributed in different parts of plants, regardless of the fact that their distribution is dependent on other external factors. Factors like stage of maturity, climate, cultivar, and farming practices contribute to the discrepancy of carotenoid quantity in plants (Prado, Veggi, & Meireles, 2014).

Table 8.1 Physicochemical Properties of Main Carotenoids Found in Human Plasma

Carotenoid	Chemical Formula	MW (g/mol)	Chemical Structure	Solvent	W/length absorbed, λ_{max} (nm)	Light reflected	Polarity	Characteristics
Lycopene	$C_{40}H_{56}$	536.87		Chloroform Acetone Ethanol Petroleum ether Hexane Tetrahydrofuran	458,484,518 448,474,505 446,472,503 421,445,474 444,470,500 451,478,511	Red	Polarity increases ↓	• Carotenes • Primary carotenoid • Acyclic
β-Carotene	$C_{40}H_{56}$	536.87		Chloroform Acetone Ethanol Hexane	435,461,485 429,452,478 425,450, 478 425,470,477	Orange		• Isomer of α-carotene • Carotenes • Primary carotenoid • Bicyclic
α-Carotene	$C_{40}H_{56}$	536.87		Chloroform Acetone Ethanol Hexane	433,457,484 424,448, 478 423,444,473 422,445473	Orange		• Isomer of β-carotene • Carotenes • Primary carotenoid • Bicyclic
β-Cryptoxanthin	$C_{40}H_{56}O$	552.85		Chloroform Ethanol Petroleum ether	435,459,485 428,450,478 425,449,476	Orange		• Xanthophyll • Secondary carotenoid • Bicyclic
Zeaxanthin	$C_{40}H_{56}O_2$	568.87		Chloroform Acetone Ethanol Petroleum ether	433,452,479 430,452,479 428,450,478 424,449,476	Yellow		• Isomer of lutein • Xanthophyll • Secondary carotenoid • Bicyclic
Lutein	$C_{40}H_{56}O_2$	568.87		Chloroform Ethanol Petroleum ether	435,458,485 422,445,475 421,445,474	Yellow		• Isomer of zeaxanthin • Xanthophyll • Secondary carotenoid • Bicyclic

MW, molecular weight.

Table 8.2 Various Carotenoids at Different Parts of Plants

Part of Plant	Botanical Name	Common Name	Carotenoid	References
Root	*Daucus carota* L.	Carrot	α-carotene, β-carotene	Fiskselova et al. (1997)
	Ipomoea batatas	Sweet potato	β-carotene	Islam et al. (2016)
	Dioscorea spp.	Yam	α-carotene, β-carotene, lutein, zeaxanthin	Ukom et al. (2014)
	Xanthosama maffa	Cocoyam	α-carotene, β-carotene, lutein, zeaxanthin	Ukom et al. (2014)
Stem	*Cuscuta australis*	Dodder	α-carotene, β-carotene, lutein	Baccarini, Bertossi, and Bagni (1964)
	Allium fistulosum L.	Onion	β-carotene, lutein	Kopsell et al. (2010)
Leaf	*Spinacia oleracea*	Spinach	Lutein, β-carotene	Larsen and Christensen (2005)
	Lactuca sativa	Lettuce	Lutein, β-carotene	Larsen and Christensen (2005)
	Brassica oleracea var. *sabellica*	Kale	Lutein, β-carotene	Khachik, Steck, and Pfander (1999)
	Rosmarinus officinalis	Rosemary	β-carotene	Labban, Mustafa, and Ibrahim (2014)
Flower	*Crocus sativus* L.	Saffron	Lycopene, α-carotene, β-carotene, zeaxanthin	Heydari and Haghayegh (2014)
	Rosa sempervirens L.	Rose	β-carotene	Ghazghazi et al. (2012)
	Canna indica	Canna	β-carotene	Srivastava and Vankar (2015)
	Dendranthema grandiflorum Ramat.	Chrysanthemum	α-carotene, β-carotene, lutein,β-cryptoxanthin, zeaxanthin	Dendranthema et al. (2015)
	Tagetes ereectal	Marigold	Lutein	Zhang et al. (2011)
Fruit	*Solanum lycopersicum*	Tomato	Lycopene, β-carotene	Cucu et al. (2012)
	Curcurbita moschata	Pumpkin	α-carotene, β-carotene, lutein, lycopene, β-cryptoxanthin, zeaxanthin	Seo et al. (2005)
	Citrullus lanatus	Watermelon	Lycopene, β-carotene	Seifi et al. (2013)
	Carica papaya	Papaya	Lycopene	Seifi et al. (2013)
	Malpighia emarginata	Acerola	β-carotene, lutein, β-cryptoxanthin	Mezadri, Perez-Gelvez, and Hornero-Mendez (2004)
	Actinida sp.	Kiwi	Lutein, β-carotene	Ampomah-Dwamena et al. (2009)
	Capsicum annuum	Red capsicum/ paprika	β-carotene, zeaxanthin, β-cryptoxanthin	Mortensen (2006)
	Citrus sinensis	Orange	α-carotene, β-carotene, lutein, β-cryptoxanthin,	Dias, Camões, and Oliveira 2009)

Table 8.2 Various Carotenoids at Different Parts of Plants *Continued*

Part of Plant	Botanical Name	Common Name	Carotenoid	References
	Pyrus communis L.	Pear	β-carotene, lutein, β-cryptoxanthin,	Dias et al. (2009)
	Prunus persica L.	Peach	α-carotene, β-carotene, lutein, β-cryptoxanthin	Dias et al. (2009)
	Malus domestica	Apple	α-carotene, β-carotene, lutein, β-cryptoxanthin	Dias et al. (2009)
	Prunus avium	Cherry	α-carotene, β-carotene, lutein, β-cryptoxanthin	Dias et al. (2009)
	Diospyros virginiana	Persimmon	α-carotene, β-carotene, lycopene, lutein, β-cryptoxanthin	Zhou et al. (2011)
Seed/ Grain	*Oryza sativa*	Black rice	α-carotene, β-carotene	Nakornriab, Sriseadka, and Wongpornchai (2008)
	Phalaris canariensis L.	Glabrous canary seed	β-carotene	Mellado-Ortega & Hornero-Méndez (2015)
	Zea mays	Corn	α-carotene, β-carotene, lutein, β-cryptoxanthin, zeaxanthin	Rios et al. (2014)

8.2.4 AGROINDUSTRIAL WASTE AS AN EMERGING SOURCE FOR CAROTENOIDS

Mass-scale production of food crops on farms and in factories generates large amounts of by-products during processing. Furthermore, disposal of these by-products creates another hurdle as plant materials are susceptible to microbial spoilage, which is further aggravated by legal restrictions. In addition, drying, storage, or shipment of these wastes are costly. Worldwide, about 5.6 million tonnes of fruits and vegetables waste are discarded annually due to deficient storage and processing facilities (Shilpi, Shivhare, & Basu, 2013). The so-called green technology is an efficient, inexpensive, and environmentally friendly way to handle agroindustrial wastes through reutilization. Food wastes are traces of high organic load that cannot be introduced into the food chain such as seeds, skin, peel, and pomace. Indeed, this food waste can be reutilized for the functional compounds including carotenoids and further developed as marketable products (Galanakis, 2012).

8.3 FUNCTIONAL AND TECHNOLOGICAL PROPERTIES OF CAROTENOIDS

Regular human cellular metabolism continuously generates free radicals; almost 95% of them are used for body metabolism and 5% of oxygen is converted as reactive oxygen species (ROS). The latter damages the major molecules in the body including DNA, protein, carbohydrate, and lipid

(Kowluru & Mishra, 2015). The damaged macromolecules cause dysfunction or alteration of various stress−response genes, which initiates further formation of ROS, e.g., hydroxyls radicals or reactive nonradical compounds like singlet oxygen and hydrogen peroxide (Goltz et al., 2013). At this point, the antioxidant effects from the ingested carotenoid significantly neutralize the ROS, which further demolishes the oxidative stress in the body.

8.3.1 ANTIOXIDANT AND PROOXIDANT EFFECTS OF CAROTENOIDS

Color pigments eventually fade or are bleached when exposed to oxidizing species or radicals. When carotenoids interact with ROS such as singlet oxygen (O_2), direct energy transfer between both molecules will occur during physical quenching. Subsequent to the energy transfer, the radical species turns from an excited state to a ground state oxygen status. Furthermore, the carotenoid species receives the energy and turns to triple excited carotene. The gained energy will be dissipated to the solvent and the carotenoid returns to the ground state (Stahl & Sies, 2003). The antioxidant mechanism of carotenoids with ROS can occur in at least three possible ways: (1) adduct formation, (2) electron transfer system, and (3) allylic hydrogen abstraction. The concept of prooxidant was postulated when a reverse effect is shown in the antioxidant activity of carotenoids. This effect was first proven by Burton and Ingold in 1984 who reported that at a low partial pressure of oxygen (pO_2), β-carotene acts as a chain-breaking antioxidant, while at higher pO_2, the carotenoid underwent autooxidation and loses its antioxidant potency (prooxidant behavior; Young & Lowe, 2001). Concomitantly, a number of studies have reported the adverse effect of increasing lung cancer among smokers due to the consumption of β-carotene supplements (Gallicchio et al., 2008; Tanvetyanon & Bepler, 2008). There is no standard dose of β-carotene that increases tumor progression in lungs as factors such as age, serum cholesterol level, food intake, and plasma response vary among individuals. For instance, 40 mg of β-carotene intake (7 years) by nonsmoking women reduced the plasma response in blood, while in another study 50 mg of β-carotene among cancer patients showed no effect on tumor progression for several years (Goralczyk, 2009). Additionally, high doses of carotenes such as lycopene and β-carotene in plasma concentration have been shown to result in skin diseases known as lycopenodermia and carotenodermia, which cause deep orange or yellow patches on the skin (Singh et al., 2015). The antioxidant and prooxidant reactions of carotenoids are discussed in detail by Kong et al. (2010). Fig. 8.2 summarizes both the antioxidant and prooxidant behaviors of carotenoids.

8.3.2 PROVITAMIN A ACTIVITIES

Provitamin A refers to the carotenoids that contain one or two beta-ionone rings at the end of their structure such as α-carotene, β-carotene, and β-cryptoxanthin. Through usual metabolic processes, this group of carotenoids is converted into vitamin A. The conversion to vitamin A involves the action of a cleavage enzyme known as mono or dioxygenase, which cleaves the double bonds in the polyene chain into two molecules of retinal. They are then further metabolized to derivatives like retinal and retinoic acid, which promote body growth, visual function, differentiation of epithelial tissue, and also embryonic development for pregnant mothers (Handelman, 2001). Nevertheless, the human need for vitamin A is modest (200 μg/d for a healthy adult), and a higher

Adduct formation: Antioxidant effect

$CAR + R^{\bullet} \longrightarrow R\text{-}CAR^{\bullet}$

The free radical (ROO●) add at any place of carotenoid forming resonance stabilized carbon-centered peroxyl radicals.

Adduct formation: Prooxidant effect

(i) $CAR + ROO^{\bullet} \longleftrightarrow ROO\text{-}CAR^{\bullet}$

At high oxygen concentration, (ROO-Car•) act reversibly due to oxygen tension

(ii) $ROO^{-} CAR^{\bullet} + O_2 \longrightarrow ROO\text{-}CAR\text{-}OO^{\bullet}$

With further oxygen presence, additional radicals will be generated by cleaving the peroxyl bond. The generated radical will act as prooxidant or initiate lipid peroxidation by acting with lipid, RH, shown in (iii). As an effect of lipid peroxidation, another peroxyl will be formed with oxygen as shown in (iv).

(i) $ROO^{-} CAR^{+} O_2 \longrightarrow ROO\text{-}CAR\text{-}OO^{\bullet}$
(ii) $R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$

But there are possibilities for the peroxyl radical to be terminated due to occurrence of another peroxyl radical, thus forming inactive product as shown below:

(vi) $ROO^{-} CAR + O_2 \longrightarrow$ inactive products

Electron Transfer System: Antioxidant effect

Exposure to smoking, generates NO_2., an environmental pollutant or powerful oxidant like trichloromethylperoxyl CCl_3O_2. leads to the formation of carotenoid cation radical ($CAR^{+\cdot}$), or anion radical ($CAR^{-\cdot}$) or an alkyl radical ($CAR^{\cdot}$)

$NO_2^{\bullet} + CAR \longrightarrow NO_2 + CAR +\bullet$

$CCl_3O_2^{\bullet} + CAR \longrightarrow [CCl_3O_2\text{-}CAR]^{\bullet} \longrightarrow CCl_3O_2 + CAR +\bullet$

Meanwhile, reaction of carotenoid with superoxide radical (O2.-) forms anion radical as shown below:

$CAR + O_2\text{-}^{\bullet} \longleftrightarrow CAR\text{-}^{\bullet} + O_2$

Allylic H abstraction: Antioxidant effect

Reaction of carotenoid as hydrogen donor reduces the radical of the compound.

$CAR + ROO^{\bullet} \longrightarrow CAR^{\bullet} + ROOH$

FIGURE 8.2

Antioxidant and prooxidant behavior of carotenoids.

dose of vitamin A is toxic. It was reported that about 80% of low-income nations are dependent on provitamin A carotenoids as a supplement for vitamin A (Stahl & Sies, 2003).

8.3.3 OTHER BIOLOGICAL ROLES OF CAROTENOIDS

The great diversity of carotenoids is not confined to plants but also includes human systems. Carotenoids play versatile biological roles that contribute therapeutic effects to a number of chronic diseases. Various studies have investigated the role of carotenoids to reduce the risk of chronic

diseases. The adverse effects of chemotherapy treatment are numerous. The intake of certain carotenoids can reduce the development of tumor cell. β-carotene, α-carotene, and lycopene are the best singlet oxygen scavengers. Dietary intake shows desirable effects on various types of cancers including prostate breast, colon, and ovarian cancers (Singh et al., 2015). As a fat soluble pigment, lycopene is largely accumulated in the fatty adipose tissue that surrounds the heart. The presence of lycopene prevents the oxidation of low-density lipoprotein (LDL) in adipose tissue and thus, reduces the myocardial infection. Provitamin A carotenoids and lycopene protect the LDL and prevent the oxidation process from taking place. The deleterious effect of high-dose β-carotene supplements has been shown to not only increase lung cancer but also cardiovascular disease. Thus standard dietary intake is best (Arab & Steck, 2000). Human immunodeficiency virus (HIV) attack causes progression in oxidative stress and ROS, while suppressing antioxidant levels in the body. Hence, deteriorating risk of this virus is evidently seen through antioxidant-rich nutritional supplements particularly carotenoids. According to Lettow et al. (2004), low provitamin A in human serum results in higher loading of HIV in adults with pulmonary tuberculosis than adults with high amounts of carotenoids in plasma. Lutein and zeaxanthin are the two carotenoids widely pronounced for eye-related diseases such as cataract and age-macular disease. For skin, photooxidative damage is known to affect cellular lipid, DNA, and protein, which eventually causes skin aging, formation of photodermatoses, and skin cancer. Carotenoids with the best scavenging ability such as lycopene efficiently reduce skin damage (Stahl & Sies, 2005). Bone homeostasis is regulated by bone-resorbing osteoclasts and bone-forming osteoblasts. A recent study by Ozaki et al. (2015) showed that appropriate intake of carotenoid-rich food (particularly β-cryptoxanthin) maintains bone health and minimizes the risk of osteoporosis by inactivating bone-resorbing osteoclast. Other biological roles such as immune enhancement, gap-junction communication, cell−cell signaling, antiinflammatory effects, and interaction with growth and sex hormones are the biochemical mechanisms underlying the disease prevention of carotenoids. In plants, carotenoids provide photoprotection from excess light by scavenging singlet oxygen and ROS during photosynthesis. Fig. 8.3 summarizes the biological functions of carotenoids and their role in the prevention of some diseases.

8.4 NUTRITION VALUES, HEALTH BENEFITS, AND CLINICAL EVIDENCE

8.4.1 ALPHA-CAROTENE AND BETA-CAROTENE

Both α-carotene and β-carotene are primarily regarded as precursors of vitamin A. Common orange-, yellow-, and green-colored fruits and vegetables such as carrot, pumpkin, apricot, sweet potato, and beans are rich sources of these carotenoids. The conversion efficacy of β-carotene to vitamin A (retinol) is theoretically higher than α-carotene and β-cryptoxanthin. For instance, one mole of β-carotene yields two moles of retinol, while the other two provitamin A carotenoids are only half as active as β-carotene (Tang, 2014). Due to the high percentage of distribution of β-carotene in plant sources (e.g., palm oil; β-carotene: 60% while α-carotene: 30%) (Murakoshi et al., 1992), the coexistence of α-carotene and its medicinal values are often less pronounced.

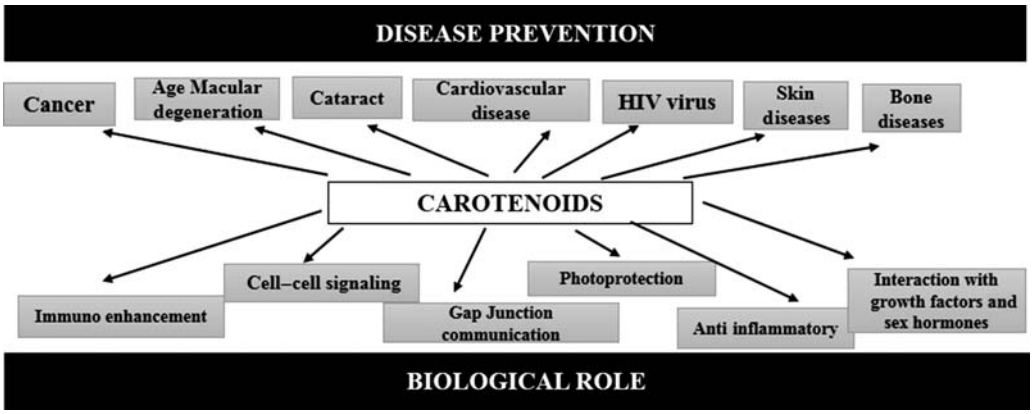


FIGURE 8.3

Summary of general biological functions of carotenoids.

Modified and redrawn from Rao, A.V & Rao, L.G. (2007). Carotenoids and human health. Pharmacological Research, 55(3), 207–216.

In addition to their therapeutic role as vitamin A precursors, these carotenoids are positively related to the prevention of several cancers including skin and breast cancers. Considering its high scavenging potency, β-carotene has been evaluated for its potential to minimize the risk of lung cancer in a few clinical studies. Unlike other cancers, β-carotene was found to have an adverse effect on lung cancer by increasing the risk of cancer several folds. Cigarette smoking causes high oxidative stress in the lungs, which triggers the prooxidant behavior of β-carotene (Gallicchio et al., 2008). In other studies, the proliferation of human malignant tumor cells was more effectively suppressed by α-carotene than by β-carotene (Gallicchio et al., 2008; Murakoshi et al., 1992). Additional functions including the ability to stimulate cell communication and immune system enhancement have also been reported for these carotenoids (Singh et al., 2015).

8.4.2 LYCOPENE

Lycopene can be found abundantly in tomatoes, pink guava, watermelon, pink grapefruit, and papaya. The distribution of lycopene in these fruits is masked by green chlorophyll in the initial stage. As the chlorophyll degrades, the pigment changes from green to white until the concentration of chlorophyll is reduced. Upon chlorophyll reduction, changes in ultrastructure occur due to the biosynthesis of lycopene, causing pigment coloration to change from white to red (Shi & Maguer, 2000). A number of in vitro, in vivo, and ex vivo studies have been carried out to investigate the effects of lycopene against oxidative stress. It has been found that high consumption of lycopene can significantly reduce the risk of cancers including breast, liver, cervical, prostate, and ovarian cancers (Singh et al., 2015). Among a number of cancer studies, lycopene has been shown to play

a much-established role in the prevention of prostate cancer. Another study found that men with the highest content of lycopene in adipose tissue had 48% less chance of developing cardiovascular-related diseases than those with low lycopene content in blood (Giovannucci et al., 1995). Accumulation of free radicals through ultraviolet (UV) radiation causes lipid oxidation at skin level, thus forming wrinkles. Skin oxidative damage is prevented when lycopene is applied. In fact, clinical evidence shows that lycopene is able to protect skin from sunburn due to UV-light damage (Camara et al., 2013). Additionally, lycopene promotes gap-junctional communication between cells and triggers the synthesis of connexin-43 (Shi & Maguer, 2000). Finally, lycopene-incorporated products are widely distributed in various forms as tablets, capsules, softgels, powders, and drinks.

8.4.3 β-CRYPTOXANTHIN

Apart from the alpha and beta carotenes, β-cryptoxanthin is also grouped as a provitamin A carotenoid. Citrus fruits such as oranges, mangoes, papaya, and peaches have been found to contain β-cryptoxanthin in excess. Due to the domination of other provitamin A carotenoids, the health benefits of this pigment are scarcely reported. However, β-cryptoxanthin has the capacity to act as therapeutic agent, particularly for bone-related diseases. Few clinical studies have concluded that β-cryptoxanthin intake increases calcium content and alkaline phosphatase activity in cortical bone and metaphyseal tissues in vitro, leading to a stimulatory effect on bone resorption, which eventually minimizes the risk of osteoporosis (Yamaguchi, 2008). Besides being recognized as a provitamin A precursor, this pigment has also been found to promote cell-to-cell communication and to enhance immune system and anticarcinogenic promoter functions (Burri, 2015). Oxaliplatin (third-generation platinum) combined with drugs like leucovorin is a chemotherapy-based treatment, which results in many undesirable side effects for colorectal cancer patients. When β-cryptoxanthin is combined with oxaliplatin, similar growth inhibition of colon cells with reduced dose of oxaliplatin was observed, thus minimizing the associated side effects of treatment (Milan et al., 2015). Commercial β-cryptoxanthin products are marketed as tablets, capsules, and soft-gel combinations with other carotenoid supplements. In order to ensure uniform water distribution of β-cryptoxanthin solubilized in oil, there is also a patented formula with the addition of emulsifier (optimum: polyglyceric fatty acid ester) in the mixture (Takahashi, 2010).

8.4.4 LUTEIN AND ZEAXANTHIN

Lutein and zeaxanthin are mainly found in leafy green vegetables (e.g., spinach, kale, and broccoli) maize, persimmons, etc. These carotenoids primarily serve as antioxidants by protecting plants from photoinduced free radical damages. In the visible light spectrum, blue light is much prone to induce photo-oxidative damage by generating ROS (Alves-Rodrigues & Shao, 2004). Hence, oxidative stress happens much frequently in the eyes than the skin. Thus these stereoisomers are widely known to promote healthy vision including age-related macular degeneration and eye cataracts. Lutein is dispersed all over the retina while zeaxanthin is accumulated at the central macula. Lutein and zeaxanthin have the ability to increase macular pigmentation of ocular tissues, which prevents

damaging blue light from entering the eye lens, thus reducing tissue damage in the eyes (Boon et al., 2010). Both isomers have also been found to be effective against light-induced skin damage, and when combined, they lower the risk of coronary heart disease, stroke, and breast cancer.

Table 8.3 shows a few examples of studies involving human or animal models to evaluate the intake of carotenoids to treat a number of chronic diseases. This epidemiological evidence involves cancer, cardiovascular, diabetes, macular degeneration, and bone-related diseases.

8.5 STABILITY, BIOAVAILABILITY, AND BIOACCESSIBILITY OF CAROTENOIDS

8.5.1 STABILITY OF CAROTENOIDS

The first-class carotenoid species are highly unsaturated and occur in all-trans-configuration. They have a higher tendency to undergo isomerization (from E-configuration to Z-configuration) and oxidation either in the form of enzymatic oxidation or autoxidation (atmospheric influences). Factors such as heat, light, oxygen, and dehydration cause bleaching or loss of a compound's original color. In addition, various experimental studies have shown that metal ions, enzyme activities, pH, and the presence of oxidants or reducing agents affect structural integrity (Boon et al., 2010). Many reports have noted that the stability of carotenoids is largely affected during three different stages: postharvesting, food processing, and storage. However, detailed studies reporting the influence of each factor on individual carotenoids and its comprehensible mechanism have scarcely been reported.

8.5.1.1 Effect of pre- and postharvesting on carotenoid stability

The biosynthesis of carotenoids in plants is dependent on several factors including (1) cultivars, (2) stage of maturity, (3) climatic effects, (4) farming practice, and (5) cleaning and packaging. Due to these factors, the carotenoid concentration in fruits and vegetables varies significantly (Rita et al., 2005). For instance, 12 different cultivars of potato tubers were reported to contain lutein in the range of 0.16−4.66 mg/kg and 0.04−0.62 mg/kg for β-carotene (Hamouz, Pazderu, Lachman, & Kotikova, 2016). The maturation or ripening process of fruits and vegetables is significantly influenced by carotenogenesis, the biosynthesis of carotenoids. For example, "bitter berries" with different ripening degree (A, B, C) contain β-carotene in the range of 0.02−0.16 mg/100 g, but α-carotene (0.15 mg/100 g) and lycopene (1.84 mg/100 g) were only detected at the highly ripened stage (Dri et al., 2010). In 2009, Cardosso et al. investigated the carotenoid content of six different leafy vegetables harvested in spring and winter. Interestingly, it was discovered that plants harvested in spring contained higher amounts of α- and β-carotene than plants harvest during the winter season. As for farming practices, Lopet et al. (2007) investigated the effect of three different agricultural practices known as conventional, integrated, and organic practice on red sweet peppers. According to this finding, organic farming practice resulted in higher content of total carotenoids like β-carotene, β-cryptoxanthin, and zeaxanthin. The last factor was investigated by Hussein et al. (2000), who have tried five different packaging style on broccoli (nonsealed bag, pillow-packed bag, sliced and pillow packed, sliced and squeezed packed, and lastly sliced and vacuum packed).

Table 8.3 Clinical Evidence of Carotenoids Effect on Chronic Diseases

Disease		Subject	Experimented Carotenoid	Effective Dose	Outcome	References
Cancer	Lung	Smokers and nonsmokers	α-carotene, β-carotene, lutein, lycopene, β-cryptoxanthin, zeaxanthin	NA	• β-carotene increases the risk of lung cancer • Inverse association of lung cancer risk was shown by other carotenoids in small intake except lutein	Gallicchio et al. (2008)
	Prostate	Human males	Lycopene	10131−1115012 μg/d	• Lycopene inhibits angiogenesis of prostate cancer cells by regulating vascular endothelial growth factor	Zu et al. (2014)
	Breast	Human females	α-carotene, β-carotene, lutein, lycopene, β-cryptoxanthin, zeaxanthin	α-carotene: 672 μg/d;β-carotene: 6004 μg/d	• α-carotene, β-carotene significantly reduces 9% risk of breast cancer • No significant association of lutein, lycopene, β-cryptoxanthin, zeaxanthin with breast cancer	Wang et al. (2014)
		Breast mammary cancer/carcinoma/ neoplasm cell	α-carotene, β-carotene, lutein, lycopene, β-cryptoxanthin, zeaxanthin	α-carotene: 300−2000 μg/d;β-carotene: 1500−7000 μg/d	• α-carotene significantly reduces the risk of breast cancer • β-carotene reduces the risk of breast cancer with marginal significance	Hu et al. (2012)
	Colorectal	Humans aged more than 50 years old	α-carotene, β-carotene, lutein, lycopene, β-cryptoxanthin, zeaxanthin	Lutein: 300 μg/d	• Lutein was the only carotenoid that was inversely related to the risk of tumor growth • β-carotene slightly increases the risk of colon tumors	Slattery et al. (2000)
	Ovarian	Human females with menopausal status	α-carotene, β-carotene, lutein, lycopene, β-cryptoxanthin	α-carotene: 308−1378 μg/dLycopene: 4743−15262 μg/d	• Lycopene strongly reduces the risk for premenopausal female • Only provitamin A carotenoids show inverse relationship with cancer risk	Cramer et al. (2001)
Coronary heart disease		Humans aged 61−82 years old	α-carotene, β-carotene, lutein, lycopene,	Content in plasma-lutein: 0.24−0.29 μmol/	• Lutein and zeaxanthin decreased the risk of atrial fibrillation • No significant association of lycopene, β-cryptoxanthin,	(Karppi et al., 2012)

		β-cryptoxanthin, zeaxanthin	LZeaxanthin:0.035−0.02 μmol/L	α-carotene, β-carotene with atrial fibrillation	
	Sparague−Dawley rats with body weight of 250−300 g	Lycopene	0.088 mg/d	• Lycopene lowers the oxidation of LDL-cholesterol which accounted to lower the risk of cardiovascular • Lycopene doesn't possess any cardioprotective role	Das et al. (2005)
Macular degeneration	Wistar rats (80−100 g body weight)-eye lens	Lycopene	200 μg/kg of body weight	• In presence of lycopene, there is a restoration in reduced glutathione level in the treated lenses • Lycopene reduces opacification and 80% of lenses remain clear	(Gupta et al., 2003)
	Humans aged 50−90 years old	α-carotene, β-carotene, lutein, lycopene, β-cryptoxanthin, zeaxanthin	Lutein/zeaxanthin: 1657−5468 μg/d	• Lutein and zeaxanthin from macular pigments reduce oxidative stress by absorbing blue light and stabilizes cell membranes • Other nonmacular pigments protect tissue against light-induced oxidative damages and free radicals	Wu et al. (2015)
Diabetes	Wistar rats (150−250 g body weight; 2 months aged)	Lycopene	4 mg/kg of body weight	• Lycopene decreases oxidative stress caused by free radicals which cause dysfunction of pancreatic β cells	Aydin and ÇelİK (2012)
Bone related disease	Male with synovitis that affects more than 2 bone joints (polyarthritis)	β-carotene, lutein, lycopene, β-cryptoxanthin, zeaxanthin	β-cryptoxanthin: 56−365 μg/d	• β-cryptoxanthin intake reduces inflammatory polyarthritis • Supplementation with β-carotene doesn't reduce the inflammatory activity	Pattison et al. (2005)

In their findings, the authors noted that β-carotene content could only be detected in fresh- and pillow-packed samples but not in the vacuum packaging. Naturally carotenoids are protected in the plant tissue. When harvesting, the cutting, chopping, and shredding processes increase exposure of carotenoids to oxygen, sunlight, and enzymes, which catalyze the oxidation. In this stage, the stability of carotenoids is also modified due to poor temperature management and relative humidity during harvesting. After harvesting, delayed cooling process of the plant parts might lead to alteration in chemical compositions. For instance, a 24-h delayed cooling process of tomatoes resulted in a 5−12% loss in ascorbic-acid composition (Lee & Kader, 2000). A similar effect can be expected in the lycopene concentration of fruit as well. Furthermore, long-term exposure of the harvested product under sunlight could degrade carotenoid content in plants (photodegradation). The stability of carotenoids from *Russula alutacea* was found to deteriorate drastically when exposed to direct light for 7 continuous days (Zhao et al., 2014).

8.5.1.2 Effects of processing and storage on carotenoid stability

The food-processing stage exerts diverse effects on the bioavailability and bioaccessibility of carotenoids. Thermal operations like cooking, blanching, frying, drying, pasteurization, and dehydration of fruits and vegetables are commonly applied in industrial practices (Martínez-Hernández et al., 2015). Initially, thermal treatments were accepted due to their efficiency in deactivating microbial activity due to heat stress, but it was found that the nutritional value, functional properties, and sensory qualities of the processed food were influenced greatly by the high temperature (Rodríguez-Roque et al., 2015). Even common operations such as cutting, chopping, slicing, or peeling can cause loss in cellular integrity. When the cell is no longer intact, modification of cell enzymatic activity and respiration will take place (Gonzalez & Barrett, 2010). A comparison study of thermally (65−95°C) and nonthermally treated tomatoes and carrots revealed that the nonthermally treated plant samples contained higher amounts of carotenoid concentrations than the thermally treated samples (Palmero et al., 2014). Another study compared the carotenoid content in raw, canned, and home-processed tomatoes (blanching in hot boiling water) to illustrate the different effects of heating. Heating caused partial degradation of carotenoid pigments and promoted isomerization of β-carotene and lycopene content in the samples (D'evoli, Lombardi-Boccia, & Lucarini, 2013). Storage conditions also play an essential role in preserving the carotenoid content of processed food. Factors like exclusion of oxygen (vacuum packaging), protection from light (packaged in dark containers/plastic), and storage at low temperature prevents carotenoid decomposition. In normal storage methods, the processed food is maintained at ambient or reduced temperature, which minimizes the degrading potency of the carotenoids. However, long-term storage can result in great losses of carotenoid compounds. This was analogous to another finding that highlighted that long-term storage (12 months) of refrigerated tomato juice depleted more than 35% of lycopene content in the extract (Jayathunge et al., 2015).

8.5.2 BIOAVAILABILITY AND BIOACCESSIBILITY

Although few reports note reduction in carotenoid content during the three processing stages, the isomerization and oxidation process show reversible effects on the bioavailability of carotenoids. For instance, cis-lycopene demonstrated higher bioavailability than trans-lycopene because

cis-isomers solubilize easily in bile-acid micelles (Lin, 2005). Carotenoids absorbed from the human diet are acquired using two different approaches:

i. intake of the integral part of fruits and vegetables in foods, and
ii. formulated products in the form of capsules or tablets after their extraction and purification from fruits or vegetables (Liu et al., 2015).

Carotenoid ingestion through the intake of raw fruits and vegetables are recommended due to the absence of synthetic formulation. However, ingested lipophilic carotenoids from raw plant material were found to exhibit poor bioavailability, which reduces the functional value of the compounds. Factors such as restricted release of carotenoids from the plant matrix, low solubility of carotenoids in the gastrointestinal fluid, and low permeability across intestinal epithelial cells or transformation of either enzymatic or chemical reaction that occur within the gastrointestinal tract drastically affect the oral bioavailability (McClements & Xiao, 2014). In this context, bioavailability refers to the "fraction of ingested carotenoid that can be utilized by body for physiological functions and storage." In another source, the term bioaccessibility is defined as the fraction of a carotenoid that is released to the gastrointestinal tract for the absorption in intestine. Therefore bioaccessibility is a part of bioavailability. Absorption of carotenoids actively occurs in the small intestine of the body. However, there is no animal model that completely mimics human absorption and metabolism of carotenoids. Hence, for experimental purposes, in vitro digestion models are built to evaluate the digestion and assimilation processes that modulate carotenoid bioaccessibility and bioavailability (Carilho et al., 2014). Four essential steps are necessary for effective carotenoid absorption:

1. carotenoid release from the food matrix,
2. incorporation of carotenoids into bile-salt micelles,
3. absorption by epithelial cells, and
4. incorporation into the chylomicrons with secretion into the lymphatic system.

In vitro models are designed to mimic the digestion process occurring in the gastrointestinal tract. Compared to the human gastrointestinal tract, the designed model does not consider muscle contraction, which provides mechanical forces and fluid motions to initiate breakdown of food, chemical digestion, absorption, and transport to the body (Fernández-García et al., 2012). There have been numerous discussions on the factors that influence the bioaccessibility and bioavailability of carotenoids in literature. Detailed explanations on the carotenoid absorption process in the human body are reported in Hof et al. (2000). Fig. 8.4 illustrates the combination of both the absorption process in the human body and the associated factors, which affect the bioavailability and bioaccessibility of carotenoids.

8.6 FOOD-PROCESSING TECHNOLOGIES FOR CAROTENOID STABILITY AND BIOACCESSIBILITY

Carotenoid bioaccessibility can be extended by eliminating chances for possible physical or chemical degradations within food or even in the gastrointestinal tract after ingestion. Since ancient times, common practices such as fermentation, drying under sunlight, or salting processes have

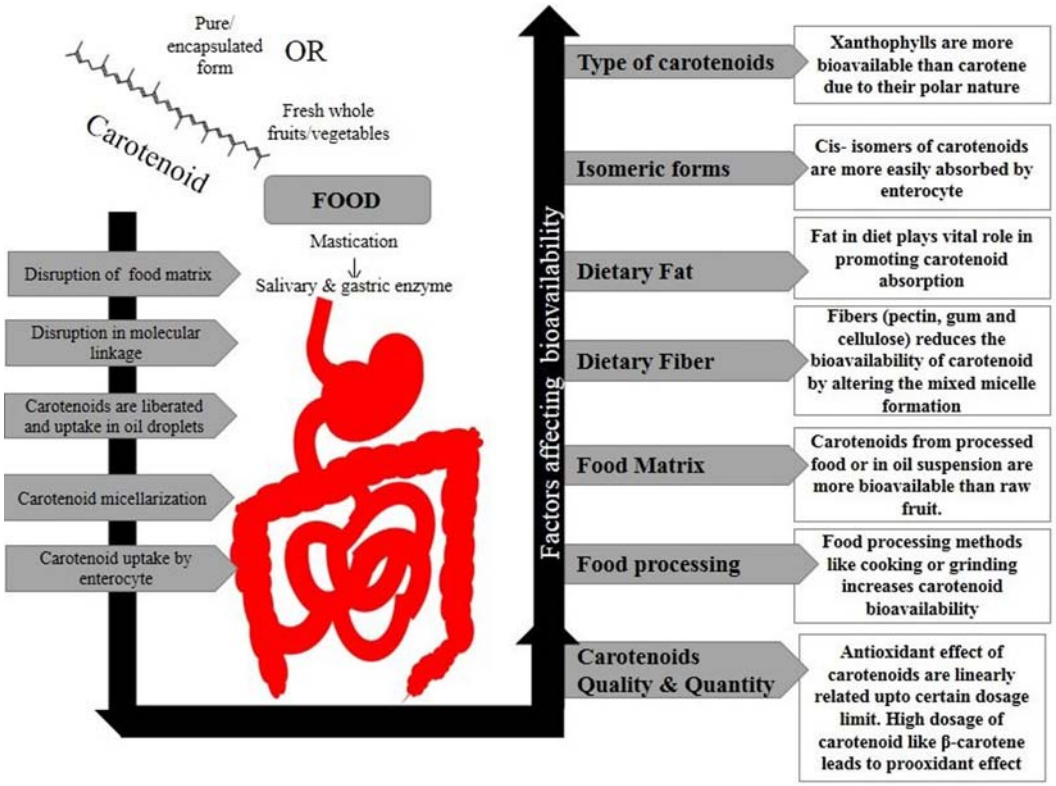


FIGURE 8.4

Carotenoids absorption in the human body and factors that affect the bioavailability of carotenoids.

Modified and redrawn from Hof, V.H., et al. (2000). Dietary factors that affect the bioavailability of carotenoids. The Journal of Nutrition, 103*(3), 503–506.*

been widely applied to preserve food material. In industrial practice, bulky food-processing technology is often linked to carotenoid release from the plant matrix while preserving its stability. Thermal food processes such as boiling, cooking, blanching, frying, and drying are mostly used to soften the plant cell wall while inactivating the microorganism activity that reduces the shelf-life of food products. However, the heat stresses from thermal processes induce isomerization, oxidation, and degradation, thus affecting the stability and bioavailability of carotenoids in food products (Martínez-Hernández et al., 2015). In order to counteract the increased consumer demand for highly nutritious food with fresh like sensory, various studies have been conducted to introduce alternate food-processing technologies that eliminate heat stress. The emergence of various innovative non-thermal technologies underlying high pressure or emulsion principles have avoided deleterious heat effects on nutrients and organoleptic properties of food.

Fig. 8.5 illustrates the available and potential food-processing technologies that can be used to increase carotenoid bioaccessibility.

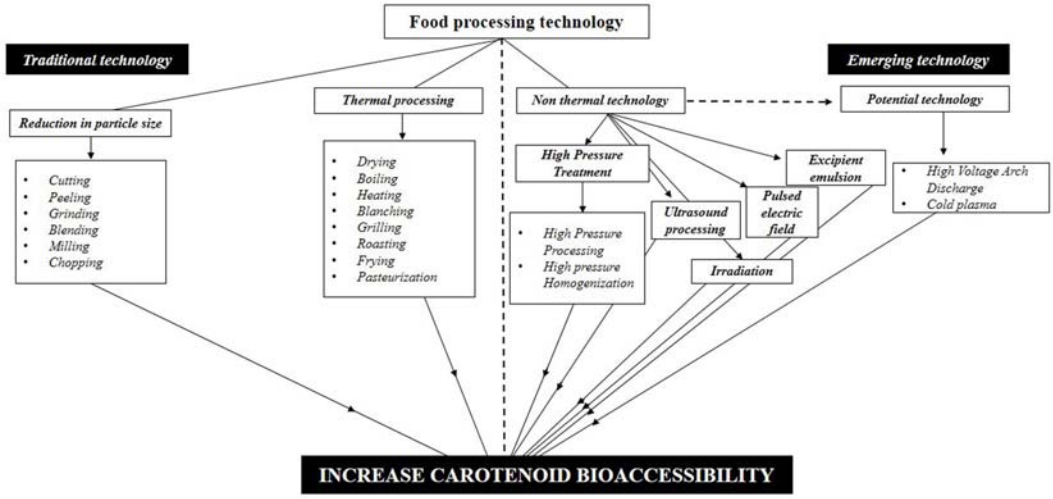


FIGURE 8.5

Available and potential food-processing technologies to increase carotenoid bioaccessibility.

8.6.1 EMERGING TECHNOLOGIES

8.6.1.1 High-pressure processing

High-pressure processing (HPP) is also called as high hydrostatic pressure processing since water is used as the main medium to transmit pressure. High hydrostatic pressure improves the mass transfer rate of carotenoids by increasing the plant-cell permeability, which eventually allows diffusion of the molecules in phase transition (Oroian & Escriche, 2015). Elevated pressures ranging from 100 to 1000 MPa are commonly employed to induce pressure stress on the plant matrix for a short period of time from a few seconds to over 20 minutes. The applied pressure acts instantaneous and uniformly in all directions of the reaction medium and therefore provides equal distribution of pressure to the sample. This method indirectly inactivates vegetative microorganisms by damaging their membranes without affecting the quality of the food in terms of texture, flavor, and color (Martínez-Hernández et al., 2015). Furthermore, this processing method does not require a heating step and hence degradation of thermosensitive biological molecules including carotenoids is not expected. In the fruit-juice industry, HPP is also known as cold pasteurization. When refrigerated smoothies of orange, papaya, melon, and milk were compared to nontreated, thermally pasteurized, and highly pressurized samples (450 MPa and 600 MPa), it was found out that HPP-processed smoothies exhibited an abrupt increase of 25% of total carotenoid content (α-carotene, β-carotene, and lycopene) with slightly noticeable color differences (Andrés, Villanueva, & Tenorio, 2016). Furthermore, the effect of HPP on lycopene extraction from tomato was found to relatively increase the proportion of cis-lycopene, although lycopene is naturally present in all trans-forms. Application of high-pressure (690 MPa) energy induces conformational change in lycopene all-trans isomer. The applied pressure stress causes the extracted lycopene to be much compact and smaller in size which obeys the characteristic of cis-lycopene (Varma, Karwe, & Lee, 2010). The effects of HPP on *Capsicum annuum* L. seedlings were tested for 0.1, 50, 100, 200, and 300 MPa for 5 min. Among the tested pressure, 50 MPa-treated samples yielded a threefold higher amount of

total carotenoid content than the lowest value (Islek, Altuner, & Alpas, 2015). Apart from highlighting the type of food matrix that influences the carotenoid bioaccessibility, this finding further suggests that higher than optimum pressure may increase compressibility on the cell membrane and result in carotenoid destruction. In another study, the storage stability of β-carotene, α-carotene, and lutein from carrot juice were evaluated after HPP treatment. The carotenoid rich extract, in particular β-carotene exhibited reduction of stability by 11.1% after 20 days of storage at 4°C. It was concluded that HPP-treated carrot juice is much more prone to geometrical isomerization and susceptibility to oxidation (Zhang et al., 2015a).

8.6.1.2 High-pressure homogenization

High-pressure homogenization (HPH) technology involves a pressurizing fluid that flows quickly through a narrow-gap valve, which escalates its velocity and results in depressurization with constant cavitation and high shear stress. The plant particles, cells, and macromolecules are dispersed in the fluid, undergoing high mechanical stress, transitory and deformed (Palmero et al. 2015). This technology has been well accepted for its capacity to promote desirable changes in the physical properties of plant matrix such as particle size, pulp sedimentation turbidity, distribution, color, and many more. Conventional HPH reaches pressures up to 40 MPa, but advanced HPH equipment increases the pressure system 10-fold higher than a conventional system (400 MPa). Industrial applications that use HPH are vastly related to produce stable emulsified based products, in the pharmaceutical, nutraceutical, and cosmetic industries (Martínez-Hernández et al., 2015). Various studies have shown that HPH increases carotenoid bioaccessibility. Desmodesmus sp. F51, a highly thermotolerant microalgae, was disrupted using various disruption methods including autoclaving, bead-beating, osmotic shock, sonication, and HPH. Among the tested methods, HPH was found to be the most applicable way to increase carotenoid bioaccessibility, especially for lutein (Xi et al., 2016). The amount of lutein accessed using the HPH method was found to be independent of the cell density of the samples, suggesting uniform pressure distribution across the plant sample. There is a large number of studies evaluating the bioaccessibility of carotenoids from HPH-treated tomatoes. According to a study by Panozzo et al. (2013), HPH reduced lycopene in vitro bioaccessibility in tomatoes, which was a consequence of the pressure applied on the tomato. All-trans lycopene in tomato present as crystalline structure by nature. Intense HPH treatment causes deformation, reduction in size and increase in surface area of fibers present in the sample. The exposed surface area increases the absorption of water and viscosity of the sample. This phenomenon hampers the release of crystalline lycopene. This finding was in agreement with a study by Svelander et al. (2011) who compared the bioaccessibility of carotenoids between carrot and tomato emulsions. For carrot emulsion, HPH treatment significantly increased the micellar incorporation of α-carotene and β-carotene, but HPH-treated tomato emulsion did not affect the in vitro bioaccessibility of lycopene compared to control. Despite HPP (100 Mpa, 1 cycle), the carrot emulsion was observed to be present as clusters of intact cell, while in tomato emulsion, no intact cells were observed at processing condition of 10 MPa for 10 cycles. Poor lycopene solubility in dietary fat emulsion as well as the possibility of pectin release that increases the consistency of tomato pulp during processing could also limit the bioaccessibility of lycopene (Thakur, Singh, & Handa, 1995).

8.6.1.3 Pulsed electric field

Pulsed electric field (PEF) is another innovative technology that enhances the mass transfer rate of carotenoid by reducing cell-membrane integrity by softening the plant tissue, as well as by

influencing the texture and electroporation of the plant (Oroian & Escriche, 2015). This technology involves the application of high-voltage pulses (20−80 kV cm-1) in liquid or semisolid food between two electrodes for a short period of time. PEF was found to be effective against various pathogenic and spoiling food microorganisms and enzymes but preserved the color, flavor, and nutrition properties of carotenoids. PEF treatments also require only short treatment times to inactive microbes at low temperatures that do not affect the quality of food (Martínez-Hernández et al., 2015). High intensified PEF enhances electroporation (the process of breaking pores in plant matrix), which causes a higher impact to loss membrane integrity, inactivation of proteins, and leakage of cellular contents of microorganisms that lead to microbial inactivation (Tiwari, O'donnell, & Cullen, 2009). Most of the research conducted on PEF has mainly been on fruit beverages. For example, Roque et al. (2015) compared the bioaccessibility of carotenoids from fruit beverages in three different emulsions. The carotenoid digestibility was found to be 15% higher in HPH treated-milk emulsion samples than in untreated samples. Similarly, carotenoid accessibility was reported to be higher in PEF-treated carrot pomace samples in sunflower oil at field strength of 0.6 kV/cm and 5 Hz than in the untreated sample (Roohinejad, Everett, & Oey, 2014). When this technology was applied to carotenoid accessibility in microalgae samples (*Chlorella vulgaris*), carotenoid extraction was higher in the PEF-treated samples preincubated for 1 h. In this way, the chloroplast of the plant sample undergoes plasmolysis due to osmolytic disequilibrium, which eases the diffusion of carotenoid pigment in the electric field (Luengo et al., 2015). In both studies, it was noted that lower pulse electric field frequencies result in higher carotenoid accessibility. At lower frequency, there is more time for the sample membrane to be charged between pulses, which initiates higher pore formation for the carotenoid release.

8.6.1.4 Ultrasound processing

This technology is based on the energy generated by sound waves ranging from 20 kHz to 10 MHz, which is known as the "sponge effect." Under critical temperature and pressure, the radical formed during sonication affects the physicochemical of ultrasound treatment results, which leads to loss of food quality such as off-flavors and degradation of compounds. Apart from food processing ultrasound applications are also versatile in extraction, emulsification, and homogenization (Pingret, Fabiano-Tixier, & Chemat, 2013). Ultrasonic processing was shown to reduce the in vitro bioaccessibility of lycopene compared to untreated samples. The sonication energy causes rapid compression and expansion of plant cells, which results in bubble formation around the sample. At this point, tomato pulp is partially deesterified and the pectin molecules (an integral part of the plant) are released, which eventually leads to formation of gel-like substances due to hydrogen and hydrophobic interactions (Anese et al., 2015). In another study, ultrasonically processed mango samples showed no significant influence on the carotenoid content of the sample compared to the untreated sample (Santos, Fernandes, Oliveira, & Miranda, 2015). An interesting finding was observed when pasteurized carrot juice was subjected to sonication at different time intervals. The concentration of β-carotene was found to increase up to 95 μg/g, which resulted in an oxidation reaction that increased the peroxide content of the sample. This was due to the prooxidant effect shown by β-carotene at higher concentration (Shanmugam & Ashokkumar, 2015).

8.6.1.5 Irradiation processing

The irradiation process is another nonthermal technology that involves exposure of food to ionizing or nonionizing energy and is known as cold pasteurization. The main goal of this technology is to

destroy and inactivate microorganism activity in the food that reduces its shelf-life. Ionizing radiation is generated by electron beams, gamma rays (Cobalt-60), and X-rays. Nonionizing radiation includes UV rays, microwaves, and visible light that do not carry energy to ionize atoms. The dose level of applied radiation plays a vital role in reducing the microbial load. Ionizing radiation radiates from the range of 1000 eV to 30 MeV. When radiation passes through the food materials, it not only inactivates microorganism growth but also weakens the chemical bonds in food. Correspondingly, a study conducted by Fan and Sokorai (2007) revealed that radiation induced losses in the texture of green peas, while the population of acid-producing microorganisms such as lactic acid were reduced significantly in the sample. As a result, the pH level of the product dropped to 4 and degraded the green chlorophyll and total carotenoids in green peas and frozen corn (Fan & Sokorai, 2007). Sprout samples radiated at 1 and 2 kGy exhibited no changes in total carotenoid content compared to control for 8 storage days. Furthermore, in another study the effect of radiation treatment (0, 2.5, 5, 7.5, and 10 kGy) was compared to thermal treatment on strawberry and papaya-blended nectar. As the irradiation dose increased, the β-cryptoxanthin content in the nectar blend reduced at 5 kGy and 7.5 kGy but increased overall by 7.4% at 10 kGy. It was concluded that increased radiation dose up to 7.5 kGy negatively affected β-cryptoxanthin, β-carotene, and lycopene content in the sample in all ratio compositions. However, at 10 kGy, the carotenoid content increased significantly compared to the sample radiated at 7.5 kGy. At higher radiation, the polygalacturonase enzyme in the plant cell hydrolyzed the glycosidic bonds and degraded the pectin. Therefore the nectar sample became much more susceptible to releasing carotenoid and hence became more bioavailable (Swada, Keeley, Ghane, & Engeseth, 2016).

8.6.1.6 Excipient emulsion

While most of the reported technologies work based on pressure stress researchers have discovered another way to increase carotenoid bioaccessibility through excipient emulsions. By definition, excipient is a component that is not bioactive but when it is coingested with another pharmaceutical preparation, the efficacy of the emulsion increases (McClements & Xiao, 2014). Emulsions are generally thermodynamically unstable and their stability is influenced mainly by several processes known as creaming, flocculation, coalescence, and Ostwald ripening. Carotenoid-incorporated excipient food is prepared using any of these processes to enhance the food bioavailability (Gerding et al., 2016). Carotenoids in raw food are usually present in the form of crystalline. For effective bioaccessibility, this crystalline molecule has to be effectively released from the matrix, solubilized, and absorbed in the intestine (Zhang et al., 2015a). To enhance the efficacy, a new class of food has been designed to enhance the oral bioaccessibility of carotenoids and is known as excipient food. This class of food uses lipids, surfactants, carbohydrates, proteins, and salt to increase the bioaccessibility of carotenoids upon consumption. In agreement to these, the bioaccessibility of β-carotene was found to increase from 14% to 86%, as the long-chain fatty acid increases from 0% to 100% (Trujillo, Qian, Bellosa, & McClements, 2013). Furthermore, the bioavailability of carotenoid-rich (lycopene and astaxanthin) nanoemulsions of linseed oil was found to be inversely correlated with the droplet size of the emulsion and positively correlated to the zeta potential of the emulsion (Gerding et al., 2016). Different food products such as beverages, syrup, candies desserts, soups, and spreads are regarded as desirable and effective excipient food. In the pharmaceutical industries, dosage form products either as capsules, pills, or syrups are well-accepted by the public (McClements & Xiao, 2014).

Table 8.4 Effect of Nonthermal Technologies on Bioavailability and Stability of Carotenoids

Nonthermal Method	Sample	Carotenoid	Sample Preparation	Operating Condition	Remarks	References
High-pressure homogenization (HPH)	Carrot, tomato	β-carotene, α-carotene, lycopene	Samples were peeled and washed in cold water	Pressure = 100 MPa Flow rate = 10 Lh^{-1} Storage = −80°C (till further analysis)	• Carrot and tomato: HPH increases bioaccessibility of β-carotene, α-carotene but not lycopene	Svelander et al. (2011)
Pulsed electric field (PEF)/ High-intensity pulsed electric field (HIPEF)	Orange	β-carotene, α-carotene, lutein, β-cryptoxanthin, zeaxanthin	Fruits maintained in 4°C and extract obtained using squeezer	Flow rate = 200 ml/min Frequency = 80 Hz Electric field = 35 kv/cm Temperature <50°C Time = 750 μs	• Did not alter total carotenoid content in the extract • Less effective than high-pressure treatment *(exhibits approximately 50% of high-pressure treatment accessibility for all the carotenoids)*	Oreno et al. (2005)
	Kiwi, orange, pineapple, mango	β-carotene, α-carotene, lutein, β-cryptoxanthin, zeaxanthin	Fruits were washed, peeled, and juice extracted.	Flow rate = 60 ml/min Frequency = 200 Hz Electric field = 35 kv/cm Temperature <35°C Time = 1800 μs	• The concentration of the total carotenoids increases with respect to untreated samples. For example: lutein and zeaxanthin concentration increases 23 and 28% each	Rodríguez-Roque et al. (2015)
High-pressure processing (HPP)/High hydrostatic pressure processing (HHP)	Avocado	β-carotene, α-carotene, lutein, β-cryptoxanthin, zeaxanthin	Washed, peeled, macerated, and vacuum deaerated	Pressure = 600 MPa Temperature = 23°C Storage = 4°C (40days)	• Carotenoid content was found to be 56% higher than unprocessed sample • Total carotenoids remained constant for first 10 days and depleted significantly at 15th day of refrigerated storage	Jacobo-Velázquez & Hernández-Brenes (2012)
	Orange	β-carotene, α-carotene, lutein, β-cryptoxanthin, zeaxanthin	Fruit was maintained in 4°C and extract obtained using squeezer	Pressure = 400 MPa Temperature = 40°C Time = 1 min	• Yielded highest amount of carotenoid accessibility when compared with thermal and PEF method	Oreno et al. (2005)
	Kiwi, orange, pineapple, mango	β-carotene, α-carotene, lutein, β-cryptoxanthin, zeaxanthin	Fruits were washed, peeled, and juice extracted	Pressure = 400 MPa Temperature = 40°C	• The concentration of the total carotenoids increases with respect to untreated samples	Rodríguez-Roque et al. (2015)

(*Continued*)

Table 8.4 Effect of Nonthermal Technologies on Bioavailability and Stability of Carotenoids *Continued*

Nonthermal Method	Sample	Carotenoid	Sample Preparation	Operating Condition	Remarks	References
Ultrasound processing (UP)	Tomato	Lycopene	Tomato pulp was sieved to separate seed and tomato pulp	Frequency = 24 kHz Amplitude = 100 μm Acoustic power = 71 W Acoustic Energy = 1462 J/cm^3	• UP increased the viscosity of tomato pulp • Compared to untreated sample, UP-treated sample shows high bioaccessibility while lycopene degradation tendency was much less	Anese et al. (2015)
Excipient emulsion	Carrot	β-carotene, α-carotene	Carrots mixed in excipient emulsion	Excipient emulsion: Homogenization of oil phase (8% weight) with aqueous phase. (92% weight) Oil: corn oil, fish oil, MCT oil	• The bioaccessibility in raw food relatively lower than lipid-based emulsions • The bioaccessibilities of carotenoids from carrot was found to be higher in lipid emulsion of corn oil > fish oil > MCT oil	Zhang et al. (2015b)
	Yellow pepper	β-carotene, α-carotene, β-cryptoxanthin	Yellow peppers (1) cut into small pieces in raw form and (2) boiled for 5 min	Excipient emulsion: Homogenization of oil phase: 900 psi Oil phase: LCT, MCT, orange oil	• Excipient emulsion fabricated from LCT gave a higher carotenoid bioaccessibility than fabricated from MCT (raw and boiled form).	Liu et al. (2015)

The effects of nonthermal technologies on carotenoid bioaccessibility and stability are presented in Table 8.4.

8.6.2 POTENTIAL TECHNOLOGIES TO INCREASE CAROTENOID BIOACCESSIBILITY

High-voltage arc-discharge technology involves electrical and mechanical effects on plant samples through the generation of strong dynamic shockwaves from an electrical arc. A plasma channel formed by high current or voltage electrical discharge between two submerged electrodes directly transmits energy into the aqueous solution. The dynamic shockwaves introduced in the system cause eventually water bubbles cavitation that lead to strong second shocks within a short time period. This secondary shockwave causes particle fragmentation and damages cellular structure (Boussetta et al., 2012; Oroian & Escriche, 2015). Cold plasma is another innovative unthermal food-processing method. The main aim of this technology is to inactivate microbial growth on the surface of food products. Cold plasma consists of ionized gas that carries electrons, photons, free radicals, and highly reactive species. This ionized gas initiates destruction of cell membrane and microbial DNA under cold temperature (Thirumdas, Sarangapani, & Annapure, 2014). Until recently, studies on high-voltage arc-discharge and cold-plasma application to increase carotenoid bioaccessibility were scarcely reported, which created a challenge for innovations in the field of carotenoid processing.

8.7 RECOVERY OF CAROTENOIDS FROM AGRO-INDUSTRIAL WASTE

During processing, plant samples undergo a few stages of processing including chopping, crushing, sieving, and refining. After the multistaged impact on the sample, the resulting waste (coarse pulp, seed, and peel) would have lost their plant integrity hence, no longer intact by structure. Therefore extraction of bioactive compounds including carotenoid from these samples will not require exhaustive extraction protocols. The following are some examples of agro-industrial waste reported for carotenoid extraction.

8.7.1 TOMATO

In industry, processed tomatoes generate about 10–40% of by-products containing peel, seeds, and pulp known as tomato pomace, which is widely known as a rich source of β-carotene and lycopene. Upon monosonication (50 kPa, 45°C), the dried tomato pomace was found to contain 14 mg of total carotenoid for every 100 g of dry-weight sample (Luengo, Abanto, Condon, Alvarez, & Raso, 2014).

8.7.2 MANGO

Mango, which is ranked as the fifth most important fruit in the world, produces 15–20 g of peel as by-product for every 100 g of fruit. Supercritical fluid extraction at 30 MPa/40°C from mango peel

revealed that it contained 17 mg of β-carotene for every 100 g of dry weight sample (Mendoza, Paula, Paviani, Cabral, & Correa, 2015).

8.7.3 CASHEW PENDUNCLE

Cashew apple bagasse is a waste from cashew penduncle juice processing. As reported the weight of the bagasse waste after processing corresponds to 90% of the total weight of the fruit. According to Macedo, Robrigues, Pinto, and Brito (2015), the crushed cashew stalk contains almost 5 mg of total carotenoid content for every 100 g sample.

8.7.4 BANANA

Almost 40% of the weight of bananas is from the banana peels disposed of when processing the fruit for powder, jam, biscuits, and bar. It was found through solvent extraction that the banana peels contain almost 5 mg of carotenoid content including β-carotene for every 100 mg of dry-weight sample (Nagarajaiah & Prakash, 2011).

8.7.5 PINK GUAVA

Pink guava is widely recognized as great source of lycopene. In the pink guava puree industry, almost 25% of pink guava fruit containing the seed and coarse pulp are disposed of as waste. Using hexane as extraction solvent, this waste was found to be 17 mg of lycopene content for 100 g of dry-weight sample (Kong & Ismail, 2011).

8.7.6 CARROT

The carrot-juice processing industry only utilizes 60−70% of carrot for juice production and the remaining carrot-pulp waste is usually subject to disposal. Carotenoid extraction from the pulp was done through blending with hexane and acetone solvent. The carotenoid powder was spray dried and found to contain a considerable amount of up to (0.598 mg) lutein, α-carotene (3.68 mg), and β-carotene (5.08 mg) for every 100 g of dry-weight sample (Chen & Tang, 1998).

8.8 EXTRACTION, SEPARATION, ANALYSIS, AND QUANTIFICATION OF CAROTENOIDS

8.8.1 CLASSIC AND CONVENTIONAL CAROTENOID EXTRACTION METHODS

Carotenoids are traditionally extracted using low-pressure extraction methods such as agitation, shaking and homogenization, centrifugal extraction, and Soxhlet extraction. The latest methods apply heat or agitation force, which increases the diffusion of carotenoids through the interface of the solid matrix−organic solvent (Singh et al., 2015). Although these methods are simple for execution, they have several well-known disadvantages. Mainly, low-pressure extraction methods require a large amount of organic solvents such as hexane and chloroform for extraction, which is a

disadvantage since most of the organic solvents ideal for carotenoid extraction are banned or only allowed in tolerable concentrations for food production in many countries (Prado et al., 2014). Other disadvantages include the time it takes to process, lack of automation, and poor carotenoid recovery (Singh et al., 2015; Strati & Oreopoulou, 2014).

8.8.2 ADVANCED EXTRACTION METHODS

The above disadvantages have encouraged technological innovations to meet the needs of both industry and society. In line with green extraction principles, extraction processes that use renewable resources, minimize the use of solvent, and eliminate petroleum-based solvents are economical and ensure high-quality extracts have been developed. A number of emerging technologies such as ultrasonic-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, PEF, subcritical water extraction, supercritical fluid extraction, enzyme-assisted extraction (Nagarajan et al., 2016), and instant controlled pressure-dropped extraction have been introduced. Table 8.5 provides the main advantages and disadvantages of these new technologies over traditional methods.

8.8.3 GREEN SOLVENT FOR ADVANCED EXTRACTION METHODS

Methods of carotenoid extraction are normally conducted using petroleum origin solvents such as hexane, ethyl acetate, methylene chloride, and propanol. These nonpolar solvents are mostly toxic and exhibit adverse effects on human health. Due to the lipophilic nature of carotenoids, some researchers have also attempted to use edible oil to extract carotenoid from plant samples. For example, Li, Tixier, Tomao, Cravotto, and Chemat (2013) used sunflower oil to extract β-carotene from carrot. It was found that use of edible oil for extraction is green, inexpensive, and easy to use and resulted in higher yield than hexane extraction. On the other hand, use of edible oil raises several concerns about carotenoid extraction. First, extraction using edible oil may be inefficient due to the high viscosity of the medium. Additionally, evaporation of the oil-enriched carotenoid solution requires a high boiling point, which would obviously degrade or induce isomerization of the extracted compound. Furthermore, close monitoring is required to ensure the extracted carotenoid does not undergo lipid oxidation and acidification.

Other potential green solvents commonly used for plant-based extraction include biobased solvents, supercritical fluid, ionic liquids (IL), and hydrotropic solvents. Biobased solvents like ethanol exhibit low selectivity for hydrophobic compounds due to their polar nature. To date, only supercritical fluid is actively used for carotenoid extraction. However, pricey instrument setup and the inability to solubilize large-molecular weight compounds including carotenoid make this solvent unattractive (Nagarajan et al., 2016). In most cases, IL are considered as green solvents due to their advantages including high conductivity, nonflammability, low melting point, low volatility, and thermal stability. However, these criteria are also considered to be insufficient to categorize this solvent as green as some IL are not ecofriendly and require toxic and laborious purification protocols (Grosso, Valentao, Ferreres, & Andrade, 2015). Hydrotropic solvent is another alternative green solvent that is widely accepted among researchers today. Hydrotropes are organic salts that exist as small amphiphilic molecule by structure with great hydrophilic character. This solvent is known to demonstrate the ability to increase solubility of hydrophobic substances in water up to

Table 8.5 Principles, Advantages, and Disadvantages of Emerging Carotenoid Extraction Technology

Extraction Method	Principle	Advantage	Disadvantage	Reference
Ultrasonic assisted extraction (UAE)	Sound waves at a frequency greater than the human hearing range disrupt the plant matrix. The solvent penetrates the solid matrix and releases the extract.	Simple, less time consuming, high extraction yield, low solvent consumption	• High intensity ultrasound generates heat that initiates thermal degradation. • Toxic organic solvent	Kumcuoglu, Yilmaz, and Tavman (2014)
Microwave assisted extraction (MAE)	Moisture of plant cell is evaporated by the heat from microwaves. The pressure formed in the cell alters the porosity and induces migration of the solvent into the cell.	Simple, less time consuming, high extraction yield	• Thermal degradation. • Require additional separation method to remove solid particles • Toxic organic solvent	Singh et al. (2015)
Pressurized Liquid Extraction (PLE)	High pressure enhances the diffusion efficiency of the solvent into plant matrix.	Faster extraction, less amount of solvent, high extraction yield	• Thermal degradation • Toxic solvent	Singh et al. (2015)
Pulsed Electric Field (PEF)	The plant cells are ruptured due to the exposure of high-intensity electric field released across solvents. External field alters the membrane of plant samples to release carotenoids.	High extraction yield	• Process parameters have to be designed prior to extraction	Buckow, Ng, and Toepfl (2013)
Subcritical Water Extraction (SWE)	Carotenoids are extracted in hot water under critical temperature (100−374°C) and also maintained at high pressure (10−60 bar).	High extraction yield, no use of toxic solvent, less time consuming	• Dependent on variability of dielectric constant of different types of carotenoids • High tendency for thermal degradation	Joana Gil-Chávez et al. (2013)
Supercritical fluid extraction (SFE)	CO_2 in supercritical state kept in contact with plant matrix. CO_2 penetrates the solid matrix and dissolves lycopene from the solid phase.	High selectivity, shorter time extraction, nontoxic organic solvent, nonflammable	• Expensive equipment setup • Multiple staged extraction process • Large molecular weight of carotenoids inhibits solubility in CO_2 gas	Prado et al. (2014)
Enzyme assisted extraction technique (EAE)	Enzyme allows degradation of plant cell wall under mild process condition and hence eases carotenoid extraction from the plant matrix.	High extraction yield	• Require parameters adjustment like pH, solvent incorporation, and enzyme concentration • Cost of enzyme is expensive	Strati and Oreopoulou (2014)
Instant controlled pressure drop assisted extraction (DIC)	Work based on thermo-mechanical effect by exposing the plant material under saturated steam followed by sudden drop in pressure toward vacuum. DIC causes rupture of plant membrane and instantaneously cools the product.	No thermal degradation, high extraction yield, no use of toxic solvent	• Expensive instrumental setup • Require multiple stage of preextraction steps	Joana Gil-Chávez et al. (2013)

210-fold. Other pronounced advantages of this solvent include the fact that they are reusable, economical, nonflammable, highly selective, and nontoxic (Nagarajan et al., 2016). Numerous bioactive compounds have been successfully recovered using both IL and hydrotropic solvents, but to the best of our knowledge extraction of carotenoids using these solvents is scarcely reported.

8.8.4 SAPONIFICATION

After extraction, the final product does not necessarily have to be in pure form. The extracted carotenoids may have been masked by other subextracted plant components like chlorophyll, lipids, fatty acids, and esters. Due to the presence of hydroxyl groups, xanthophylls are often present in esterified form (Sarkar et al., 2012). Retrieving carotenoids is conducted with saponification to eliminate chlorophylls or undesired lipids that might cause intercalation during spectrophotometric analysis. The saponification process can be directly performed during sample homogenization before or even after extraction. During this stage, a solvent such as methanolic sodium or potassium hydroxide is added and stirred under atmosphere of nitrogen to remove the lipid or chlorophyll phase from the extract (Larsem & Christensen, 2005). However, the saponification step can be omitted in nonchlorophyll plant species such as carrot or tomato as the defatting process shows only negligible differences in the total carotenoid content. Unnecessary saponification can also lead to structural alteration or degradation of carotenoids (Singh et al., 2015).

8.8.5 ANALYSIS AND QUANTIFICATION OF CAROTENOIDS

Prior to quantification, it's absolutely crucial to perform several identification tests on the extracted carotenoid. The most fundamental identification tests on carotenoids are performed to determine:

a. the maximum absorption wavelengths via UV-vis spectrophotometric,
b. nature of functional groups through Fourier Transform Infrared Spectroscopy (FTIR) analysis, and
c. conjugation of double bonds are tested using NMR analysis.

The obtained spectral fine structure and the cis−trans peak intensity must be in agreement with the commercially purchased carotenoid standards or positive control. The maximum UV-vis wavelength absorption for each carotenoid is given in Table 8.2. In FTIR analysis, the vibrational mode, symmetric stretching, and asymmetric stretching of each individual carotenoid differ in structure. For instance, β-carotene contains C = C, C-C, C = O, O-H groups, which are represented by peaks in the region of ($1571\ cm^{-1}$), ($1166\ cm^{-1}$), ($1687\ cm^{-1}$), and ($3372\ cm^{-1}$), respectively. Furthermore, CH_2 asymmetrical, CH_2 symmetrical, C = C acyclic olefin, and C = C groups in lycopene are represented by peaks in the region of $2924\ cm^{-1}$, $2854\ cm^{-1}$, $1643\ cm^{-1}$, and $1510\ cm^{-1}$. Despite having the same functional group, the symmetrical and asymmetrical stretches lead to broad differences in the absorption strength of the peak, which falls in different regions. Structural elucidation of carotenoids in NMR is done by splitting 1H NMR and 13C NMR. A typical 1H NMR spectrum provides information on chemical shift, multiplet structure, and spacing between the resonances (LaFountain et al., 2013).

The chromatographic technique adopted for carotenoids must be compatible with the polarity range. Since carotenoids are sensitive molecule, chromatographic techniques that require long-term

exposure are regarded as unreliable for analysis. For instance, thin-layer chromatography requires carotenoid separation on highly exposed Thin Layer Chromatography (TLC) plate, while gas chromatography principally works under high temperature that initiates thermal degradation of carotenoids. Reversed-phase HPLC analysis has always been the preferred way to quantify carotenoids. The monomeric octyl (C8) and octadecyl (C18) are the most widely used stationary phase for reversed-phase chromatography (HPLC). C18 columns are specifically designed for weak hydrophobic interaction and compatible with most organic solvent, hence short-chain carotenoids analysis aptly suits the column. However, this column failed to resolve the long-chained carotenoids and its cis-isomers analysis. To counter this, C30 column was introduced for the separation of less polar carotenoids like lycopene and β-carotene (Singh et al., 2015). The right choices of isocratic or gradient mobile-phase considering polarity, viscosity, and volatility will result in reliable trans-cis peak separation using this column. The quantification of carotenoids is based on the linear relation of the weight of the injected standard and the elucidated peak area (Lee & Chen, 2001). Most common liquid chromatography systems are coupled with UV or Photodiode Array (PDA) systems. Apart from identifying the carotenoids, they do not provide the molecular structure information of the injected sample. In this case, mass spectrometry (MS) instruments provide detailed information on the molecular structure. MS analysis confirms the identification of carotenoids by providing molecular mass and fragmentation patterns, although some carotenoids contain similar chemical composition and structure. There are various LC-MS detection systems widely used for carotenoid analysis including electron impact, electrospray ionization (ESI), fast atom bombardment, or atmospheric pressure chemical ionization (APCI) (Carilho et al., 2014). Among these, APCI demonstrates the strongest signal strength for carotenoid elution. When compared to ESI, APCI exhibited 100-fold higher sensitivity than other methods for testing carotenoids from plant samples (Rivera et al., 2013).

8.8.6 IN VITRO ANTIOXIDANT EVALUATION METHODOLOGIES

Unlike hydrophilic antioxidants, antioxidant assays available for hydrophobic compounds are limited. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is the most commonly used antioxidant assay for plant extract. In this assay, a molecule or antioxidant with weak A-H bonding will react with a stable free radical DPPH• (2,2-diphenyl-1-picrylhydrazyl, $\lambda_{max} = 517$ nm) causing discoloration of the molecule. The ferric-reducing antioxidant power assay evaluates the reducing potency of the antioxidant to react on ferric tripyridyltriazine ($Fe_{3+}-TPTZ$) complex. When antioxidant donates a hydrogen atom to ferric complex, the radical chain reaction breaks and produces blue-color ferrous $\lambda_{max} = 593$ nm (Alam, Bristi, & Rafiquzzaman, 2013). 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay is another popularly used antioxidant assay, particularly for lipophilic extract. In the presence of an antioxidant, the ABTS + radical, which has a strong absorption band at 734 nm, will be scavenged, which turns the reaction mixture from blue to green. The oxygen radical absorbance capacity assay works based on the measurement of fluorescent signaling by adding fluorescein. The use of APPH (2,2-azobis 2-amidopropane dihydrochloride) in the assay generates free radical upon thermal decomposition. In the presence of antioxidant, the free radical is scavenged, which retains the fluorescence intensity. The emission intensity is measured at 517 and 485 nm using a spectrofluorometer. Linoleic acid incorporated into β-carotene-linoleic acid bleaching acid is an unsaturated fatty acid that is prone to oxidation

and thus forms ROS or lipid radicals. Formation of ROS will eventually trigger oxidation of β-carotene, which is a yellowish color. In the presence of an antioxidant, the oxidation of β-carotene is inhibited and the yellowish-orange coloration is retained ($\lambda_{max} = 470$ nm) (Thaipong et al., 2006).

8.9 CHALLENGES DURING CAROTENOID ANALYSIS

Prior to extraction, the plant samples are usually subjected to drying to remove the moisture content. To date, the effects of drying on carotenoid isomerization have not been established. However, carotenoid degradation that occurs during drying is highly dependent on the protective activity of the antioxidant present in the extracellular matrix. Long-term exposure of sample drying is not recommended for carotenoid-rich samples as they are susceptible to oxidative degradation (Enrique et al., 2013). In usual phenomena, carotenoid content analysis will show variation. This is because this molecule contains conjugated double bonds in its structure, which accounts for the instability of the compound. Hence, a few precautions are needed prior to carotenoid analysis. In order to avoid thermal stress on the extract, use of a rotary evaporator with low boiling point is highly recommended. Lighting effect can be reduced by performing extraction under dim light and using amber glassware or aluminum foil covering the extract. Oxidation of the extract can be avoided by incorporating antioxidants such as butylated hydroxytoluene (BHT) or sodium ascorbate in the extraction system (Martinez et al., 2007). Challenges associated with measuring and comprehending carotenoid bioavailability can be categorized into four categories as reported by Faulks and Southon (2005): release, solubility, measuring and interpreting plasma response, and interpreting differences in interindividual responses. The first two challenges suggest that carotenoids from complex food either in the form of protein-carotenoid complex or semicrystalline to be solubilized in lipid emulsion or mixed micelles for effective absorption in enterocyte. The third challenge points out that measurement of total carotenoid based on blood plasma can be ineffective. This is because plasma response studies does not take into account of presence of carotenoids in eyes and LDL. Hence, the remaining lutein and zeaxanthin dispersed in between HDL and LDL is more likely being measured. Lastly, plasma response for carotenoid is known to be different among participants. Hence, to avoid these challenges it is suggested that the rate and dispersal of carotenoids be measured by modeling the chylomicron carotenoid clearance rate as this model does not contain sequestered carotenoids re-exported by liver.

8.10 CONCLUSION

The health-promoting properties of carotenoids have gotten the attention of researchers and industrialists. The cost of natural resources can be reduced by reutilizing the agricultural waste yielded from food processing, especially fruits. The adapted food-processing and extraction technology must ensure minimum oxidization and isomerization of carotenoids. Quantification and analysis techniques promise reliable results with high accuracy and precision. In vitro antioxidant methodologies hint at the scavenging and reducing antioxidant potency of carotenoids.

In line with the growing convergence of pharmaceuticals and food companies, a carotenoid formula with high antioxidant capacity, stability, and bioavailability will succeed. There are numerous unexplored avenues for carotenoid analysis where special attention should be given to the search for low-cost natural sources to meet the demand of populations that lack the proper diet, especially rural areas.

REFERENCES

Alam, N., Bristi, N., & Rafiquzzaman, M. D. (2013). Review on invivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, *21*, 143–152.

Alves-Rodrigues, A., & Shao, A. (2004). The science behind lutein. *Toxicology Letters*, *150*(1), 57–83.

Amaya Delia, B. R. (2016). Natural food pigments and colorants. *Current opinion in Food Science*, *7*, 20–26.

Ampomah-Dwamena, C., et al. (2009). The kiwifruit lycopene beta-cyclase plays a significant role in carotenoid accumulation in fruit. *Journal of Experimental Botany*, *60*(13), 3765–3779.

Andrés, V., Villanueva, M. J., & Tenorio, M. D. (2016). The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. *Food Chemistry*, *192*, 328–335.

Anese, M., et al. (2015). Effect of ultrasound treatment, oil addition and storage time on lycopene stability and in vitro bioaccessibility of tomato pulp. *Food Chemistry*, *172*, 685–691.

Arab, L., & Steck, S. (2000). Lycopene and cardiovascular disease. *The American Journal of Clinical Nutrition*, *71*(6), 1691–1695.

Aydin, M., & Çelik, S. (2012). Effects of lycopene on plasma glucose, insulin levels, oxidative stress, and body weights of streptozotocin-induced diabetic rats. *Turkish Journal of Medical Science*, *42*, 1406–1413.

Baccarini, A., Bertossi, F., & Bagni, N. (1964). Carotenoid pigments in the stem of *Cuscuta australis*. *Phytochemistry*, *4*(1945), 349–351.

Boon, C. S., et al. (2010). Factors influencing the chemical stability of carotenoids in foods. *Critical Reviews in Food Science and Nutrition*, *50*(6), 515–532.

Boussetta, N., et al. (2012). Scale-up of high voltage electrical discharges for polyphenols extraction from grape pomace: Effect of the dynamic shockwaves. *Innovative Food Science & Emerging Technologies*, *16*, 129–136.

Britton, G. (1995). Structure and properties of carotenoids in relation to function. *FASEB Journal*, *9*(15), 1551–1558.

Buckow, R., Ng, S., & Toepfl, S. (2013). Pulsed electric field processing of orange Juice: A review on microbial, enzymatic, nutritional, and sensory quality and stability. *Comprehensive Reviews in Food Science and Food Safety*, *12*(5), 455–467.

Burri, B. J. (2015). Beta-cryptoxanthin as a source of vitamin A. *Journal of the Science of Food and Agriculture*, *95*(9), 1786–1794.

Camara, M., et al. (2013). Lycopene: A review of chemical and biological activity related to beneficial health effects. *Studies in Natural Products Chemistry*, *40*, 383–426.

Cardoso, P. C., Lucia, C. M., Stringheta, P. C., Chaves, J. B., & Ana, H. M. (2009). Carotene and provitamin A content of vegetables sold in Viçosa, MG, Brazil, during spring and winter. *Brazilian Journal of Pharmaceutical Science*, *45*, 3.

Carilho, K., et al. (2014). Review of methods for analysis of carotenoids. *Trends in Analytical Chemistry*, *56*, 49–73.

Carrasco, U., Tudesca, V., & Zevallos Cisneros (2002). Edible coating effects on the sensory quality of green bell pepper fruits (Capsicum annuum L.) during storage. *Arch Latinoam Nutrition*, *52*, 84–90.

Cazzonelli, C. I. (2011). Carotenoids in nature: insights from plants and beyonds. *Functional Plant Biology*, *38*, 833−847.
Chen, B. H., & Tang, Y. C. (1998). Processing and stability of carotenoid powder from carrot pulp waste. *Journal of Agricultural and Food Chemistry*, *46*, 2312−2318.
Choksi, P. M., & Joshi, V. Y. (2007). A review on lycopene—Extraction, purification, stability and applications. *International Journal of Food Properties*, *10*(2), 289−298.
Cramer, D., et al. (2001). Carotenoids, antioxidants and ovarian cancer risk in pre- and post menopausal women. *International Journal of Cancer*, *94*(1), 128−134.
Cucu, T., et al. (2012). A simple and fast HPLC method to determine lycopene in foods. *Food Analytical Methods*, *5*(5), 1221−1228.
Das, S., et al. (2005). Lycopene, tomatoes, and coronary heart disease. *Free Radical Research*, *39*(4), 449−455.
Dendranthema, C., et al. (2015). Anthocyanin and carotenoid contents in different cultivars of Chrysanthemum (*Dendranthema grandiflorum* Ramat.) flower. *Molecules*, *20*(4), 11090−11102.
D'evoli, L., Lombardi-Boccia, G., & Lucarini, M. (2013). Influence of heat treatments on carotenoid content of cherry tomatoes. *Foods*, *2*(3), 352−363.
Dias, M. G., Camões, M. F. G. F. C., & Oliveira, L. (2009). Carotenoids in traditional Portuguese fruits and vegetables. *Food Chemistry*, *113*(3), 808−815.
Dri, B. D., et al. (2010). Effects of different maturity stages on antioxidant content of Ivorian Gnagnan (*Solanum indicum* L.) berries. *Molecules*, *15*, 7125−7138.
Esteban, R., et al. (2015). Versatility of carotenoids: An integrated view on diversity, evolution, functional roles and environmental interactions. *Environmental and Experimental Botany*, *119*, 63−75.
Fan, X., & Sokorai, K. J. B. (2007). Effects of ionizing radiation on sensorial, chemical, and microbiological quality of frozen corn and peast. *Journal of Food Protection*, *70*(8), 1901−1908.
Faulks, R. M., & Southon, S. (2005). Challenges to understanding and measuring carotenoid bioavailability. *Biochimica et Biophysica Acta (BBA)*, *1740*(2005), 95−100.
Fernández-García, E., et al. (2012). Carotenoids bioavailability from foods: From plant pigments to efficient biological activities. *Food Research International*, *46*(2), 438−450.
Fiskselova, M., et al. (1997). Extraction of carrot (*Daucus carota* L.) carotenes under different conditions. *Czech Journal of Food Science*, *26*(4), 268−274.
Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology*, *26*(2), 68−87.
Gallicchio, L., et al. (2008). Carotenoids and the risk of developing lung cancer: A systematic. *The American Journal of Cinical Nutrition*, *88*, 372−383.
Gerding, D. S., et al. (2016). High carotenoid bioaccessibility through linseed oil nanoemulsions with enhanced physical and oxidative stability. *Food Chemistry*, *199*(2016), 463−470.
Ghazghazi, H., et al. (2012). Leaf essential oil, leaf methanolic extract and rose hips carotenoids from Rosa sempervirens L. growing in North of Tunisia and their antioxidant activities. *Journal of Medicinal Plants Research*, *6*(4), 574−579.
Giovannucci, E., et al. (1995). Intake of carotenoids and retinol in relation to risk of prostate cancer. *Journal of National Cancer Institute*, *87*(23), 1767−1776.
Goltz, S. R., et al. (2013). Carotenoid bioavailability from raw vegetables and a moderate amount of oil in human subjects is greatest when the majority of daily vegetables are consumed at one meal. *Nutrition Research (New York, N.Y.)*, *33*(5), 358−366.
Goralczyk, B. (2009). β-Carotene and lung cancers in smokers: Review of hypotheses and status of research. *Nutrition and Cancer*, *61*(6), 767−774.
Grosso, C., Valentao, P., Ferreres, F., & Andrade, P. B. (2015). Alternative and efficient extraction methods for marine-derived compounds. *Marine Drugs*, *13*, 3182−3230.

Gupta, S. K., et al. (2003). Lycopene attenuates oxidative stress induced experimental cataract development: an in vitro and in vivo study. *Nutrition*, *19*(9), 794–799.
Hamouz, K., Pazderu, K., Lachman, J., & Kotikova, C. Z. (2016). Effect of cultivar, fresh color, locality and year on carotenoid content in potato tubers. *Plant Soil Environment*, *62*(2), 86–91.
Handelman, G. J. (2001). The evolving role of carotenoids in human biochemistry. *Nutrition*, *17*(10), 818–822.
Heydari, S., & Haghayegh, G. H. (2014). Extraction and microextraction techniques for the determination of compounds from saffron. *Canadian Chemical Transactions*, *2*(2), 221–247.
Hof, V. H., et al. (2000). Dietary factors that affect the bioavailability of carotenoids. *The Journal of Nutrition*, *103*(3), 503–506.
Hu, F., et al. (2012). Carotenoids and breast cancer risk: a meta-analysis and meta-regression. *Breast Cancer Research and Treatment*, *131*(1), 239–253.
Hussein, A., et al. (2000). Effects of processing and packaging on vitamin C and b-carotene of ready to use vegetables. *Food Research International*, *33*, 131–136.
Islam, S. N., et al. (2016). Carotenoids and β-carotene in orange fleshed sweet potato: A possible solution to vitamin A deficiency. *Food Chemistry*, *199*, 628–631.
Islek, C., Altuner, E. M., & Alpas, H. (2015). The effect of high hydrostatic pressure on the physiological and biochemical properties of pepper (*Capsicum annuum* L.) seedlings. *High Pressure Research*, *35*(4), 396–404.
Jacobo-Velázquez, D. A., & Hernández-Brenes, C. (2012). Stability of avocado paste carotenoids as affected by high hydrostatic pressure processing and storage. *Innovative Food Science & Emerging Technologies*, *16*, 121–128.
Jayathunge, K. G. L. R., et al. (2015). Impact of long-term storage at ambient temperatures on the total quality and stability of high-pressure processed tomato juice. *Innovative Food Science & Emerging Technologies*, *32*, 1–8.
Joana Gil-Chávez, G., et al. (2013). Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: An overview. *Comprehensive Reviews in Food Science and Food Safety*, *12*(1), 5–23.
Karppi, J., et al. (2012). Low serum lycopene and β-carotene increase risk of acute myocardial infarction in men. *European Journal of Public Health*, *22*(6), 835–840.
Khachik, F., Steck, A., & Pfander, H. (1999). Isolation and structural elucidation of (13Z, 13 'Z, 3R, 3 'R, 6 'R) -lutein from marigold flowers, kale, and human plasma. *Journal of Agricultural and Food Chemistry*, *47*, 455–461.
Khoo, H.-E., et al. (2011). Carotenoids and their isomers: Color pigments in fruits and vegetables. *Molecules (Basel, Switzerland)*, *16*(2), 1710–1738.
Kirthi, K., Amita, S., Priti, S., Kumar, A. M., & Jyoti, S. (2014). Colorful world of microbes: Carotenoids and their applications. *Advances in Biology*, *2014*, 1–13.
Kong, et al. (2010). Revealing the power of the natural red pigment lycopene. *Molecules*, *15*, 959–987.
Kong, K. W., & Ismail, A. (2011). Lycopene content and lipophilic antioxidant capacity of byproducts from *Psidium guajava* fruits produced during puree production industry. *Food and Bioproducts Processing*, *89*, 53–61.
Kopsell, D. A., et al. (2010). Characterization of nutritionally important carotenoids in Bunching onion. *HortScience*, *45*(3), 463–465.
Kowluru, R. A., & Mishra, M. (2015). Oxidative stress, mitochondrial damage and diabetic retinopathy. *Biochimica et Biophysica Acta*, *1852*(11), 2474–2483.
Kumar, Y., Patel, K. K., & Kumar, V. (2015). Pulsed electric field processing in food technology. *International Journal of Engineering Studies and Technical Approach*, *01*(2), 6–17.
Kumcuoglu, S., Yilmaz, T., & Tavman, S. (2014). Ultrasound assisted extraction of lycopene from tomato processing wastes. *Journal of Food Science and Technology*, *51*(12), 4102–4107.

Labban, L., Mustafa, U. E., & Ibrahim, Y. M. (2014). The effects of Rosemary (*Rosmarinus officinalis*) leaves powder on glucose level, lipid profile and lipid perodoxation. *International Journal of Clinical Medicine*, *5* (3), 297−304.

Lafountain, A. M., et al. (2013). Nuclear magnetic resonance analysis of carotenoids from the burgundy plumage of the Pompadour Cotinga (*Xipholena punicea*). *Archives of Biochemistry and Biophysics*, *539*(2), 133−141.

Larsen, E., & Christensen, L. (2005). Simple saponification method for the quantitative determination of carotenoids in green vegetables. *Journal of Agricultural and Food Chemistry*, *53*, 6598−6602.

Lee, M., & Chen, B. (2001). Separation of lycopene and its cis isomers by liquid chromatography. *Chromatographia*, *54*, 613−617.

Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, *20*(3), 207−220.

Lettow, M., et al. (2004). Micronutrient malnutrition and wasting in adulys with pulmonary tuberculosis with and without HIV co-infection in Malawi. *BMC Infectious Diseases*, *4*(61), 1−8.

Li, Y., Tixier, A. S., Tomao, V., Cravotto, G., & Chemat, F. (2013). Green ultrasound-assisted extraction of carotenoids based on the bio-refinery concept using sunflower oil as an alternative solvent. *Ultrasonics Sonochemistry*, *20*(1), 12−18.

Lin, C. (2005). Stability of carotenoids in tomato juice during storage. *Food Chemistry*, *90*, 837−846.

Liu, X., et al. (2015). Increasing carotenoid bioaccessibility from yellow peppers using excipient emulsions: Impact of lipid type and thermal processing. *Journal of Agricultural and Food Chemistry*, *63*(38), 8534−8543.

Lopez, et al. (2007). Effects of agricultural practices on color, carotenoids composition, and minerals contents of sweet peppers, cv. Almuden. *Journal of Agriculture and Food Chemistry*, *55*, 8158−8164.

Luengo, E., et al. (2015). A comparative study on the effects of milisecond and microsecond pulsed electric field treatments on the permeabilization and extraction of pigments from *Chlorella vulgaris*. *Journal of Membrane Biology*, *248*, 883−891.

Luengo, E., Abanto, S. E., Condon, S., Alvarez, I., & Raso, J. (2014). Improving the extraction of carotenoids from tomato waste by application of ultrasound under pressure. *Separation and Purification Technology*, *136*(2014), 130−136.

Macedo, M., Robrigues, R. D., Pinto, G. A. S., & Brito, E. S. (2015). Influence of pectinolyttic and cellulotyc enzyme complexes on cashew bagasse maceration in order to obtain carotenoids. *Journal of Food Science Technology*, *52*(6), 3689−3693.

Major, N., & Herceg, Z. (2013). Aroma profile and sensory properties of ultrasound-treated apple juice and nectar. *Food Technology and Biotechnology*, *51*(1), 101−111.

Martinez-Antonio, J. M., Brahm, P. M., Gonzalez, A. B., & Stinco, C. M. (2015). A comprehensive review on the colorless carotenoids phytoene and phytofluene. *Archieves of Biochemistry and Biophysics*, *572*(2015), 188−200.

Martinez-Antonio, J. M., & Heredia, I. M. (2007). Review: Analysis of carotenoids in orange juice. *Journal of Food Composition and Analysis*, 638−663.

Martínez-Hernández, G. B., et al. (2015). Processing, packaging, and storage of tomato products: Influence on the lycopene content. *Food Engineering Reviews*, 1−24.

Massoud, R., Belgheisi, S., & Massoud, A. (2016). Effect of high pressure homogenization on improving the quality of milk and sensory properties of yogurt: A review. *International Journal of Chemical Engineering and Applications*, *7*(1), 66−70.

McClements, D. J., & Xiao, H. (2014). Excipient foods: designing food matrices that improve the oral bioavailability of pharmaceuticals and nutraceuticals. *Food & Function*, *5*(7), 1320−1333.

Mellado-Ortega, E., & Hornero-Méndez, D. (2015). Carotenoids in cereals: an ancient resource with present and future applications. *Phytochemistry Reviews*, 873−890.

Mendoza, M. P., Paula, J. T., Paviani, L. C., Cabral, F., & Correa, H. M. (2015). Extracts from mango peel byproduct obtained by supercritical CO_2 and pressurized solvent process. *Food Science and Technology*, *62*(2015), 131–137.

Mezadri, T., Perez-Gelvez, A., & Hornero-Mendez, D. (2004). Carotenoid pigments in acerola fruits (Malpighia emarginata DC.) and derived products. *European Food Research and Technology*, *220*(1), 63–69.

Millan, C. S., et al. (2015). β-cryptoxanthin synergistically enhances the antitumoral activity of oxaliplating through DNP73 negative regulation in colon cancer. *Clinical Cancer Research*, *21*(19), 4398–4409.

Mortensen, A. (2006). Carotenoids and other pigments as natural colorants. *Pure and Applied Chemistry*, *78* (8), 1477–1491.

Murakoshi, M., et al. (1992). Potent preventive action of α-carotene against carcinogenesis: Spontaneous liver carcinogenesis and promoting stage of lung and skin carcinogenesis in mice are suppressed more effectively by α-carotene than by β-carotene 1. *Cancer Research*, *52*, 6583–6587.

Nagarajaiah, S. B., & Prakash, J. (2011). Chemical composition and antioxidant potential of peels from three varieties of banana. *Asian journal of Food Agro-Industry*, *4*(1), 31–46.

Nagarajan, J., et al. (2016). Extraction of phytochemicals using hydrotropic solvents. *Separation Science and Technology*, Available at http://dx.doi.org/10.1080/01496395.2016.1143842.

Nakornriab, M., Sriseadka, T., & Wongpornchai, S. (2008). Quantification of carotenoid and flavonoid components in brans of some Thai Black Rice cultivators using supercritical fluid extraction and HPLC-Mass spec. *Journal of Food Lipids*, *15*, 488–503.

Oreno, Ä. N., et al. (2005). Impact of high pressure and pulsed electric fields on bioactive compounds and antioxidant activity of orange juice in comparison with traditional thermal processing. *Journal of Agricultural and Food Chemistry*, *53*, 4403–4409.

Oroian, M., & Escriche, I. (2015). Antioxidants: Characterization, natural sources, extraction and analysis. *Food Research International*, *74*, 10–36.

Ozaki, et al. (2015). Daily intake of β-cryptoxanthin prevents bone loss by preferential disturbance of osteoclastic activation in ovariectomized mice. *Journal of Pharmacological Science*, *129*(1), 72–77.

Palmero, P., et al. (2014). Role of carotenoid type on the effect of thermal processing on bioaccessibility. *Food Chemistry*, *157*, 275–282.

Palmero, P., et al. (2015). Enzymatic cell wall degradation of high-pressure-homogenized tomato puree and its effect on lycopene bioaccessibility. *Journal of the Science of Food and Agriculture*, *96*, 254–261.

Panozzo, A., et al. (2013). Microstructure and bioaccessibility of different carotenoid species as affected by high pressure homogenisation: a case study on differently colored tomatoes. *Food Chemistry*, *141*(2013), 4094–4100.

Pattison, D. J., et al. (2005). Dietary β-cryptoxanthin and inflammatory polyarthritis: Results from a population-based prospective study. *The American Journal of Cinical Nutrition*, *81*, 451–455.

Pingret, D., Fabiano-Tixier, A. S., & Chemat, F. (2013). Degradation during application of ultrasound in food processing: A review. *Food Control*, *31*(2), 593–606.

Prado, J. M., Veggi, P. C., & Meireles, M. A. A. (2014). Extraction methods for obtaining carotenoids from vegetables - Review. *Current Analytical Chemistry*, *10*(1), 29–66.

Rao, A. V., & Rao, L. G. (2007). Carotenoids and human health. *Pharmacological Research*, *55*(3), 207–216.

Rios, S. D. A., et al. (2014). Color of corn grains and carotenoid profile of importance for human health. *American Journal of Plant Sciences*, *5*(3), 857–862.

Rita, S. A., et al. (2005). Acceleration effect of sulfides on photodegradation of carotenoids by UVA irradiation. *Bioscience, Biotechnology and Biochemistry*, *69*(9), 1786–1789.

Rivera, S. M., Christou, P., & Garayoa, R. C. (2014). Identification of carotenoids using mass spectrometry. *Mass Spectrometry Reviews*, *33*(5), p. 353−372.

Rodríguez-Roque, M. J., et al. (2015). Food matrix and processing influence on carotenoid bioaccessibility and lipophilic antioxidant activity of fruit juice-based beverages. *Food & function*, *7*(1), 380−389.

Roohinejad, S., Everett, D. W., & Oey, I. (2014). Effect of pulsed electric field processing on carotenoid extractability of carrot puree. *International Journal of Food Science and Technology*, *49*, 2120−2127.

Rutz, J. K., Borges, C. D., Zambiazi, R. C., Rosa, C. G., & Silva, M. M. (2016). Elaboration of microparticles of carotenoids from natural and synthetic sources for applications in food. *Food Chemistry*, *202*(2016), 324−333.

Saini, R. K., Nile, S. H., & Park, S. W. (2015). Carotenoids from fruits and vegetables: Chemistry, analysis, occurrence, bioavailability and biological activities. *Food Research International*, *76*(3), 735−750.

Santos, J. G., Fernandes, F. A., Oliveira, L. S., & Miranda, M. R. (2015). Influence of ultrasound on fresh cut mango quality through evaluation of enzymatic and oxidative metabolism. *Food Bioprocess Technology*, *8*, 1532−1542.

Sarkar, C., et al. (2012). An efficient condition of Saponification of Lutein ester from marigold flower. *Annals of Biological Research*, *3*(3), 1461−1466.

Seifi, M., et al. (2013). Extraction of lycopene from tomato paste by ursodeoxycholic acid using the selective inclusion complex method. *Journal of Food Science*, *78*(11), 1680−1685.

Seo, J. S., et al. (2005). Extraction and chromatography of carotenoids from pumpkin. *Journal of Chromatography A*, *1073*(1−2), 371−375.

Shanmugam, A., & Ashokkumar, M. (2015). Characterization of ultrasonically prepared flaxseed oil enriched beverage/carrot juice emulsion and process-induced changes to the functional properties of carrot juice. *Food Bioprocess Technology*, *8*, 1258−1266.

Shi, J., & Maguer, M. L. (2000). Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Critical Reviews in Food Science and Nutrition*, *40*(1), 1−42.

Shilpi, A., Shivhare, U. S., & Basu, S. (2013). Supercritical CO_2 extraction of compounds with antioxidant activity from fruits and vegetables waste -A review. *Focusing on Modern Food Industry*, *2*(1), 44−62.

Singh, A., Ahmad, S., & Ahmad, A. (2015). Green extraction methods and environmental applications of carotenoids-A review. *Royal Society Chemistry Advance*, *5*(77), 62358−62393.

Slattery, M. L., et al. (2000). Carotenoids and colon cancer. *The American Journal of Clinical Nutrition*, *71*(2), 575−582.

Srivastava, J., & Vankar, P. S. (2015). Carotenoids: as natural food colorant from Canna flowers. *Pigment & Resin Technology*, *44*(1), 13−18.

Stahl, W., & Sies, H. (2003). Antioxidant activity of carotenoids. *Molecular Aspects of Medicine*, *24*(6), 345−351.

Stahl, W., & Sies, H. (2005). Bioactivity and protective effects of natural carotenoids. *Carotenoids and Dietary Lipids*, *1740*(2), 101−107.

Strati, I. F., & Oreopoulou, V. (2014). Recovery of carotenoids from tomato processing byproducts − A review. *Food Research International*, *65*(2014), 311−321.

Svelander, C. A., et al. (2011). High pressure homogenization increases the in vitro bioaccessibility of α- and β-carotene in carrot emulsions but not of lycopene in tomato emulsions. *Journal of Food Science*, *76*(9), 215−225.

Swada, J. G., Keeley, C. J., Ghane, M. A., & Engeseth, N. J. (2016). Synergistic potential of papaya and strawberry nectar blends focus on specific nutrients and antioxidants using alternative thermal and non thermal processing techniques. *Food Chemistry*, *199*(2016), 87−95.

Takashi (2010) Extract solution comprising a β-cryptoxanthin components and food and drink soaps. US Patent 0063163 A1.

Tang, G. (2014). Vitamin A value of plant food provitamin A - evaluated by the stable isotope technologies. *International Journal for Vitamin and Nutrition Research*, 25–29.

Tanvetyanon, T., & Bepler, G. (2008). Beta-carotene in multivitamins and the possible risk of lung cancer among smokers versus former smokers: A meta-analysis and evaluation of national brands. *Cancer*, *113* (1), 150–157.

Thaipong, K., et al. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, *19*, 669–675.

Thakur, B. R., Singh, R. K., & Handa, A. K. (1995). Effect of homogenization pressure on consistency of tomato juice. *Journal of Food Quality*, *18*(5), 389–396.

Thirumdas, R., Sarangapani, C., & Annapure, U. S. (2014). Cold plasma: A novel nonthermal technology for food processing. *Food Biophysics*, *10*(1), 1–11.

Tiwari, B. K., O'donnell, C. P., & Cullen, P. J. (2009). Effect of non thermal processing technologies on the anthocyanin content of fruit juices. *Trends in Food Science & Technology*, *20*(3), 137–145.

Trujillo, L. S., Qian, C., Bellosa, O., & McClements, D. J. (2013). Modulating β-carotene bioaccessibility by controlling oil composition and concentration in edible nanoemulsions. *Food Chemistry*, *139*(2013), 878–884.

Ukom, A. N., et al. (2014). Proximate composition and carotenoid profile of yams (Dioscorea spp.) and cocoyam (Xanthosoma maffa (Scoth)) root tubers from Nigeria. *American Journal of Food and Nutrition*, *4*(1), 1–10.

Varma, S., Karwe, M. V., & Lee, T. C. (2010). Effect of high hydrostatic pressure processing on lycopene isomers. *International Journal of Food Engineering*, *6*(5), 1–20.

Wang, L., et al. (2014). Specific carotenoid intake is inversely associated with the risk of breast cancer among Chinese women. *The British Journal of Nutrition*, *111*(9), 1686–1695.

Woodside, J. V., et al. (2015). Carotenoids and health in older people. *Maturitas*, *80*(1), 63–68.

Wu, J., et al. (2015). Intakes of lutein, zeaxanthin, and other carotenoids and age-related macular degeneration during 2 decades of prospective follow-up. *JAMA Ophthalmology*, *133*(12), 1415–1424.

Xie, Y., et al. (2016). Disruption of thermo-tolerant Desmosemus sp. F51 in high pressure homogenization as a prelude to carotenoids extraction. *Biochemical Engineering Journal*, *109*(2016), 243–251.

Yamaguchi, M. (2008). β-Cryptoxanthin and Bone Metabolism: The preventive role in osteoporosis. *Journal of Health Science*, *54*(4), 356–369.

Young, A. J., & Lowe, G. M. (2001). Antioxidant and prooxidant properties of carotenoids. *Archives of Biochemistry and Biophysics*, *385*(1), 20–27.

Zhang, K. S., et al. (2011). Supercritical carbon dioxide extraction of carotenoids from marigold flower using orthogonal test design. *Advanced Materials Research*, *396–398*, 2253–2255.

Zhang, Y., et al. (2015a). Quality comparison of carrot juices processed by high pressure processing and high temperature short time processing. *Innovative Food Science and Emerging Technologies*, (*in press*).

Zhang, R., et al. (2015b). Enhancing nutraceutical bioavailability from raw and cooked vegetables using excipient emulsions: Influence of lipid type on carotenoid bioaccessibility from carrots. *Journal of Agricultural and Food Chemistry*, *63*(48), 10508–10517.

Zhao, Y., et al. (2014). Stability of carotenoids in *Russula alutacea* for extraction. *Advanced Materials Research*, *962*, 1239–1243.

Zhou, C., et al. (2011). Carotenoids in fruits of different persimmon cultivars. *Molecules (Basel, Switzerland)*, *16*(1), 624–636.

Zu, K., et al. (2014). Dietary lycopene, angiogenesis, and prostate cancer: a prospective study in the prostate-specific antigen era. *Journal of the National Cancer Institute*, *106*(2), 1–10.

CHAPTER

FOOD AROMA COMPOUNDS

9

Urszula Tylewicz[1], Raffaella Inchingolo[2] and Maria Teresa Rodriguez-Estrada[1,2]

[1]Alma Mater Studiorum-Università di Bologna, Cesena, Italy [2]Alma Mater Studiorum-Università di Bologna, Bologna, Italy

9.1 INTRODUCTION

Aroma compounds are one of the main food sensory characteristics that impact consumer preference and acceptance. Such compounds can be naturally present in foods as a consequence of physiological and/or enzymatic processes, as well as generated by microorganisms during fermentation processes. However, aroma compounds can also be produced and/or modified due to chemical, biochemical, or microbial changes during their extraction, processing, and storage, thus affecting the overall food quality, sensory profile, and shelf-life. During processing, food aroma compounds are generated by enzymatic activity, fermentation, lipid oxidation, and thermal reactions (Maillard reaction, caramelization). While thermal technologies increase food safety, they also accelerate the aforementioned chemical reactions and thus induce several modifications in the macro- and microcomponents. Heat-generated flavors are in fact the most abundant group of volatile compounds (VOCs) in food and some of them can be actually used as process markers. Recent advances in the food industry have led to the development of new processing technologies (e.g., nonthermal technologies, nanotechnology), which can affect food aroma compounds differently depending on the composition and microstructure of the food.

This chapter discusses the main natural and technology-derived food aroma compounds, with a focus on novel extraction and delivery strategies as well as on the effects of innovative food-processing technologies on the food aroma profile.

9.1.1 CLASSES OF FOOD AROMA COMPOUNDS

Among the >7000 VOCs isolated from foods, only ~5% of them actually contribute to food aroma (Belitz, Grosch, & Schieberle, 2009). Aroma compounds are present in food in a wide concentration range and their impact on food aroma not only depends on their concentration, but also on their odor thresholds. The thresholds of some odorous compounds in aqueous systems can be found in the literature (http://www.leffingwell.com/odorthre.htm; http://www.leffingwell.com/ald1.htm; Ma, Chyau, & Pan, 2004; Meynier, Genot, & Gandemer, 1998). Both threshold values and concentrations of the single VOCs will contribute to the overall VOC equilibrium, which will determine the final food aroma and flavor acceptance. Aroma compounds are mainly comprised of

Nutraceutical and Functional Food Components. DOI: http://dx.doi.org/10.1016/B978-0-12-805257-0.00009-0

organic molecules present in the liquid or gaseous state, and are characterized by a low molecular weight (<400 Da; Soccol, Medeiros, Vandenberghe, Soares, & Pandey, 2008), most of them having a lipophilic character. The main aroma compound classes are discussed as follows.

9.1.2 ACIDS

Organic acids are one of the most important odor compound classes, as they contribute differently to food aroma according to their dimensions and corresponding threshold values. Most fatty acids are derived from enzymatic or chemical lipid hydrolysis, while short-chain linear or branched fatty acids generally originate from the metabolism of amino acids or carbohydrates. Organic acids can also be generated by aldehyde oxidation. Certain small organic acids (such as acetic, succinic, and lactic acids) are used as indicators of the alcoholic fermentation performed by *Saccharomyces cerevisiae*.

9.1.3 ALCOHOLS

Primary and secondary alcohols confer important characteristics to food aroma profiles and can be synthesized from diverse metabolic pathways starting from carbohydrates or amino acids. They can also originate from carbonyl group (ketone or aldehyde) reduction and long-chain polyunsaturated fatty acid oxidation.

9.1.3.1 Amines and other nitrogen compounds

Volatile or nonvolatile amines generally derive from the decarboxylation of amino acids due to proteolysis. The odor of volatile amines is usually defined as alcoholic, fruity, or varnishy; however, some of them can be associated with extensive food degradation, conferring fishy or putrid notes. Primary and secondary amines have much higher detection threshold values than tertiary ones (Molimard & Spinnler, 1996). On the other hand, amino acid deamination releases ammonia, which results in an ammoniacal taste and odor in some ripened protein foods (such as salami or cheese).

9.1.3.2 Carbonyl compounds

Carbonyl compounds are comprised of aldehydes and ketones. Aldehydes can be formed by amino acid deamination or transamination, Strecker degradation, microbial activity during fermentation, and fatty acid oxidation. Most aldehydes have a fruity or floral aroma with a fresh note, but the elongated alkyl group augments the oily or fatty note. Ketones are mainly derived from lipid oxidation, as well as from the citrate and glucose metabolism. Ketones with a 5−13 carbon atom structure usually provide fruity or musty notes, while some smaller ketones (such as acetoin and diacetyl) possess a buttery flavor.

9.1.3.3 Esters

Esters are generally formed by esterification reactions between fatty acids (short or medium chain) and alcohols (methanol or ethanol) that are derived from carbohydrate fermentation or from amino acid catabolism. Such reactions are catalyzed by esterases. Fruity or floral notes are usually attributed to esters, some of which exhibit perception thresholds about 10-fold lower than those of their corresponding alcohols (Carballo, 2012).

9.1.3.4 Lactones

Lactones are cyclic esters formed by the condensation of an acid and an alcohol. Lactones may form in foods by microbial action, extensive lipid oxidation (thermoxidation or autoxidation), or heating. Lactones can also form from amino acids. Lactones are mainly responsible for fruity notes. In general, γ-lactones have lower detection thresholds than δ-lactones, and the values tend to increase with increasing chain length (Carballo, 2012).

9.1.3.5 Oxygen-containing heterocyclic compounds

Furanones and pyranones are oxygen-containing heterocyclic compounds responsible for both caramelized and Maillard flavors. The most common aroma notes of these compounds are caramel-like, sweet, fruity, nutty, or burnt. They are formed when carbohydrates are subjected to non-enzymatic browning reactions. The formation mechanism usually involves the cyclization of non-nitrogen-containing browning intermediates that are derived from sugar dehydration or Strecker degradation.

9.1.3.6 Pyrazines

Pyrazines are heterocyclic, nitrogen-containing compounds that significantly impact the flavor of many roasted, toasted, or fermented foods. They are mainly associated with non-enzymatic browning reactions (Strecker degradation), and are able to provide diverse flavors, which greatly vary according to the type of alkyl substituents. They are generated by Maillard reaction in thermally treated food and are associated with roasty, toasty, or nutty flavors. In raw vegetables, they contribute to their green notes.

9.1.3.7 Sulfur compounds

Sulfur compounds originate from the synthesis and degradation routes of sulfur amino acids, in particular methionine and cysteine. These compounds are extremely volatile, and their perception threshold values are quite low (Molimard & Spinnler, 1996). Their flavors have been associated with raw and cooked cabbage, cauliflower, and garlic (Carballo, 2012). The Maillard reaction also generates thiazoles and thiophenes, which are heterocyclic sulfur-containing compounds with similar sensory properties.

9.1.3.8 Terpenes

Terpenes are hydrocarbons based on the five-carbon isoprene unit (2-methyl-1,3-butadiene), with structures that may be open chain, closed chain, saturated, or unsaturated, as well as contain O, N, or S. They are commonly associated with the flavor of spices, herbs, and citrus products. Terpenes are in fact the primary constituents of the essential oils (concentrated hydrophobic liquids containing volatile aroma compounds) of many of plants, spices, flowers, and citrus products.

9.2 EXTRACTION, RECOVERY, AND APPLICATIONS

In recent decades, innovative food has become a priority in industrially manufactured products, such as confectionery and bakery products, teas and beverages. Consumer demand for organic and natural ingredients has encouraged food companies to create environmentally friendly, novel,

economic, and natural aroma compounds. "Natural" aroma compounds are directly extracted from plants/vegetables, while "synthetic natural" compounds are chemically synthesized but have identical chemical structure to their natural counterparts. Their synthesis starts with a natural compound available in large amounts at low cost, or with a basic chemical one. Some examples of synthetic natural compounds are vanillin, citral, and menthol. However, expensive processes and purity requirements imposed on synthetic natural aroma compounds have often led to a low production rate of those compounds (Belitz et al., 2009). In addition, the composition and content of flavor and aromatic compounds largely vary according to the origin of the raw materials from which they are obtained, so it becomes particularly difficult and inconvenient to synthesize them (Lee & Lee, 2003). Therefore it has become extremely important that those compounds are selectively extracted from the original raw material with a proper (conventional or novel) approach. The literature reports different examples of extraction techniques, among which some of them, such as distillation or solvent extraction, have been used for many years (Azmir et al., 2013). To overcome some of the drawbacks of conventional methods, novel techniques aimed at improving the sustainability of the process and at reducing environmental impact by decreasing energy consumption, processing time, and solvent use, as well as by utilizing agri-food waste, by- and/or coproducts to isolate added-value compounds, have been developed. Of course, these objectives should not disregard the goal of obtaining high-quality, high-yield, and safe extracts. However, the main drawback of novel technologies is that they have a high degree of complexity and uncertainty about the extract composition and some of the process parameters (i.e., efficiency, yield, cost, industrial applicability), so the industry has to decide which emerging technology is more suitable to replace a traditional extraction method, according to market needs and economical possibilities. Supercritical/subcritical fluid extraction (SFE), subcritical water extract, ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) are some of the emerging extraction technologies that actually meet some of these requirements (Li, Fabiano-Tixier, & Chemat, 2014). Table 9.1 shows some detailed examples of novel extraction techniques and their comparison with conventional techniques for the recovery of essential oils.

9.2.1 CONVENTIONAL EXTRACTION TECHNOLOGIES

A brief description of the most common conventional techniques is given in this section to better understand the advantages of novel technologies.

9.2.1.1 Distillation

In general, all the extraction processes that use hot steam to remove the aromatic compounds are called *distillation*. Further condensation of these compounds produces the essential oils and water, which are then separated, clarified, and stored (Belitz et al., 2009; Lee & Lee, 2003). The hydrodistillation (HD) process heats the sample in water at a temperature close to 100°C with the aim to produce vapors (steam and vapors containing VOCs), which are then directed to a condenser and separated in a funnel (Sadgrove & Jones, 2015). Essential oil is comprised of a complex mixture of lipophilic VOCs, generally present at low concentrations. In contrast, to enrich or isolate a single aroma compound that represents the dominant constituent in the essential oil, the fractional distillation is used (e.g., menthol from peppermint; Belitz et al., 2009). Depending on the type and form of raw materials (powders, roots, seeds, or leaves), the performance of the distillation process can

Table 9.1 Examples of novel extraction techniques and their comparison with conventional techniques for the recovery of essential oils

Matrix (Rif.)	Optimized Operating Mode Of Extraction Techniques				Novel vs. Conventional Techniques	Useful Notes for Industrial Application
		Novel		Conventional		
Rosmarinus officinalis L. (Filly et al., 2014)		**SFME**	**MAC-75 (pilot scale)**	**HD**	– **EOs Composition**: Similar – **Yield (%)**: 0.54 (SFME), 0.50 (MAC-75) vs. 0.57 (HD) – **Extraction Time**: HD > SFME, MAC-75 – Oxygenated compounds (%): 36.2 (SFME), 32 (MAC-75) vs. 33.9 (HD) – **Monoterpene hydrocarbons (%)**: 58.3 (SFME), 62.7 (MAC-75) vs. 66 (HD) – **Aromatic profile**: MAC-75 ≈ SFME	– **Compounds identified**: 45 – **Principal VOCs**: limonene, α-pinene, camphor, camphene, bornyl acetate, β-pinene, borneol, eucalyptol, α-terpineol, β-caryophyllene and terpin-4-ol – **Overall Advantages**: Quicker, less energy consumption, reduced costs, environmentally friendly (no use of solvent and water)
	Extraction time	30 min	30 min	90 min		
	Sample weight	150 g	3 Kg	1 Kg sample + 7 L of water		
	Equipment parameters	150 W	3 KW	–		
	Processing conditions	100°C, P_{atm}	–	–		
Shatin pomelo peels (Teaka) (Chen et al., 2016)		**SFME**		**HD**	– **Composition**: similar – **Yield (%, w/w)**: • 150 W, 30 min (12.6); 300 W, 30 min (25.3) > HD • 150 W, 90 min > 150 W, 30 min • 300 W, 90 min > 300 W, 30 min – **Oxygenated compounds**: • 150 W, 90 min; 300 W, 90 min > HD • HD > 450 W, 90 min	– **Principal VOCs** : limonene, β-pinene, linalool, α-terpineol, nerolidol and ester compounds – **Advantages**: higher yield and better quality of EOs vs. HD; no consumption of solvent and water – **Drawbacks**: thermal degradation at high microwave power (450 W) and temperature above 106°C
	Extraction time	− 30 min − 90 min		180 min		
	Sample weight	120 g		1:6 (matrix: water)		
	Equipment parameters	− 150 W − 300 W − 450 W		–		
	Processing conditions	100−106°C		100°C		
Mentha piperita (Gavahian et al., 2015)		**MAHD**	**OAHD**	**HD**	– **Composition**: similar – **Yield (%, v/w)**: 2.17 (MAHD), 2.29 (OAHD) vs. 2.29 (HD) – **Rate of increasing temperature vs. extraction time**: MAHD, OAHD (5.6 times) > > HD – **Mint glands structure**: smoother surfaces (MAHD, OAHD) vs. wrinkled surfaces (HD) – **Specific gravity, refractive indices, sensory color perceptions of EOs**: similar – **Energy consumption**: HD > MAHD > OAHD	– **Compounds identified**: 34 – **Principal VOCs**: neoiso-menthol, iso-menthone and menthofuran – **Environmentally friendly technique**: OAHD > MAHD > HD
	Extraction time	16.50 min	19.71 min	55.88 min		
	Sample weight	30 g + 0.5 L salted water (1% NaCl, w/v)	30 g + 0.5 L salted water (1% NaCl, w/v)	30 g + 0.5 L water		
	Equipment parameters	500 W	220 V, 50 Hz	–		
	Processing conditions	100°C	100°C	100°C		

(*Continued*)

Table 9.1 Examples of novel extraction techniques and their comparison with conventional techniques for the recovery of essential oils *Continued*

Matrix (Rif.)	Optimized Operating Mode Of Extraction Techniques				Novel vs. Conventional Techniques	Useful Notes for Industrial Application
		Novel		Conventional		
***Satureja macrosiphonia* leaves** (Seidi Damyeh et al., 2015)		**MAHD**	**UAHD**	**HD**	– **Composition**: similar – **Yield (%, v/w)**: 1.82 (MAHD), 1.80 (UAHD) vs. 1.98 (HD) – **Rate of increasing temperature vs. extraction time**: MAHD > OAHD > HD – **Oxygenated monoterpenes (%)**: 88.08 (MAHD) > 72.32 (OAHD) > 70.92 (HD) – **Sesquiterpenes (%)**: 4.91 (HD) > 3.64 (OAHD) > 1.50 (MAHD) – **Specific gravity, refractive indices**: similar – **VOCs**: Significant differences in their percentage among the methods, which might be due to the conversion of some components (such as linalool and terpinene-4-ol). – **Energy consumption**: HD > OAHD > MAHD	– **Compounds identified**: 39 – **Principal VOCs**: *cis*-sabinene hydrate, linalool, borneol and terpinene-4-ol – **Advantages**: MAHD could be a selective extraction method of hydrated components (such as *cis*-sabinene hydrate) – **MAHD & OAHD**: effective heating and fast energy transfer, faster extraction process and low operating costs.
	Extraction time	12.11 min	84.67 min	181.33 min		
	Sample weight	30 g + 0.6 L salted water (0.3% NaCl)	30 g + 0.6 L salted water (0.3% NaCl)	30 g + 0.6 L salted water (0.3% NaCl)		
	Equipment parameters	1000 W	120 V	–		
	Processing conditions	100°C	100°C	100°C		
Hemp inflorescences (*Cannabis sativa L.)* (Da Porto et al., 2014)		**Sc-CO_2**		**HD**	– **Extraction yield Sc-CO_2 (I, II)**: S1 > S2, cuticular waxes precipitated in S1 – **Yield (%,w/w)**: 0.67 (S2, I) > 0.34 (S2, II) > 0.24 (HD) – **Hydrocarbon sesquiterpenes** (caryophyllene, β-farnesene, α-humulene) **and oxygenated sesquiterpenes** (caryophyllene oxide, β-eudesmol, β-bisabolol and α-bisabolol) **(%):** 64.24 (HD) > 63.89 (S2, II) > 45.56 (S2, I) – **Terpenes (%):** (S2, I) similar to hemp inflorescences	– **Principal VOCs**: α-pinene, β-pinene, myrcene, terpinolene, caryophyllene, α-humulene and caryophyllene oxide – **Conditions for obtaining superior aromatic extract quality:** (I) 10 MPa, 40°C – **Cuticular waxes**: collected in S1 – **Advantages:** less energy consumption, recovery of fractions with different composition and biological properties suitable for cosmetic and/or food industry.
	Extraction time	–		–		
	Sample weight	1500 g		150 g		
	Processing conditions	i. E: 10 MPa, 40°C (on-line fractionation **S1**: 7 MPa, 25°C; **S2**: 5 MPa, 15°C) ii. E: 14 MPa, 40°C (on-line fractionation **S1**: 7 MPa, 25°C; **S2:** 5 MPa, 15°C) CO_2 flow rate: 3 kg/h		–		

Melissa officinalis L. (Bogdanovic et al. 2016)		**Sc-CO_2**	**HD**	– **Yield (%):** 0.83-1.39 (I, II, III) vs. 0.03 (HD) – **Monoterpenes** (citronellal, neral, geranial) **(%):** 26.4 (HD) > SC-CO_2 – **Sesquiterpenes** (caryophyllene, caryophyllene oxide) **(%):** 45.5 (HD) > SC-CO_2 – **Unsaturated fatty acid, diterpenes:** SC-CO_2 > HD – **Eugenol and thymol:** not found in HD and SC-CO_2 (E); SC-CO_2 (S2, III) > SC-CO_2 (S2, I e II) – **Antioxidant capacity:** S2 II > S2 III > E > HD > S2 I	A method of two-step supercritical fractional extraction increased the amount of isolated compounds with greater molecular mass (heavy alcohols and waxes), as well as some important phenolic compounds present in the monoterpenes fraction (eugenol and thymol) that were not present in the first step of the extraction.
	Extraction time	4.5 h	4 h		
	Sample weight	50 g of milled lemon balm folium	50 g of milled lemon balm folium		
	Processing conditions	i. **E**: 10 MPa, 40°C (**S2**: 30 MPa, 25°C **(T < cT)**) subcritical condition ii. **E**: 10 MPa, 40°C (**S2**: 30 MPa, 40°C **(Sc-CO_2)**) iii. **E**: 10 MPa, 40°C (**S2**: 30 MPa, 100°C **(Sc-CO_2)**) CO_2 flow rate: 0.5 kg/h at 10 MPa and 0.3 kg/h at 30 MPa	–		
Jasminum sambac (L.) Ait (Ye et al., 2015)		**SFE-DME**	**SE-petroleum**	– **Extraction yield (%)**: 5.00 – **Yields of key components**: • benzyl acetate: SFE (7) > SE (1) • linalool : SFE (2) > SE (1) – **Caryophyllene, α-caryophyllene**: SE > SFE	– **Superior quality of fragrant volatiles:** SFE-DME – **Key compounds of EOs:** benzyl acetate and linalool – **Reduction of components with sweeter smell:** caryophyllene and α-caryophyllene – **Advantages:** high yield and quality of extracted aroma compounds from jasmine flowers; suitable for food applications (high quality jasmine tea processing without climate or timing limitation and with lower labor cost)
	Extraction time	53 min	–		
	Sample weight	Activated carbon (equivalent to 1155 g of original jasmine flowers) (308 g)	Activated carbon with aroma (300 g)		
	Processing conditions	Solvent-to-material ratio of 3.5:1 Subcritical condition 44 °C	– –		

(*Continued*)

Table 9.1 Examples of novel extraction techniques and their comparison with conventional techniques for the recovery of essential oils *Continued*

Matrix (Rif.)	Optimized Operating Mode Of Extraction Techniques			Novel vs. Conventional Techniques	Useful Notes for Industrial Application
		Novel	**Conventional**		
Lemon, orange, mandarin, and grapefruit peels (Omar et al., 2013)		**Sc-CO_2 (VOCs) Sc-CO_2-EtOH (polyphenols)**	**FUSE cyclohexane (VOCs) or ethanol (polyphenols)**	– **Extraction repeatability of volatile compounds (%)**: FUSE, 2−20; SFE 4−19 – **Extraction repeatability of total phenol and the antioxidant capacity (%)**: FUSE, 1−7; SFE, 1−9 – **Yield (%)**: FUSE > SFE	– **Monoterpenes:** α-pinene, camphene, β-pinene, *p*-cymene, limonene, eucalyptol, γ-terpinene, linalool and citral. – **Stability of aromas and antioxidant capacity:** aromas were stable stored at 4°C for 6 weeks and the antioxidant capacity for 13 weeks. – **Advantages:** agro-food wastes used as source of both VOCs and phenolic compounds, which can be used in cosmetics, pharma, or food processing.
	Extraction time	5 min	5 min		
	Sample weight	0.16−0.24 g	0.25 g		
	Processing conditions	P = 10 MPa (for volatile compounds); 17 MPa (for polyphenols) T = 35°C CO_2 flow rate: 1 mL/min	Cycle = 5 s^{-1} Amplitude = 3%		

Abbreviations: E: extraction vassel; EOs: essential oils; FUSE: focused ultrasound extraction; HD: hydrodistillation; MAHD: microwave assisted hydrodistillation; OAHD: ohmic assisted hydrodistillation; Sc-CO_2: supercritical fluid extraction-carbon dioxide; SE: solvent extraction; SFE-DME: subcritical fluid extraction-dimethyl ether; SFME: solvent free microwave extraction; S1, S2: on-line fractionation separator; VOCs: volatile compounds. Roman numbers in bracket indicate different experimental conditions performed separately.

be enhanced by choosing between steam distillation, hydrodistillation, or a combination of both (Lee & Lee, 2003). In the distillation method, it is important to monitor the processing temperature, pressure, and duration, in order to avoid the degradation reactions (thermal decomposition, oxidation, hydrolysis, loss of esters, and pyrolysis) that would cause loss of aroma compounds and thus reduction of the essential oil yield (Belitz et al., 2009). An example is the essential oil obtained from citrus fruits, which contains terpene hydrocarbons that are readily autoxidized and polymerized ("resin formation"). Therefore the fractional distillation could represent a way to remove these undesirable compounds (Belitz et al., 2009).

9.2.1.2 Solvent extraction

To obtain the highest extraction yields without thermal degradation, the conventional solvent extraction is a well-known technology utilized for the production of oleoresins and essential oils. This conventional method involves the use of liquid solvent to extract the soluble compounds from solid material (solid/liquid extraction) or liquid blend (liquid/liquid extraction; Lee & Lee, 2003). In this method, several solvent extraction cycles are performed to attain the best yield. In particular, the solvent percolates through the extraction vessel that contains the raw material to extract the compounds of interest and thereafter it returns into the boiler, where it is heated and distilled off to continue the cycle. In this process, the "oil extract" can be a resin or an absolute extract in which the volatile fraction can account for more than 10%. This method is preferred when the content of essential oil is low or the aroma compounds are destroyed by distillation or lost due to their high water solubility (Belitz et al., 2009). Consequently, it is important to choose the right solvent according to the polarity of the VOCs. In agreement with Good Manufacturing Practices (GMP), the solvents that can be used for the production of foodstuffs and food ingredients are propane, butane, butyl acetate, ethyl acetate, ethanol, carbon dioxide, acetone, and nitrous oxide (Lee & Lee, 2003).

9.2.1.3 Cold expression

The pure essential oil contained in the fruit skin is usually obtained by expression, which is commonly called cold pressing. This conventional technique does not employ heating to extract essential oils from citrus peels. The principle of this methodology is based on extraction by mechanical pressing of fruit, followed by filtering. A temperature range of 60−80°C is used in this process to facilitate extraction; however, it is lower than that used in the solvent extraction technique, which results in lower extraction yield. The main limitation of this method is extract deterioration, which can occur by hydrolysis, dissolution of oxygenated compounds, and microorganism carryover (Lee & Lee, 2003; Li et al., 2014).

9.2.1.4 Enfleurage

Another conventional extraction technique is enfleurage, which is primarily used to extract the aroma compounds from flower petals. In particular, flower petals are placed on a glass plate, covered with a lard layer, and then pressed, thus allowing the aroma compounds to be absorbed into the lard. The process continues until the lard becomes fully charged. Afterward, the oil is extracted using ethanol. Likewise the enfleurage, during the maceration method, the flowers are directly dipped in hot fat, which absorbs essential oils.

9.2.2 NOVEL EXTRACTION TECHNOLOGIES

9.2.2.1 Supercritical fluid extraction

Supercritical fluid extraction (SFE) utilizes a particular state of supercritical fluids in which both liquid and gas properties coexist. This distinctive state is obtained when the supercritical fluid goes beyond its critical point (pressure > critical pressure (cp), temperature > critical temperature (cT); Azmir et al., 2013; Capuzzo, Maffei, & Occhipinti, 2013). Supercritical fluids result in higher extraction yields in a shorter time, due to their high solvent power and high penetration capability in plant materials. In the supercritical state, the solvatation power of the supercritical fluid depends on the temperature and pressure used.

Among the available carriers, CO_2 can be considered the best choice to extract aroma compounds by SFE, because it is odorless, colorless, highly pure, safe, cost effective, nonflammable, and uses recyclable gas (Azmir et al., 2013; Capuzzo et al., 2013). Supercritical fluid CO_2 ($ScCO_2$, 32°C and 7.38 MPa) is suitable for extracting lipophilic compounds, such as oleoresins and essential oils, because of its low polarity; this fact can become, at the same time, a challenge to overcome if the extraction yield of the polar compounds is to be increased. Polar compounds can be extracted by SFE either by modifying the $ScCO_2$ polarity with a cosolvent or by using multistep extraction with different operating conditions to achieve selective extraction of compounds with diverse polarity (Bogdanovic et al., 2016; Capuzzo et al., 2013). The advantages of $ScCO_2$ are the production of an aroma extract similar to that of the original plant when the process conditions are liquid state and low temperature, as well as the ability to completely remove the supercritical fluid from the extract when it is depressurized at room temperature. At the same time, this technique removes undesirable compounds, such as waxes and pigments (Azmir et al., 2013; Capuzzo et al., 2013).

SFE has drawn the attention of food industry and has been used for different purposes, such as decaffeination, production of hops extracts, recovery of food aromas and flavors, and removal of contaminants (Khosravi-Darani, 2010). This technique is often used to obtain a specific composition of active components in extract (Bogdanovic et al., 2016). Da Porto, Decorti, and Natolino (2014) performed $ScCO_2$ on hemp inflorescences at two different operating modes: a pressure of 10 MPa at 40°C and a pressure of 14 MPa, at 40°C. At constant temperature, the increase of pressure enhanced the CO_2 density and its solvent power, thus improving the solubility of some compounds, such as hydrocarbon sesquiterpenes and oxygenated sesquiterpenes. Online multistep extraction of VOCs was achieved in two separators where temperature and pressure were decreased (S1: 7 MPa, 25°C; S2: 5 MPa, 15°C). As noted above, the fractionation allowed the recovery of undesirable compounds like cuticle waxes in the first separator, due to their lower solubility under the conditions used (S1: 7 MPa, 25°C); in the second separator, the VOCs were collected (Da Porto et al., 2014). Bogdanovic et al. (2016) developed a two-step extraction method to separate different compounds from lemon balm. The $ScCO_2$ extraction (E) performed in the extractor vessel (E: 10 MPa, 40°C) allowed the recovery of the essential oil fraction, which was mostly comprised of highly volatile aromatic compounds. The second extraction step was carried out using different operating temperatures but all at a pressure of 30 MPa (higher than the critical pressure). Thus the second fractionation was necessary to deeply penetrate the plant matrix and, depending on the adopted temperature (25, 40, or 100°C), the solvent selectivity was changed. In particular, the $ScCO_2$ (40°C) application in the second separator was able to increase the recovery of high-molecular-weight

compounds (such as heavy alcohols and waxes), whereas the application of temperatures higher than 25°C in the second separator allowed the extraction of some important phenolic compounds (such as eugenol [only at 100°C] and thimol), which were not present in the first extract vessel or in a hydrodistillation extract.

Other types of solvents can be used to extrapolate target compounds from raw materials by employing subcritical conditions. For instance, dimethyl ether was chosen as the subcritical fluid to extract fragrant volatiles from activated carbon that had absorbed jasmine flower aroma compounds. The jasmine VOC extract obtained with the subcritical solvent was of better quality than the solvent extract, as it contained a lower amount of spicy ingredients (like caryophyllene and α-caryophyllene) responsible for unpleasant odors. In addition, this technique led to a higher extraction yield (Ye et al., 2015).

9.2.2.2 Ultrasound-assisted extraction

Regarding green extraction technology, ultrasound-assisted extraction (UAE) was developed to improve process efficiency and to reduce extraction time with lower energy consumption. The ultrasound waves (frequencies >20 kHz) create expansion and compression cycles in the food matrix. In a liquid, the collapse of cavitation bubbles gives rise to high-speed micro-jets of liquid that impact the plant cell wall, which contains the essential oil (Baiano, 2014; Li et al., 2014). This mechanical rupture facilitates the mass transfer and release of plant essential oil. To achieve this objective, the operating parameters (such as ultrasonic frequency and intensity, temperature and treatment time) are determinant. The operation mode of this technique uses low temperature and pressure and thus diminishes the extent of thermal degradation of the extract's compounds. Therefore UAE results in high-quality aroma, with high extraction yield. However, this technology has some limitations, which include the presence of a dispersed phase in the system, as well as the choice of sonotrode, which can affect the extract stability due to metal release after prolonged utilization (Baiano, 2014; Li et al., 2014).

Ultrasound-assisted extraction of essential oils from clove was carried out by Tekin, Akalin, and Şeker (2015). They optimized the extraction process parameters (temperatures, extraction times, and plant concentrations) in order to get a higher yield of clove extract. The study showed that the extraction temperature was the most influencing parameter on the VOCs extraction yield. At higher temperature, the ultrasonic cavitations rose due to an increase in solvent vapor pressure, as well as to a decrease in both the viscosity and surface tension of the liquid phase. These phenomena result in more effective decomposition of plant cells and faster mass transfer between plant and solvent (Tekin et al., 2015).

Based on UAE, other techniques (such as focused ultrasound extraction (FUSE)) have been developed to expand the use of ultrasound in the extraction area. In FUSE, the ultrasound energy is focused on the ultrasound probe tip (typically made of titanium), which is immersed in the solvent solution (Omar, Alonso, Garaikoetxea, & Etxebarria, 2013). According to the authors, FUSE led to a higher yield of essential oil VOCs and phenolic compounds extracted from different types of citrus peel food waste as compared to $ScCO_2$, which was ascribed to the diverse solvent power of the mixture chosen (cyclohexane and ethanol) for its extraction by FUSE (Omar et al., 2013).

9.2.2.3 Microwave-assisted extraction

Microwave is used to distill plant materials, since it is able to achieve more effective and selective heating by using a noncontact heating source. In this way, the extraction process is completed in a few minutes instead of hours. In general, the process parameters (temperature, time, microwave frequencies, and power) depend on the experimental protocol, the nature of the raw materials, and the need for solvent or water for the extraction. Over the past few years, different microwave extraction techniques have been proposed with the aim to reduce the environmental impact and human exposure to solvents, as well as to obtain a high-quality extract with reduced degradation (thermal, hydrolysis, etc.) (Filly et al., 2014; Li et al., 2014). The first MAE technology proposed was compressed air microwave distillation (CAMD) (Craveiro, Matos, Alencar, & Plumel, 1989), followed by vacuum microwave hydrodistillation (VMHD), which was optimized for the extraction of thermal-sensitive compounds as it uses mild operating conditions (Mengal et al., 1993). Recently, microwave turbo hydrodistillation and simultaneous microwave distillation were able to achieve considerable reduction of treatment time and solvent used (Ferhat, Tigrine-Kordjani, Chemat, Meklati, & Chemat, 2007; Périno-Issartier, Abert-Vian, Petitcolas, & Chemat, 2010). All these MAE techniques are faster than conventional extraction (CE) methods (e.g., hydrodistillation) and exhibit similar extract yield and composition to those obtained by CE.

Among all MAE technology, solvent-free microwave extraction (SFME) represents the greenest technique (Filly et al., 2014; Li et al., 2014). The operating principle consists of the microwave-assisted dry distillation of fresh plant materials, where the apparatus selectively heats the *in situ* water of plant materials, which leads to the enlargement of the plant cells, up to the rupture of oleiferous glands. The continuous condensation system connected to the outer part of the microwave oven allows the recovery of the evaporated water−oil mixture at atmospheric pressure (Li et al., 2014). SFME could be negatively impacted by the different solubility of the volatile components of essential oils present in the plant cell water; moreover, their diverse dipole moments (low or high) could also affect the interaction with microwaves (Filly et al., 2014). SFME represents a suitable potential application for VOCs extraction from aromatic herbs with considerably good efficiency (Filly et al., 2014). In a recent study (Chen, Hu, Yao, & Liang, 2016), the essential oils from citrus peel were processed by SFME and the results were compared with those obtained with an HD method. Although the findings showed higher yield and better quality of essential oil extracted by SFME, it highlighted the occurrence of thermal degradation at higher microwave temperature (Chen et al., 2016). It is possible to overcome this problem by choosing the right operation temperature or selecting an appropriate apparatus. Depending on the extraction goal, the microwave apparatus can be used in closed mode, where it is possible to apply high pressures and temperatures for a fast and efficient extraction, or in the open mode for extracting thermo-labile compounds (Baiano, 2014).

Among the novel and green techniques, microwave hydrodiffusion and gravity (MHG) has been used to extract orange peel essential oil, which showed similar extraction yields to those achieved with steam distillation extraction (Boukroufa, Boutekedjiret, Petigny, Rakotomanomana, & Chemat, 2015). Boukroufa et al. (2015) used the wastewater recovered after essential oil separation as solvent to extract polyphenols and pectin from MHG residues.

9.2.2.4 Ohmic-assisted hydrodistillation

Ohmic-assisted hydrodistillation (OAHD) is another green extraction technique that has been recently proposed for the extraction of essential oils from aromatic plants. The principle of this

technology is based on the application of ohmic heating for greater and faster disruption of cells, which depends on the electrical conductivity of the material. The higher the heating rate achieved, the lower the processing time required (Gavahian, Farahnaky, Farhoosh, Javidnia, & Shahidi, 2015). This method was applied for the first time for the extraction of essential oils from *Zataria multiflora Boiss* (shirazi thyme; Gavahian et al., 2011), and later used for *Thymus vulgaris L.* (common thyme; Gavahian, Farahnaky, Javidnia, & Majzoobi, 2012), *Myrtle communis* (Gavahian & Farahnaky, 2013), *Cymbopogon atratus* (lemon grass), *Cymbopogon nardus* (citronella grass), *Backhousia citriodora* (lemon myrtle), *Syzygium aromaticum* (clove; Hamzah et al., 2011), *Satureja macrosiphonia* (Seidi Damyeh, Niakousari, Golmakani, & Saharkhiz, 2015), and *Mentha piperita* (Gavahian et al., 2015). Since direct heating by OAHD is limited to the electrical conductivity and distilled water is an exceptional electrical insulator, salted water is applied as the liquid phase in this process, so that materials such as oil droplets in mixed systems (with low electrical conductivity) can be processed.

9.2.2.5 Enzyme-assisted extraction

In this section, both conventional and modern extraction techniques are discussed in terms of general technology principles. However, pretreatment of raw material to enhance the extraction yield of the desirable aroma compounds could be an advantageous complementary approach. With this aim, enzyme-based extraction technology (EAE) can help destabilizing the structural integrity of plant cells. Enzymes are able to catalyze reactions with specificity and region-selectivity in aqueous solution under mild processing conditions, which makes EAE an environmentally friendly extraction method. The efficiency of EAE in food applications can be maximized when the following parameters are known: the nature of the enzyme, the action mechanism, optimum concentration, pH, time, and temperature of incubation (Sowbhagya & Chitra, 2010). Zhang et al. (2014) optimized vanillin production from natural green vanilla pods using pectinase (from *Aspergillus niger*)-assisted extraction combined with prefreezing and thawing. The pectinase amount, reaction temperature, time, and pH were kept at 84 mg, 50°C, 7.1 h, and 4.2, respectively, in order to get the maximum vanillin production (4.62% ± 0.14%). In this study, the combined action of prefreezing and thawing with EAE improved transformation of glucovanillin to vanillin and the production of natural vanillin from green vanilla.

In a recent work (Dutta & Bhattacharjee, 2015), EAE was also used in combination with Sc-CO_2 to enhance the yield of piperine-enriched extract from black pepper with a good combination of phytochemical properties (antioxidant activity, total phenolic content, reducing power, and anti-inflammatory activity). Optimized amounts of lyophilized enzyme (2 mg, 5 mg, and 10 mg) and enzyme exposure times to Sc-CO_2 (2.25 h and 4.25 h) were used to test the effectiveness of α-amylase (from *Bacillus licheniformis*) in both batch and continuous modes. Considering that starch is one of the major constituents of black-pepper coat, the utilization of enzymes improved the extraction of piperine. Regarding the enzyme activity, it was increased by 2.13- and 1.25-fold in the continuous and batch modes, respectively. Despite the higher enzyme activity, the continuous mode showed lower extraction yield from pepper materials, probably due to the lack of enzyme incubation time. These research results thus highlight that enzyme incubation time is required for starch hydrolysis in black-pepper matrix, and than an enhanced oleoresin yield was obtained when EAE was carried out in the batch mode (Dutta & Bhattacharjee, 2015).

Enzyme pretreatment of plant material for the extraction of flavor components results in enhancement of aroma recovery, since it increases the permeability of the cell wall and thus leads

to higher yield of the target compounds. On the other hand, this technique has some limitations, such as high cost of processing large volumes of raw material, current availability of enzyme preparations and, more importantly, the difficulty to scale EAE up to industrial level (Baiano, 2014; Sowbhagya & Chitra, 2010).

9.2.3 POTENTIAL FOOD-GRADE DELIVERY STRATEGY FOR AROMATIC COMPOUNDS

In recent years, the food industry has shown increasing interest in implementing food-grade delivery systems for added-value aromatic compounds, in response to growing consumer demand for natural food ingredients. Added-value food ingredients are used in place of synthetic compounds of natural extracts that have some health-promoting benefits. However, the development of ingredient incorporation systems should take into consideration important parameters such as bioavailability, physicochemical stability, thermal protection, and consumer acceptability in order to attain an effective delivery solution. At present, optimal formulation and processing strategies are needed for the successful protection and inclusion of naturally derived aromatic compounds. Efficacious, natural ingredients cannot only replace synthetic aroma, flavors, colorants, or preservatives, but they can also be employed for their potential antioxidant and probiotic health benefits. Compound incorporation by emulsion or inclusion could be a good approach for food formulation design; however, one of the difficulties faced during product formulation is that the overall system should be generally recognized as safe (GRAS). The optimized approach for food formulation also requires a suitable processing strategy, which will depend on the type of molecules to be incorporated, the formulation itself, and the type of food product. Moreover, from an industrial standpoint, it should also be inexpensive and reproducible for large-scale production (Đorđević et al., 2014).

9.2.4 OPTIMIZED APPROACH TO FORMULATION

In general, emulsion formulation requires the use of oil, water, surfactant, and co-surfactant. These components are organized in such a way as to obtain a suspension of small particles dispersed in a liquid medium. If the oil droplets are distributed in an aqueous phase, or vice versa, the systems are called oil-in-water (O/W) and water-in-oil (W/O) emulsions, respectively. In a food formulation, these emulsions are useful to carry lipophilic or hydrophilic compounds in aqueous- or lipid-based food. Depending on the specific goal, emulsions with smaller droplets (such as microemulsions and nanoemulsions) can present several advantages concerning physical stability, high optical clarity (useful for transparent beverages), and/or enhanced bioavailability of lipophilic components. However, when dealing with emulsion formulation, it is difficult to distinguish between these two systems as they use the same ingredients, but differ in processing method, stability, and functionality (McClements, 2012; Salvia-Trujillo, Martín-Belloso, & Mcclements, 2016).

Cyclodextrins (CD) are naturally occurring cyclic oligosaccharides that contain 6−8 glucose molecules. They are organized in a cylinder-shaped structure where the external part is hydrophilic and the cavity is a hydrophobic zone displaying diverse diameters along the structure. Among cyclodextrins, the β-CD is the most widely used, due to its lack of toxicity as well as for its cavity size and production costs. The main advantage of using this oligosaccharide is that it can encapsulate a wide variety of lipophilic molecules via hydrophobic interactions (so-called molecular inclusion complexation), thus increasing their aqueous solubility. Thus cyclodextrins can be introduced

in real food systems to protect hydrophobic added-value aromatic compounds (flavor, color, antioxidant) against induced degradation, as well as to perform controlled release of aromatic compounds (Đorđević et al., 2014; Madene, Jacquot, Scher, & Desobry, 2006; Zhang et al., 2015; Zuidam & Heinrich, 2010).

9.2.4.1 Microemulsions

Microemulsions are typically transparent, low-viscosity, isotropic dispersions with small particle size that can form spontaneously if a kinetic energy barrier is overcome. Considering their predisposition to form spontaneously, microemulsions could be easily developed for industrial applications when the main compounds (oil, water, surfactant) are put together and an external energy is applied (such as heating) to overcome the kinetic energy barrier or mass transport limitation (McClements, 2012). Furthermore, microemulsions are thermodynamically stable colloidal systems, which make them attractive delivery systems for bioactive molecules in functional food formulation. As long as the storage conditions or the chemical composition do not change, microemulsions will be thermodynamically stable and will have a long shelf-life, which is critical for ensuring the protection and further delivery of bioactive compounds. However, microemulsion formulation requires a higher surfactant-to-oil ratio than nanoemulsions do, which represents a limitation for their use in food systems considering the scarce availability of food-grade emulsifiers (Chatzidaki, Mitsou, Yaghmur, Xenakis, & Papadimitriou, 2015; Đorđević et al., 2014; McClements, 2012). Therefore academic and industrial investigations on food-grade microemulsions designed with emulsifiers and intended to deliver added-value natural aromatic compounds can help meet consumer demand for natural food ingredients.

9.2.4.2 Nanoemulsions

The use of nanoemulsions as potential delivery systems for lipophilic food ingredients is an emerging and promising method. An O/W nanoemulsion is defined as a thermodynamically unstable emulsion system in which small oil particles ($r < 100$ nm) are suspended in an aqueous solution-defined continuous phase (McClements, 2012). To produce nanoemulsions with droplet sizes in the nano-range, an external energy input is required to generate a colloidal dispersion because of its instability. The small droplet dimensions of the nanoemulsions give them unique properties such as improved transport of bioactive compounds through the biological membrane and increased active surface area, which raises their functionality. However, nanoemulsions are susceptible to physical changes during prolonged storage due to coalescence or Ostwald ripening phenomena. Therefore relatively long kinetic stability of the nanoemulsion can be obtained by optimizing its formulation (e.g., particle size distribution, type of oil, and emulsifier; Đorđević et al., 2014; Salvia-Trujillo et al., 2016). The lack of detailed information on enhanced functionality and stability of nanoemulsions further justifies scientific investigation for application of nanoemulsion technology in the food industry.

Essential oils can contain a complex mixture of compounds that are attractive for food ingredient formulation, due to their flavoring, antimicrobial, or antioxidant properties. In addition, their incorporation into food products is promising, because they are considered natural compounds that can replace the synthetic counterparts with the same functionality. In particular, essential oils have been praised for their antimicrobial activity due to the interaction of their phenolic compounds with the microbial cell membranes. However, the incorporation of essential oils into food products is

limited due to their flavor profile, physical state, low solubility, physicochemical stability, handling characteristics, toxicity, and costs.

In a recent work, Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, and Martín-Belloso (2015) investigated how the formulation of a nanoemulsion loaded with essential oils (lemongrass, clove, tea tree, thyme, geranium, marjoram, palmarosa, rosewood, sage, or mint) affects its physicochemical properties. In a second phase, the authors explored if those delivery systems improved the bactericide action of aromatic compounds, with respect to coarse emulsions (both stabilized with Tween 80 and sodium alginate). In general, the changes observed after microfluidization of the coarse emulsion (droplet size, *z*-potential, viscosity, whiteness index) depended on the type of essential oils loaded, which could have diverse molecular structure, concentration of VOCs, viscosity, interfacial tension, or different affinity with the surfactant. Moreover, the nanoemulsions exhibited increased and faster inactivation of *Escherichia coli*, resulting from fast release of antimicrobial aromatic compounds, which was particularly evident in the lemongrass or clove oil-loaded nanoemulsions (Salvia-Trujillo et al., 2015). Therefore essential oil loaded in a nanoemulsion can be a useful industrial food application, due to the transportation and delivery of the natural bioactive and aromatic compounds (e.g., terpenes, eugenol, citral, linalool). This example shows the advantages of using food-grade nanoemulsions incorporating essential oils, especially related to their antimicrobial activity as well as to the critical physico-chemical parameters that can affect their formulation.

Another major concern with nanoemulsions is their long-term stability as related to their thermodynamic destabilization. To address this issue, the long-term stability (56 days, 25°C) of food-grade nanoemulsions containing essential oils (oregano, thyme, lemongrass, or mandarin) stabilized by high methoxyl pectin and Tween 80 was determined (Guerra-Rosas, Morales-Castro, Ochoa-Martínez, Salvia-Trujillo, & Martín-Belloso, 2016). The results showed that long-term stability depended on the essential oil type as determined by the physico-chemical measurements (droplet size, droplet size distribution, and creaming index). The lemongrass and mandarin essential oil-pectin nanoemulsions showed high stability, while the oregano and thyme formulation destabilized after 56-day storage (at room temperature) probably due to Ostwald ripening. Another reason for their destabilization could be either a lower electrostatic repulsion between droplets, which depends on the molecular species absorbed at oil-in-water interface, or a lower pectin adsorption, which is related to the oil type (Guerra-Rosas et al., 2016). Considering the long-term stability of pectin-nanoemulsions containing essential oils, these nanoemulsions can be dispersed in food products and beverages to supply them with added-value natural aromatic compounds and to overtake some problems which could be linked to the solubility and/or aroma intensity of these essential oils.

9.2.4.3 Multilayer emulsion

More complex emulsions are emerging with structure that improves the physical/chemical stability of the system and/or the protection and target delivery of the encapsulated compounds in unusual environment/conditions, such as the mouth or stomach.

In multilayer emulsion, the interfacial membrane is engineered with more layers of molecules using a layer-by-layer technique. This technique is based on the electrostatic interaction between oppositely charged molecules that are absorbed around the droplet and thus lead to the modification of surface charge, thickness, permeability, rheology, and environmental responsiveness of the interfacial layer. Therefore the absorption of molecules is affected by pH, ionic strength, droplet

characteristics (particle concentration and size), solvent, nature of emulsifiers, and biopolymers. To formulate multilayer emulsions, food-grade emulsifiers and biopolymers are widely used. However, the main drawback of successful implementation of multilayer emulsion methods is the formation of irreversible flocs during processing, which may be attributed to several obstacles (e.g., polyelectrolyte concentration (low or too high), slow absorption rate), which can be overcome by preparing relatively dilute emulsions (<5 wt%).

Considering their good stability, multilayer emulsions are suitable for the encapsulation of lipophilic active compounds due to their chemical stability, especially when they are within laminated coating. Therefore the food industry can design a customized interfacial membrane to improve the functional performances of the system; however, it must be considered that its preparation requires more costly processes than conventional emulsion (Mao & Miao, 2015; McClements, 2015).

In addition to their sensory role, food flavors can also exhibit diverse biological activities, such as antimutagenic or antianalgesic effects. However, the flavor incorporation can be limited in foods or beverages due to its solubility or stability and thus it is sometimes necessary to incorporate these compounds in an emulsion system. Another example of food aroma largely used in the food/beverage or pharmaceutical industry is vanillin. The microencapsulation of vanillin by multilayer emulsion may improve its functionality and stability, as well as protect and control its release. Recently, Noshad, Mohebbi, Shahidi, and Koocheki (2015) microencapsulated vanillin using a layer-by-layer polyelectrolyte method followed by spray drying and evaluated the structure, physico-chemical properties of the microcapsules, as well as the flavor volatiles released from microcapsules into water (37°C and 80°C). The natural biopolymers used to surround the vanillin-containing microcapsules at the interfacial membrane were soy protein isolate (SPI, primary emulsion), OSA starch (secondary emulsion), and chitosan (tertiary emulsion). As shown by Fourier transform infrared spectroscopy (FTIR), the biopolymers at the interface interacted between them to build the multilayer emulsion. All formulations showed Newtonian behavior and the addition of chitosan to the third layer at the interface increased the viscosity and resulted in larger droplet size with irregular shapes of emulsion as compared to the others. As the authors noted, the one- and two-layer emulsions showed smooth surface, which may be due to faster film formation and could also explain the better encapsulation efficiency. In addition, the tertiary emulsion had the lowest moisture content, which is probably due to its higher viscosity. However, all emulsions showed lower solubility or hygroscopicity values and the same encapsulation efficiency. The low solubility is desirable in flavor encapsulation, since it allows controlled release into the medium, avoiding alterations in ionic strength, and it also makes capsule packaging and handling easier. The work by Noshad et al. (2015) highlighted the possibility of using the multilayer technique to encapsulate vanillin and protect it from high temperatures (even at 80°C). From an industrial standpoint, the three-layer microcapsules had more release-retarding efficacy, which is of utmost importance for the controlled release of bioactive compounds.

9.2.5 PROCESSING STRATEGIES FOR THE ENCAPSULATION OF NATURAL COMPOUNDS

Added-value natural compounds are normally more prone to degradation reactions during processing/handling, so it is necessary to use a suitable technology to avoid their deterioration. Spray drying and freeze drying were evaluated as microencapsulation technologies for the preparation of

turmeric oleoresine delivery systems, using different coating materials (gum arabic, maltodextrin plus modified starch, and a mixed system that contained all three coating materials), with the aim to improve the curcumin's stability (Cano-Higuita, Malacrida, & Telis, 2015). This hydrophobic yellow-orange polyphenol is an important compound used in food formulation as food aroma, stabilizer in jellies, or natural dye in cheeses, pickles, mustards, cereals, soups, ice creams, and yogurts (Mangolim et al., 2014). In the study by Cano-Higuita et al. (2015), it was demonstrated how the drying processing conditions could affect the curcumin retention, as this polyphenol was more retained in the freeze-dried microcapsule than in the spray-dried one. Among the coating systems tested, those containing gum arabic emulsifier, alone and in combination, presented higher cumin retention (Cano-Higuita et al., 2015). As the curcumin is not only a yellow dye but also an antioxidant compound that is light-sensitive, a shelf-life study (25°C) was carried out under light exposure for 8 weeks. The different microencapsulation technology also influenced the loss of curcumin, showing different degradation rates during the storage time probably due to the different particle microstructure obtained as well as to the diverse process parameters used. Throughout the shelf-life study, spray-dried particles lost curcumin to a lesser extent than freeze-dried particles, and both systems generated unpleasant odors and color change. The coating material comprised of gum arabic alone (for freeze drying) and in combination with modified starch and maltodextrin (for spray drying) was more effective at retaining the curcumin during shelf-life study and at preventing color modification during processing (Cano-Higuita et al., 2015). The different encapsulation results should be considered when developing certain food formulations, like mustard, enriched with particular aroma and color compounds. The use of these encapsulation systems to control the functional properties of turmeric oleoresin in terms of its optimized release, dosage, and stability in the food system is important.

In a recent work (Mangolim et al., 2014), different technologies (freeze drying, coprecipitation, and solvent evaporation) were used to encapsulate the hydrophobic polyphenol curcumin with β-cyclodextrins (β-CD). The complex formation was evaluated using several techniques (FT-IR, FT-Raman, X-ray diffraction, photoacustic spectroscopy). The inclusion complexation of the hydrophobic polyphenols with β-CD facilitated their application in the food system as compared to the direct addition of the pure curcumin, which exhibited different limitations (solubility, stability, gut absorption). Thus it is necessary to find a way to stabilize this molecule before applying it in a food system. According to Mangolim et al. (2014), the coprecipitation method allows the curcumin and β-CD to form an inclusion complexation similar to that obtained with others technologies. Furthermore, analytical methods showed that curcumin exists in keto-enol tautomeric form and that the β-CD-curcumin inclusion complex was formed due to the H-bonding between one or both of the aromatic rings of curcumin with the inner CD cavity. In addition, the successful coprecipitation resulted in a higher complexation efficiency, as 74% of the curcumin quantity that was initially added remained within the newly formed complex. Therefore the coprecipitation method for curcumin complexation could be a solution to its low water solubility and high sensitivity to alkaline conditions. Indeed, the authors found an increase in its water solubility, as well as improved stability of the curcumin-β-CD complex at several critical conditions tested (light, pH, storage, and heating), than for the curcumin alone.

On the other hand, the food application of curcumin complex was also studied in vanilla ice cream, to be used as natural yellow polyphenol ingredient compound. In this context, the curcumin-β-CD complex showed good performance in the vanilla ice cream, as it dispersed better

than pure curcumin. The inclusion complex formulation provided good color intensity and sensory acceptance, even though it used 83% less colorant than the pure dye system (Mangolim et al., 2014). Since color is an important aspect of food formulation, as it makes the final product more attractive and acceptable to consumers, the preparation of curcumin-β-CD complex by a simple and inexpensive technology like coprecipitation could represent a stable, cost-effective alternative for the food industry.

9.3 EFFECTS OF FOOD-PROCESSING TECHNOLOGIES ON THE CONTENT OF AROMATIC COMPOUNDS AND SENSORY PROFILE

In recent years, food industries have faced increasing demand to develop and to improve processing technologies and preservation techniques with minimal impact on the taste, texture, and nutritional value of food products. Thermal treatment has proven to be very effective for food preservation in terms of microbial inactivation. However, food handling and processing, including improper harvesting and heat treatment, play a significant role in degradation or loss of nutrients and aroma compounds, thus resulting in off-flavor or off-odor in the final products (Evrendilek, Avsar, & Evrendilek, 2016). Therefore recently a series of nonthermal emerging processing methods were explored as alternative ways to obtain safe products with sensory and nutritional attributes similar to those of fresh products. These methods include high-voltage pulsed electric field (PEF), ultrahigh hydrostatic pressure (HHP), ultraviolet radiation, gamma irradiation, ultrasound (US), and nonconventional chemical reagents.

9.3.1 PULSED ELECTRIC FIELD

Pulsed electric field (PEF) involves permeabilization of biological membranes that results from the application of short, high-power electrical pulses (ms or μs) to a product placed in between electrodes. Depending on the level of electric field strength applied, the process could be effective in microbial inactivation or could be used to improve mass transfer in processes such as extraction or drying. In fact, PEF treatment has been demonstrated to be a viable alternative to high-temperature inactivation of microbial load in liquid foods such as fruit juices, green tea extracts, and milk (Cregenzán-Alberti, Halpin, Whyte, Lyng, & Noci, 2015; Jaeger, Schulz, Karapetkov, & Knorr, 2009; Kempkes, 2010; Timmermans et al., 2014; Zhao et al., 2008). Further explanations of the basic principles of this technique can be found in the research studies carried out by Barba et al. (2015), Soliva-Fortuny, Balasa, Knorr, and Martín-Belloso (2009), and Toepfl, Mathys, Heinz, and Knorr (2006).

9.3.1.1 Juices

Cserhalmi et al. (2006) and Hartyáni et al. (2011) evaluated the effect of PEF treatment on the aroma compounds of fruit juices. They reported that PEF treatment (28 kV/cm, 50 pulses) did not significantly decrease the quantity of ethyl-esters (mainly ethyl-butyrate and linalool) in orange juice. Similarly, no significant changes were observed in grapefruit and tangerine juice aroma compounds (Cserhalmi et al., 2006; Hartyáni et al., 2011). In the case of lemon juice, no marked

decrease was detected in the amount of neral and geranial, which are responsible for lemon odor. The concentration of known off-flavor compounds (α-terpineol and terpinen-4-ol) remained constant (Cserhalmi et al., 2006). An increase in the main terpene components (limonene and valencene by 22 and 5%, respectively) was observed in orange juice after PEF processing, probably due to their release by such treatment (Hartyáni et al., 2011); however, a 45% degradation of decanal was noted (Hartyáni et al., 2011). In contrast, Cserhalmi et al. (2006) observed that the levels of orange juice VOCs (namely decanal and valencene) were analogous in both untreated and treated samples. According to the instrumental sensory analysis performed by electronic nose, only half of the juice control samples was ranked in the correct group, suggesting that the applied PEF treatment (28 kV/cm, 50 pulses) did not cause drastic changes in the overall aroma profile of treated samples (Hartyáni et al., 2011). In addition, a combination of thermosonication (55°C, 10 min) and PEF (40 kV/cm, 100 μs) did not induce significant changes in orange juice flavor as compared to the high-temperature, short-time (HTST) pasteurized product (94°C, 26 s). However, further analysis carried out by the sensory panel showed a blander taste in HTST orange juice with respect to the TS/PEF treated, while the latter had a metallic flavor (Walkling-Ribeiro, Noci, Cronin, Lyng, & Morgan, 2009). Caminiti, Noci, Morgan, Cronin, and Lyng (2012) observed a lower score for flavor perception and consequently lower acceptance of an orange and carrot juice blend treated with PEF (24 kV/cm, 18 Hz, 93 μs) combined with manothermosonication (MTS) (400 kPa, 35°C, 1000 W, 20 kHz), as compared to the pasteurized one. In contrast, Rivas, Rodrigo, Martínez, Barbosa-Cánovas, and Rodrigo (2006) found that PEF treatment (25 kV/cm, both with 280 μs and 333 μs) promoted favorable changes in the odor and taste of an orange and carrot juice blend with respect to conventional HTST treatment (98°C, 21 s).

Regarding apple juice aroma, Aguilar-Rosas, Ballinas-Casarrubias, Martin-Belloso, Nevarez-Moorillon, and Ortega-Rivas (2007) identified eight odor-active volatiles as the most important contributors. PEF treatment at 35 kV/cm was compared to HTST pasteurization in terms of retention/decrease of selected aroma compounds. Both treatments promoted a decrease in aroma compounds of apple juice, but some compounds (such as hexanal and hexyl acetate) remained almost unchanged (7−8% of loss, respectively) in the PEF-treated juices compared to the fresh, untreated sample. Moreover, PEF treatment resulted in higher retention of these compounds and other VOCs (acetic acid, butyl hexanoate, ethyl and methyl butyrate, hexyl acetate) than HTST did. Ethyl acetate was the only compound that exhibited higher losses in PEF-treated juice (Aguilar-Rosas et al., 2007).

The composition of the VOCs in apple ciders is even more complex than that of apple juice, since it depends on several factors such as fruit variety, maturity, and storage conditions (Azhu Valappil, Fan, Zhang, & Rouseff, 2009). Immediately after PEF treatment (23 kV/cm, 150 μs) or thermal processing (76°C, 1.3 s), the apple cider aroma compounds did not significantly change, but significant volatile differences between treatments were observed after 4 weeks of storage at 4°C. In general, PEF treatment decreased hexyl acetate concentrations during storage in all apple cider samples compared to untreated samples. 1-hexanol increased in PEF-treated samples, due the action of the residual esterases present in apple cider pulp. However, PEF-treated cider was, in general, able to better maintain the levels of butyl acetate, 2-methylbutyl acetate, 2-(E)-hexanal, hexanal, and benzaldehyde as compared to the HTST treatment (Azhu Valappil et al., 2009). Evrendilek (2016) studied the effect of PEF treatment time on aroma active compounds in sour cherry juice, as well as in apricot and peach nectars. While only one of the 17 identified compounds in the sour

cherry juice changed (6%), 50% and 100% of the aroma compounds in apricot and peach nectar underwent changes, respectively. In particular, the major component found in sour cherry juice was hexanol, which was not significantly impacted by PEF treatment. Concerning the apricot nectar, the most abundant compounds (3-hexyloxylan-2-one and 3,7-dimethylocta-1,6-dien-3-yl-2-aminobenzoate) decreased only when the longest treatment time (210 μs) was applied. In peach nectar, the most important changes were observed in octadecanoic acid concentration (raised by 251%) and heptadecane (reduced by 37%) (Evrendilek, 2016). Regarding the sensory analysis of these juice and nectar samples, the increasing treatment time did not adversely impact the flavor of both sour cherry juice and apricot nectar, while peach nectar showed a higher flavor score; consequently, the overall acceptability was higher for PEF-treated samples (Evrendilek, 2016). In another work by this group (Evrendilek et al., 2016), a lower mean loss of aroma active compounds of PEF-treated samples occurred at a PEF treatment time of 131 μs, which was also confirmed by better flavor and overall acceptability of PEF-treated peach nectar for 131 μs (Evrendilek et al., 2016).

9.3.1.2 Juice-milk beverage

Sampedro, Geveke, Fan, and Zhang (2009) identified 12 VOCs in an orange juice-milk beverage (OJMB), which could be divided into two groups according to their molecular weight (MW): the low MW group (β-pinene, α-pinene, β-myrcene, limonene, α-phellandrene, 3-carene, and 4-carene) and the relatively high MW group (nonanal, decanal, caryophyllene, dodecanal, and valencene). In general, thermal pasteurization of the OJMB (60−90°C, 1 min) promoted high VOCs loss, especially those belonging to the first group: β-pinene (48%), α-pinene (42%), and 3-carene (41%). Different PEF treatment conditions (15, 20, 25, 30 kV/cm; initial T: 25, 40, 65°C; 50 μs) were studied and their efficacy on the VOCs retention were checked (Sampedro et al., 2009). PEF treatment of 15 kV/cm at all three temperatures and of 20 kV/cm at 25 and 45°C increased the content of several compounds (such as β-pinene, limonene, 3-carene, 4-carene, nonanal, decanal, and dodecanal). This phenomenon was explained by the presence of suspended solids in the pulp, which can be extracted easier by PEF technology due to the increased membrane permeabilization. The most sensitive compounds to the PEF treatment were β-myrcene, α-pinene, and caryophyllene, and the least sensitive ones were limonene, 4-carene, and nonanal. The same authors evaluated the effects of high hydrostatic pressure (HHP; 450−650 MPa, 30 or 50°C) on the aroma compounds of OJMB (see Section 9.3.3).

9.3.1.3 Green tea

Zhao, Yang, Wang, and Lu (2009) investigated the effects PEF treatments on the main bioactive components (polyphenols, catechins, and free amino acids), color, and flavor of green tea infusions. PEF efficiently retained polyphenols, catechins, and the original color of green tea infusions at electric field strength from 20 to 40 kV/cm for 200 μs. No significant effects of PEF treatment at 20 or 30 kV/cm on flavor compounds of green tea infusions were noted. However, when the highest PEF treatment (40 kV/cm, for 200 μs) was applied, about a 10% loss of VOCs in green tea infusions was reported.

9.3.1.4 Wine must

Garde-Cerdán, Marsellés-Fontanet, Arias-Gil, Ancín-Azpilicueta, and Martín-Belloso (2008) studied the effects of PEF treatment (35 kV/cm, 1000 Hz, 1 ms) and storage conditions (5 and 23°C) on

the evolution of VOCs in white wine aged in bottles (6 months) without SO_2 addition. PEF treatment of wine showed a concentration increase of the esters that had a high influence on wine aroma (such as isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate), as compared to the standard fermentation wine; however, their concentration decreased during aging at both storage conditions. The concentration of ethyl esters of organic acids (such as ethyl lactate, diethyl succinate, and diethyl malate), which seem to play only a limited role in the organoleptic characteristics of healthy wines, was only detected in PEF-treated wine and increased when stored at room temperature with respect to the samples kept at 5°C. The concentration of ethyl acetate was lower in wines stored at both temperatures, but it was higher in the wine obtained by standard fermentation. However, it should be noted that the level found was far below the concentration at which this compound negatively affects wine aroma (150 mg/L).

Garde-Cerdán et al. (2008) observed that the concentration of total alcohols slightly increased in the wines aged in bottles at room temperature, while it did not change in wines that were kept at 5°C. However, it was always below the negative threshold (300 mg/L), suggesting that these compounds did not mask the wine aroma. Isoamyl alcohols were detected at higher concentrations in wine obtained with PEF-treated must, while 2-phenyl-ethanol was higher in wine produced with the standard fermentation. Isoamyl alcohols and 2-phenyl-ethanol were produced by *Saccharomyces* and non-*Saccharomyces* yeasts, respectively. The concentration of 2-phenyl ethanol, which gives a positive, rose aroma to wine, similarly increased in both wines during aging in bottles. Other VOCs (3-(methylthio)-1-propanol, *n*-hexanol, and benzyl alcohol) evolved in a similar way in both PEF-treated and control wines, as their concentration was slightly higher at the end of aging (Garde-Cerdán et al., 2008).

Garde-Cerdán et al. (2013) studied the effect of diverse PEF treatments on VOCs composition of must obtained from three different red grape varieties (Graciano, Tempranillo, and Grenache). In general, the aroma compounds of grape juice were enhanced by PEF application in Grenache, but no significant improvement was observed in the other two varieties. In fact, the response to the PEF treatment differed according to the grape variety. PEF treatments at low energies (10 μs−300 Hz; 10 μs−400 Hz and 20 μs−300 Hz) decreased the total monoterpenoid concentration in the Graciano variety, while the presence of these compounds was similar to that of the control sample under the highest energy (20 μs−400 Hz). This trend was also observed for linalool, geranyl acetone, and γ-geraniol. Nerol and neral were only affected by 10 μs−400 Hz treatment, showing a slight increase compared to the untreated sample, while α-terpineol and citronellol, geraniol, and *p*-cymene were not impacted by PEF treatment. In the case of Tempranillo, total monoterpenoids were higher in the treated samples, but when they were considered individually, most monoterpenoids (linalool, neral, geranyl acetone, and γ-geraniol) did not change after PEF treatment. In general, the Grenache variety had a higher quantity of monoterpenoids with respect to the other two varieties and most of them (linalool, citronellol, nerol, geraniol, isogeraniol, and γ-geraniol) increased after PEF treatment; however, other monoterpenoids present in this variety were not affected by PEF treatment or showed only small variations. Concerning the total C_{13} norisoprenoids, their concentration in the Graciano variety decreased only after the lowest PEF treatment (10 μs−300 Hz), which reflected the trend of (E)-β-damascenone, the most abundant C_{13} norisoprenoid in this variety. In general, PEF led to a decrease of methyl jasmonate in the Graciano variety, while other C_{13} norisoprenoids ((Z)-β-damascenone, β-ionone, β-cyclocitral) did not change during PEF treatment. In Tempranillo variety, the concentration of β-damascenones and consequently the total content of C_{13} norisoprenoids decreased

during PEF treatment. For Grenache, only β-ionone and β-cyclocitral were affected by PEF treatments, showing a slight increase. Concerning esters, the lower energy PEF treatments (10 μs−300 Hz) favored their presence in Graciano variety, while intermediate (10 μs−400 Hz or 20 μs−300 Hz) and high energy PEF treatments (20 μs−400 Hz) promoted their occurrence in Tempranillo and Grenache varieties, respectively. The increase of ester content could be ascribed to the application of PEF on grape skin. The content of benzenoid compounds, which give the wine a desirable aroma, was only slightly affected by PEF treatments in both Graciano and Tempranillo varieties, while PEF favored an increase of their concentration in Granache. Finally, the least influenced compounds by PEF treatment were the C6 ones, especially in Graciano and Tempranillo varieties. In Grenache, an increase of hexanal, (Z)-3-hexen-1-ol, and (E)-2-hexen-1-ol was observed when the lowest energy was used for PEF treatment (Garde-Cerdán et al., 2013).

9.3.2 IONIZING IRRADIATION

Irradiation is the process that exposes food to ionizing radiation, such as gamma and electron beam, in order to enhance its safety and consequently its shelf-life. The aim is to destroy microorganisms or insects that may be present in the food, and in some cases, to improve the food functional properties or to eliminate toxins, all while limiting the impact on their sensory and nutritional characteristics (Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012). Although food irradiation is considered a nonthermal process, it is often associated with development of off-flavors in some fresh fruits and vegetables. Thus it is important to choose the right radiation dose to enhance the positive effects and minimize the negative ones (Stewart, 2009).

9.3.2.1 Whole and fresh-cut fruit and vegetables

Prakash, Lim, Duong, Caporaso, and Foley (2010) treated raw almonds with 1, 1.5, 2, 2.5, and 3 kGy and studied the effects of these treatments on the microbial inactivation (*Salmonella*) and sensory characteristics of almonds. Panelists detected a significantly lower almond flavor in the irradiated samples (2.98 and 5.25 kGy) as compared to the control ones. In addition, the irradiated almonds had a significantly higher metallic/chemical/rancid/oxidized/fatty taste than the control samples; however, no significant differences among irradiated samples were observed. Although the 5.25 kGy irradiation was efficient in Salmonella inactivation, it made the almonds unacceptable from a sensory standpoint. Thus irradiation alone is not a suitable pasteurization treatment for raw almonds. Taipina, Lamardo, Rodas, and Del Mastro (2009) evaluated the effect of gamma irradiation (^{60}Co, doses of 1 and 3 kGy) on vitamin E content and sensory properties of pecan nuts (*Carya illinoensis*). Neither irradiation treatment promoted any changes in vitamin E content, and irradiation at 1 kGy did not induce significant changes in sensory attributes (appearance, aroma, texture, and flavor). In the case of fresh-cut spinach, irradiation at doses up to 2 kGy may enhance microbial safety without affecting consumer acceptance or overall antioxidant values of irradiated spinach (Fan and Sokorai, 2011).

9.3.3 HIGH HYDROSTATIC PRESSURE

The use of high hydrostatic pressure (HHP) or high-pressure processing (HPP) as a food-preservation technology has gained momentum in the past few years. By applying elevated pressures to foodstuffs

(from 300 to 1000 MPa), this technique achieves microbial inactivation or alters food attributes, attaining the desired food characteristics. Process temperature during pressure treatment can range from −20°C (to minimize any effects of adiabatic heat) to 121°C. The key advantage of HHP is the possibility to perform the process at room or refrigerated temperatures, thus eliminating thermally induced cooked off-flavors and providing special benefits for heat-sensitive products. In fact, as compared with thermal processing, HHP produces foods with better appearance, texture, and nutritional profile, as it causes minimal changes in the food freshness characteristics by avoiding thermal degradation (Knorr et al., 2011; Koutchma, 2014; Oey, Lille, Van Loey, & Hendrickx, 2008).

9.3.3.1 Juices

The effects of different processing methods such as pasteurization (90°C, 3 min), sterilization (121°C, 4 min), and HHP (400 MPa, 20 min, <30°C) on flavor characteristics of pennywort juices was evaluated by Wongfhun, Gordon, and Apichartsrangkoon (2010). In general, the total concentration of VOCs in fresh juice was significantly higher than in processed juice; however, HHP led to higher retention of volatile acyclic alcohols, aldehydes, and oxygenated monoterpenoids than pasteurization and sterilization. Ketones (including 2-butanone, 2-nonanone, 5-methyl-2-hexanone, and 3-nonen-2-one) were not present in fresh pennywort juice, but after processing, higher concentrations of 2-nonanone and 3-nonen-2-one in sterilized juice as compared to the HHP processed juice were found, which indicates that such ketones were generated at high temperatures during sterilization. Conclusively, HHP treatment maintained the juice flavor better than pasteurization and sterilization treatments (Wongfhun et al., 2010).

The volatile profile of a plum purée processed by HHP (400 and 600 MPa for 1, 150, and 300 s) was evaluated by González-Cebrino, García-Parra, and Ramírez (2016). Forty VOCs were identified in plum purée, among which only 23 were significantly influenced by HHP. However, such modifications represented only a small change in the total aroma of the original purée (1.8%). For instance, among aldehydes, the concentration of hex-2-enal increased in all HP treatment conditions, while dodecanal only increased after the most intense treatment (600 MPa, 300 s). In the case of alcohols, the two most abundant compounds (hexan-1-ol and hexen-3-ol) presented a decreasing trend, which was significant only for hexen-3-ol when the juice was subjected to more moderate conditions (400 MPa at 1 and 150 s). However, a general increase of oct-1-en-3-ol and non-1-en-3-ol levels was observed. Most esters (e.g., ethyl acetate, hexyl acetate, hexyl butanoate, pentylacetate) decreased after the high-pressure treatments. Among the four isolated terpenes (β-cyclocitral, β-ionone, β-damascenone, geranylacetone), only β-ionone rose after the softest HHP treatment (González-Cebrino et al., 2016).

9.3.3.2 Juice-milk beverages

Sampedro et al. (2009) studied the effects of different HHP conditions (initial T: 30 and 50 °C; pressure: 450, 500, 550, 600 and 650 MPa) on VOCs of orange juice-milk beverage (OJMB). At 30°C, only β-myrcene and α-phellandrene decreased at any pressure level (∼30−50%) among the assayed monoterpenes, and a slight reduction of limonene, α-pinene, β-pinene, and 3-carene contents was observed at 650 MPa, but lower pressure promoted an increase of such compounds. Moreover, other VOCs (such as 4-carene, nonanal, caryophylene, dodecanal, and valencene) displayed an increase after treatment at all pressure values at 30°C, which could be ascribable to their HHP-promoted release from the orange pulp. By increasing the initial temperature to 50°C, the

average loss was increased in all compounds and was directly related to the applied pressure. At 650 MPa and 50°C, the VOCs loss was the same as the one reached after the thermal treatment (85−90°C). Overall, the VOCs most sensitive to the HHP treatment were β-myrcene, α-phellandrene, and 3-carene, while valencene, limonene, and caryophyllene were the least sensitive ones (Sampedro et al., 2009).

9.3.3.3 Milk and cheese

Vazquez-Landaverde, Torres, and Qian (2006) reported the effects of different HHP treatments (482, 586, and 620 MPa), temperatures (25 and 60°C), and holding times (1, 3, and 5 min) on the generation of VOCs in milk. The results showed that pressure, temperature, and time, as well as their interactions, all had significant effects on VOCs production in milk. HHP at 25°C caused minimal changes on the aroma compounds of milk, whereas at 60°C VOCs formation was different from that observed at atmospheric pressure conditions. High-temperature heat treatment (25, 60, and 80°C for 1, 3, and 5 min) promoted the formation of both aldehydes and methyl ketones, whereas HHP at high temperature only favored the formation of aldehydes (e.g., hexanal concentration in samples with the strongest HHP treatment was almost 3-fold that of samples treated at atmospheric pressure). HHP also influenced the formation of sulfur compounds, as a significant increase in hydrogen sulfite (H_2S) was observed when subjected to HHP treatment even at 25°C, which was shown to be pressure-dependent. The H_2S levels in HHP-treated samples were generally higher than those of the corresponding heat-treated samples. Methanethiol concentrations were drastically diminished under pressure treatments, which could be due to its conversion into other compounds (Vazquez-Landaverde et al., 2006).

In mature raw goat milk cheeses with paprika on the rind, HHP treatment at 400 MPa and 600 MPa did not significantly influence most of the identified VOCs (Delgado, González-Crespo, Cava, & Ramírez, 2011). The small induced differences in some aroma compounds, were anyway flattened during refrigerated storage for 30 days. Among organic acids (the main VOCs class in this food product), butanoic, hexanoic, heptanoic, and decanoic acids were significantly higher after 400 MPa, as compared to the control and to the 600 MPa treatment samples. These differences could be due to higher enzymatic inactivation at 600 MPa than at 400 MPa, thus promoting the inactivation of lipases, which are responsible for lipolysis. The level of some carboxylic acids, which are important flavor compounds in goat cheese (butanoic acid (rancid cheese-like odor), hexanoic acid (goat odor), octanoic acid (rancid, pungent odor) and decanoic acid (sour, aged cheese odor), were lower in HHP-treated cheeses than in control ones after 30 days of refrigerated storage. Concerning alcohols, a reduction of 1-propanol and 2-pentanol concentration was observed in HHP-treated samples after 1 month of refrigerated storage. Ketones and esters contents were not affected by HHP treatment (Delgado et al., 2011).

Keenan, Brunton, Mitchell, Gormley, and Butler (2012) studied the effects of thermal and HHP (450 MPa/5 min/20°C or 600 MPa/10 min/20°C) on the VOCs and descriptive sensory attributes of fruit smoothies during their shelf-life of 30 days at 4°C. Six aroma compounds were detected, with limonene being the most abundant one. Pressure treatment did not significantly impact the concentration of these compounds, but thermal processing did affect them. 2-Hexanal, the second most abundant aroma compound, was not influenced by any processing treatment, but slightly higher concentrations of both butylacetate and amylacetate were detected in 600 MPa-treated samples. Refrigerated storage led to an overall decrease in the VOCs content of all smoothies, especially

trans-2-hexanal, which is associated with fresh/green/grassy flavor notes. Sensory analysis and principal component analysis of the descriptive sensory data revealed that attributes such as pink color, fresh color, apple aroma, fresh aroma, and fresh flavor were more associated with both fresh and processed smoothies at day 0, but they were not significantly affected by the treatments.

9.3.3.4 Wine

Santos et al. (2016) evaluated the effects of HHP treatment (at 500 and 600 MPa at 20°C for 5 and 20 min) on the phenolic compound composition and sensory profile of red wine after 5 months of storage. The pressure treatment promoted a decrease (directly related to the strength of the HHP conditions) of monomeric anthocyanins (13−14%), phenolic acids (8−11%), and flavonols (14−19%) after storage, as compared to unpressurized wine. The aroma of HHP-treated wines was characterized by a higher cooked fruit and sulfur aroma and a lower odor of fruity aromas compared to the unpressurized wine. Moreover, the 600 MPa wine samples had higher metallic notes than the control and 500 MPa samples. All these changes promoted a lower overall aroma assessment in HHP-treated wines. Similar results were obtained by Santos et al. (2013) in HHP-treated SO_2-free red wines. The wines treated at 425 MPa had a similar aroma as the SO_2-added wines, but the wine pressurized at 500 MPa had a higher cooked fruit odor and spice aroma. The control wines displayed a lower fruity and floral aroma, but they had a more pronounced metallic and leather aroma than the other wines.

9.3.4 ULTRASOUND

Ultrasounds (US) are air vibrations with frequencies ranging from 20 kHz to 100 MHz that generate rapid compression and expansion, which is usually referred to as the sponge effect. They induce cavitation, which is the formation of small bubbles at the points of low pressure in the sound wave. Once these bubbles reach a certain size range, they will collapse, often violently (cavitational collapse), thus increasing the local temperature and pressure. This phenomenon leads to an increase of mass transport in different processes (e.g., extractions, drying, freezing), but it can also promote microbial inactivation and modification of several sensory attributes (texture, color, taste, and odor) (Kentish & Feng, 2014; Nowacka, Tylewicz, Laghi, Dalla Rosa, & Witrowa-Rajchert, 2014; Schössler, Jäger, & Knorr, 2012).

9.3.4.1 Juices

Šimunek et al. (2013) studied the effects of different US processing parameters (amplitude, temperature, and time) on the aroma profile and sensory properties of apple juice and nectar. In general, ultrasonic treatment had an effect on aroma, leading to the formation of new compounds (e.g., butyl acetate, ethyl 2-methylbutyrate, 1-decanol) or the disappearance of others that were originally present in the fresh samples (e.g., 2,3-dimethyl-2-hexanol, 2-ethylhexyl salicylate). In apple juice, ethyl dodecanoate was drastically reduced by the pasteurization process (80°C, 2 min) from 41.38% (fresh juice) to 7.98% (pasteurized one). The effect of US treatment on the concentration of this compound depended on all three considered factors. US treatment at an amplitude of 120 mm at 60°C for 9 min increased the level of ethyl dodecanoate (61.85%), while a noticeable drop (3.75%) of ethyl dodecanoate was observed in the sample subjected to 90 mm and 40°C for 6 min. Similar behavior was noted in apple nectar, as it increased significantly after US treatment at 60 mm/60°C/

9 min from 0.11% in fresh samples to 66.49% in US-treated ones. Concerning the ethyl hexanoate content, in general, both pasteurization and US treatment of apple juice promoted its increase (from 17.43 in untreated samples to 25.17 % or 55.01% in pasteurized and US-90 mm, 20°C treated samples, respectively). However, under other treatment conditions (e.g., US-60 mm, 60°C, 3 min, and US-120 mm, 60°C, 3 min), the presence of ethyl hexanoate was reduced or not even detected. However, in apple nectar, both pasteurization and US treatment decreased the concentration of ethyl hexanoate. The diverse behavior of US-treated samples could be due to the combination of acoustic wave properties and the chemical changes caused by imploding cavitation bubbles, which leads to the subsequent hydrolysis of water to form free radicals that will participate in chemical reactions. As for the sensory analysis of the samples, rating differences in taste, odor, aroma, and color depended on the US treatment and the type of juice. The most accepted US-treated samples were apple juice at 60 mm and 40°C for 6 min and apple nectar at 120 mm and 20°C for 3 min (Šimunek et al., 2013).

Bastianello et al. (2016) identified 56 different aroma compounds in cloudy apple juice. Most of the aroma compounds showed the same trends in challenge (inoculation of the juice with two spoilage yeasts, *R. glutinis* and *C. parapsilosis*) and storage tests (4°C, 28 days). However, the content of some molecules (such as 2-methylbutyl acetate and hexyl acetate) decreased in US-treated (400 W, 24 KHz, <35 °C) juice as compared to the untreated juice. In the challenge test, a different trend was observed; for instance, the level of 1,3-octanediol was higher in US-treated juice than in fresh one, but it was lower in inoculated US juice than in inoculated fresh juice. The sonication process effectively delayed the oxidative process, which is related to the yeast metabolism. In fact, some alcohols (i.e., 3-methyl-1-butanol and phenylethyl alcohol) and some unpleasant carboxylic acids that are related to yeast metabolism showed increased levels in inoculated and nonfresh apple juice, but they were stable in US-treated apple juice. In general, the US treatment led to a shelf-life extension of apple juice under refrigeration (14 days), being able to keep its characteristic aroma (Bastianello et al., 2016).

Gómez-López, Orsolani, Martínez-Yépez, and Tapia (2010) analyzed the sensory characteristics of calcium-enriched orange juice subjected to US treatment (20 KHz, three wave amplitudes 59.5 mm (50%), 71.4 mm (60%), and 89.25 mm (75%) applied continuously for 2, 4, 6, 8, and 10 min). The sensory quality of the juice slightly deteriorated after US treatment, but during 10-day storage, it degraded faster in controls than in treated samples. In fact, the shelf-life in terms of off-odor comparison was extended for 4 extra days in US-treated orange juice (from 6 days in control juices to 10 days in US-treated ones).

9.3.4.2 Milk

Engin and Yuceer (2012) evaluated the effect of US application (US intensity 135 J/mL) on the aroma-active compounds and flavor characteristics of milk. In general, 41 different aroma compounds are found in milk among which aldehydes, ketones, esters, and acids were the major VOCs found in the analyzed milk. In both pasteurized (65°C, 30 min) and US-treated milk, maltol, which is responsible for the burnt sugar aroma, was detected, but US prevented the formation of other heat-generated aroma-active compounds, including 2-acetyl-1-pyrroline and 2-acetyl-2-thiazoline, compared to the pasteurized milk. The intensities of butyric, pentanoic, and hexanoic acids, which are responsible for the cheesy or sour aroma note, were higher in US-treated milk than in other milk samples, probably because it is related to the effects of cavitation and disruption on the fat

globule membrane due to US application (Engin and Yuceer, 2012). Riener, Noci, Cronin, Morgan, and Lyng (2009) observed that the application of high-intensity ultrasound (power output of 400 W; frequency 24 kHz) generated benzene, toluene, 1,3−butadiene, 5-methyl-1,3-cyclopentadiene, and a series of aliphatic 1-alkenes. These US-generated substances, predominantly hydrocarbons, are believed to be of pyrolytic origin and are generated by high localized temperatures as a consequence of cavitation phenomena.

9.3.4.3 Wine

Kulkarni et al. (2015) studied the combined effect of non-*Saccharomyces* yeast strain addition and US application (50 kHz; 10 min per day in 6 weeks of storage at 23°C) on polysaccharides release, aroma profiles, and organoleptic properties of red wine aged in lees. In general, US treatment of non-*Saccharomyces* and *Saccharomyces* red wines did not promote clear changes on fermentation VOCs that could be ascribed to wine aging in lees presence. The only differences that were observed involved acetaldehyde, ethyl lactate, 2-methyl-1-butanol, and isoamyl acetate. Acetaldehyde was formed due to either temperature increase during US treatment or phenolic compound oxidation. During phenolic compound oxidation, hydrogen peroxide can be released, which could lead to the oxidation of ethanol to acetaldehyde. Ethyl lactate concentration also increased in all yeast strains, which could be ascribed to the esterases release during autolysis; however, the highest increase was observed in *S. pombe* (up to 80 mg/L) as compared to the other yeast strains (70−75 mg/L). Concerning the sensory analysis, the aroma and organoleptic characteristics in terms of overall aroma rating were in general higher for wines aged on lees and subjected to US treatment (for all the strains studied) than for control samples. Luo, Schmid, Grbin, and Jiranek (2012) showed that high-power ultrasonics (HPU) can modify the wine flavor and aroma profile, which could be related to the generation of active free radicals during cavitation.

9.3.5 COLD PLASMA OR OZONE TREATMENT

Cold plasma or atmosphere cold plasma (ACP) and ozone processing are emerging technologies that offer many potential applications in food processing. Cold plasma is a gas that involves the coexistence, in a free space, of electrons and free ions in thermic motion. It is considered the "fourth state of matter" and is generated by applying energy to a gas mixture, causing gas ionization and the formation of active components (such as radicals, charged particles, and UV radiations) (Tappi et al., 2016; Zainal, Redzuan, & Misnal, 2015).

Ozone (O_3) is an unstable gas that decays naturally into diatomic oxygen, thus leaving no chemical residues. Ozone is a strong antimicrobial agent with several potential applications in the food industry. In fact, it is a very effective germicide against viruses, bacteria, spores, and stored grain insects and can cause irreversible oxidative damage to the fatty acids present in the cell membrane and cellular proteins (Tiwari & Muthukumarappan, 2012). Although the latter could represent an advantage from a food safety standpoint, it could become a disadvantage for the overall product quality as it could promote lipid and protein oxidation.

Alves Filho et al. (2016) evaluated the effect of ACP (70 kV for different treatment times—15, 30, 45, and 60 sec) and ozone treatments (different ozone loads: 0.057, 0.128, and 0.230 mg O_3/mL) on key compounds, including aromatic compounds, in orange juice using the ^{1}H-NMR technique. Diverse aromatic compounds were identified including niacin, histidine, formic acid,

5-HMF, phenylalanine, and tyrosine. The control juice presented the highest amount of formic acid (8.36 ppm) and tyrosine (6.96 and 7.24 ppm). Principal component analysis of the data showed that cold plasma and ozone treatment had different effects on diverse groups of compounds (aromatic or aliphatic). In general, all three different ozone processing loads similarly affected the aromatic compounds signals, while atmosphere cold plasma influenced more the aliphatic signals; however, no significant quantitative changes were detected in the orange juice as a whole.

Sekhon et al. (2010) evaluated the effects of O_3 at 175 ppm for 48 h on the VOCs contents in dry-cured ham. More than 40 aroma compounds were detected in the control and O_3 fumigated sample; however, 15 compounds were selected for further study based on aroma intensity (>5 in at least 1 treatment) and reproducibility. In O_3 fumigation, the control sample (without any fumigation) had more intense unpleasant, sulfur, putrid, burnt, gasoline, and rubbery odors than the fumigated hams. The fumigated samples (175 ppm) had more intense green, sweet, fermented, milky, cocoa, baked potato, citrusy, fruity, smoky, and sweet ham odors than the nonfumigated control samples. Moreover, there were high concentrations of 3-methyl butanal, methional, 2,6-dimethoxy phenol, and total VOCs in the 175-ppm samples compared to the control ham. A sulfur compound such as benzothiazole, which is responsible for the gasoline and rubber-like odor (generated by Maillard reactions), was detected in both control and O_3 fumigated samples, and had a greater contribution to the ham aroma in the control sample. The O_3 treatment promoted an increase in methional concentration (baked potato, grassy odor) in the lean muscle fraction compared to the nonfumigated control, which could potentially have a negative effect on the flavor and sensory qualities of the product. Three aldehydes (hexanal, heptanal, and 3-methyl butanal) were detected in the headspace of the dry-cured hams fumigated with O_3. While heptanal and hexanal did not show any concentration differences, the 3-methyl butanal content increased in both the lipid and muscle fractions of the fumigated samples as compared to the nonfumigated control, suggesting that O_3 induced some oxidation processes. The consumer test indicated that consumers were unable to distinguish between the control hams and the O_3-fumigated ones.

9.3.6 UV LIGHT

UV-light technology is based on the emission of electromagnetic waves with different wavelengths and frequencies. In general, the wavelength for UV technology ranges from 100 to 400 nm, but the most interesting range for food processing is from 200 to 280 nm (called UV-C) as it is usually responsible of bacteria and viruses inactivation. Short UV-C is almost completely absorbed in air within a few hundred meters (Koutchma, Forney, & Moraru, 2009). However, UV treatment is a well-known lipid-oxidation promoter, so it can compromise product stability and quality, depending on the exposure wavelength/time/intensity used.

9.3.6.1 Juices

Caminiti et al. (2012) studied the effects of different nonthermal technologies (UV, HILP, PEF) applied together with MTS technology (400 kPa, 35°C, 1000 W, 20 kHz) on the sensory characteristics of an orange and carrot juice blend. In all cases, the pasteurized control juice blend scored higher for flavor attributes compared to the products treated by the selected nonthermal combinations. Between the nonthermal techniques, the UV treatment (10.6 J/cm^2) was preferred in terms of juice flavor (4.8), but it was not significantly different from the pasteurized samples. In another

study, Caminiti et al. (2011) found that UV (5.3 J/cm^2) + MTS (5 bar, 43 C, 750 W, 20 kHz) and HILP (3.3 J/cm^2) + MTS combinations applied to an apple and cranberry juice blend had an adverse impact on the odor and flavor of the product, while UV + PEF (34 kV/cm, 18 Hz, 93 μs) and HILP + PEF treatments did not affect those sensory attributes.

9.3.6.2 Milk

UV treatment (UV intensity = 13.87 J/mL) of bovine milk caused an increase in most aldehydes and ketones. Diacetyl with buttery note and aldehydes, which are major oxidation products of unsaturated fatty acids (hexanal, (*E*,*Z*)-2,6-nonadienal and (*E*,*E*)-2,4-nonadienal), were found in higher quantity in UV-treated milk than in pasteurized samples. Instead, heat treatment resulted in an overall increase in heat-generated aroma compounds and Maillard reaction products. However, the sensory analysis, in terms of flavor, did not show significant differences between UV-treated milk and pasteurized one (Engin & Yuceer, 2012).

Matak et al. (2007) performed a sensory and chemical analysis of UV-treated goat milk, which was exposed to UV for a cumulative exposure time of 18 s and targeted UV dose of 15.8 + /-1.6 mJ/cm^2. As UV dose increased, the main aldehydes (pentanal, hexanal, and heptanal) also rose in milk. In fact, a triangle test showed differences between the odor of raw milk and the UV-irradiated one.

9.3.6.3 Fresh-cut fruit

George, Razali, Santhirasegaram, and Somasundram (2015) evaluated the effects of ultraviolet (UV-C) and medium-heat (70°C) treatments on the quality aspects of fresh-cut Chokanan mango and Josephine pineapple. The fresh-cut fruits were exposed to UV-C for 0, 15, 30, and 60 min, while heat treatments were carried out at 70°C for 0, 5, 10, and 20 min. In the sensory analysis of the differently treated fruits, the UV-C treated samples scored closest to controls in terms of aroma and taste, thus suggesting better retention of both sensory attributes after treatment. Heat treatment longer that 5 min resulted in low sensory scores, which was unacceptable to consumers. Since aroma and taste are correlated, change or deterioration of these attributes may be due to the changes in the total soluble solids and titratable acidity observed in analyzed samples. Overall acceptability of the treated fruits, considering the four attributes (appearance, texture, aroma, and taste), showed that UV-C treated samples were more acceptable than the heat-treated ones. Moreover, the results of other qualitative analytical parameters also supported the fact that use of UV-C treatment in these fresh fruit products promotes better retention of their quality, effective microbial inactivation, and enhancement of health-promoting compounds.

9.4 AROMATIC COMPOUNDS AS NATURAL ADDITIVES IN FOOD PRODUCTS

9.4.1 LEGISLATION

EU legislation defines flavorings as products added to food in order to impart or modify odor and/or taste (http://ec.europa.eu/food/safety/food_improvement_agents/flavourings/index_en.htm). Furthermore, flavorings are classified as flavoring substances, flavoring preparations, thermal

process flavorings, smoke flavorings, flavor precursors, and other flavorings. Regulation (EC) No. 1334/2008 on flavorings and certain food ingredients with flavoring properties for use in/on foods was adopted in December 2008 and entered into force in January 2009. This EU regulation was issued to ensure the protection of consumers and lays down the general requirements for safe use of flavorings. It also provides definitions for different types of flavorings. Although smoke flavorings are a specific category of flavorings included in this regulation, they are governed by a specific set of three regulations (Regulation (EC) No. 2065/2003, Regulation (EC) No. 627/2006, Regulation (EU) No. 1321/2013). The Union list of flavoring substances, approved for use in and on foods, was adopted in October 2012 and was introduced in Annex I of this regulation (and subsequent amendments). The regulation also indicates the substances for which an evaluation and approval is required. Furthermore, it includes a list of prohibited compounds and states the maximum addition levels for those substances that may be harmful to human health. The regulation also sets out the rules for labeling of flavorings and describes the specific requirements for use of the term "natural."

9.4.2 AROMA COMPOUNDS ADDITION AND APPLICATION

Aromatized foods have undergone significant development in the past several decades due to the significant increase in industrially produced food, which are often subjected to aroma losses during processing and storage. In some cases, aromatization is required because raw materials are unavailable and/or are too expensive.

Flavor compounding is the result of mixing 20–50 selected raw materials (organic chemicals, essential oils, extracts, oleoresins, or processed flavors) at different ratios to create a unique aroma formulation (Sun-Pan, Kuo, & Wu, 2006). Flavor compounding requires knowledge of the raw materials' natural, physical, and sensory properties. The aroma formulation also depends on the application and thus consideration must be given to whether a food item will be further processed, thermally treated, or only subjected to storage. For example, if a strawberry flavor is to be formulated for a cake mix, heat-stable compounds with high boiling points will be selected (Sun-Pan et al., 2006). However, it is possible that further adjustments to the initial flavor formulation may be required, as other food product ingredients can also release aroma compounds and thus mask and/or modify the sensory profile provided by the original flavor formula. In the cake mix example, a pineapple note (instead of a strawberry one) was perceived after cooking, which was ascribed to the overlapping of the strawberry flavor with the oily-fatty notes from other cake mix ingredients (butter, sugar, and milk) (Sun-Pan et al., 2006).

9.4.2.1 Process flavors

Process flavors include reaction flavors, fat flavors, hydrolysates, autolysates, enzyme-modified flavors, etc. (Sun-Pan et al., 2006). The International Organization of the Flavor Industry (IOFI) has guidelines for the production and labeling of thermal process flavorings (http://www.iofi.org/), which are based on the Codex Alimentarius Guidelines (CAC/GL 66–2008) (FAO/WHO, 2008). In these guidelines, the reactants are specified and it is also stated that flavorings, flavoring substances, flavor enhancers, and process flavor adjuncts shall be added once processing is completed. Furthermore, the processing conditions should not exceed 180°C for 15 min (or proportionately longer at lower temperatures), and the pH should be below 8.0.

9.4.2.2 Flavors application and future developments

The choice of the type and amount of flavor, as well as the food processing step at which it will be added, are key aspects to consider for flavor applications. Another parameter to take into account is the extent of evaporation loss of each flavor ingredient, which varies according to the type of food-processing technology used. Moreover, some food components (such as starch, lipids, and proteins), can trap flavor compounds and reduce their volatilities. It is well known that the texture and microstructure of different food systems affect the retention and release of aroma compounds during processing and storage (de Roos, 2003). In complex solid and partly solid products, flavor compounds are entrapped in the hydrophilic or the lipid phase across the matrix (de Roos, 2006). The food system can also influence aroma release by physicochemical mechanisms, such as oil−water and water−air partition.

Considering the increasing demand for natural flavors by industry and consumers, the utilization of biotechnology for the production of flavoring compounds appears to be a good alternative (Gounaris, 2010). Over the past couple of decades, this approach has shown to be efficient, effective, as well as economical, giving good aroma yields. Dairy and meat-like aromas are some of the successful applications of biotechnological flavor production (Akacha & Gargouri, 2015). Suitable aromatization processes are also necessary to warrant the formation of the desired sensory profile and thus consumer acceptance of new raw materials that are being introduced in the food market to diversify or expand traditional food sources.

Moreover, the creation of new flavors represents both a challenge and an opportunity for innovative food applications and products, which should also consider adequate aroma delivery systems to warrant the controlled release of the aroma compounds. Since the latter can also exhibit diverse biological effects, aromatization will not only impact the sensory profile of food, but it can also enhance the food product stability and/or health-promoting characteristics, thus resulting in the development of high-quality food products. Finally, further research should focus on maintaining and/or enhancing the aroma profile of foods processed by emerging nonthermal technologies, without compromising their overall quality and safety.

REFERENCES

Aguilar-Rosas, S. F., Ballinas-Casarrubias, M. L., Martin-Belloso, O., Nevarez-Moorillon, G. V., & Ortega-Rivas, E. (2007). Thermal and pulsed electric fields pasteurization of apple juice: Effects on physicochemical properties and flavour compounds. *Journal of Food Engineering*, *83*(1), 41−46.

Akacha, N. B., & Gargouri, M. (2015). Microbial and enzymatic technologies used for the production of natural aroma compounds: Synthesis, recovery modeling, and bioprocesses. *Food and Bioproducts Processing*, *94*, 675−706.

Alves Filho, E. G., Almeida, F. D. L., Cavalcante, R. S., De Brito, E. S., Cullen, P. J., Frias, J. M., & Rodrigues, S. (2016). ^{1}H NMR spectroscopy and chemometrics evaluation of nonthermal processing of orange juice. *Food Chemistry*, *204*, 102−107.

Azhu Valappil, Z., Fan, X., Zhang, H. Q., & Rouseff, R. L. (2009). Impact of thermal and nonthermal processing technologies on unfermented apple cider aroma volatiles. *Journal of Agricultural and Food Chemistry*, *57*(3), 924−929.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, *117*(4), 426−436.

Baiano, A. (2014). Recovery of biomolecules from food wastes — A review. *Molecules*, *19*(9), 14821−14842.

Barba, F. J., Parniakov, O., Pereira, S. A., Wiktor, A., Grimi, N., Boussetta, N., & Vorobiev, E. (2015). Current application and new opportunities for the use of pulsed electric fields in foods science and industry. *Food Research International*, *77*(4), 773−798.

Bastianello, E., Montemurro, F., Fasolato, L., Balzan, S., Marchesini, G., Contiero, B., & Novelli, E. (2016). Volatile compounds and microbial development in sonicated cloudy apple juices: preliminary results. *CyTA − Journal of Food*, *14*(1), 65−73.

Belitz, H., Grosch, W., & Schieberle, P. (2009). Aroma compounds. In H. Belitz, W. Grosch, & P. Schieberle (Eds.), *Food chemistry* (4th ed). Berlin, Heidelberg: Springer-Verlag.

Bogdanovic, A., Tadic, V., Arsic, I., Milovanovic, S., Petrovic, S., & Skala, D. (2016). Supercritical and high pressure subcritical fluid extraction from lemon balm (*Melissa officinalis L.*, Lamiaceae). *The Journal of Supercritical Fluids*, *107*, 234−242.

Boukroufa, M., Boutekedjiret, C., Petigny, L., Rakotomanomana, N., & Chemat, F. (2015). Bio-refinery of orange peels waste: a new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. *Ultrasonics Sonochemistry*, *24*, 72−79.

Caminiti, I. M., Noci, F., Morgan, D. J., Cronin, D. A., & Lyng, J. G. (2012). The effect of pulsed electric fields, ultraviolet light or high intensity light pulses in combination with manothermosonication on selected physicochemical and sensory attributes of an orange and carrot juice blend. *Food and Bioproducts Processing*, *90*(3), 442−448.

Caminiti, I. M., Noci, F., Muñoz, A., Whyte, P., Morgan, D. J., Cronin, D. A., & Lyng, J. G. (2011). Impact of selected combinations of nonthermal processing technologies on the quality of an apple and cranberry juice blend. *Food Chemistry*, *124*(4), 1387−1392.

Cano-Higuita, D. M., Malacrida, C. R., & Telis, V. R. N. (2015). Stability of curcumin microencapsulated by spray and freeze drying in binary and ternary matrices of maltodextrin, gum arabic and modified starch. *Journal of Food Processing and Preservation*, *39*(6), 2049−2060.

Capuzzo, A., Maffei, M., & Occhipinti, A. (2013). Supercritical fluid extraction of plant flavors and fragrances. *Molecules*, *18*(6), 7194−7238.

Carballo, J. (2012). The role of fermentation reactions in the generation of flavor and aroma of foods. In B. M. Mehta, A. Kamal-eldin, & R. Z. Iwanski (Eds.), *Fermentation effects on food properties*. Boca Raton, FL: CRC Press.

Chatzidaki, M. D., Mitsou, E., Yaghmur, A., Xenakis, A., & Papadimitriou, V. (2015). Formulation and characterization of food-grade microemulsions as carriers of natural phenolic antioxidants. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *483*, 130−136.

Chen, Q., Hu, Z., Yao, F. Y.-D., & Liang, H. (2016). Study of two-stage microwave extraction of essential oil and pectin from pomelo peels. *LWT-Food Science and Technology*, *66*, 538−545.

Craveiro, A. A., Matos, F. J. A., Alencar, J. W., & Plumel, M. M. (1989). Microwave oven extraction of an essential oil. *Flavour and Fragrance Journal*, *4*(1), 43−44.

Cregenzán-Alberti, O., Halpin, R. M., Whyte, P., Lyng, J. G., & Noci, F. (2015). Study of the suitability of the central composite design to predict the inactivation kinetics by pulsed electric fields (PEF) in Escherichia coli, Staphylococcus aureus and Pseudomonas fluorescens in milk. *Food and Bioproducts Processing*, *95*, 313−322.

Cserhalmi, Z., Sass-Kiss, Á., Tóth-Markus, M., & Lechner, N. (2006). Study of pulsed electric field treated citrus juices. *Innovative Food Science and Emerging Technologies*, *7*, 49−54.

Da Porto, C., Decorti, D., & Natolino, A. (2014). Separation of aroma compounds from industrial hemp inflorescences (*Cannabis sativa L.*) by supercritical CO_2 extraction and on-line fractionation. *Industrial Crops and Products*, *58*, 99–103.

Delgado, F. J., González-Crespo, J., Cava, R., & Ramírez, R. (2011). Effect of high-pressure treatment on the volatile profile of a mature raw goat milk cheese with paprika on rind. *Innovative Food Science and Emerging Technologies*, *12*(2), 98–103.

De Roos, K. B. (2003). Effect of texture and microstructure on flavour retention and release. *International Dairy Journal*, *13*(8), 593–605.

De Roos, K. B. (2006). Understanding and controlling the behaviour of aroma compounds in thermally processed foods. *Trends in Food Science & Technology*, *17*(5), 236–243.

Đorđević, V., Balanč, B., Belščak-Cvitanović, A., Lević, S., Trifković, K., Kalušević, A., & Nedović, V. (2014). Trends in encapsulation technologies for delivery of food bioactive compounds. *Food Engineering Reviews*, *7*(4), 452–490.

Dutta, S., & Bhattacharjee, P. (2015). Enzyme-assisted supercritical carbon dioxide extraction of black pepper oleoresin for enhanced yield of piperine-rich extract. *Journal of Bioscience and Bioengineering*, *120*(1), 17–23.

Engin, B., & Yuceer, Y. K. (2012). Effects of ultraviolet light and ultrasound on microbial quality and aroma-active components of milk. *Journal of the Science and Food Agriculture*, *92*, 1245–1252.

Evrendilek, G. A. (2016). Change regime of aroma active compounds in response to pulsed electric field treatment time, sour cherry juice apricot and peach nectars, and physical and sensory properties. *Innovative Food Science and Emerging Technologies*, *33*, 195–205.

Evrendilek, G. A., Avsar, Y. K., & Evrendilek, F. (2016). Modeling stochastic variability and uncertainty in aroma active compounds of PEF-treated peach nectar as function of physical and sensory properties, and treatment time. *Food Chemistry*, *190*, 634–642.

Fan, X., & Sokorai, K. J. B. (2011). Changes in quality, liking, and purchase intent of irradiated fresh-cut spinach during storage. *Journal of Food Science*, *76*(6), S363–S368.

FAO/WHO. (2008). Codex alimentarius guidelines for the use of flavorings (Reference CAC/GL 66-2008).

Ferhat, M. A., Tigrine-Kordjani, N., Chemat, S., Meklati, B. Y., & Chemat, F. (2007). Rapid extraction of volatile compounds using a new simultaneous microwave distillation: solvent extraction device. *Chromatographia*, *65*(3–4), 217–222.

Fernandes, A., Antonio, A. L., Oliveira, M. B. P. P., Martins, A., & Ferreira, I. C. F. R. (2012). Effect of gamma and electron beam irradiation on the physicochemical and nutritional properties of mushrooms: A review. *Food Chemistry*, *135*(2), 641–650.

Filly, A., Fernandez, X., Minuti, M., Visinoni, F., Cravotto, G., & Chemat, F. (2014). Solvent-free microwave extraction of essential oil from aromatic herbs: from laboratory to pilot and industrial scale. *Food Chemistry*, *150*, 193–198.

Garde-Cerdán, T., González-Arenzana, L., López, N., López, R., Santamaría, P., & López-Alfaro, I. (2013). Effect of different pulsed electric field treatments on the volatile composition of Graciano, Tempranillo and Grenache grape varieties. *Innovative Food Science and Emerging Technologies*, *20*, 91–99.

Garde-Cerdán, T., Marsellés-Fontanet, A. R., Arias-Gil, M., Ancín-Azpilicueta, C., & Martín-Belloso, O. (2008). Effect of storage conditions on the volatile composition of wines obtained from must stabilized by PEF during aging without SO_2. *Innovative Food Science and Emerging Technologies*, *9*, 469–476.

Gavahian, M., & Farahnaky, A. (2013). A novel technology for extraction of essential oil from Myrtus communis: Ohmic-assisted hydrodistillation. *Journal of Essential Oil Research*, *25*(4), 257–266.

Gavahian, M., Farahnaky, A., Farhoosh, R., Javidnia, K., & Shahidi, F. (2015). Extraction of essential oils from Mentha piperita using advanced techniques: Microwave versus ohmic assisted hydrodistillation. *Food and Bioproducts Processing*, *94*, 50–58.

Gavahian, M., Farahnaky, A., Javidnia, K., & Majzoobi, M. (2012). Comparison of ohmic-assisted hydrodistillation with traditional hydrodistillation for the extraction of essential oils from *Thymus vulgaris L.* *Innovative Food Science & Emerging Technologies*, *14*, 85–91.

Gavahian, M., Farahnaky, A., Majzoobi, M., Javidnia, K., Saharkhiz, M. J., & Mesbahi, G. (2011). Ohmic-assisted hydrodistillation of essential oils from Zataria multiflora Boiss (Shirazi thyme). *International Journal of Food Science & Technology*, *46*(12), 2619–2627.

George, D. S., Razali, Z., Santhirasegaram, V., & Somasundram, C. H. (2015). Effects of ultraviolet light (UV-c) and heat treatment on the quality of fresh-cut Chokanan mango and Josephine pineapple. *Journal of Food Science*, *80*(2), S426–S434.

Gómez-López, V. M., Orsolani, L., Martínez-Yépez, A., & Tapia, M. S. (2010). Microbiological and sensory quality of sonicated calcium-added orange juice. *LWT–Food Science and Technology*, *43*(5), 808–813.

González-Cebrino, F., García-Parra, J., & Ramírez, R. (2016). Aroma profile of a red plum purée processed by high hydrostatic pressure and analysed by SPME–GC/MS. *Innovative Food Science and Emerging Technologies*, *33*, 108–114.

Gounaris, Y. (2010). Biotechnology for the production of essential oils, flavours and volatile isolates. A review. *Flavour and Fragrance Journal*, *25*, 367–386.

Guerra-Rosas, M. I., Morales-Castro, J., Ochoa-Martínez, L. A., Salvia-Trujillo, L., & Martín-Belloso, O. (2016). Long-term stability of food-grade nanoemulsions from high methoxyl pectin containing essential oils. *Food Hydrocolloids*, *52*, 438–446.

Hamzah, M. H., Sudin, S. S., Tengku Ab Mutalib, T. N. A., Malek, N. F. H. A., Yusof, N., Jamaludin, H., & Zainal Abidin, Z. (2011). Preliminary study of ohmic heated hydro distillation for essential oil's plant extraction. In *Research and Development (SCOReD) IEEE Student Conference* (pp. 211–214).

Hartyáni, P., Dalmadi, I., Cserhalmi, Z., Kántor, D.-B., Tóth-Markus, M., & Sass-Kiss, Á. (2011). Physical–chemical and sensory properties of pulsed electric field and high hydrostatic pressure treated citrus juices. *Innovative Food Science & Emerging Technologies*, *12*(3), 255–260.

http://ec.europa.eu/food/safety/food_improvement_agents/flavourings/index_en.htm.

http://www.iofi.org/.

Jaeger, H., Schulz, A., Karapetkov, N., & Knorr, D. (2009). Protective effect of milk constituents and sublethal injuries limiting process effectiveness during PEF inactivation of Lb. rhamnosus. *International Journal of Food Microbiology*, *134*(1–2), 154–161.

Keenan, D. F., Brunton, N. P., Mitchell, M., Gormley, R., & Butler, F. (2012). Flavour profiling of fresh and processed fruit smoothies by instrumental and sensory analysis. *Food Research International*, *45*(1), 17–25.

Kempkes, M. A. (2010). Pulsed electric field (PEF) systems for commercial food and juice processing. In C. J. Doona, K. Kustin, & F. E. Feeherry (Eds.), *Case studies in novel food processing technologies: Innovations in processing, packaging, and predictive modelling*. Cambridge: Woodhead Publishing Ltd.

Kentish, S., & Feng, H. (2014). Applications of power ultrasound in food processing. *Annual Review of Food Science and Technology*, *5*, 263–284.

Khosravi-Darani, K. (2010). Research activities on supercritical fluid science in food biotechnology. *Critical Reviews in Food Science and Nutrition*, *50*(6), 479–488.

Knorr, D., Froehling, A., Jaeger, H., Reineke, K., Schlueter, O., & Schoessler, K. (2011). Emerging technologies in food processing. *Annual Review of Food Science and Technology*, *2*, 203–235.

Koutchma, T. (2014). *Adapting high hydrostatic pressure (HPP) for food processing operations*. Cambridge, MA: Academic Press.

Koutchma, T. N., Forney, L. J., & Moraru, C. I. (2009). *Ultraviolet light in food technology: Principles and applications*. Boca Raton, FL: CRC Press Taylor & Francis Group.

Kulkarni, P., Loira, I., Morata, A., Tesfaye, W., González, M. C., & Suárez-Lepe, J. A. (2015). Use of non-Saccharomyces yeast strains coupled with ultrasound treatment as a novel technique to accelerate aging on lees of red wines and its repercussion in sensorial parameters. *LWT-Food Science and Technology*, *64*, 1255–1262.

Lee, Y., & Lee, Y. Y. (2003). Flavor and aroma substances. In C. Tzia, & G. Liadakis (Eds.), *Extraction optimization in food engineering*. New York: Marcel Dekker Inc.

Li, Y., Fabiano-Tixier, A.-S., & Chemat, F. (2014). Essential oils: From Conventional to Green extraction. In Y. Li, A.-S. Fabiano-Tixier, & F. Chemat (Eds.), *Essential oils as reagents in green chemistry*. New York: Springer International Publishing.

Luo, H., Schmid, F., Grbin, P. R., & Jiranek, V. (2012). Viability of common wine spoilage organisms after exposure to high power ultrasonics. *Ultrasonics Sonochemistry*, *19*(3), 415–420.

Ma, N. T., Chyau, C. C., & Pan, B. S. (2004). Fatty acid profile and aroma compounds of the lipoxygenase-modified chicken oil. *Journal of the American Oil Chemists' Society*, *81*(10), 921–926.

Madene, A., Jacquot, M., Scher, J., & Desobry, S. (2006). Flavour encapsulation and controlled release - a review. *International Journal of Food Science and Technology*, *41*(1), 1–21.

Mangolim, C. S., Moriwaki, C., Nogueira, A. C., Sato, F., Baesso, M. L., Neto, A. M., & Matioli, G. (2014). Curcumin-β-cyclodextrin inclusion complex: stability, solubility, characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy, and food application. *Food Chemistry*, *153*, 361–370.

Mao, L., & Miao, S. (2015). Structuring food emulsions to improve nutrient delivery during digestion. *Food Engineering Reviews*, *7*(4), 439–451.

Matak, K. E., Sumner, S. S., Duncan, S. E., Hovingh, E., Worobo, R. W., Hackney, C. R., & Pierson, M. D. (2007). Effects of ultraviolet irradiation on chemical and sensory properties of goat milk. *Journal of Dairy Science*, *90*(7), 3178–3186.

Mcclements, D. J. (2012). Nanoemulsions versus microemulsions: Terminology, differences, and similarities. *Soft Matter*, *8*(6), 1719–1729.

Mcclements, D. J. (2015). *Food emulsions: Principles, practices, and techniques*. Boca Raton, FL: CRC Press.

Mengal, P., Behn, D., Bellido Gil, M., & Mompon, B. (1993). VMHD: extraction of essential oil by microwave. *Parfums, Cosmétiques, Aromes*, *114*, 66–67.

Meynier, A., Genot, C., & Gandemer, G. (1998). Volatile compounds of oxidized pork phospholipids. *Journal of the American Oil Chemists' Society*, *75*(1), 1–7.

Molimard, P., & Spinnler, H. E. (1996). Compounds involved in the flavor of surface mold-ripened cheeses: Origins and properties. *Journal of Dairy Science*, *79*(2), 169–184.

Noshad, M., Mohebbi, M., Shahidi, F., & Koocheki, A. (2015). Effect of layer-by-layer polyelectrolyte method on encapsulation of vanillin. *International Journal of Biological Macromolecules*, *81*, 803–808.

Nowacka, M., Tylewicz, U., Laghi, L., Dalla Rosa, M., & Witrowa-Rajchert, D. (2014). Effect of ultrasound treatment on the water state in kiwifruit during osmotic dehydration. *Food Chemistry*, *144*, 18–25.

Oey, I., Lille, M., Van Loey, A., & Hendrickx, M. (2008). Effect of high pressure processing on colour, texture and flavour of fruit and vegetable-based food products: a review. *Trends in Food Science & Technology*, *19*(6), 320–328.

Omar, J., Alonso, I., Garaikoetxea, A., & Etxebarria, N. (2013). Optimization of focused ultrasound extraction (fuse) and supercritical fluid extraction (sfe) of citrus peel volatile oils and antioxidants. *Food Analytical Methods*, *6*(4), 1244–1252.

Périno-Issartier, S., Abert-Vian, M., Petitcolas, E., & Chemat, F. (2010). Microwave turbo hydrodistillation for rapid extraction of the essential oil from Schinus terebinthifolius Raddi Berries. *Chromatographia*, *72*(3–4), 347–350.

Prakash, A., Lim, F. T., Duong, C., Caporaso, F., & Foley, D. (2010). The effects of ionizing irradiation on Salmonella inoculated on almonds and changes in sensory properties. *Radiation Physics and Chemistry*, *79*(4), 502–506.

Regulation (Ec) No 1334/2008 of 16 December 2008. *Official Journal of the European Union*. L 354/34.
Regulation (Ec) No 2065/2003 of 10 November 2003. *Official Journal of the European Union*. L 309/1.
Regulation (Ec) No 627/2006 of 21 April 2006. *Official Journal of the European Union*. L 109/3.
Regulation (Eu) No 1321/2013 of 12 December 2013. *Official Journal of the European Union*. L 333/54.
Riener, J., Noci, F., Cronin, D. A., Morgan, D. J., & Lyng, J. G. (2009). Characterization of volatile compounds generated in milk by high intensity ultrasound. *International Dairy Journal*, *19*(4), 269–272.
Rivas, A., Rodrigo, D., Martínez, A., Barbosa-Cánovas, G. V., & Rodrigo, M. (2006). Effect of PEF and heat pasteurization on the physical–chemical characteristics of blended orange and carrot juice. *LWT-Food Science and Technology*, *39*(10), 1163–1170.
Sadgrove, N., & Jones, G. (2015). A contemporary introduction to essential oils: chemistry, bioactivity and prospects for Australian agriculture. *Agriculture*, *5*(1), 48–102.
Salvia-Trujillo, L., Martín-Belloso, O., & Mcclements, D. J. (2016). Excipient nanoemulsions for improving oral bioavailability of bioactives. *Nanomaterials*, *6*(1), 17.
Salvia-Trujillo, L., Rojas-Graü, A., Soliva-Fortuny, R., & Martín-Belloso, O. (2015). Physicochemical characterization and antimicrobial activity of food-grade emulsions and nanoemulsions incorporating essential oils. *Food Hydrocolloids*, *43*, 547–556.
Sampedro, F., Geveke, D. J., Fan, X., & Zhang, H. Q. (2009). Effect of PEF, HHP and thermal treatment on PME inactivation and volatile compounds concentration of an orange juice–milk based beverage. *Innovative Food Science and Emerging Technologies*, *10*(4), 463–469.
Santos, M. C., Nunes, C., Cappelle, J., Gonçalves, F. J., Rodrigues, A., Saraiva, J. A., & Coimbra, M. A. (2013). Effect of high pressure treatments on the physicochemical properties of a sulphur dioxide-free red wine. *Food Chemistry*, *141*(3), 2558–2566.
Santos, M. C., Nunes, C., Jourdes, M., Teissedre, P.-L., Rodrigues, A., Amado, O., & Coimbra, M. A. (2016). Evaluation of the potential of high pressure technology as an enological practice for red wines. *Innovative Food Science and Emerging Technologies*, *33*, 76–83.
Schössler, K., Jäger, H., & Knorr, D. (2012). Effect of continuous and intermittent ultrasound on drying time and effective diffusivity during convective drying of apple and red bell pepper. *Journal of Food Engineering*, *108*(1), 103–110.
Seidi Damyeh, M., Niakousari, M., Golmakani, M. T., & Saharkhiz, M. J. (2015). Microwave and ohmic heating impact on the *in situ* hydrodistillation and selective extraction of *Satureja macrosiphonia* essential oil. *Journal of Food Processing and Preservation*. Available from http://dx.doi.org/10.1111/jfpp.12644.
Sekhon, R. K., Schilling, M. W., Phillips, T. W., Aikins, R. M. J., Hasan, M. M., Nannapaneni, R., & Mikel, W. B. (2010). Effects of carbon dioxide and ozone treatments on the volatile composition and sensory quality of dry-cured ham. *Journal of Food Science*, *75*(5), C452–C458.
Šimunek, M., Režek Jambrak, A., Petrović, M., Juretić, H., Major, N., Herceg, Z., & Vukušić, T. (2013). Aroma profile and sensory properties of ultrasound-treated apple juice and nectar. *Food Technology and Biotechnology*, *51*(1), 101–111.
Soccol, C. R., Medeiros, A. B. P., Vandenberghe, L. P. S., Soares, M., & Pandey, A. (2008). *Production of aroma compounds. Current developments in solid-state fermentation*. New York: Springer.
Soliva-Fortuny, R., Balasa, A., Knorr, D., & Martín-Belloso, O. (2009). Effect of pulsed electric fields on bioactive compounds in foods: a review. *Trends in Food Science & Technology*, *20*(11–12), 544–556.
Sowbhagya, H. B., & Chitra, V. N. (2010). Enzyme-assisted extraction of flavorings and colorants from plant materials. *Critical Reviews in Food Science and Nutrition*, *50*(2), 146–161.
Stewart, E. M. (2009). Food irradiation. In R. H. Stadler, & D. R. Lineback (Eds.), *Process-induced food toxicants*. Hoboken, NJ: John Wiley & Sons Inc.

Sun-Pan, B., Kuo, J.-M., & Wu, C.-M. (2006). Flavor compounds in foods. In Z. E. Sikorski (Ed.), *Chemical and functional properties of food components* (3rd ed.). Boca Raton, FL: CRC Press.

Taipina, M. S., Lamardo, L. C. A., Rodas, M. A. B., & Del Mastro, N. L. (2009). The effects of gamma irradiation on the vitamin E content and sensory qualities of pecan nuts (Caryaillinoensis). *Radiation Physics and Chemistry*, *78*(7–8), 611–613.

Tappi, S., Gozzi, G., Vannini, L., Berardinelli, A., Romani, S., Ragni, L., … Rocculi, P. (2016). Cold plasma treatment for fresh-cut melon stabilization. *Innovative Food Science and Emerging Technologies*, *33*, 225–233.

Tekin, K., Akalin, M. K., & Şeker, M. G. (2015). Ultrasound bath-assisted extraction of essential oils from clove using central composite design. *Industrial Crops and Products*, *77*, 954–960.

Timmermans, R. A. H., Nierop Groot, M. N., Nederhoff, A. L., Van Boekel, M. A. J. S., Matser, A. M., & Mastwijk, H. C. (2014). Pulsed electric field processing of different juices: Impact of pH and temperature on inactivation of spoilage and pathogenic micro-organisms. *International Journal of Food Microbiology*, *173*, 105–111.

Tiwari, B. K., & Muthukumarappan, K. (2012). Ozone in fruit and vegetable processing. In C. O'Donnell, B. Tiwari, P. J. Cullen, & R. G. Rice (Eds.), *Ozone in food processing*. Oxford: Wiley-Blackwell.

Toepfl, S., Mathys, A., Heinz, V., & Knorr, D. (2006). Review: Potential of high hydrostatic pressure and pulsed electric fields for energy efficient and environmentally friendly food processing. *Food Reviews International*, *22*(4), 405–423.

Vazquez-Landaverde, P. A., Torres, J. A., & Qian, M. C. (2006). Effect of high-pressure-moderate-temperature processing on volatile profile of milk. *Journal of Agricultural Food Chemistry*, *54*(9), 9184–9192.

Walkling-Ribeiro, M., Noci, F., Cronin, D. A., Lyng, J. G., & Morgan, D. J. (2009). Shelf life and sensory evaluation of orange juice after exposure to thermosonication and pulsed electric fields. *Food and Bioproducts Processing*, *87*(2), 102–107.

Wongfhun, P., Gordon, M. H., & Apichartsrangkoon, A. (2010). Flavour characterisation of fresh and processed pennywort (*Centella asiatica L.*) juices. *Food Chemistry*, *119*(1), 69–74.

www.leffingwell.com/odorthre.htm.

www.leffingwell.com/ald1.htm.

Ye, Q., Jin, X., Zhu, X., Lin, T., Hao, Z., & Yang, Q. (2015). An efficient extraction method for fragrant volatiles from Jasminum sambac (L.) ait. *Journal of Oleo Science*, *64*(6), 645–652.

Zainal, M. N. F., Redzuan, N., & Misnal, M. F. I. (2015). Brief review: Cold plasma. *Jurnal Teknologi*, *74*(10), 57–61.

Zhang, W., Li, X., Yu, T., Yuan, L., Rao, G., Li, D., … Mu, C. (2015). Preparation, physicochemical characterization and release behavior of the inclusion complex of trans-anethole and β-cyclodextrin. *Food Research International*, *74*, 55–62.

Zhang, Y., Mo, L., Chen, F., Lu, M., Dong, W., Wang, Q., … Gu, F. (2014). Optimized production of vanillin from green vanilla pods by enzyme-assisted extraction combined with prefreezing and thawing. *Molecules*, *19*(2), 2181–2198.

Zhao, W., Yang, R., Lu, R., Wang, M., Qian, P., & Yang, W. (2008). Effect of PEF on microbial inactivation and physical–chemical properties of green tea extracts. *LWT-Food Science and Technology*, *41*(13), 425–431.

Zhao, W., Yang, R., Wang, M., & Lu, R. (2009). Effects of pulsed electric fields on bioactive components, colour and flavour of green tea infusions. *International Journal of Food Science and Technology*, *44*(2), 312–321.

Zuidam, N. J., & Heinrich, E. (2010). Encapsulation of aroma. In N. J. Zuidam, & E. Heinrich (Eds.), *Encapsulation technologies for active food ingredients and food processing*. New York: Springer.

CHAPTER

INTERACTION OF COMPOUNDS 10

Mauro D. Santos[1], Rui P. Queirós[1], Silvia A. Moreira[1], Zhenzhou Zhu[2], Francisco J. Barba[3] and Jorge A. Saraiva[1]

[1]Universidade de Aveiro, Aveiro, Portugal [2]Wuhan Polytechnic University, Wuhan, China
[3]Universitat de València, Burjassot, Valencia, Spain

10.1 INTRODUCTION

Today's lifestyles have increased the incidence of chronic diseases, such as diabetes, cancer, and cardiovascular diseases, which has encouraged the development of so-called functional foods. In addition to the nutrients and energy needed by the body, functional foods provide physiological benefits like health-promoting or disease-preventing properties, due to the presence of bioactive compounds (Kaur & Das, 2011). The term nutraceutical refers to any substance, either a food or part of a food, that provides medical or health benefits, including the prevention and treatment of diseases. In contrast to functional foods, nutraceuticals are usually consumed in the form of pills or capsules (Kaur & Das, 2011). However, the average consumer prefers natural products over chemical ones, even if they are chemically identical, since people want to consume food with the desired health benefits rather than take medicine separately.

Today, food products with functional properties are easily accepted by consumers due to their high nutritional value and high bioactive compound content. These type of products are usually preserved by thermal treatments (i.e., pasteurization or sterilization) that accelerate the loss of compounds with organoleptic properties and nutritional value due to the use of high temperatures. Emerging nonthermal processing technologies, such as high-pressure processing (HPP), pulsed electric field (PEF), and high-pressure homogenization (HPH), among others, promise to overcome these problems (Galanakis, 2012; Galanakis, 2013; Galanakis, Barba, & Prasad, 2015). This chapter provides a general overview of the effects these technologies can have on the interactions between different food components, such as proteins, polysaccharides, lipids, and phenolic compounds.

10.2 HIGH-PRESSURE PROCESSING

HPP is a new nonthermal technology for food processing capable of microbial inactivation, avoiding spoilage and allowing food safety. It can inactivate enzymes with minimal effects on nutritional and sensory components, which are normally affected during heat treatment (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). HPP affects noncovalent bonds (ionic and

Nutraceutical and Functional Food Components. DOI: http://dx.doi.org/10.1016/B978-0-12-805257-0.00010-7

hydrophobic interactions) and to a much lesser extent covalent bonds, which leads to alterations in larger molecules, such as proteins (by alteration of secondary, tertiary, and quaternary structures), lipids, polysaccharides, and membranes by changes in components arrangement and architecture. Low-molecular-weight compounds such as peptides, vitamins, and flavor and pigmentation compounds are much less affected (Rastogi et al., 2007). These characteristics allow the development of new products with potential different and improved functional properties (Farr, 1990).

Table 10.1 presents a review of the principal studies that demonstrate the effect of HPP on the interactions between food compounds (e.g., lipid−protein, protein−protein, and protein−polysaccharide).

10.2.1 EFFECT OF HPP ON LIPID−PROTEIN INTERACTIONS

Ye, Anema, and Singh (2004) studied the interactions between milk fat globule membrane and whey proteins after treatment with high pressure in the range of 100−800 MPa for 30 min at 20°C. This work showed that with pressure increase, milk fat globule membrane associates with β-lactoglobulin by hydrophobic and sulfhydryl-disulfide interactions, showing a maximum value of 0.75 mg/g of fat. At high pressures (> 700 MPa), other milk proteins such as α-lactalbumin and κ-casein also interact by sulfhydryl-disulfide exchange with the thiol group being provided by β-lactoglobulin or by milk fat globule membrane proteins their self.

10.2.2 EFFECT OF HPP ON PROTEIN−PROTEIN INTERACTIONS

Proteins can be affected by high-pressure treatment due to modifications in structure caused by denaturation and refolding, compressibility, dissociation−association events, and modifications of enzymatic activity (Gross & Jaenicke, 1994). These effects are related to the rupture of noncovalent bond interactions between proteins, with reformation of molecular bonds after pressure release. Various types of interactions can affect protein structure, but the most sensitive to pressure is the hydrophobic interaction (Rastogi et al., 2007).

HPP can modify the form and volume of casein micelles by alteration of the balance between electrostatic repulsion and hydrophobic interactions. Huppertz, Fox, and Kelly (2004) verified the effect of HPP at 100, 250, 400, and 600 MPa, at 20°C for 30 min in bovine milk casein content. The authors reported a maximum increase of soluble caseins after treatment at 250 MPa and related these results to the higher solubilization of calcium phosphate at this pressure level, which is responsible for casein aggregation, neutralization of charged phosphoserine groups, and disruption of hydrophobic bonds. They also reported higher solids content for HPP-treated whey at 400 and 600 MPa (similar to that obtained for untreated milk) than at 250 MPa due to interactions between denatured β-lactoglobulin and casein micelles (Huppertz et al., 2004). These results indicate a reversible casein-solvation state (fewer interactions with water as pressure increased), indicating that HPP-treated milk can have different composition, structure, and properties than untreated milk.

Alvarez, Ramaswamy, and Ismail (2007) studied the effect of high pressure on whey protein profile by subjecting whey proteins (α-lactalbumin and β-lactoglobulin) to 450, 550, and 650 MPa and to a particular three-cycle treatment at 400 MPa. While the α-lactalbumin structure remained compact after pressure release, β-lactoglobulin appeared to show more charges than in the unpressurized whey, which may be due to interactions between hydrophobic portions of the molecule that became accessible to the solvent after HPP. β-Lactoglobulin has five cysteine groups in its

Table 10.1 Overview of the Studies of High-Pressure Processing (HPP) Effect on Compound Interactions

Product	Treatment	Interaction	References
Broad bean	200 and 400 MPa, 25°C, 20 min	After pressure treatment occurs dissociation of electrostatic protein-polysaccharide complexes, being restored stronger after pressure release	Galazka, Dickinson, and Ledward, (2000b)
Milk	100, 250, 400, and 600 MPa (300 MPa/min), 20°C, 30 min	Increased casein solubilization after HPP due to neutralization of negative charges of phosphoserine groups and disruption of hydrophobic interactions	Huppertz et al. (2004)
Milk	100−800 MPa, 20°C, 30 min	Pressure caused denaturation of whey proteins and milk fat globule membrane, allowing thiol-disulfide interactions, and aggregation	Ye et al. (2004)
Apple juice	200−400 MPa, 20°C, for 0−10 min	Interactions between phenolic compounds and pectin methylesterase can affect its enzymatic activity	Baron et al. (2006)
Whey	450, 550, and 650 MPa, 4−10°C, 10 min	Partial denaturation caused by high pressure allows interaction between Ca^{2+} ions and α-lactalbumin and consequently decreases water accessibility to proteins	Alvarez et al. (2007)
Egg white	400−700 MPa (100 MPa/min), 10−60°C, 20 min	Due to protein−protein interactions (such as solubility and exposed thiol groups) foams with high volume and average density after pressure treatment at pH 8.8 were obtained	Van Der Plancken et al. (2007)
Skim milk	200−600 MPa (6 MPa/s), 20°C, 30 min	Pressure treatment before acidification can promote gels firmness, at adequate pH, by hydrophobic and thiol-disulfide interactions	Anema (2010)
Soybean	200 and 400 MPa (3.4 MPa/s), 20°C, 10 min	HP denatured protein (soybean protein isolate) interacted with calcium, enhancing stiffer protein gels than with thermal treatment	Speroni et al. (2010)
Dry-cured ham	600 MPa, 32°C, 6 min	Increase in saltiness perception due to break of interactions between Na^{+} ions and proteins, leaving ions more accessible	Clariana et al. (2011)
Abalone	200, 350, 450, and 550 MPa (17 MPa/s), 20°C, 3, 5, and 10 min	Interaction between proteins and starch increases the emulsifying capacity at pressures over 350 MPa applied for 3 and 5 min	Barrios-Peralta et al. (2012)
Walnut	300, 400, 500, and 600 MPa (120 MPa/min), room temperature, 20 min	Interactions between hydrophobic groups and water, and between proteins lead to a decrease of solubility of walnut protein	Qin et al. (2013)

conformation: four involved in disulfide bonds and one residue free. As a result of HPP, this free cysteine residue will be accessible for the formation of new covalent bonds, producing a new protein conformation or polymerization (Galazka, Sumner, & Ledward, 1996). Alvarez et al. (2007) also reported that HPP affects the interactions between α-lactalbumin fraction and commercial whey isolate calcium ions, decreasing the accessibility of water to some protein side chains, and subsequently decreasing its solubility. These results showed that whey proteins can respond differently to HPP, turning difficult to obtain and compare rheological and nutraceutical properties after whey pressure treatment. Anema (2010) studied the effect of pH on milk pressure treatment at 200−600 MPa, at 20°C, for 30 min and obtained results similar to Alvarez et al. (2007), i.e., α-lactalbumin was more pressure resistant than β-lactoglobulin. After pressurization, particle sizes seemed to be larger. In particular, when whey is under pressure, calcium phosphate solubility increases due to a decrease of hydrophobic interactions. Thereafter, when pressure is released, those interactions are quickly retaken, leading to a nonnative reassociation of the denatured proteins. This aggregation reaction is higher for lower pH values, while denaturation occurs faster at higher pH values.

The foaming capacity of egg-white protein is an important property for the preparation of various food products. Van Der Plancken, Van Loey, and Hendrickx (2007) subjected egg white to a pressure range of 400−700 MPa, at 10−60°C for 20 min. By increasing pressure (at high pH—8.8), it was possible to obtain foams with higher volumes than those heat-treated, independent of the pressure or temperature used. However, at lower pH (7.6), HPP (600−700 MPa) led to reduced foaming ability, with the formation of low-volume foams. This effect was due to a decrease of protein solubility by protein aggregate formation, which avoided the incorporation of air and generated denser foams by hydrophobic interactions.

Soybean proteins are a very rich source of nutrients, with great functional properties, such as the ability to form gels when calcium is present. When soybean was submitted to pressure levels of 200 and 400 MPa (for 10 min at 20°C), pressure-denatured proteins interacted with the present calcium, which resulted in the formation of protein complexes by hydrophobic and electrostatic interactions as well as hydrogen bonds with higher stiffness than the gels formed without calcium (Speroni, Jung, & De Lamballerie, 2010). Another interesting effect of HPP-induced interactions with ions was reported by Clariana et al. (2011), who studied HPP-treated dry-cured ham (600 MPa, at 32°C, for 6 min). After HPP, sample saltiness perception was higher without any changes in salt content, which was due to the fact that high pressure can affect the interaction between Na^+ ions and proteins, making sodium ions more accessible and making the food product taste saltier (without increasing the salt concentration).

More recently, Qin et al. (2013) studied a solution of walnut protein, which was subjected to HPP at 300, 400, 500, and 600 MPa, at room temperature, for 20 min. They observed a decrease in protein solubility after pressurization, due to protein denaturation with consequent exposure of hydrophobic groups to the aqueous media. This exposure can lead to protein−protein interactions and subsequent formation of aggregates by hydrophobic exchanges and disulfide bonds.

10.2.3 EFFECT OF HPP ON PROTEIN−POLYSACCHARIDE INTERACTIONS

In the food industry it is common to use proteins and polysaccharides to change and control food properties, such as structure, stability, and texture. Proteins are known for their emulsifying and

foaming properties and polysaccharides for their water-holding capacity. Macromolecular interactions between these two biopolymers (determined by relative concentration and solution conditions) can affect the state of a colloidal system depending on their nature (attractive or repulsive), being manifested in alterations of emulsion properties (Dickinson & James, 2000).

Pressure treatment of broad bean at 200 or 400 MPa (for 20 min at 25°C) can affect protein (7S and 11S) structure and surface hydrophobicity due to the release of protein core sites, which promotes the formation of aggregates by hydrophobic interactions (Galazka et al., 2000b). When carrageenan was added to the protein solution, a decrease of aggregate formation was observed, which featured the presence of polysaccharide and its capacity to bond to hydrophobic protein sites, avoiding its complexation after pressure treatment.

Barrios-Peralta, Pérez-Won, Tabilo-Munizaga, and Briones-Labarca (2012) studied the effect of HPP on the interaction of abalone proteins with potato starch and agar components at pressure levels of 200, 350, 450, and 550 MPa, at 20°C, for 3, 5, and 10 min, respectively. The authors reported that noncovalent protein−polysaccharide (protein−agar and protein−starch) interactions were modified due to pressure-induced electrostatic interactions, increasing the emulsifying capacity at 350 MPa, for 3−5 min. These results were supported by the study of Galazka, Dickinson, and Ledward (2000a) who showed that a mixture of commercial ovalbumin with dextran sulfate forms reversible electrostatic complexes (being the strength of the interaction dependent of the charge of the polysaccharide) with improved emulsifying efficiency after pressure treatment at 600 MPa. These results are related to the G' parameter, which provides an indication of emulsion gel strength. It has been shown that a mixture of protein and polysaccharide (such as pectin) can improve emulsion gel strength after pressure treatment (Dickinson & James, 2000).

10.2.4 EFFECT OF HPP ON PROTEIN−PHENOLIC COMPOUND INTERACTIONS

Phenolic compounds are beneficial components mainly found in fruit and vegetables, and are partly known for their antioxidant potential. Baron, Dénes, and Durier (2006) studied the effect of high-pressure treatment (200−400 MPa, 20°C, for 0−10 min) on apple juice and showed a potential interaction between phenolic compound oxidation and pectin methylesterase (PME) activity. A decrease of PME activity after pressurization at levels below 300 MPa was reported, while at 400 MPa it was enhanced.

These results were related to the higher phenolic compound content (such as hydroxycinnamic acid, procyanidins, and catechins) after juice pressurization at 200−300 MPa, for 10 min, which inhibited PME, decreasing its activity in this pressure range compared to the control.

10.3 PULSED ELECTRIC FIELD

PEF is an emerging nonthermal technology that is based on the application of moderate or high voltage pulses (from 20 to 60 kV/cm) during short periods of time (μs or ms), which allows the pasteurization of liquid or semiliquid food without impairing sensory and nutritional value (Barba et al., 2015). However, PEF induces redox electrochemical reactions in the interface around the anode and cathode, which can lead to the formation of reactive oxygen species (e.g., hydrogen

peroxide or hydroxyl radicals), which in turn can react with several compounds that play a significant role in their degradation (Ma & Wang, 2013; Ruenroengklin, Yang, Lin, Chen, & Jiang, 2009). Nevertheless, the effects of PEF on the interaction between nutraceutical and functional food components is an area that is not well-studied. The available information in literature is scarce since most studies focus on the effects on individual food components rather than on interactions between them (Odriozola-Serrano, Aguiló-Aguayo, Soliva-Fortuny, & Martín-Belloso, 2013; Soliva-Fortuny, Balasa, Knorr, & Martín-Belloso, 2009). Due to the lack of research and the complexity and nature of the interactions in food matrix, the interactions promoted by PEF on the following nutrients/compounds are generalized here.

10.3.1 EFFECT OF PEF ON PROTEINS

Protein response to PEF comprises several phenomena depending on the PEF system and parameters used (field strength, pulse duration, number of pulses, and pulse shape), as well as the type and concentration of protein. These phenomena consist of disulfide bond cleavage, exposure of hydrophobic amino acid residues, and unfolding of the tertiary and secondary structure. This phenomena can origin macroscopic changes, for instance, several types of protein interactions including self-aggregation and solubility (Zhao, Yang, Tang, Zhang, & Hua, 2009). As noted, PEF can induce changes that expose sulfhydryl groups, which results in protein unfolding or ionization. This can cause changes in protein conformation where reactive sulfhydryl groups can form disulfide bonds between proteins, consequently promoting their solubility or aggregation. Moderate parameters (e.g., electric field strength of 25 kV/cm for 400 μs) do not seem to have great impact on the physicochemical properties of proteins (Zhao, Tang, Lu, Chen, & Li, 2013; Wu, Zhao, Yang, & Chen, 2014). For these type of treatments, the proteins partially unfold and subunit dissociation occurs, increasing solubility (Li, Chen, & Mo, 2007). For more intense treatments, the above phenomena take place and thus solubility or aggregation occurs. The most common interaction between proteins caused by strong PEF conditions is polymerization/aggregation induced by noncovalent (e.g., electrostatic interactions, hydrogen bonds, and hydrophobic interactions) and disulfide bonds (Zhao, Yang, & Zhang, 2012; Wu, Zhao, Yang, & Yan, 2015).

Liquid egg, milk, and soymilk-based products are the most commonly studied when it comes to the effects of PEF in protein-based foods (Zhao et al., 2013; Zhao et al., 2012). Two protein systems, β-lactoglobulin concentrate and egg white, were tested by Perez and Pilosof (2004). When β-lactoglobulin was subjected to 12.5 kV/cm and 15 s between pulses, small amounts of covalent-linked aggregates were formed. In egg-white proteins, the treatments induce partial polymerization of the major proteins (e.g., lysozyme, ovalbumin, and conalbumin). An increasing number of pulses increased the degree of polymerization. On the other hand, Li et al. (2007) assessed the effects of PEF on soybean protein isolates, and reported that treatments between 20 and 30 kV/cm during 72−288 μs partially unfolded the proteins, thus increasing their interaction with water. More intense treatments (e.g., above 30 kV/cm and 288 μs) caused slight denaturation and stronger molecular polarization, promoting protein aggregation. Zhao et al. (2009) evaluated the interactions caused by PEF in egg-white proteins. It was shown that moderate conditions such as 25 and 30 kV/cm for 400 μs did not produce insoluble aggregates. On the other hand, more intense conditions such as an electric strength of 35 kV/cm for 400 and 800 μs induced the formation of insoluble protein aggregates through the formation of disulfide bonds. Sui, Roginski, Williams, Versteeg, and

Wan (2011) studied the effects of PEF on whey protein isolate using 30 and 35 kV/cm for 19.2 and 211 μs and concluded that these conditions do not promote protein aggregation. More recently, Zhao et al. (2012) studied the interaction between ovalbumin and bovine serum albumin induced by PEF. Treatments above 25 kV/cm for 400 μs were sufficient to induce self-aggregation of ovalbumin, but no self-aggregation of bovine serum albumin was observed. It is noteworthy that these treatments induce interaction and aggregation between both proteins. Wu et al. (2015) applied PEF treatments, 25 kV/cm from 200 to 800 μs, to a multiprotein system having ovalbumin, ovotransferrin, and lysozyme. They concluded that the aggregate formation is intrinsically connected to different protein concentrations, since an electric field of 25 kV/cm for 800 μs did not promote aggregates in some concentrations. Overall, low-to-intermediate intensity treatments seem to have little effect on protein interaction. However, more intense treatments may lead to a higher protein solubility and protein aggregation.

10.3.2 EFFECT OF PEF ON LIPIDS

PEF can induce electrical chemical reactions that influence lipids structure and subsequently promote interaction with oxygen (Zeng, Han, & Zi, 2010). Another hypothesis is that PEF treatment generates hydrogen radicals, which may be related to the oxidation process (Zhao et al., 2011). According to Zeng et al. (2010), peanut oil subjected to 20−50 kV/cm for 40 μs elevated the peroxide value as a function of increasing electric field. However, the oxidation rate decreased during storage compared to untreated samples. Zhao et al. (2011) suggested the use of oleic acid as a model for monounsaturated fatty acids, and the effects of an electrical field of 25, 30, and 35 kV/cm for 400 μs were assessed. The peroxide value increased by increasing field strength, but no significant differences among the conditions were found. Contrary to the findings of Zeng et al. (2010), the lipid oxidation of the treated oleic acid was greater than that of the nontreated samples during storage. Abenoza et al. (2012) studied the effects of PEF treatments from 1 to 2 kV/cm for 3 μs on olive oil. The treatments promoted an increase in oxidation, although the peroxide value remained within the legal limits. However, the increase in the peroxide value was less pronounced than that noted above. The difference in the results compared to those obtained for peanut oil and oleic acid may be due to the intensity of the treatments, since the objective in the olive oil was improvement of extraction rather than pasteurization.

10.3.3 EFFECT OF PEF ON L-ASCORBIC ACID

It is common knowledge that vitamin C is essential to human health. Rodríguez-Roque et al. (2015) studied the concentration and bioavailability of vitamin C in mixtures of water, milk, or soymilk with fruit juices treated with a 35 kV/cm electric field strength and 1800 μs total treatment time. They concluded that PEF reduced vitamin C concentration in a range of 8 to 15% and that the reduction might be due to the binding of proteins and/or other vitamins and metal ions, which can increase its degradation rates. Odriozola-Serrano, Soliva-Fortuny, and Martín-Belloso (2009) assessed the retention of vitamin C in strawberry juice after PEF treatment (35 kV/cm for 1000 μs) and no considerable losses were found. Low losses could possibly be attributed to the interaction with oxygen (oxidation), which was promoted by the temperature rise induced by PEF.

10.3.4 EFFECT OF PEF ON PHENOLIC COMPOUNDS

In a study conducted by Rodríguez-Roque et al. (2015) the effects of PEF on phenolic compounds were considered. It was found that the total phenolic content can decrease or increase depending on the base used (water, milk, or soymilk). Since enzymes can both be activated or inactivated by PEF treatments, depending on the treatment conditions, type, concentration of enzyme, etc. (Terefe, Buckow, & Versteeg, 2015), Rodríguez-Roque et al. (2015) suggested that these variations can result from the interaction of the phenolic compounds with enzymes related to their loss or participation in their biosynthesis. Sánchez-Vega, Elez-Martínez, and Martín-Belloso (2015) noted enzyme activity as an additional cause for the reduction of phenolic compounds in broccoli juice after 35 kV/cm from 500 to 2000 μs. Furthermore, PEF can cause changes in phenol structure and phenolic derivatives can be formed due to the loss of functional groups between phenols and sugars. PEF also diminishes the bioavailability of phenolic compounds in beverages that contain higher amounts of proteins due to covalent or noncovalent bonds between these compounds, leading to precipitation (multisite or multidentate interactions; Rodríguez-Roque et al., 2015).

Likewise, anthocyanins may be affected by PEF treatment depending on the type of product and treatment conditions. Zhang et al. (2006) studied the degradation of cyanidin-3-glucoside in an aqueous-methanol solution when exposed to low-intensity PEF treatments (1.2, 2.2, and 3.3 kV/cm). They reported that the degradation of anthocyanins increases with increasing treatment intensity, and that these changes could be related to structural modifications and interconversion of different anthocyanin species. In another study, Odriozola-Serrano et al. (2009) evaluated the effects of an electric field of 35 kV/cm for 1000 μs on anthocyanins concentration in strawberry juice and verified losses up to 17%. This reduction may be related to others factors and not only due to the treatment because anthocyanins can interact with peroxides resulting from the oxidation of ascorbic acid. Other supposition is the interaction with enzymes that are responsible for the degradation of anthocyanins, such as peroxidase or β-glucosidase that are not fully inactivated by PEF (Odriozola-Serrano et al. 2009; Terefe et al. 2015).

10.3.5 EFFECT OF PEF ON CAROTENOIDS

There is a possibility that PEF treatments can induce carotenoid conversions, resulting in an increase of some carotenoids (due to some precursors) over other compounds, which may explain why some carotenoids increase after PEF treatments while others decrease (Odriozola-Serrano et al., 2013). Sánchez-Vega et al. (2015) verified that an electric field of 15 kV/cm for 500 μs reduced the content of lutein and β-carotene by 35.5% and 49.3%, respectively. It is known that lower electric field strengths and small treatment times can result in reduced inactivation of enzymes (Terefe et al., 2015), while the interaction between oxidative enzyme and carotenoids can result in a decrease of the latter (Sánchez-Vega et al., 2015).

10.4 HIGH-PRESSURE HOMOGENIZATION

Usually, HPH refers to a pressure level between 150 and 200 MPa, and ultrahigh-pressure homogenization (UHPH) refers to a pressure range between 350 and 400 MPa (Dumay et al., 2013).

Different applications have been studied for this new technology, e.g., microorganism and enzyme inactivation, extraction of metabolites/compounds from plant and animal cells, reduction of particles size in fluids for viscosity modification, and production of stable emulsions. The processing of vegetable and animal source food products by UHPH induces mechanical forces, shear stress, turbulence, shock, and cavitation phenomena that occur through the high-pressure valve, leading to mechanical disruption and alteration of cell membranes, influencing matrix characteristics (Kleinig & Middelberg, 1998; Lopez-Sanchez, Svelander, Bialek, Schumm, & Langton, 2011). Since the bioaccessibility and bioavailability of nutrients are more important than the concentration present itself and depend on different factors (e.g., food matrix and compound preservation over food-processing phases), UHPH can contribute positively to enhanced product quality (Knockaert, Lemmens, Van Buggenhout, Hendrickx, & Van Loey, 2012; Svelander, Lopez-Sanchez, Pudney, Schumm, & Alminger, 2011).

10.4.1 EFFECT OF HPH ON TOCOPHEROLS

When applying UHPH in an almond beverage a total tocopherol loss of about 80−95% was observed (Toro-Funes, Bosch-Fuste, Veciana-Nogues, & Vidal-Carou, 2014b). This loss mainly concerned α-tocopherol (from 46.60 ± 2.44 mg/L to values ranging between 7.92 ± 0.48 and 3.15 ± 0.12 mg/L), and δ-tocopherol was not found in any treated sample (from 2.56 ± 0.31 mg/L to below the detection limit). On the other hand, γ-tocopherol remained at 100% and 60% after 200 MPa treatment at 55°C and 65°C, respectively. In addition, it was noted that the destroying effect was more predominant when increasing temperature instead of pressure. A similar conclusion was also found when treating soymilk with UHPH. In this case, the samples with the higher total tocopherols content were the nontreated ones, and mild treatment (200 MPa/55°C T_{inlet}) resulted only in a reduction of 20%. The samples obtained with UHPH treatment at 300 MPa/75°C T_{inlet} showed a decrease of 50% compared to the nontreated sample. The decrease of tocopherol content using UHPH can be correlated to the occurrence of heating phenomena when samples go through the high-pressure valve gap, e.g., in some cases the temperature can reach 129°C (Toro-Funes et al., 2014b).

10.4.2 EFFECT OF HPH ON POLYAMINES

Polyamines (e.g., spermine and spermidine) remain stable against UHPH treatment, as has been noted for almond beverage and soymilk (Toro-Funes, Bosch-Fuste, Veciana-Nogues, & Vidal-Carou, 2014a; Toro-Funes et al., 2014b). In other cases, a decrease of polyamines content was observed as a function of temperature treatment at 300°C for 6−12 min (Cirilo et al., 2003). However, this temperature is much higher than that commonly used in UHPH treatments.

10.4.3 EFFECT OF HPH ON PHYTOSTEROLS

UHPH was shown to increase the extractability of phytosterols in an almond beverage as a function of pressure and temperature (300 MPa, 75°C T_{inlet}) increase (Toro-Funes et al., 2014b). However, phytosterol stability decreases when subjected to high temperatures over an extended time period. The mechanical forces, shear stress, turbulences, shock, and cavitation phenomena induced by

UHPH treatment could lead to mechanical disruption and alteration of cell membranes (Dumay et al., 2013; Kleinig & Middelberg, 1998), which allows the extraction of phytosterols that are integral lipid components of plant cell membranes.

A similar pattern was observed in UHPH soymilk (Toro-Funes et al., 2014a) in which total phytosterol content was statistically higher than that in the nontreated sample. The increase of these compounds could be related to the mechanical forces of UHPH, which reduce fat globule size and other dispersed particles allowing the release of phytosterols from fat globules (Desrumaux & Marcand, 2002; Toro-Funes et al., 2014a). It was also observed that the higher extraction capacity was verified at the highest T_{inlet} (75°C) regardless of pressure level.

10.4.4 EFFECT OF HPH ON MILK CASEIN MICELLES

UHPH can enhance the binding capacity of several hydrophobic compounds (e.g., aromatic molecules, vitamins, and drugs) to individual caseins or casein micelles by modifying the casein micelles structure. Thus the enrichment of milk-protein dispersions with vitamins or others compounds with biological activity can be obtained and enhanced by UHPH (Dumay et al., 2013).

Enhancement of the binding efficiency by 1.5 and 3 (compared to control dispersions at atmospheric pressure) of α-tocopherol acetate to phosphocasein micelles was observed by Chevalier-Lucia, Blayo, Gràcia-Julià, Picart-Palmade, and Dumay (2011), when 250 MPa (T_{inlet} 34°C) and 300 MPa (T_{inlet} 14°C) treatments were applied, respectively. When these phosphocasein micelles were deposed onto Caco-2 cell monolayers to assess possible cellular damages using lactate dehydrogenase release assay or inflammation using interleukin-8 secretion, the cellular responses were the same as native-like phosphocasein micelles (Chevalier-Lucia et al., 2011; Dumay et al., 2013).

10.4.5 EFFECT OF HPH ON L-ASCORBIC ACID

Orange juice processed by UHPH revealed a significantly higher amount of L-ascorbic acid than the thermal-pasteurized samples (Velázquez-Estrada, Hernández-Herrero, Rüfer, Guamis-López, & Roig-Sagués, 2013). In this study, a decrease of L-ascorbic acid content of about 2%, 5%, and 11% was found for UHPH treatments of 100, 200, and 300 MPa, respectively (45°C, 70°C, and 94°C were the highest temperatures achieved during the processing period, respectively). On the other hand, when UHPH (100, 200, and 300 MPa, T_{inlet} of 4°C and 20°C) was applied to clear apple juice, no effects on ascorbic acid and dehydroascorbic acid functionality were observed (Suarez-Jacobo et al., 2011).

10.5 COLD-PLASMA PROCESSING

Another nonthermal emerging technology is atmospheric pressure plasma, also known as cold, or cool, plasma. The term plasma was defined in 1923, when specific oscillations in ionized gas, determined by electron density and electron mass, were observed (Surowsky, Schlüter, & Knorr, 2014). It refers to a quasi-neutral ionized gas, constituted by highly energetic species in permanent interaction, such as photons, positive and negative ions, and free electrons and radicals (Pankaj

et al., 2014; Segat, Misra, Cullen, & Innocente, 2015). These nonthermal plasmas have a low degree of ionization, mainly impelled by electrons, which are responsible for atomic and molecular excitation, dissociation, and production of radicals. The final state is formed by an active gaseous medium (rich in reactive oxygen species, ROS) that can be used with safety and that will not cause thermal damage to the surrounding material. Therefore they are capable of inactivating microbes since they react with almost all cell components (Surowsky et al., 2014). Due to its different properties cold plasma can be used in various fields like textile, electronics, life sciences, and packaging (Thirumdas, Sarangapani, & Annapure, 2014), but in food technology cold plasma is typically applied for microbial decontamination and sterilization of foods. Cold plasma can also be used as an alternative to traditional thermal food pasteurization techniques, decreasing the impact of color changes, off-flavor production, and nutritional losses. However, its main disadvantages include low penetration depth (enabling bacteria migration into the food tissue) and the effect on food components such as vitamins and polyphenols. Although cold-plasma processing is an important methodology, there is a lack of information on the promoted interactions in food components.

10.5.1 EFFECT OF COLD PLASMA ON PROTEIN–PROTEIN INTERACTIONS

Segat et al. (2015) studied the effect of cold-plasma treatment (70 kV for 1 up to 60 min) on whey protein isolate and reported an increase in protein unfolding for 30 and 60 min treatments, which led to an increase in surface hydrophobicity exposure. For both treatments, higher distributions of particles was observed due to general molecular unfolding and network formation among proteins (Segat et al., 2015). These results suggest that cold-plasma processing can lead to protein aggregation. Contrasting results were reported by Tammineedi, Choudhary, Perez-Alvarado, and Watson (2013) who studied the effect of cold-plasma treatment (13.56 MHz radio frequency up to 10 min) on the allergenicity of α-casein and whey proteins. According to their results, no effect or interactions were observed in these compounds after treatment, indicating that higher plasma power may yield better results toward reducing the allergenicity of major milk allergens (Tammineedi et al., 2013). The foaming and emulsifying capacities of whey protein isolate were also significantly improved after cold-plasma treatment (15 min), since the partial unfolding of proteins in the beginning of treatment allowed the generation of a more flexible structure able to arrange itself at the air–water interface in order to form the foam. However, after 30 and 60 min, this property decreased abruptly due to the formation of protein aggregates by disulfide bond formation (Segat et al., 2015).

10.5.2 EFFECT OF COLD PLASMA ON ASCORBIC ACID INTERACTIONS

Cold-plasma treatment of orange juice has been used to reduce its vitamin C content. For example, Shi et al. (2011) subjected orange juice to 20 kV, 60 kHz, for 20 s and reported a lower concentration of dehydroascorbic acid due to its interaction with ROS. However, when the sample was added to other substances, such as carbohydrates, the losses were significantly decreased due to the interaction of oxygen atoms with water on aqueous solutions and consequent stabilization under treatment (Chemistry, Ferrier, & Blattner, 2001; Surowsky et al., 2014).

10.5.3 EFFECT OF COLD PLASMA ON PHENOLIC COMPOUNDS

Although phenolic compounds are responsible for many of the functional properties found in food, their interactions after cold-plasma treatment has not been thoroughly investigated. However, the presence of ROS and charged particles can direct reaction pathways, eventually leading to the appearance of new secondary metabolites. Grzegorzewski, Ehlbeck, Schlüter, Kroh, and Rohn (2011) studied lamb's lettuce (*Valerianella locusta*) after exposure to a cold-plasma treatment (35 W for 40 s). According to their results, degradation was not induced by thermal or photochemical processing, but due to interactions with the radical constituents of plasma. Nevertheless, the destruction of diosmetin and quercetin was correlated to their ability to scavenge free radicals, leading to an erosion of food epidermal tissue layers (where flavonoids and other components accumulate; Grzegorzewski et al., 2011). Indeed, their degradation occurs by the action of OH radicals that initiate one-electron oxidation of polyphenols, leading to low-molecular-weight phenolics.

10.6 ULTRASOUND TECHNOLOGY

Ultrasound (US) technology is recognized as an innovative technology for food processing, and is used for several purposes, e.g., emulsification, crystallization, homogenization, cutting, hydrolysis, extraction, and microbial inactivation. US has both positive (e.g., modification of viscosity and homogenization parameters) and negative (e.g., off-flavors, undesired physical parameters) impacts on food products. These changes are due to critical temperature and pressure conditions that allow the formation of radicals during sonocavitation (Pingret, Fabiano-Tixier, & Chemat, 2013).

10.6.1 EFFECT OF ULTRASOUND ON CAROTENOIDS

In carrot juice processed by US (24 kHz, 50−58°C, 120 μm, 10 min), a considerable increase (2.71−3.44%) of total carotenoid content was observed by increasing the processing temperature. This increase was correlated to the mechanical disruption of cell walls (Martinez-Flores, Garnica-Romo, Bermudez-Aguirre, Pokhrel, & Barbosa-Canovas, 2015). On the other hand, lycopene content of watermelon juice processed by US (20 kHz, 25−45°C, 24.4−61 μm, 2−10 min, pulses of 5 s—on/off) was shown to decrease (Rawson et al., 2011). In particular, increase of processing time, amplitude, and temperature caused lycopene degradation and led to the identification of oxidation compounds such as acetone, methyl-heptenone, laevunilic aldehyde, and glyoxal, affecting food product characteristics (Cole & Kapur, 1957a; Cole & Kapur, 1957b; Rawson et al., 2011). In another study, treatment of tomato pulp with US (24 kHz, 100 μm, 15−60 min, <90°C) caused a progressive loss of cell integrity as a function of processing time increase, without a significant difference in lycopene content (Anese, Mirolo, Beraldo, & Lippe, 2013). However, a decrease of in vitro bioaccessibility was observed due to the entrapment of lycopene in a stronger fiber network making it less accessible to digestive enzymes and bile salts (Anese et al., 2013).

10.6.2 EFFECT OF ULTRASOUND ON ASCORBIC ACID

US processing (20 kHz, 25−45°C, 24.4−61 μm, 2−10 min, pulses of 5 s—on/off) has also been shown to degrade ascorbic acid in watermelon juice (Rawson et al., 2011), which was attributed to the development of pyrolysis and oxidation by $OH^{\bullet}$ radicals formed during cavitation. In a similar study conducted on orange juice (20 kHz, 5−30 °C, 24.4−61.0 μm, 0−10 min, pulses of 5 s—on/off), ascorbic acid content decreased around 10% (Valdramidis, Cullen, Tiwari, & O'donnell, 2010). In this case, several degradation mechanisms were proposed, e.g., thermal effects produced by bubble implosion, mechanical stresses produced microstreaming and implosion shockwaves, and free radical production. In summary, the effect of US in ascorbic acid was attributed to thermolysis (inside bubbles) and triggering of the Maillard reaction and/or the reaction with OH^{-} and production of oxidative products on the surface of bubbles (Valdramidis et al., 2010). On the other hand, Aadil, Zeng, Han, and Sun (2013) observed an increase in vitamin C content when grapefruit juice was treated with US (28 kHz, 20°C, 30−90 min). This trend was attributed to the removal of entrapped oxygen due to cavitation.

10.6.3 EFFECT OF ULTRASOUND ON TOTAL PHENOLICS

It is known that the effect of antioxidants is dependent on the degree of hydroxylation, and thus the reactive radicals formed during cavitation are considered a disadvantage for preserving the bioactivity of food components (e.g., phenols). However, this chemical effect of US (radical formation) can enhance the antioxidant activity of other components by increasing the extent of hydroxylation (i.e., flavonoids; Soria & Villamiel, 2010; Wan et al., 2005).

When watermelon juice was processed by US (20 kHz, 25−45°C, 24.4−61 μm, 2−10 min, pulses of 5 s—on/off), a decrease of total phenolics was observed as a function of processing temperature and processing time (Rawson et al., 2011). On the other hand, Aadil et al. (2013) found an increase in total phenol content in sonicated grapefruit juice (28 kHz, 20°C, 30−90 min). This tendency was attributed to a possible breakage of cell wall induced by cavitation pressure and to the addition of hydroxyl group produced by sonication to the aromatic ring of phenolic compounds.

10.6.4 EFFECT OF ULTRASOUND ON ANTHOCYANINS

Strawberry juice processed by US (20 kHz, 24.4−61 μm, 2−10 min, pulses of 5 s—on/off) presented a content decrease of about 3.2%, possibly due to thermolysis and combustion occurring inside the bubbles or reaction with hydroxyl radicals, leading to the formation of oxidation products on the bubble surface (Tiwari, O'donnell, Patras, & Cullen, 2008).

10.6.5 EFFECT OF ULTRASOUND ON PROTEINS

The molecular structure of proteins influences their functional properties and their inter- and intramolecular interactions. Therefore the sonolysis of water can be used to induce crosslinking of protein molecules in an aqueous medium by the generated radicals and superoxides (Soria & Villamiel, 2010). For instance, when bovine serum albumin was processed with US (Gülseren, Güzey, Bruce, & Weiss, 2007), the cavitation-generated hydrogen peroxide changed its chemical

structure by oxidizing its free sulfhydryl groups, leading to sulfinic and sulfonic acid formation. In this study, the protein aggregates detected were possibly formed due to noncovalent interactions such as electrostatic and hydrophobic interactions.

US has also been applied to meat tenderization. Using US, the release of myofibrillar proteins is easier and leads to improved water binding capacity, tenderness, as well as cohesiveness of meat products (Mcclements, 1995).

10.6.6 EFFECT OF ULTRASOUND ON TOTAL VOLATILES

Although US technology can be used to homogenize and decrease the microbial load of certain food emulsions, e.g., milk, US processing (24 kHz, 45°C, 2.5−20 min) sometimes leads to the formation of volatiles (e.g., benzene, toluene, 1,3−butadiene, 5-methyl-1,3-cyclopentadiene, and a series of aliphatic 1-alkenes) generated by pyrolysis (Riener, Noci, Cronin, Morgan, & Lyng, 2009).

10.7 OHMIC HEATING

Ohmic heating (OH) consists of heating foods through the passage of electric current (i.e., using food as an electrical resistor; Knirsch, Alves Dos Santos, Martins De Oliveira Soares Vicent, & Vessoni Penna, 2010). With the current's passage there is a transformation of electric energy into thermal energy and thus heat is generated inside the food differently than conventional thermal processing in which heat transfer occurs from the food exterior to interior (Varghese, Pandey, Radhakrishna, & Bawa, 2014). Therefore the food products are heated particularly fast and more homogeneously, minimizing their exposure to heat, which may increase the quality of the processed products by maintaining their initial characteristics (Knirsch et al., 2010). OH can be applied to several foods for food pasteurization (e.g., fruit juices and/or purees), vegetable blanching, and cooking of meat products. Its advantages include shorter processing times with higher yields, small impact on nutrients, less cooking time, and good energy efficiency (Evrendilek et al., 2011; Varghese et al., 2014; Yildiz-Turp, Sengun, Kendirci, & Icier, 2013).

Since foods are heated exceptionally fast, the impact caused by heat is reduced, minimizing quality changes. Furthermore, researchers believe that in addition to the thermal effects, electric effects may exist due to the electric field, current, and frequency applied. These effects can impact foods at molecular and cellular level and promote nutrient interactions. However, more studies are needed to understand these phenomena (Evrendilek et al., 2011; Knirsch et al., 2010). While most known effects of OH are due to thermal effects and there are few known interactions between nutrients due to the electrical effects, the mechanisms induced by electric changes must be further studied.

When OH is conducted at low electric field frequencies the electrolysis of water can occur in the vicinity of stainless-steel electrodes yielding hydrogen and oxygen. The generated oxygen may interact with food components, particularly vitamins, amino acids, and lipids, promoting their oxidation. Additionally, the corrosion of the electrodes can possibly occur leading to the generation of metal ions, such as Fe^{2+} and Fe^{3+}, that can catalyze the oxidation of some vitamins. Furthermore,

these metal ions could form chelated complexes (e.g., with citric acid; Assiry, Sastry, & Samaranayake, 2003).

10.7.1 EFFECT OF OHMIC HEATING ON ASCORBIC ACID

The application of low electric field frequencies (10−1000 Hz) on acerola pulp resulted in an increase in the oxidation of the ascorbic acid compared to the oxidation obtained at a higher frequency (10 Hz). The authors justified this increase with electrochemical degradation, promoted by the electrolysis and corrosion of the electrodes that occur a lower frequencies, as previously mentioned. Furthermore, high electric field frequencies resulted in lower oxidation of ascorbic acid than that from conventional heating, strengthening this conclusion (Mercali, Jaeschke, Tessaro, & Marczak, 2012; Mercali, Schwartz, Marczak, Tessaro, & Sastry, 2014). Similar results were reported for *Aloe vera* gel juice, where the decrease of ascorbic acid was more pronounced when processed with OH than in the samples processed by conventional heat (Saberian, Hamidi Esfahani, & Abbasi, 2015).

10.7.2 EFFECT OF OHMIC HEATING ON PROTEINS

OH can also increase the oxidation of proteins in pork meat, although no association with electrolysis has been found. Nonetheless this interaction with oxygen can promote further interactions as the carbonyl groups can react with free amino groups forming amide bonds (Dai et al., 2014). Overall, most nutrient interactions promoted by OH are related to the thermal effects, but little is known about the interactions promoted by the possible electrical effects. Therefore more studies are needed to fully understand the impact of nonthermal effects of OH on food constituents.

10.8 CONCLUSION

The demand for improved quality and safe food products with high nutritional value and functional and exceptional sensorial attributes has led to the development and application of new food-processing technologies. Traditional thermal processing cannot meet all consumer requirements, due to the high temperatures used and longer processing times, which lead to changes in food characteristics.

As discussed, HPP processing, PEF, HPH, cold plasma, US, and OH have the capability of enhancing food quality, improving, for instance, the bioavailability of compounds and its organoleptic and sensorial characteristics. Some of these technologies are being increasingly applied worldwide in food industries (e.g., HPP).

The effect of these processing technologies in foods has been the focus of several studies, and positive and negative consequences have been noted depending on the food properties and processing conditions. Nonetheless, the possible interactions between food compounds promoted by these technologies is still poorly known (e.g., between polysaccharides, proteins, phenolic compounds, lipids) and further research is needed.

ACKNOWLEDGMENTS

M. D. Santos, R. P. Queirós, and S. A. Moreira acknowledge the financial support of Fundação para a Ciência e Tecnologia (Portugal), via doctoral fellowships (SFRH/BD/103145/2014, SFRH/BD/111002/2015 and SFRH/BD/110430/2015, respectively). Thanks are also due to FCT/MEC for the financial support of the QOPNA research Unit (FCT/ID/QUI/00062/2013), through national funds, and where applicable, cofinanced by the FEDER, within the PT2020 Partnership Agreement.

REFERENCES

Aadil, R. M., Zeng, X. A., Han, Z., & Sun, D. W. (2013). Effects of ultrasound treatments on quality of grapefruit juice. *Food Chemistry*, *141*, 3201−3206.

Abenoza, M., Benito, M., Saldaña, G., Álvarez, I., Raso, J., & Sánchez-Gimeno, A. C. (2012). Effects of pulsed electric field on yield extraction and quality of olive oil. *Food and Bioprocess Technology*, *6*, 1367−1373.

Alvarez, P. A., Ramaswamy, H. S., & Ismail, A. A. (2007). Effect of high-pressure treatment on the electrospray ionization mass spectrometry (ESI-MS) profiles of whey proteins. *International Dairy Journal*, *17*, 881−888.

Anema, S. G. (2010). Effect of pH at pressure treatment on the acid gelation of skim milk. *Innovative Food Science & Emerging Technologies*, *11*, 265−273.

Anese, M., Mirolo, G., Beraldo, P., & Lippe, G. (2013). Effect of ultrasound treatments of tomato pulp on microstructure and lycopene in vitro bioaccessibility. *Food Chemistry*, *136*, 458−463.

Assiry, A., Sastry, S. K., & Samaranayake, C. (2003). Degradation kinetics of ascorbic acid during ohmic heating with stainless steel electrodes. *Journal of Applied Electrochemistry*, *33*, 187−196.

Barba, F. J., Parniakov, O., Pereira, S. A., Wiktor, A., Grimi, N., Boussetta, N., & Vorobiev, E. (2015). Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Research International*, *77*, 773−798.

Baron, A., Dénes, J.-M., & Durier, C. (2006). High-pressure treatment of cloudy apple juice. *LWT - Food Science and Technology*, *39*, 1005−1013.

Barrios-Peralta, P., Pérez-Won, M., Tabilo-Munizaga, G., & Briones-Labarca, V. (2012). Effect of high pressure on the interactions of myofibrillar proteins from abalone (*Haliotis rufescens*) containing several food additives. *LWT - Food Science and Technology*, *49*, 28−33.

Chemistry, R. S., Ferrier, R. J., & Blattner, R. (2001). *Carbohydrate chemistry*. Royal Society of Chemistry.

Chevalier-Lucia, D., Blayo, C., Gràcia-Julià, A., Picart-Palmade, L., & Dumay, E. (2011). Processing of phosphocasein dispersions by dynamic high pressure: Effects on the dispersion physico-chemical characteristics and the binding of α-tocopherol acetate to casein micelles. *Innovative Food Science & Emerging Technologies*, *12*, 416−425.

Cirilo, M. P. G., Coelho, A. F. S., Araújo, C. M., Gonçalves, F. R. B., Nogueira, F. D., & Glória, M. B. A. (2003). Profile and levels of bioactive amines in green and roasted coffee. *Food Chemistry*, *82*, 397−402.

Clariana, M., Guerrero, L., Sárraga, C., Díaz, I., Valero, Á., & García-Regueiro, J. A. (2011). Influence of high pressure application on the nutritional, sensory and microbiological characteristics of sliced skin vacuum packed dry-cured ham. Effects along the storage period. *Innovative Food Science & Emerging Technologies*, *12*, 456−465.

Cole, E. R., & Kapur, N. S. (1957a). The stability of lycopene. I.-Degradation by oxygen. *Journal of the Science of Food and Agriculture*, *8*, 360−365.

Cole, E. R., & Kapur, N. S. (1957b). The stability of lycopene. II.—Oxidation during heating of tomato pulps. *Journal of the Science of Food and Agriculture*, *8*, 366−368.

Dai, Y., Lu, Y., Wu, W., Lu, X.-M., Han, Z.-P., Liu, Y., & Dai, R.-T. (2014). Changes in oxidation, color and texture deteriorations during refrigerated storage of ohmically and water bath-cooked pork meat. *Innovative Food Science & Emerging Technologies*, *26*, 341−346.

Desrumaux, A., & Marcand, J. (2002). Formation of sunflower oil emulsions stabilized by whey proteins with high-pressure homogenization (up to 350 MPa): effect of pressure on emulsion characteristics. *International Journal of Food Science & Technology*, *37*, 263−269.

Dickinson, E., & James, J. D. (2000). Influence of high-pressure treatment on beta-lactoglobulin-pectin associations in emulsions and gels. *Food Hydrocolloids*, *14*, 365−376.

Dumay, E., Chevalier-Lucia, D., Picart-Palmade, L., Benzaria, A., Gràcia-Julià, A., & Blayo, C. (2013). Technological aspects and potential applications of (ultra) high-pressure homogenisation. *Trends in Food Science & Technology*, *31*, 13−26.

Evrendilek, G. A., Baysal, T., Icier, F., Yildiz, H., Demirdoven, A., & Bozkurt, H. (2011). Processing of fruits and fruit juices by novel electrotechnologies. *Food Engineering Reviews*, *4*, 68−87.

Farr, D. (1990). High pressure technology in the food industry. *Trends in Food Science & Technology*, *1*, 14−16.

Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology*, *26*, 68−87.

Galanakis, C. M. (2013). Emerging technologies for the production of nutraceuticals from agricultural by-products: A viewpoint of opportunities and challenges. *Food and Bioproducts Processing*, *91*, 575−579.

Galanakis, C.M., Barba, F.J., Prasad, K.N. 2015. Cost and safety issues of emerging technologies against conventional techniques. In: C. M. E. Galanakis (Ed.), *Food waste recovery: processing technologies and industrial techniques*, Academic Press.

Galazka, V. B., Dickinson, E., & Ledward, D. A. (2000a). Effect of high pressure on ovalbumin-polysaccharide interactions. *High Pressure Research*, *19*, 125−130.

Galazka, V. B., Dickinson, E., & Ledward, D. A. (2000b). Influence of high pressure on interactions of 11S globulin *Vicia faba* with ι-carrageenan in bulk solution and at interfaces. *Food Hydrocolloids*, *14*, 551−560.

Galazka, V. B., Sumner, I. G., & Ledward, D. A. (1996). Changes in protein-protein and protein-polysaccharide interactions induced by high pressure. *Food Chemistry*, *57*, 393−398.

Gross, M., & Jaenicke, R. (1994). Proteins under pressure. The influence of high hydrostatic pressure on structure, function and assembly of proteins and protein complexes. *European Journal of Biochemistry*, *221*, 617−630.

Grzegorzewski, F., Ehlbeck, J., Schlüter, O., Kroh, L. W., & Rohn, S. (2011). Treating lamb's lettuce with a cold plasma − Influence of atmospheric pressure Ar plasma immanent species on the phenolic profile of *Valerianella locusta*. *LWT - Food Science and Technology*, *44*, 2285−2289.

Gülseren, İ., Güzey, D., Bruce, B. D., & Weiss, J. (2007). Structural and functional changes in ultrasonicated bovine serum albumin solutions. *Ultrasonics Sonochemistry*, *14*, 173−183.

Huppertz, T., Fox, P. F., & Kelly, A. L. (2004). Dissociation of caseins in high pressure-treated bovine milk. *International Dairy Journal*, *14*, 675−680.

Kaur, S., & Das, M. (2011). Functional foods: An overview. *Food Science and Biotechnology*, *20*, 861−875.

Kleinig, A. R., & Middelberg, A. P. J. (1998). On the mechanism of microbial cell disruption in high-pressure homogenisation. *Chemical Engineering Science*, *53*, 891−898.

Knirsch, M. C., Alves Dos Santos, C., Martins De Oliveira Soares Vicent, A. A., & Vessoni Penna, T. C. (2010). Ohmic heating − a review. *Trends in Food Science & Technology*, *21*, 436−441.

Knockaert, G., Lemmens, L., Van Buggenhout, S., Hendrickx, M., & Van Loey, A. (2012). Changes in β-carotene bioaccessibility and concentration during processing of carrot puree. *Food Chemistry*, *133*, 60−67.

Li, Y., Chen, Z., & Mo, H. (2007). Effects of pulsed electric fields on physicochemical properties of soybean protein isolates. *LWT - Food Science and Technology*, *40*, 1167−1175.

Lopez-Sanchez, P., Svelander, C., Bialek, L., Schumm, S., & Langton, M. (2011). Rheology and microstructure of carrot and tomato emulsions as a result of high-pressure homogenization conditions. *Journal of Food Science*, *76*, E130−E140.

Ma, S., & Wang, Z. H. (2013). Pulsed electric field-assisted modification of pectin from sugar beet pulp. *Carbohydrate Polymers*, *92*, 1700−1704.

Martinez-Flores, H. E., Garnica-Romo, M. G., Bermudez-Aguirre, D., Pokhrel, P. R., & Barbosa-Canovas, G. V. (2015). Physico-chemical parameters, bioactive compounds and microbial quality of thermosonicated carrot juice during storage. *Food Chemistry*, *172*, 650−656.

Mcclements, D. J. (1995). Advances in the application of ultrasound in food analysis and processing. *Trends in Food Science & Technology*, *6*, 293−299.

Mercali, G. D., Jaeschke, D. P., Tessaro, I. C., & Marczak, L. D. F. (2012). Study of vitamin C degradation in acerola pulp during ohmic and conventional heat treatment. *LWT - Food Science and Technology*, *47*, 91−95.

Mercali, G. D., Schwartz, S., Marczak, L. D., Tessaro, I. C., & Sastry, S. (2014). Effect of the electric field frequency on ascorbic acid degradation during thermal treatment by ohmic heating. *Journal of Agricultural and Food Chemistry*, *62*, 5865−5870.

Odriozola-Serrano, I., Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2013). Pulsed electric fields processing effects on quality and health-related constituents of plant-based foods. *Trends in Food Science & Technology*, *29*, 98−107.

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Impact of high-intensity pulsed electric fields variables on vitamin C, anthocyanins and antioxidant capacity of strawberry juice. *LWT - Food Science and Technology*, *42*, 93−100.

Pankaj, S. K., Bueno-Ferrer, C., Misra, N. N., Milosavljević, V., O'donnell, C. P., Bourke, P., & Cullen, P. J. (2014). Applications of cold plasma technology in food packaging. *Trends in Food Science & Technology*, *35*, 5−17.

Perez, O. E., & Pilosof, A. M. R. (2004). Pulsed electric fields effects on the molecular structure and gelation of β-lactoglobulin concentrate and egg white. *Food Research International*, *37*, 102−110.

Pingret, D., Fabiano-Tixier, A.-S., & Chemat, F. (2013). Degradation during application of ultrasound in food processing: A review. *Food Control*, *31*, 593−606.

Qin, Z., Guo, X., Lin, Y., Chen, J., Liao, X., Hu, X., & Wu, J. (2013). Effects of high hydrostatic pressure on physicochemical and functional properties of walnut (*Juglans regia* L.) protein isolate. *Journal of the Science of Food and Agriculture*, *93*, 1105−1111.

Rastogi, N. K., Raghavarao, K. S., Balasubramaniam, V. M., Niranjan, K., & Knorr, D. (2007). Opportunities and challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition*, *47*, 69−112.

Rawson, A., Tiwari, B. K., Patras, A., Brunton, N., Brennan, C., Cullen, P. J., & O'donnell, C. (2011). Effect of thermosonication on bioactive compounds in watermelon juice. *Food Research International*, *44*, 1168−1173.

Riener, J., Noci, F., Cronin, D. A., Morgan, D. J., & Lyng, J. G. (2009). Characterisation of volatile compounds generated in milk by high intensity ultrasound. *International Dairy Journal*, *19*, 269−272.

Rodríguez-Roque, M. J., De Ancos, B., Sánchez-Moreno, C., Cano, M. P., Elez-Martínez, P., & Martín-Belloso, O. (2015). Impact of food matrix and processing on the in vitro bioaccessibility of vitamin C, phenolic compounds, and hydrophilic antioxidant activity from fruit juice-based beverages. *Journal of Functional Foods*, *14*, 33−43.

Ruenroengklin, N., Yang, B., Lin, H., Chen, F., & Jiang, Y. (2009). Degradation of anthocyanin from litchi fruit pericarp by H2O2 and hydroxyl radical. *Food Chemistry*, *116*, 995−998.

Saberian, H., Hamidi Esfahani, Z., & Abbasi, S. (2015). Effect of conventional and ohmic pasteurization on some bioactive components of *Aloe vera* gel juice. *Iranian Journal of Chemistry and Chemical Engineering*, *34*, 99−108.

Sánchez-Vega, R., Elez-Martínez, P., & Martín-Belloso, O. (2015). Influence of high-intensity pulsed electric field processing parameters on antioxidant compounds of broccoli juice. *Innovative Food Science & Emerging Technologies*, *29*, 70−77.

Segat, A., Misra, N. N., Cullen, P. J., & Innocente, N. (2015). Atmospheric pressure cold plasma (ACP) treatment of whey protein isolate model solution. *Innovative Food Science & Emerging Technologies*, *29*, 247−254.

Shi, X.-M., Zhang, G.-J., Wu, X.-L., Li, Y.-X., Ma, Y., & Shao, X.-J. (2011). Effect of low-temperature plasma on microorganism inactivation and quality of freshly squeezed orange juice. *Ieee Transactions on Plasma Science*, *39*, 1591−1597.

Soliva-Fortuny, R., Balasa, A., Knorr, D., & Martín-Belloso, O. (2009). Effects of pulsed electric fields on bioactive compounds in foods: a review. *Trends in Food Science & Technology*, *20*, 544−556.

Soria, A. C., & Villamiel, M. (2010). Effect of ultrasound on the technological properties and bioactivity of food: a review. *Trends in Food Science & Technology*, *21*, 323−331.

Speroni, F., Jung, S., & De Lamballerie, M. (2010). Effects of calcium and pressure treatment on thermal gelation of soybean protein. *Journal of Food Science*, *75*, E30−E38.

Suarez-Jacobo, A., Rufer, C. E., Gervilla, R., Guamis, B., Roig-Sagues, A. X., & Saldo, J. (2011). Influence of ultra-high pressure homogenisation on antioxidant capacity, polyphenol and vitamin content of clear apple juice. *Food Chemistry*, *127*, 447−454.

Sui, Q., Roginski, H., Williams, R. P. W., Versteeg, C., & Wan, J. (2011). Effect of pulsed electric field and thermal treatment on the physicochemical and functional properties of whey protein isolate. *International Dairy Journal*, *21*, 206−213.

Surowsky, B., Schlüter, O., & Knorr, D. (2014). Interactions of nonthermal atmospheric pressure plasma with solid and liquid food systems: A review. *Food Engineering Reviews*, *7*, 82−108.

Svelander, C. A., Lopez-Sanchez, P., Pudney, P. D., Schumm, S., & Alminger, M. A. (2011). High pressure homogenization increases the in vitro bioaccessibility of alpha- and beta-carotene in carrot emulsions but not of lycopene in tomato emulsions. *Journal of Food Science*, *76*, H215−H225.

Tammineedi, C. V. R. K., Choudhary, R., Perez-Alvarado, G. C., & Watson, D. G. (2013). Determining the effect of UV-C, high intensity ultrasound and nonthermal atmospheric plasma treatments on reducing the allergenicity of α-casein and whey proteins. *LWT - Food Science and Technology*, *54*, 35−41.

Terefe, N. S., Buckow, R., & Versteeg, C. (2015). Quality-related enzymes in plant-based products: effects of novel food processing technologies part 2: pulsed electric field processing. *Critical Reviews in Food Science and Nutrition*, *55*, 1−15.

Thirumdas, R., Sarangapani, C., & Annapure, U. S. (2014). Cold plasma: A novel nonthermal technology for food processing. *Food Biophysics*, *10*, 1−11.

Tiwari, B. K., O'donnell, C. P., Patras, A., & Cullen, P. J. (2008). Anthocyanin and ascorbic acid degradation in sonicated strawberry juice. *Journal of Agricultural and Food Chemistry*, *56*, 10071−10077.

Toro-Funes, N., Bosch-Fuste, J., Veciana-Nogues, M. T., & Vidal-Carou, M. C. (2014a). Effect of ultra high pressure homogenization treatment on the bioactive compounds of soya milk. *Food Chemistry*, *152*, 597−602.

Toro-Funes, N., Bosch-Fuste, J., Veciana-Nogues, M. T., & Vidal-Carou, M. C. (2014b). Influence of ultra-high-pressure homogenization treatment on the phytosterols, tocopherols, and polyamines of almond beverage. *Journal of Agricultural and Food Chemistry*, *62*, 9539−9543.

Valdramidis, V. P., Cullen, P. J., Tiwari, B. K., & O'donnell, C. P. (2010). Quantitative modelling approaches for ascorbic acid degradation and nonenzymatic browning of orange juice during ultrasound processing. *Journal of Food Engineering*, *96*, 449−454.

Van Der Plancken, I., Van Loey, A., & Hendrickx, M. E. (2007). Foaming properties of egg white proteins affected by heat or high pressure treatment. *Journal of Food Engineering*, *78*, 1410–1426.

Varghese, K. S., Pandey, M. C., Radhakrishna, K., & Bawa, A. S. (2014). Technology, applications and modelling of ohmic heating: A review. *Journal of Food Science and Technology*, *51*, 2304–2317.

Velázquez-Estrada, R. M., Hernández-Herrero, M. M., Rüfer, C. E., Guamis-López, B., & Roig-Sagués, A. X. (2013). Influence of ultra high pressure homogenization processing on bioactive compounds and antioxidant activity of orange juice. *Innovative Food Science & Emerging Technologies*, *18*, 89–94.

Wan, J., Mawson, R., Ashokkumar, M., Ronacher, K., Coventry, M. J., Roginski, H., & Versteeg, C. (2005). Emerging processing technologies for functional foods. *Australian Journal of Dairy Technology*, *60*, 167–169.

Wu, L., Zhao, W., Yang, R., & Chen, X. (2014). Effects of pulsed electric fields processing on stability of egg white proteins. *Journal of Food Engineering*, *139*, 13–18.

Wu, L., Zhao, W., Yang, R., & Yan, W. (2015). Pulsed electric field (PEF)-induced aggregation between lysozyme, ovalbumin and ovotransferrin in multi-protein system. *Food Chemistry*, *175*, 115–120.

Ye, A., Anema, S. G., & Singh, H. (2004). High-pressure-induced interactions between milk fat globule membrane proteins and skim milk proteins in whole milk. *Journal of Dairy Science*, *87*, 4013–4022.

Yildiz-Turp, G., Sengun, I. Y., Kendirci, P., & Icier, F. (2013). Effect of ohmic treatment on quality characteristic of meat: A review. *Meat Science*, *93*, 441–448.

Zeng, X.-A., Han, Z., & Zi, Z.-H. (2010). Effects of pulsed electric field treatments on quality of peanut oil. *Food Control*, *21*, 611–614.

Zhang, Y., Liao, X., Ni, Y., Wu, J., Hu, X., Wang, Z., & Chen, F. (2006). Kinetic analysis of the degradation and its color change of cyanidin-3-glucoside exposed to pulsed electric field. *European Food Research and Technology*, *224*, 597–603.

Zhao, W., Tang, Y., Lu, L., Chen, X., & Li, C. (2013). Review: Pulsed electric fields processing of protein-based foods. *Food and Bioprocess Technology*, *7*, 114–125.

Zhao, W., Yang, R., Shi, X., Pan, K., Zhang, S., Zhang, W., & Hua, X. (2011). Oxidation of oleic acid under pulsed electric field processing. *Food Research International*, *44*, 1463–1467.

Zhao, W., Yang, R., Tang, Y., Zhang, W., & Hua, X. (2009). Investigation of the protein-protein aggregation of egg white proteins under pulsed electric fields. *Journal of Agricultural and Food Chemistry*, *57*, 3571–3577.

Zhao, W., Yang, R., & Zhang, H. Q. (2012). Recent advances in the action of pulsed electric fields on enzymes and food component proteins. *Trends in Food Science & Technology*, *27*, 83–96.

Index

Note: Page numbers followed by "*f*" and "*t*" refer to figures and tables, respectively.

D

E

F

G

H

I

L

M

N

O

P

Q

R

S

T

U

Nutraceutical and Functional Food Components

Effects of Innovative Processing Techniques

Edited by
Charis M. Galanakis, PhD, R&I Director at Galanakis Laboratories, Chania, Greece

Nutraceutical and Functional Food Components: Effects of Innovative Processing Techniques explains the impacts recent food industry advances have on different parameters of food components. The effects of emerging technologies on nutritional value, physical, chemical, and functional properties, as well as on bioavailability, bioaccessibility, and bioactivity characteristics are examined by international experts. Nutraceutical and functional food applications, shelf-life, sensory characteristics are also explored so that researchers and product developers can understand the effects of nonthermal, extraction, and processing techniques. The chemistry, biochemistry, metabolism, and health-promoting activities of disparate food components are reflected in the literature.

Key Features

- Targets key food components like proteins, amino acids, peptides, carbohydrates, starch, dietary fiber, minerals, vitamins, polyphenols, carotenoids, and aroma compounds
- Describes in details the effect of emerging technologies on nutritional value, bioavailability, bioaccessibility, and bioactivity of functional ingredients
- Uses an integrated approach to provide a holistic view of the interactions between novel processing techniques and food components
- Explains how innovative and nonthermal techniques advance extraction, processing, and formulation of the above components
- Covers sustainable food applications, shelf-life, and sensory characteristics

About the Editor

Charis M. Galanakis is a dynamic and interdisciplinary scientist with a fast-expanding work that balances between food and environment, industry, and academia. His research targets mainly the separation and recovery of functional macro- and micro-molecules from different food by-products, as well as their implementation as additives in food and other products. He is the research and innovation director of Galanakis Laboratories (Chania, Greece) and the coordinator of Food Waste Recovery Group of ISEKI Food Association (Vienna, Austria). He serves an editorial board member and subject editor of *Food and Bioproducts Processing and Food Research International*, and he has edited the books entitled *Food Waste Recovery: Processing Technologies and Techniques* (Academic Press, 2015), *Innovation Strategies in the Food Industry: Tools For Implementation* (Academic Press, 2016), and *Olive Mill Waste: Recent Advances for the Sustainable Management* (Academic Press, 2017).

ELSEVIER

ACADEMIC PRESS
An imprint of Elsevier
elsevier.com

ISBN 978-0-12-805257-0